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[Fortsetzung auf der nächsten Seite]

(54) Title: NOVEL BINDER-DRUG CONJUGATES (ADCS) AND THEIR USE

(54) Bezeichnung : NEUE BINDER-WIRKSTOFF KONJUGATE (ADCS) UND IHRE VERWENDUNG

(57) Abstract: The present application relates to novel, anti-Target Epidermal Growth Factor Receptor (EGFR, GeneID1956) binder-drug conjugates (ADCs) of N,N-dialkyl auristatins, to effective metabolites of said ADCs, to methods for producing said ADCs, to the use of said ADCs for treating and/or preventing diseases, and to the use of said ADCs for producing pharmaceuticals for treating and/or preventing diseases, in particular hyperproliferative and/or angiogenic diseases, such as cancers. Such treatments can be carried out as monotherapy or in combination with other pharmaceuticals or additional therapeutic measures.

(57) Zusammenfassung: Die vorliegende Anmeldung betrifft neue, gegen das Target Epidermal Growth Factor Receptor (EGFR, GeneID1956) gerichtete Binder-Wirkstoff-Konjugate (ADCs) von N,N-Dialkylauristatinen, wirksamer Metabolite dieser ADCs, Verfahren zur Herstellung dieser ADCs, die Verwendung dieser ADCs zur Behandlung und/oder Prävention von Krankheiten sowie die Verwendung dieser ADCs zur Herstellung von Arzneimitteln zur Behandlung und/oder Prävention von Krankheiten, insbesondere von hyperproliferativen und/oder angiogenen Erkrankungen wie beispielsweise Krebserkrankungen. Solche Behandlungen können als Monotherapie oder auch in Kombination mit anderen Arzneimitteln oder weiteren therapeutischen Maßnahmen erfolgen.

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NovelBinder-Drug Conjugates (ADCs) and Use of Same

The present application relates to novelbinder-drug conjugates (antibody-drug conjugates, ADCs) of N,N-dialkylauristatins, in particular those directed against the target epidermal growth factor receptor (EGFR, gene ID 1956), active metabolites of these ADCs, methods of synthesis
5 of these ADCs, use of these ADCs for treatment and/or prevention of diseases and use of these ADCs for production of drugs for treatment and/or prevention of diseases, in particular hyperproliferative and/or angiogenic diseases, such as the various forms of cancer, for example. Such treatments may be administered as monotherapy or in combination with other drugs or other therapeutic measures.

10 Cancer is the result of uncontrolled cell growth of a wide variety of tissues. In many cases, the cells grow into the existing tissue (invasive growth) or metastasize to remote organs. Cancer occurs in a wide variety of organs and the pathology often has a tissue-specific course. The term cancer is therefore a generic term that describes a large group of specific diseases of various organs, tissues and types of cells.

15 Early-stage tumors can in some cases be removed by surgical and radiotherapeutic measures. Metastatic tumors can usually be treated only palliatively by chemotherapeutic agents. The goal here is to find the optimum combination of improving the quality of life and prolonging life.

Most of the chemotherapeutic agents administered parenterally today are not distributed to the tumor tissue or tumor cells in a targeted manner but instead are nonspecifically distributed
20 throughout the patient's body through systemic administration, i.e., at sites where exposure to the drug is often undesirable, such as in healthy cells, tissues and organs, for example. This may lead to adverse effects or even serious general toxic effects, which then often severely limit the therapeutically usable drug dosage range or necessitate complete cessation of the medication.

The improved and selective availability of these chemotherapeutic agents in the tumor cell or the
25 immediate surrounding tissue and the associated increase in effect, on the one hand, and minimization of toxic side effects, on the other hand, have therefore for many years been the focus of work in developing new chemotherapeutic drugs. There have been numerous attempts so far to develop efficient methods for introducing drugs into the target cell. However, it is still a difficult task to optimize the association between the drug and the intracellular target and to
30 minimize the intercellular distribution of the drug, e.g., to neighboring cells.

Monoclonal antibodies, for example, are suitable for targeted addressing of tumor tissue and tumor cells. The importance of such antibodies for clinical treatment of cancer has grown enormously in recent years based on the efficacy of such agents as trastuzumab (Herceptin), rituximab (Rituxan), cetuximab (Erbix) and bevacizumab (Avastin) which have been approved in the meantime for treatment of individual specific tumor conditions (see, for example, G. P. Adams and L. M. Weiner, *Nat. Biotechnol.* **23**, 1147-1157 (2005)). As a result, there has been a significant increase in interest in so-called immunoconjugates, such as the aforementioned ADCs, for example, in which an internalizing antibody directed against a tumor-associated antigen is bound covalently to a cytotoxic agent by a linking unit ("linker"). After introducing the ADCs into the tumor cell and then splitting off the conjugate, either the cytotoxic agent itself or another cytotoxic metabolite formed from it is then released inside the tumor cell, where it can manifest its effect directly and selectively. In this way, the damage to normal tissue can be kept within significantly narrower limits in comparison with conventional chemotherapy for cancer (see, for example, J. M. Lambert, *Curr. Opin. Pharmacol.* **5**, 543-549 (2005); A. M. Wu and P. D. Senter, *Nat. Biotechnol.* **23**, 1137-1146 (2005); P. D. Senter, *Curr. Opin. Chem. Biol.* **13**, 235-244 (2009); L. Ducry and B. Stump, *Bioconjugate Chem.* **21**, 5-13 (2010)).

Instead of antibodies, binders from the field of small drug molecules may be used as binders to selectively bind to a specific target, such as, for example, a receptor (see, e.g., E. Ruoslahti *et al.*, *Science*, **279**, 377-380 (1998); D. Karkanet *et al.*, *PLoS ONE* **3** (6), e2469 (June 25, 2008)). Conjugates of a cytotoxic drug and an addressing ligand having a defined cleavage site between the ligand and the drug for release of the drug are also known. One such "intended breaking point" may consist of a peptide chain, for example, which can be cleaved selectively at a certain site by a specific enzyme at the site of action (see, for example, R. A. Firestone and L. A. Telan, US Patent Application US 2002/0147138).

Monoclonal antibodies are suitable in particular for targeted address of tumor tissues and tumor cells, especially those directed against the target EGFR. The "epidermal growth factor receptor" (EGFR, gene ID 1956) is a trans-membrane glycoprotein (170 kDa) belonging to the tyrosine kinase subfamily. Although the EGF receptor is expressed in many normal cells, it is overexpressed in many forms of human cancer, including cancer of the large and small intestine, carcinomas of the head and neck, pancreatic cancer and gliomas. The extent of this overexpression correlates with a poor prognosis (Galizia, G. *et al.*, *Ann. Surg. Oncol.*, June 2006, **13**(6):823-35).

Binding of the ligand EGF to the EGF receptor leads to dimerization of the receptor and activation of the intracellular kinase domains. These kinase domains undergo autophosphory-

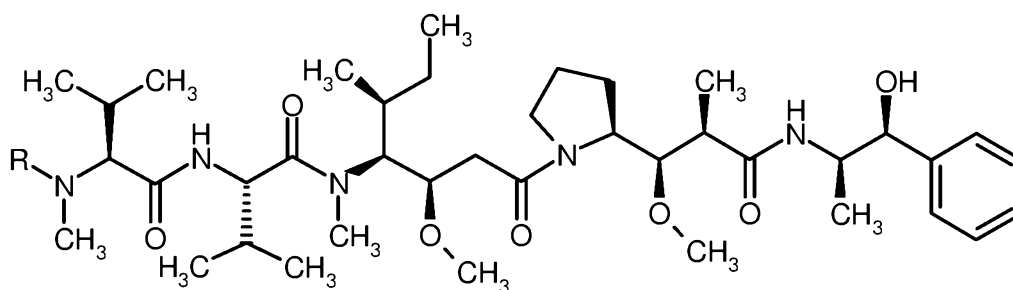
lation and thus activate pro-proliferative signal cascades (including those via mitogen-activated protein kinases (MAPKs) and Akt). These signal cascades regulate the transcription of genes involved in cell growth and cell survival, motility and proliferation.

Signal transduction by the EGF receptor also results in activation of the wild-type KRAS gene, but the presence of an activating somatic mutation in the KRAS gene within a cancer cell leads to dysregulation of the signal pathways and to resistance to EGFR inhibitory treatments (Allegra *et al.*, *J. Clin. Oncol.*, 20 April 2009, **27**(12):2091-6).

In an ADC approach, an additional antitumor effect can be achieved by the attached cytotoxic agent in addition to inhibiting the interaction between ligands and receptor.

10 The following publications describe the EGF receptor and anti-EGFR antibodies in general:
WO 00069459A1,WO2010145796A2,WO02100348A2, EP00979246B1,EP00531472B1,
Mendelsohn, J., Baselga, J., *Oncogene* (2000) **19**, 6550-6565;M.L. Janmaat and G. Giaccone,
Drugs of Today, Vol. 39, Suppl. C, 2003, pp. 61-80;Normanno. N., *et al.*, *Gene*, Jan 17, 2006,
366(1):2-16, Epidermal growth factor receptor (EGFR) signaling in cancer.

15 Auristatin E (AE) and monomethylauristatin E (MMAE) are synthetic analogs of the dolastatins,
a special group of linear pseudopeptides, which were originally isolated from marine sources,
and some of which have a very potent cytotoxic activity with respect to tumor cells (for an
overview, see, for example, G. R. Pettit, *Prog. Chem. Org. Nat. Prod.* **70**, 1-79 (1997); G. R.
Pettit *et al.*, *Anti-Cancer Drug Design* **10**, 529-544 (1995); G. R. Pettit *et al.*, *Anti-Cancer Drug*
20 *Design* **13**, 243-277 (1998)).



Auristatin E (AE): $R = CH_3$

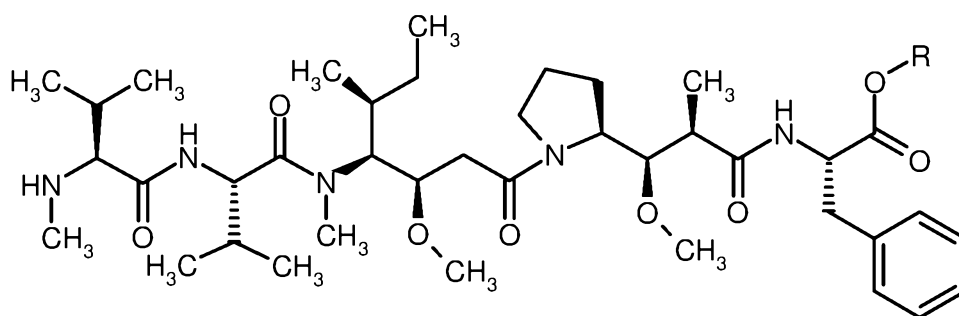
Monomethylauristatin E (MMAE): R = H

However, MMAE has the disadvantage of a comparatively high systemic toxicity. To improve the tumor selectivity, MMAE is used for targeted tumor therapy in conjunction with enzymatically cleavable valine-citrulline linkers in the ADC setting in particular

(WO 2005/081711 A2; S. O. Doronima *et al.*, *Bioconjugate Chem.* **17**, 114-124 (2006)). After proteolytic cleavage, MMAE is preferably released from the corresponding ADCs intracellularly.

However, when used in the form of antibody-drug conjugates (ADCs), MMAE is not compatible with linking units (linkers) between the antibody and the drug, which do not have any enzymatically cleavable intended breaking point (S. O. Doronina *et al.*, *Bioconjugate Chem.* **17**, 114-124 (2006)).

Monomethyl auristatin F (MMAF) is an auristatin derivative with a C-terminal phenylalanine unit having only a moderate antiproliferative effect in comparison with MMAE. This can very likely be attributed to the free carboxyl group, which has a negative effect on the cell viability of this compound because of its polarity and charge. In this context, the methyl ester of MMAF (MMAF-OMe) has been described as a prodrug derivative, which has a neutral charge and can pass through the cell membrane; it also has an increased *in vitro* cytotoxicity, which is greater by several orders of magnitude in comparison with MMAF with respect to various carcinoma cell lines (S. O. Doronina *et al.*, *Bioconjugate Chem.* **17**, 114-124 (2006)). It may be assumed that this effect is caused by the MMAF itself, which is rapidly released by intracellular ester hydrolysis after the prodrug has been incorporated into the cells.



Monomethylauristatin F (MMAF): R = H

Monomethylauristatin F-methylester (MMAF-OMe): R = CH₃

However, drug compounds based on simple ester derivatives are generally at risk of chemical instability due to a nonspecific ester hydrolysis, which is independent of the intended site of action, for example, due to esterases present in blood plasma. This can greatly restrict the usability of such compounds in treatment.

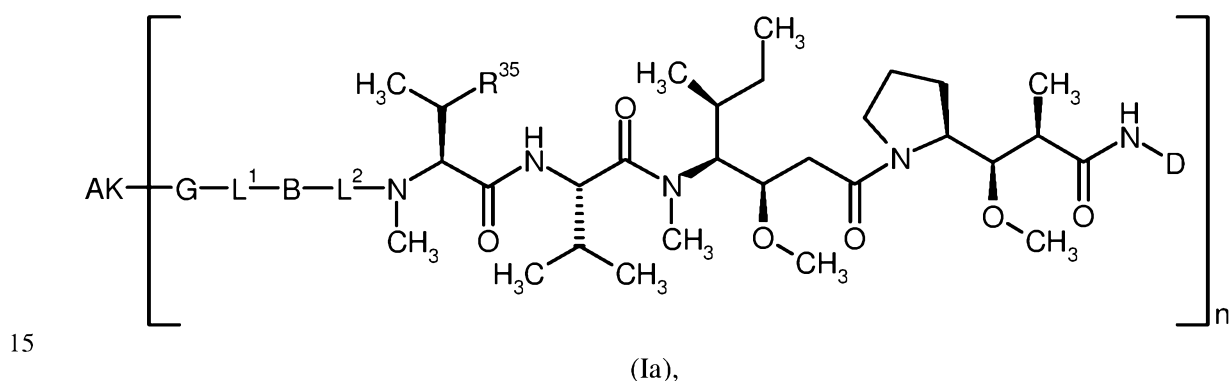
Monomethyl auristatin F (MMAF) as well as various esters and amide derivatives thereof were disclosed in WO 2005/081711 A2. Additional auristatin analogs having a C-terminal amide-substituted phenylalanine unit are described in WO 01/18032 A2. MMAF analogs involving side chain modifications of phenylalanine are claimed in WO 02/088172 A2 and WO 2007/008603

A1. WO 2007/008848 A2 describes those in which the carboxyl group of phenylalanine is modified. Auristatin conjugates linked via the C-terminus were recently described in WO 2009/117531 A1 (see also S. O. Doronina *et al.*, *Bioconjugate Chem.* **19**, 1960-1963 (2008)).

5 In addition, auristatin derivatives such as MMAE and MMAF are also substrates for transporter proteins, which are expressed by many tumor cells, which can lead to development of resistance to these drugs.

The object of the present invention was to provide novel binder-drug conjugates (ADCs) which, due to the combination of novel N,N-dialkylauristatin derivatives with suitable novel linkers and binders, have a very attractive profile of effects with regard to their specific tumor effect and/or the lower potential of the metabolites formed intracellularly as a substrate with respect to transporter proteins, for example, and are therefore suitable for treatment and/or prevention of hyperproliferative and/or angiogenic diseases, e.g., cancers.

The subject matter of the present invention is binder-drug conjugates of the general formula (Ia)



in which

n stands for a number from 1 to 50.

AK stands for a binder, preferably a chimeric humanized or human antibody, especially preferably an anti-EGFR antibody,

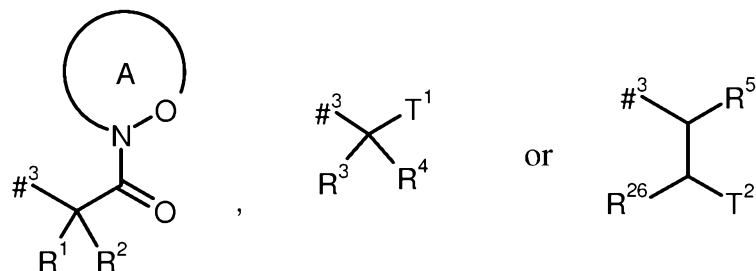
the group $\S\text{-G-L}^1\text{-B-L}^2\text{-}\S$ stands for a linker,

wherein

§ denotes the linkage site to the group AK and

§§ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

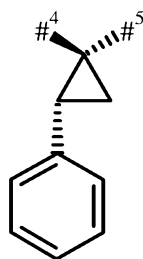
5 $\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen or methyl,

10 R^2 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

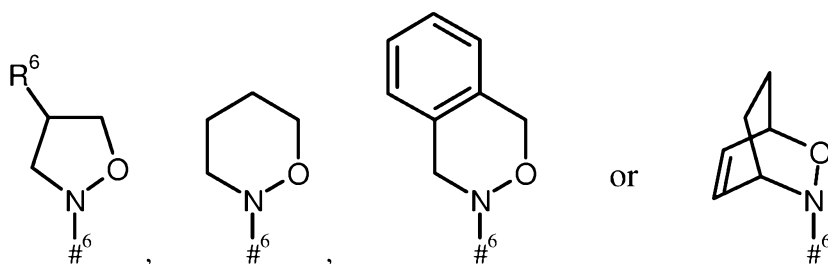


15 wherein

$\#^4$ denotes the linkage site to the vicinal nitrogen atom,

$\#^5$ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

5 $\#^6$ denotes the linkage site to the carbonyl group,

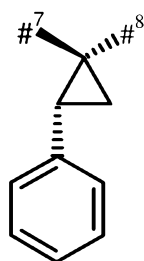
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

10 R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



15 wherein

$\#^7$ denotes the linkage site to the vicinal nitrogen atom,

$\#^8$ denotes the linkage site to the group T^1 ,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

5 R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R⁸ stands for hydrogen or methyl,

R⁹ stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

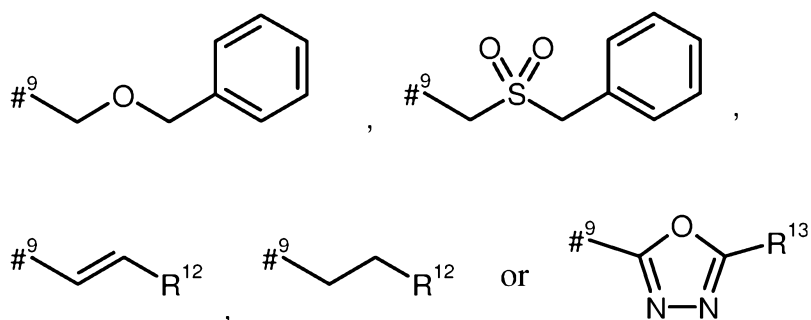
or

10 R⁸ and R⁹ together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R¹⁰ stands for benzoyl,

R¹¹ stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R⁵ stands for hydrogen, methyl or a group of the formula



wherein

#⁹ denotes the linkage site to -CHC(R²⁶)-T²,

R¹² stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula -S(O)₂OH,

20 R¹³ stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R²⁶ stands for hydrogen or hydroxyl,

T² stands for phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R³⁵ stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

5 Compounds according to the invention include the compounds of formula (I) and their salts and solvates as well as the solvates of the salts, the compounds of the formulas given below, covered by formula (I), and their salts and solvates as well as the solvates of the salts as well as the compounds covered by formula (I) and referred to below as exemplary embodiments as well as their salts and solvates as well as the solvates of the salts inasmuch as the compounds covered by
10 formula (I) and listed below are not already the salts and solvates as well as the solvates of the salts.

The compounds according to the invention may exist in different stereoisomeric forms depending on their structure, i.e., in the form of configurational isomers or optionally also as conformational isomers (enantiomers and/or diastereomers, including those in atropisomers). The present
15 invention therefore includes the enantiomers and diastereomers and their respective mixtures. The stereoisomerically uniform components can be isolated in a known way from such mixtures of enantiomers and/or diastereomers. Chromatographic methods, in particular HPLC chromatography on a chiral or achiral phase, are preferably used for this purpose.

If the compounds according to the invention can occur in tautomeric forms, then the present
20 invention also includes all tautomeric forms.

The present invention also includes all suitable isotope variants of the compounds according to the invention. Isotope variants of a compound according to the invention are understood here to refer to a compound, in which at least one atom within the compound according to the invention is exchanged with another atom of the same ordinal number but with a different atomic mass
25 than the atomic mass normally or mainly occurring in nature. Examples of isotopes that may be incorporated into a compound according to the invention include those of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, chlorine, bromine and iodine such as ²H (deuterium), ³H (tritium), ¹³C, ¹⁴C, ¹⁵N, ¹⁷O, ¹⁸O, ³²P, ³³P, ³³S, ³⁴S, ³⁵S, ³⁶S, ¹⁸F, ³⁶Cl, ⁸²Br, ¹²³I, ¹²⁴I, ¹²⁹I and ¹³¹I. Certain isotope variants of a compound according to the invention, such as in
30 particular those in which one or more radioactive isotopes are incorporated, may be beneficial for investigating the mechanism of action or the distribution of the drug in the body, for example. Compounds labeled with ³H or ¹⁴C isotopes are especially suitable for this purpose because of

their comparative ease of synthesizing and detection. In addition, the implantation of isotopes, such as deuterium, for example, may lead to certain therapeutic advantages as a result of a greater metabolic stability of the compound, such as prolonging the half-life in the body, for example, or reducing the required active dose. Therefore, such modifications of the compounds according to the invention may optionally also be preferred embodiments of the present invention. Isotope variants of the compounds according to the invention can be synthesized by the methods known to those skilled in the art, for example, according to the methods described below and the procedures given in the exemplary embodiments by using the corresponding isotopic modifications of the respective reagents and/or starting compounds.

10 Within the scope of the present invention, the preferred salts are the physiologically safe salts of the compounds according to the invention. This also includes salts that are not suitable for pharmaceutical applications per se but may be used for isolating or purifying the compounds according to the invention, for example.

Physiologically safe salts of the compounds according to the invention include acid addition salts of mineral acids, carboxylic acids and sulfonic acids, for example, salts of hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methane sulfonic acid, ethane sulfonic acid, benzene sulfonic acid, toluene sulfonic acid, naphthalene disulfonic acid, acetic acid, trifluoroacetic acid, propionic acid, lactic acid, tartaric acid, malic acid, citric acid, fumaric acid, maleic acid and benzoic acid.

20 Physiologically safe salts of the compounds according to the invention also include the salts of conventional bases such as preferably and for example, alkali metal salts (e.g., sodium and potassium salts), alkaline earth salts (e.g., calcium and magnesium salts) and ammonium salts derived from ammonia or organic amines with 1 to 16 carbon atoms, such as preferably and for example, ethylamine, diethylamine, triethylamine, ethyl diisopropylamine, monoethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, dimethylaminoethanol, procaine, 25 dibenzoylamine, N-methylpiperidine, N-methylmorpholine, arginine, lysine and 1,2-ethylene-diamine.

Within the scope of the invention, the solvates refer to forms of the compounds according to the invention which form a complex in the solid or liquid state by coordination with solvent molecules. Hydrates are a special form of solvates having coordinated molecules of water. Hydrates are the preferred solvates within the scope of the present invention.

Furthermore, the present invention also includes prodrugs of the compounds according to the invention. The term "prodrugs" here refers to compounds which may be biologically active or

inactive themselves but are converted to the compounds according to the invention during their dwell time in the body (for example, metabolically or hydrolytically).

Within the scope of the present invention, the substituents have the following meanings, unless otherwise specified:

- 5 (C₁-C₄)-Alkyl within the scope of the invention stands for a linear or branched alkyl radical with 1 to 4 carbon atoms. The following can be mentioned, preferably and for example: methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, 1-methylpropyl and *tert*-butyl.

- Alkanediyl within the scope of the invention stands for a linear α,ω -divalent alkyl radical having the number of carbon atoms indicated in each case. The following can be mentioned, preferably
10 and for example: methylene, ethane-1,2-diyl (1,2-ethylene), propane-1,3-diyl (1,3-propylene), butane-1,4-diyl (1,4-butylene), pentane-1,5-diyl (1,5-pentylene), hexane-1,6-diyl (1,6-hexylene), heptane-1,7-diyl (1,7-hexylene), octane-1,8-diyl (1,8-octylene), nonane-1,9-diyl (1,9-nonylene), decane-1,10-diyl (1,10-decylene).

- (C₃-C₇)-Cycloalkyl and/or three- to seven-membered carbocycle within the scope of the invention
15 stands for a monocyclic saturated cycloalkyl group with 3 to 7 carbon atoms. The following can be mentioned preferably and for example: cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

- The side group of an α -amino acid in the R¹⁹ meaning includes both the side groups of the naturally occurring α -amino acids and the side groups of the homologs and isomers of these
20 α -amino acids. The α -amino acid may be present in both the L- and D-configurations or as a mixture of these L- and D-forms. Examples of side groups that can be mentioned include: methyl (alanine), propan-2-yl (valine), propan-1-yl (norvaline), 2-methylpropan-1-yl (leucine), 1-methylpropan-1-yl (isoleucine), butan-1-yl (norleucine), *tert*-butyl (2-*tert*-butylglycine), phenyl (2-phenylglycine), benzyl (phenylalanine), p-hydroxybenzyl (tyrosine), indol-3-ylmethyl
25 (tryptophan), imidazol-4-ylmethyl (histidine), hydroxymethyl (serine), 2-hydroxyethyl (homoserine), 1-hydroxyethyl (threonine), mercaptomethyl (cysteine), methylthiomethyl (S-methylcysteine), 2-mercaptoethyl (homocysteine), 2-methylthioethyl (methionine), carbamoylmethyl (asparagine), 2-carbamoyl ethyl (glutamine), carboxymethyl (aspartic acid), 2-carboxyethyl (glutamic acid), 4-aminobutan-1-yl (lysine), 4-amino-3-hydroxybutan-1-yl
30 (hydroxylysine), 3-aminopropan-1-yl (ornithine), 2-aminoethyl (2,4-diaminobutyric acid), aminomethyl (2,3-diaminopropionic acid), 3-guanidinopropan-1-yl (arginine), 3-ureidopropan-1-yl (citrulline). Preferred α -amino acid side groups in the meaning of R¹⁹ include methyl (alanine), propan-2-yl (valine), 2-methylpropan-1-yl (leucine), benzyl (phenylalanine),

imidazole-4-ylmethyl (histidine), hydroxymethyl (serine), 1-hydroxyethyl (threonine), 4-aminobutan-1-yl (lysine), 3-aminopropan-1-yl (ornithine), 2-aminoethyl (2,4-diaminobutyric acid), aminomethyl (2,3-diaminopropionic acid), 3-guanidinopropan-1-yl (arginine). The L configuration is preferred in each case.

- 5 A four- to seven-membered heterocycle within the scope of the invention stands for a monocyclic saturated heterocycle having a total of four to seven ring atoms that contain one or two ring heteroatoms from the series of N, O, S, SO and/or SO₂ and are linked via a ring carbon atom or optionally a ring nitrogen atom. A five- to seven-membered heterocycle with one or two ring heteroatoms from the series N, O and/or S, especially preferably a five- or six-membered
- 10 heterocycle with one or two ring heteroatoms from the series of N and/or O is preferred. Examples include: azetidiny, oxetanyl, pyrrolidinyl, pyrazolidinyl, tetrahydrofuranyl, thiolanyl, piperidinyl, piperazinyl, tetrahydropyranyl, tetrahydrothiopyranyl, morpholinyl, thiomorpholinyl, hexahydroazepinyl and hexahydro-1,4-diazepinyl. Preferred examples include pyrrolidinyl, tetrahydrofuranyl, piperidinyl, piperazinyl, tetrahydropyranyl and morpholinyl.
- 15 In the formula for the group for which A, B, D, G, L¹, L², L⁴, R¹, R², R³, R⁴ and/or R⁵ may stand, the end point of the line at which the symbol #⁶, *, **, #³, #¹, #², ##¹, ##², ##³, ##⁴, ***, ****, #⁴, #⁵, #⁶, #⁷, #⁸ and/or #⁹ appears does not stand for a carbon atom or a CH₂ group but instead is a component of the bond to the respective atom identified, to which A, B, D, G, L¹, L², L⁴, R¹, R², R³, R⁴ and/or R⁵ is bound.
- 20 Within the scope of the present invention, it is true that for all radicals that occur several times, their meanings are independent of one another. If radicals are substituted in the compounds according to the invention, then the radicals may be substituted one or more times, unless otherwise specified. Substitution with one or two substituents that are the same or different is preferred. Substitution with one substituent is especially preferred.
- 25 Within the scope of the present invention, the terms that are used have the following meanings, unless otherwise specified:

The term "linker" is understood in the broadest sense to be a chemical unit comprising a covalent bond or a row of atoms covalently linking a binder to adrug. The term "linker" is preferably understood to be a series of atoms in the sense of the present invention, which covalently link a
- 30 binder to adrug. In addition, linkers may be divalent chemical units, such as alkylidyls, arylidyls, heteroarylidyls, heterocyclylidy, dicarboxylic acid esters, dicarboxylic acid amides.

The term "binder" is understood in the broadest sense to be a molecule, which binds to a target molecule that is present on a certain target cell population to be addressed by the binder-drug conjugate. The term "binder" is to be understood in its broadest interpretation, which also includes, for example, lectins, proteins that can bind certain sugar chains or phospholipid binding proteins. Such binders include, for example, high-molecular proteins (binder proteins), polypeptides or peptides (binder peptides), nonpeptidic molecules (e.g., aptamers (US 5,270,163; review article by Keefe, A. D. *et al.*, *Nat. Rev. Drug Discov.* 2010; **9**:537-550) or vitamins) and all other cell-binding molecules or substances. Binder proteins include, for example, antibodies and antibody fragments or antibody mimetics such as affibodies, adnectins, anticalins, DARPins, avimers, nanobodies (review article by Gebauer, M. *et al.*, *Curr. Opinion in Chem. Biol.* 2009; **13**:245-255; Nuttall, S. D. *et al.*, *Curr. Opinion in Pharmacology*, 2008; **8**:608-617). Binder peptides include, for example, ligands of a ligand-receptor pair, e.g., VEGF of the ligand receptor pair VEGF/KDR, such as transferrin of the ligand-receptor pair transferrin/transferrin receptor or a cytokine/cytokine receptor, such as TNF α of the ligand-receptor pair TNF α /TNF α receptor.

Preferred binders according to the invention include antibodies (in particular human or humanized monoclonal antibodies) or antigen binding antibody fragments that bind to EGFR. In the case of antibodies such as anti-EGFR antibodies, n (i.e., the number of toxophore molecules per antibody molecule) is preferably in the range of 1 to 10, especially preferably 2 to 8.

A "target molecule" is understood in the broadest sense to be a molecule, which is present in the target cell population and may be a protein (e.g., a receptor of a growth factor) or a non-peptidic molecule (e.g., a sugar or phospholipid). It is preferably a receptor or an antigen.

The term "extracellular" target molecule describes a target molecule, which is bound to the cell and is found on the outside of a cell or part of a target molecule, which is found on the outside of a cell, i.e., a binder may bind to an intact cell at its extracellular target molecule. An extracellular target molecule may be anchored in the cell membrane or may be part of the cell membrane. Those skilled in the art are familiar with methods for identifying extracellular target molecules. For proteins this may take place by determining the transmembrane domain(s) and though orientation of the protein in the membrane. These specifications are usually stored in the protein data banks (e.g., SwissProt).

The term "cancer target molecule" describes a target molecule, which is present on one or more types of cancer cells in comparison with noncancer cells of the same type of tissue. The cancer target molecule is preferably selectively present on one or more types of cancer cells in comparison with noncancer cells of the same tissue type, where the term "selective" describes an at least two-fold enrichment on cancer cells in comparison with noncancer cells of the same type of tissue (a

"selective cancer target molecule"). Use of cancer target cells allows selective treatment of cancer cells with the conjugates according to the invention.

The binder may be linked to the linker via a bond. Various possibilities of covalent bonding (conjugation) of organic molecules to antibodies are known from the literature. The linkage of the binder may be accomplished by means of a heteroatom of the binder. Heteroatoms of the binder according to the invention that may be used for linkage include sulfur (by means of a sulfhydryl group of the binder in one embodiment), oxygen (by means of a carboxyl or hydroxyl group of the binder according to the invention) and nitrogen (by means of a primary or secondary amine group or amide group of the binder in one embodiment). Conjugation of the toxophores to the antibodies via one or more sulfur atoms of cysteine radicals of the antibody and/or via one or more NH groups of lysine radicals of the antibody is/are preferred according to the invention. These heteroatoms may be present in the natural binder or may be introduced through chemical or molecular biological methods. According to the present invention, the linkage of the binder to the toxophore only has a low influence on the binding activity of the binder to the target molecule. In a preferred embodiment, the linkage has no effect on the binding activity of the binder to the target molecule.

The term "antibody" is understood in its broadest sense according to the present invention and includes immunoglobulin molecules, for example, intact or modified monoclonal antibodies, polyclonal antibodies or multispecific antibodies (e.g., bispecific antibodies). An immunoglobulin molecule preferably comprises a molecule having four polypeptide chains, two heavy chains (H chains) and two light chains (L chains), which are typically linked by disulfide bridges. Each heavy chain comprises one variable domain of the heavy chain (abbreviated VH) and one constant domain of the heavy chain. The constant domain of the heavy chain may comprise, for example, three domains CH1, CH2 and CH3. Each light chain comprises one variable domain (abbreviated VL) and one constant domain. The constant domain of the light chain comprises one domain (abbreviated CL). The VH and VL domains can be further subdivided into regions of hypervariability, also known as complementarity determining regions (abbreviated CDR), and regions of a lower sequence variability ("framework region," abbreviated FR). Each VH and VL region is typically made up of three CDRs and up to four FRs, for example, from the amino terminus to the carboxy terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. An antibody can be obtained from any species suitable for this, e.g., rabbit, llama, camel, mouse or rat. In one embodiment, the antibody is of human or murine origin. An antibody may be human, humanized or chimeric, for example.

The term "monoclonal" antibody refers to antibodies obtained from a population of substantially homogeneous antibodies, i.e., individual antibodies of the population are identical except for naturally occurring mutations which may occur in a small number. Monoclonal antibodies recognize

a single antigenic binding site with a high specificity. The term monoclonal antibody is not based on a specific synthesis process.

The term "intact" antibody relates to antibodies comprising both an antigen binding domain and the constant domain of the light and heavy chains. The constant domain may be a naturally occurring
5 domain or a variant thereof in which several amino acid positions have been altered.

The term "modified intact" antibody refers to intact antibodies that have been fused via their amino terminus or carboxy terminus to another polypeptide or protein that does not originate from an antibody by means of a covalent bond (for example, a peptide linkage). In addition, antibodies may also be modified by inserting reactive cysteines at defined sites to facilitate coupling to a toxophore
10 (see Junutula *et al.*, Nat. Biotechnol., August 2008; **26**(8):925-32).

The term "human" antibody denotes antibodies that can be obtained from a human or are synthetic human antibodies. A "synthetic" human antibody is an antibody that can be obtained entirely or partially by in silico synthesis sequences based on analysis of human antibody sequences. A human antibody may be coded by a nucleic acid, for example, isolated from a library of antibody sequences
15 of human origin. One example of such an antibody is given by Söderlind *et al.*, Nature Biotech. 2000, **18**:853-856.

The term "humanized" or "chimeric" antibody describes antibodies consisting of a human sequence component and a nonhuman sequence component. In these antibodies, a portion of the sequences of the human immunoglobulin (recipient) have been replaced by sequence components of a nonhuman
20 immunoglobulin (donor). The donor is frequently a murine immunoglobulin. In humanized antibodies, amino acids of the CDR of the recipient are replaced by amino acids of the donor. In some cases amino acids of the framework are also replaced by corresponding amino acids of the donor. In many cases the humanized antibody contains amino acids not present in the recipient or donor but inserted during optimization of the antibody. In chimeric antibodies, variable domains of
25 donor immunoglobulin are fused to constant regions of a human antibody.

The term complementarity determining region (CDR) as used here refers to the amino acids of a variable antibody domain, which are necessary for binding to the antigen. A variable region will typically have three CDR regions, which are identified as CDR1, CDR2 and CDR3. Each CDR region may comprise amino acids according to the definition by Kabat and/or amino acids of a
30 hypervariable loop defined according to Chotia. The definition according to Kabat includes, for example, the region of approximately amino acid positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3) of the variable light chain and 31-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3) of the variable heavy chain (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th ed. Public

Health Service, National Institutes of Health, Bethesda, MD (1991)). The definition according to Chotia comprises, for example, approximately the region of amino acid positions 26-32 (CDR1), 50-52 (CDR2) and 91-96 (CDR3) of the variable light chain and 26-32 (CDR1), 53-55 (CDR2) and 96-101 (CDR3) of the variable heavy chain (Chotia and Lesk; J. Mol. Biol. **196**:901-917(1987)). In many cases, a CDR may comprise amino acids from a CDR region as defined by Kabat and Chiotia.

Antibodies can be divided into several various classes, depending on the amino acid sequence of the constant domain of the heavy chain. There are five main classes of intact antibodies: IgA, IgD, IgE, IgG and IgM, several of which can be divided further into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The constant domain of the heavy chain corresponding to the different classes are identified as [alpha/α], [delta/δ], [epsilon/ε], [gamma/γ] and [mu/μ]. Both the three-dimensional structure and the subunit structure of antibodies are known.

The term "functional fragment" or "antigen binding antibody fragment" of an antibody/immunoglobulin is defined as a fragment of an antibody/immunoglobulin (e.g., the variable domains of an IgG), which still comprises the antigen binding domains of the antibody/immunoglobulin. The "antigen binding domain" of an antibody typically comprises one or more hypervariable regions of an antibody, e.g., the CDR, CDR2 and/or CDR3 regions. However, the "framework" region of an antibody may also play a role in binding the antibody to the antigen. The framework region forms the framework for the CDRs. The antigen binding domain preferably comprises at least amino acids 1 through 103 of the variable light chain and amino acids 5 through 109 of the variable heavy chain, more preferably amino acids 3 through 107 of the variable light chain and 4 through 111 of the variable heavy chain, with the complete variable light and heavy chains being especially preferred, i.e., amino acids 1 through 109 of the VL and 1 through 113 of the VH (numbering according to WO 97/08320).

"Functional fragments" or "antigen binding antibody fragments" of the invention comprise not conclusively Fab, Fab', F(ab')₂ and Fv fragments, diabodies, single domain antibodies (DAbs), linear antibodies, single chain antibodies (single chain Fv, abbreviated scFv) and multispecific antibodies, for example, bi- and tri-specific antibodies formed from antibody fragments (C.A.K. Borrebaeck, editor (1995), Antibody Engineering (Breakthroughs in Molecular Biology), Oxford University Press; R. Kontermann and S. Duebel, editors (2001), Antibody Engineering (Springer Laboratory Manual), Springer Verlag). Antibodies other than "multispecific" or "multifunctional" include those with identical binding sites. Multispecific antibodies may be specific for different epitopes of an antigen or specific for epitopes of more than one antigen (see, for example, WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt *et al.*, 1991, J. Immunol. **147**:60-69; US Patents 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; or

Kostelny *et al.*, 1992, *J. Immunol.* **148**:1547-1553). A F(ab')₂ or Fab molecule may be constructed so that the number of intermolecular disulfide interactions taking place between the CH1 and CL domains can be reduced or completely prevented.

5 "Functional fragment" or "antigen binding antibody fragments" may be fused to an additional polypeptide or protein which does not originate from an antibody by way of their amino terminus or carboxy terminus by means of a covalent bond (e.g., a peptide linkage). In addition, antibodies and antigen binding fragments may be modified so that reactive cysteines are inserted at defined sites to facilitate coupling to a toxophore (see Junutula *et al.*, *Nat. Biotechnol.*, August 2008, **26**(8):925-32).

10 Polyclonal antibodies can be synthesized by methods with which the average person skilled in the art is familiar. Monoclonal antibodies can be synthesized by methods with which those skilled in the art are familiar (Köhler and Milstein, *Nature*, **256**:495-497, 1975). Human and/or humanized monoclonal antibodies can be synthesized by methods with which the average person skilled in the art is familiar (Olsson *et al.*, *Meth. Enzymol.* **92**:3-16 and/or Cabilly *et al.*,
15 US 4,816,567 or Boss *et al.*, US 4,816,397).

The average person skilled in the art is familiar with various methods for synthesis of antibodies and their fragments such as, for example, by means of transgenic mice (N. Lonberg and D. Huszar, *Int. Rev. Immunol.* 1995;**13**(1):65-93) or Phage Display Technologies (Clackson *et al.*, *Nature*, August 15, 1991, **352**(6336):624-628). Antibodies according to the invention can be
20 obtained from recombinant antibody library consisting of the amino acid sequences of a plurality of antibodies created from a large number of healthy volunteer subjects. Antibodies can also be synthesized by means of known recombinant DNA technologies. The nucleic acid sequence of an antibody can be obtained by routine sequencing or is available from publicly accessible data banks.

25 An "isolated" antibody or binder has been purified to remove other constituents of the cell. Contaminating ingredients of a cell which can interfere with a diagnostic or therapeutic use may be, for example, enzymes, hormones or other peptidic or nonpeptidic components of a cell. An antibody or binder that has been purified to more than 95% by weight, based on the antibody and/or binder (determined by the Lowry method, UV-Vis spectroscopy or SDS capillary gel
30 electrophoresis, for example). Furthermore, an antibody that has been purified to the extent that at least 15 amino acids of the amino terminus or an internal amino acid sequence can be determined or which has been purified to the point of homogeneity, where homogeneity is determined by SDS-PAGE under reducing or nonreducing conditions (detection may be

performed by Coomassie blue staining or preferably by silver staining) may also be used. However, an antibody is normally synthesized by at least one purification step.

The term "specific binding" or "binds specifically" refers to an antibody or binder that binds to a predetermined antigen/target molecule. Specific binding of an antibody or binder typically describes an antibody, i.e., binder having an affinity of at least 10^{-7} M (as the K_d value; i.e., preferably those with a K_d value of less than 10^{-7} M), where the antibody, i.e., the binder, has an affinity for the predetermined antigen/target molecule that is at least twice as high as that of a nonspecific antigen/target molecule (e.g., bovine serum albumin or casein) which is not the predetermined antigen/target molecule or a closely related antigen/target molecule.

Antibodies which are specific against a cancer cell antigen can be synthesized by the average person skilled in the art using methods with which he is familiar (such as recombinant expression) or may be acquired commercially (for example, from Merck KGaA, Germany). Examples of known commercially available antibodies in cancer therapy include Erbitux® (cetuximab, Merck KGaA), Avastin® (bevacizumab, Roche) and Herceptin® (trastuzumab, Genentech). Trastuzumab is a recombinanthumanized monoclonal antibody of the IgG1 κ type which binds the extracellular domains of human epidermal growth receptor with a high affinity in a cell-based assay (K_d = 5 nM). The antibody is synthesized recombinantly in CHO cells.

The compounds of formula (I) constitute a subgroup of the compounds of formula (Ia).

The preferred subject matter of the invention is binder-drug conjugates of the general formula (Ia), wherein

n stands for a number from 1 to 50,

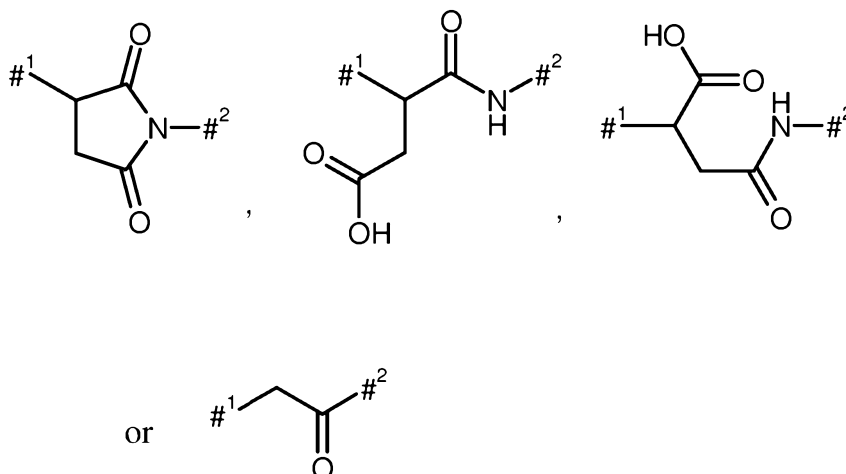
AK stands for AK₁ or AK₂

wherein

AK₁ stands for a binder (preferably for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody) which is bound to the group G by a sulfur atom of the binder,

AK₂ stands for a binder (preferably for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody) which is bound to the group G by a nitrogen atom of the binder,

G for the case when AK = AK₁ stands for a group of the formula



wherein

#¹ denotes the linkage site to the sulfur atom of the binder,

#² denotes the linkage site to the group L¹,

5 or

for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₁-C₁₀)-alkanediyl, a group of the formula



wherein

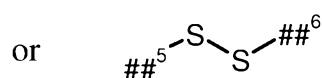
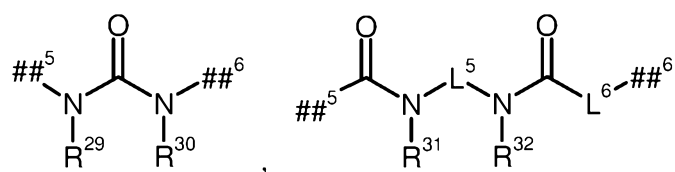
10 m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

L^{1A} stands for linear (C₂-C₁₀)-alkanediyl,

B¹ stands for a group of the formula



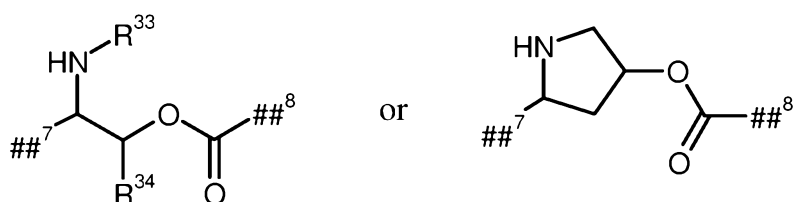
wherein

##⁵ denotes the linkage site to the group L^{1A},

##⁶ denotes the linkage site to the group L^{1B},

5 L⁵ stands for a bond or (C₂-C₄)-alkanediyl,

L⁶ stands for a bond or a group with the formula



wherein

##⁷ denotes the linkage site to the carbonyl group,

10 ##⁸ denotes the linkage site to L^{1B},

R³³ stands for hydrogen, (C₁-C₄)-alkylcarbonyl, *tert*-butoxy-carbonyl or benzyloxycarbonyl,

R³⁴ stands for hydrogen or methyl,

R²⁹ stands for hydrogen or (C₁-C₄)-alkyl,

15 R³⁰ stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{29} and R^{30} together with the atoms to which they are bound form a five- or six-membered heterocycle,

R^{31} stands for hydrogen or (C₁-C₄)-alkyl,

R^{32} stands for hydrogen or (C₁-C₄)-alkyl,

5 or

R^{31} and R^{32} together with the atoms to which they are bound form a five- or six-membered heterocycle,

L^{1B} stands for linear (C₂-C₁₀)-alkanediyl,

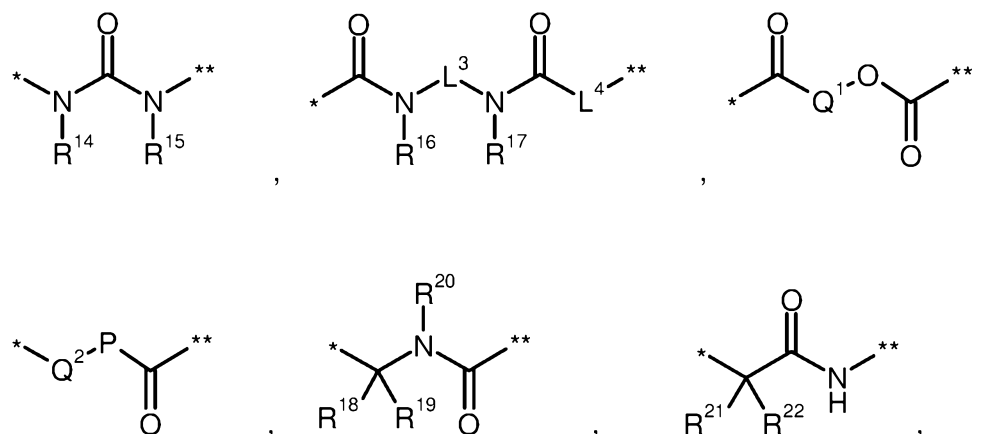
and

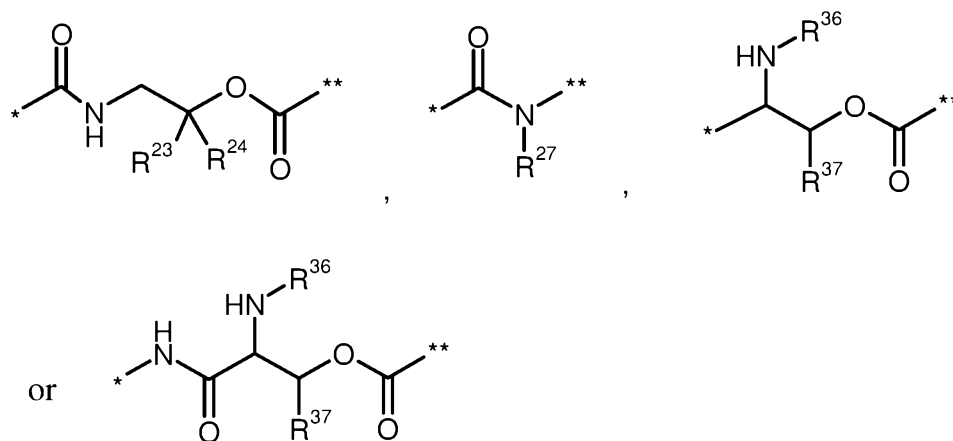
10 wherein (C₁-C₁₀)-alkanediyl may be substituted with one to four substituents selected independently from one another from the group, comprising methyl, hydroxyl and benzyl,

and

15 wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally situated between them, to form a (C₃-C₆)-cycloalkyl ring or a phenyl ring,

B stands for a bond or a group of the formula





wherein

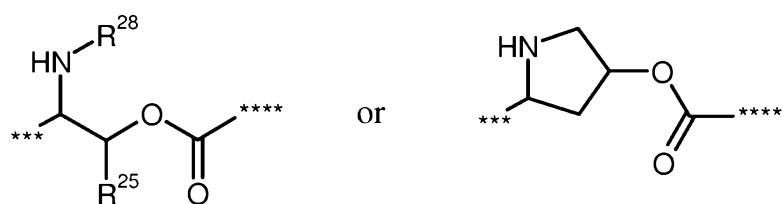
* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

5 P stands for O or NH,

L^3 stands for a bond or $(\text{C}_2\text{-C}_4)$ -alkanediyl,

L^4 stands for a bond or a group of the formula



wherein

10 *** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

R^{25} stands for hydrogen or methyl,

R^{28} stands for hydrogen, $(\text{C}_1\text{-C}_4)$ -alkylcarbonyl, *tert*-butoxycarbonyl or benzyloxycarbonyl,

15 Q^1 stands for a four- to seven-membered heterocycle,

- Q² stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,
- R¹⁴ stands for hydrogen or (C₁-C₄)-alkyl,
- R¹⁵ stands for hydrogen or (C₁-C₄)-alkyl,
- 5 or
- R¹⁴ and R¹⁵ together with the atoms to which they are bound form a five- or six-membered heterocycle,
- R¹⁶ stands for hydrogen or (C₁-C₄)-alkyl,
- R¹⁷ stands for hydrogen or (C₁-C₄)-alkyl,
- 10 or
- R¹⁶ and R¹⁷ together with the atoms to which they are bound form a five- or six-membered heterocycle,
- R¹⁸ stands for hydrogen or (C₁-C₄)-alkyl,
- R¹⁹ stands for hydrogen or the side group of α -amino acid or its homologs or isomers,
- 15 R²⁰ stands for hydrogen or (C₁-C₄)-alkyl,
- or
- R¹⁹ and R²⁰ together with the atoms to which they are bound form a pyrrolidinyl ring,
- 20 R²¹ stands for hydrogen or (C₁-C₄)-alkyl,
- R²² stands for hydrogen or (C₁-C₄)-alkyl,
- or
- R²¹ and R²² together with the atoms to which they are bound form a three- to seven-membered carbocycle,
- 25 R²³ stands for (C₁-C₄)-alkyl,

R^{24} stands for hydrogen or (C₁-C₄)-alkyl,

R^{27} stands for hydrogen or (C₁-C₄)-alkyl,

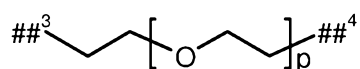
R^{36} stands for hydrogen, (C₁-C₄)-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxy-carbonyl,

5 R^{37} stands for hydrogen or methyl,

or

R^{36} and R^{37} together with the atoms to which they are bound form a pyrrolidine ring,

L^2 stands for linear (C₂-C₁₀)-alkanediyl or for a group of the formula



10 wherein

p stands for a number from 2 to 6,

##^3 denotes the linkage site to the group B,

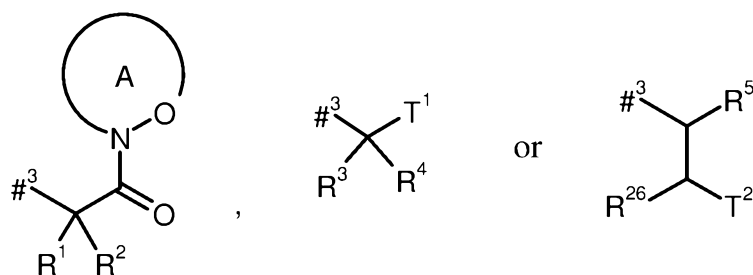
##^4 denotes the linkage site to the nitrogen atom,

15 wherein (C₂-C₁₀)-alkanediyl may be substituted with one to four substituents, selected independently of one another from the group comprising methyl, hydroxyl and benzyl,

and

wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally present between them, to form a (C₃-C₆) cycloalkyl ring or a phenyl ring,

20 D stands for a group of the formula



wherein

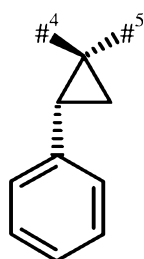
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen or methyl,

5 R² stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxy-ethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

10 R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

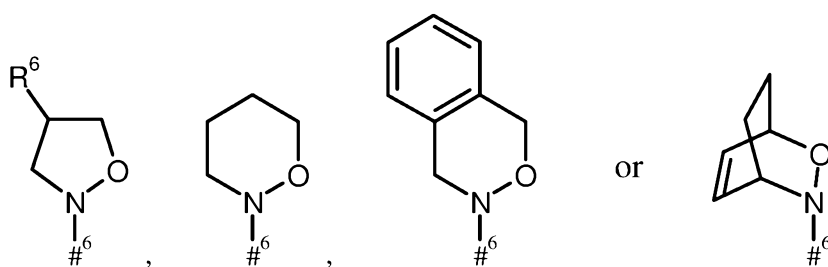


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

15 the ring A with the N-O grouping contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

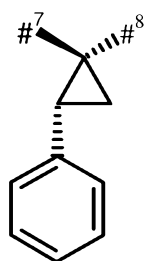
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen or methyl,

R⁴ stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

15 #⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

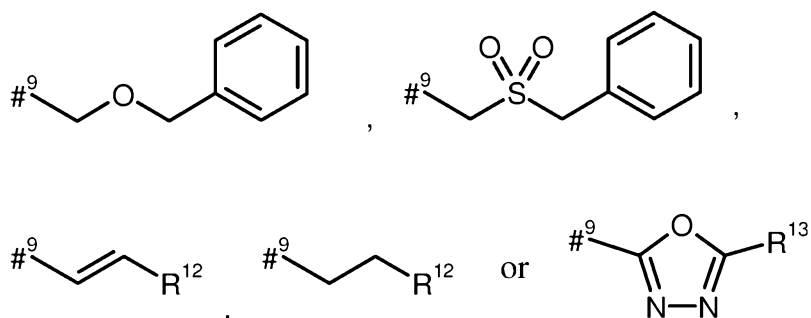
5 or

R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(\text{R}^{26})\text{-T}^2$,

15 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

20 T^2 stands for phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

Binder-drug conjugates of the general formula (Ia), wherein

n stands for an integer from 1 to 50,

AK stands for a binder, preferably a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

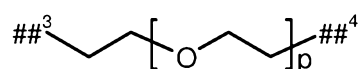
the group $\S\text{-G-L}^1\text{-B-}\S\S$ for a linker

wherein

$\S$ denotes the linkage site to the group AK and

$\S\S$ denotes the linkage site to the nitrogen atom,

L^2 stands for linear $(C_2\text{-}C_{10})$ -alkanediyl or a group of the formula



wherein

p stands for a number from 2 to 6,

$\#\#^3$ denotes the linkage site to the group B,

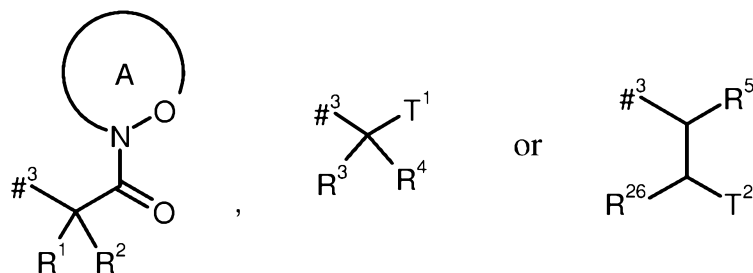
$\#\#^4$ denotes the linkage site to the nitrogen atom,

wherein $(C_2\text{-}C_{10})$ -alkanediyl may be substituted with one to four substituents, independently of one another, selected from the group comprising methyl, hydroxyl and benzyl,

and

wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4- relation to one another to form a $(C_3\text{-}C_6)$ cycloalkyl ring or a phenyl ring, including the carbon atoms optionally situated between them,

D stands for a group of the formula



wherein

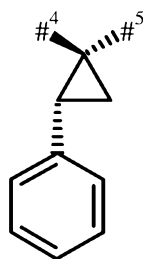
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen or methyl,

5 R² stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxy-ethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

10 R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

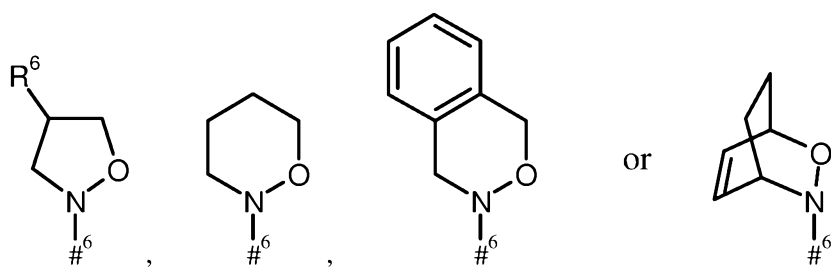


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

15 the ring A with the N-O group contained therein stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

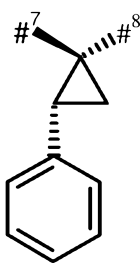
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen or methyl,

R⁴ stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

15 #⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

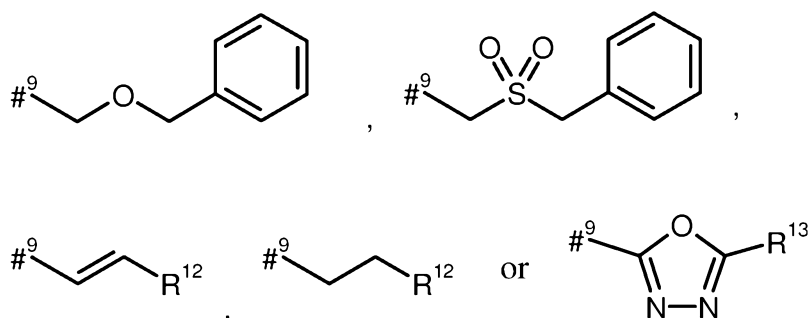
5 or

R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl, which may be substituted with methoxycarbonyl or carboxyl in the phenyl group,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(\text{R}^{26})\text{-T}^2$,

15 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

20 T^2 stands for phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention relates to binder-drug conjugates of general formula (Ia) as given above, wherein

n stands for a number from 1 to 50,

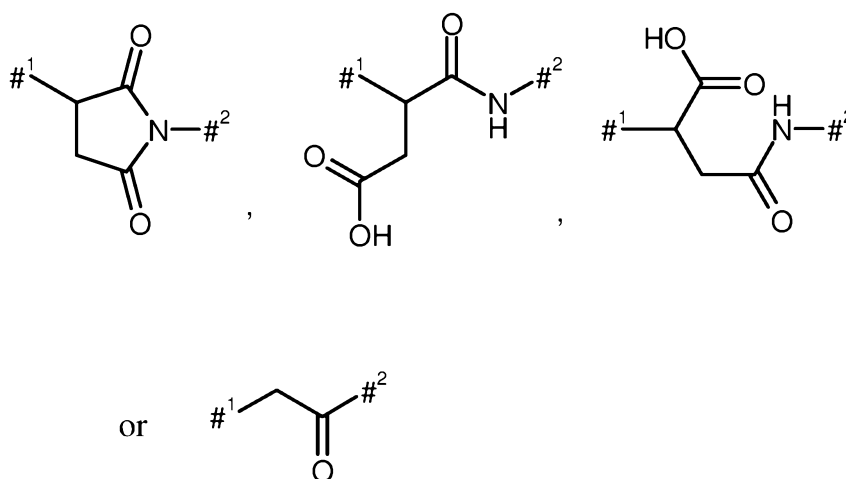
5 AK stands for AK₁ or AK₂

wherein

AK₁ stands for a binder (preferably for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody), which is bound to the group G via a sulfur atom of the binder,

10 AK₂ stands for a binder (preferably for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody) that is bound to the group G via a nitrogen atom of the binder,

G for the case when AK = AK₁, stands for a group of the formula



15 wherein

#¹ denotes the linkage site to the sulfur atom of the binder,

#² denotes the linkage site to the group L¹,

or

for the case when AK = AK₂, G stands for carbonyl,

L^1 stands for a bond, linear (C₁-C₁₀)-alkanediyl, a group of the formula



wherein

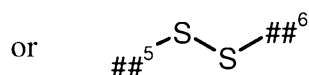
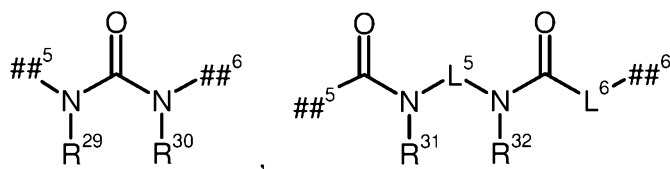
m stands for a number from 2 to 6,

5 ##^1 denotes the linkage site to the group G,

##^2 denotes the linkage site to the group B,

L^{1A} stands for linear (C₂-C₁₀)-alkanediyl,

B^1 stands for a group of the formula



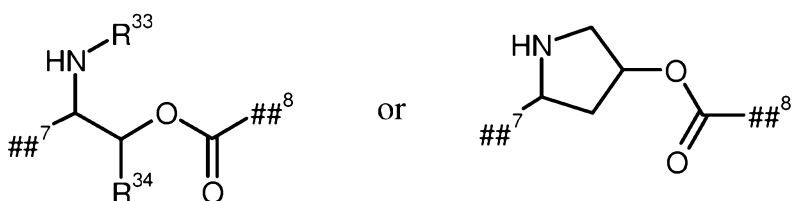
10 wherein

##^5 denotes the linkage site to the group L^{1A} ,

##^6 denotes the linkage site to the group L^{1B} ,

L^5 stands for a bond or (C₂-C₄)-alkanediyl,

L^6 stands for a bond or a group with the formula



15

wherein

$\# \#^7$ denotes the linkage site to the carbonyl group,

$\# \#^8$ denotes the linkage site to L^{1B} ,

5 R^{33} stands for hydrogen, (C_1-C_4) -alkylcarbonyl, *tert*-butyloxy-carbonyl or benzyloxycarbonyl,

R^{34} stands for hydrogen or methyl,

R^{29} stands for hydrogen or (C_1-C_4) -alkyl,

R^{30} stands for hydrogen or (C_1-C_4) -alkyl,

or

10 R^{29} and R^{30} together with the atoms to which they are bound form a five- or six-membered heterocycle,

R^{31} stands for hydrogen or (C_1-C_4) -alkyl,

R^{32} stands for hydrogen or (C_1-C_4) -alkyl,

or

15 R^{31} and R^{32} together with the atoms to which they are bound form a five- or six-membered heterocycle,

L^{1B} stands for linear (C_2-C_{10}) -alkanediyl,

and

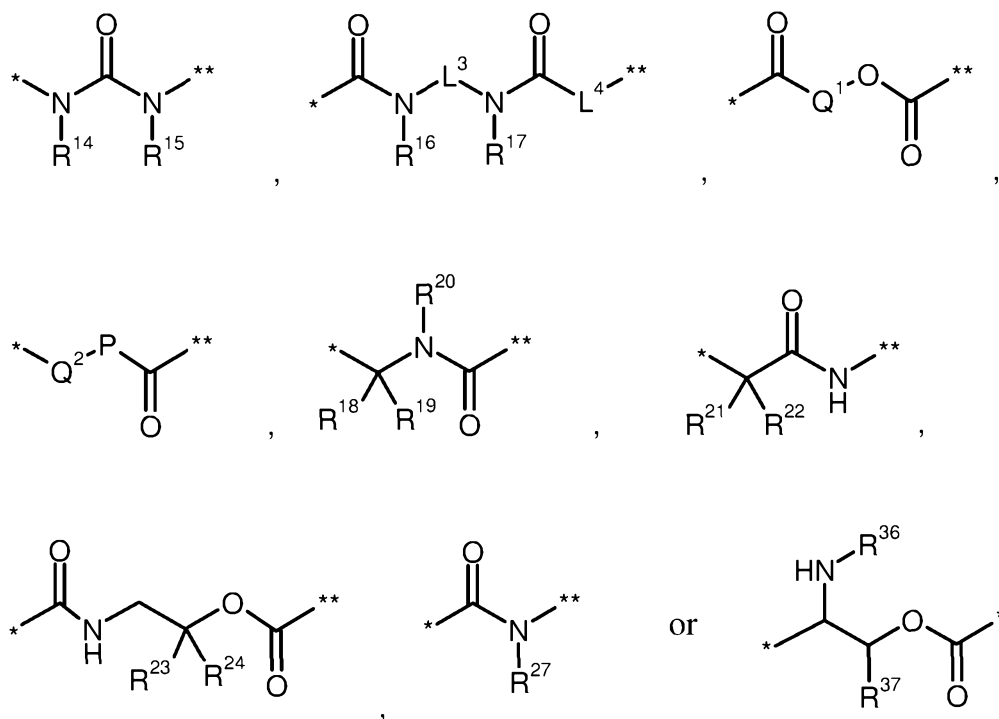
20 wherein (C_1-C_{10}) -alkanediyl may be substituted with one to four substituents selected independently of one another from the group comprising methyl, hydroxyl and benzyl

and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another may be bridge to form a (C_3-C_6) -cycloalkyl ring or a phenyl ring by inclusion of the carbon atoms optionally occurring between them,

25 B stands for a bond or a group of the formula

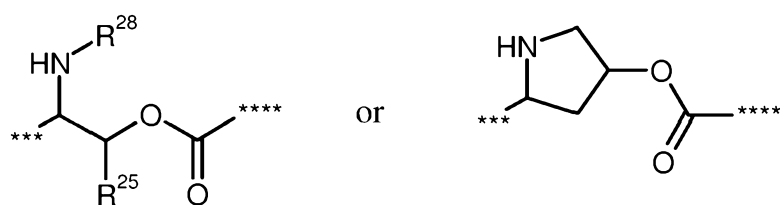
- 35 -



wherein

* denotes the linkage site to L^1 ,** denotes the linkage site to L^2 ,

5 P stands for O or NH,

 L^3 stands for a bond or (C_2-C_4) -alkanediyl, L^4 stands for a bond or a group of the formula

wherein

10 *** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 , R^{25} stands for hydrogen or methyl,

- R^{28} stands for hydrogen, (C₁-C₄)-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,
- 5 Q^1 stands for a four- to seven-membered heterocycle,
- Q^2 stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,
- R^{14} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{15} stands for hydrogen or (C₁-C₄)-alkyl,
- or
- 10 R^{14} and R^{15} together with the atoms to which they are bound may form a five- or six-membered heterocycle,
- R^{16} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{17} stands for hydrogen or (C₁-C₄)-alkyl,
- or
- 15 R^{16} and R^{17} together with the atoms to which they are bound form a five- or six-membered heterocycle,
- R^{18} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{19} stands for hydrogen or the side group of a naturally occurring α -amino acid or its homologs or isomers,
- R^{20} stands for hydrogen or (C₁-C₄)-alkyl,
- 20 or
- R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidinyl ring,
- R^{21} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{22} stands for hydrogen or (C₁-C₄)-alkyl,
- 25 or

R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{23} stands for (C₁-C₄)-alkyl,

R^{24} stands for hydrogen or (C₁-C₄)-alkyl,

5 R^{27} stands for hydrogen or (C₁-C₄)-alkyl,

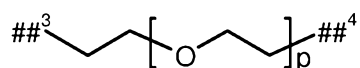
R^{36} stands for hydrogen, (C₁-C₄)-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

R^{37} stands for hydrogen or methyl,

or

10 R^{36} and R^{37} together with the atoms to which they are bound form a pyrrolidine ring,

L^2 stands for linear (C₂-C₁₀)-alkanediyl or for a group of the formula



wherein

p stands for a number from 2 to 6,

15 $##^3$ denotes the linkage site to the group B,

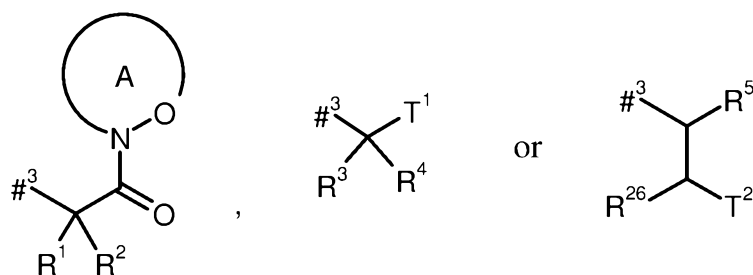
$##^4$ denotes the linkage site to the nitrogen atom,

wherein (C₂-C₁₀)-alkanediyl may be substituted with 1 to 4 substituents selected independently of one another from the group comprising methyl, hydroxyl and benzyl,

and

20 wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another to form a (C₃-C₆) cycloalkyl ring or a phenyl ring, including the carbon atoms optionally situated between them,

D stands for a group of the formula



wherein

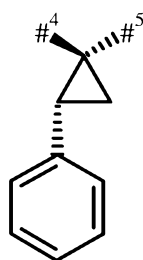
$\text{\#}^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen or methyl,

5 R^2 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

10 R^1 and R^2 together with the carbon atom to which they are attached may form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

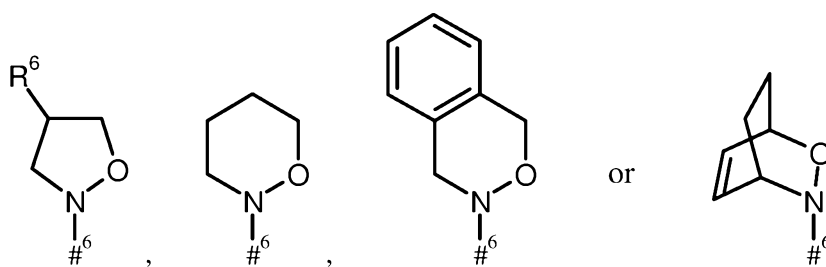


wherein

$\text{\#}^4$ denotes the linkage site to the vicinal nitrogen atom,

15 $\text{\#}^5$ denotes the linkage site to the carbonyl group,

the ring A together with the N-O group contained therein stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

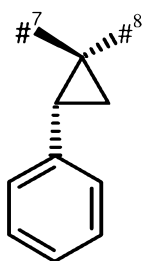
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen or methyl,

R⁴ stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached forms a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

15 #⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

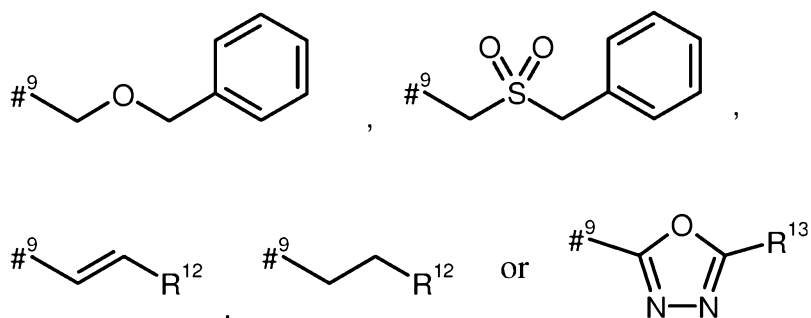
5 or

R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $\text{-CHC(R}^{26}\text{)-T}^2$,

15 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $\text{-S(O)}_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

20 T^2 stands for phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the invention relates to binder-drug conjugates of the general formula (Ia), wherein

n stands for a number from 1 to 20,

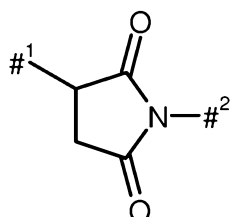
5 AK stands for AK₁ or AK₂

wherein

AK₁ stands for an antibody or an antigen binding antibody fragment which binds to EGFR and is bound to the group G via the sulfur atom of a cysteine radical of the binder,

10 AK₂ stands for an antibody or an antigen binding antibody fragment which binds to EGFR and is bound to the group G via the NH side group of a lysine radical of the binder,

G for the case when AK = AK₁ stands for a group of the formula



15 wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

or

for the case when AK = AK₂, G stands for carbonyl,

20 L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



wherein

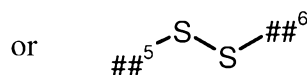
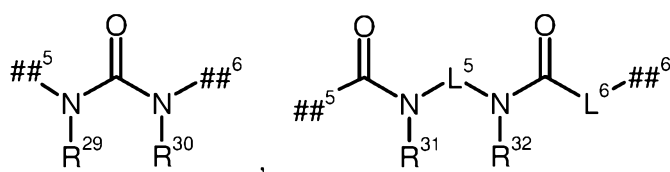
m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

5 L^{1A} stands for linear (C₂-C₆)-alkanediyl,

B¹ stands for a group of the formula



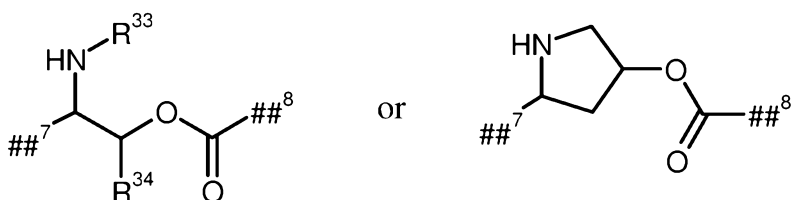
wherein

##⁵ denotes the linkage site to the group L^{1A},

10 ##⁶ denotes the linkage site to the group L^{1B},

L⁵ stands for a bond,

L⁶ stands for a bond or a group of the formula



wherein

15 ##⁷ denotes the linkage site to the carbonyl group,

##⁸ denotes the linkage site to L^{1B},

R³³ for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

R³⁴ stands for hydrogen or methyl,

R²⁹ stands for hydrogen,

R^{30} stands for hydrogen,

5 R^{31} stands for hydrogen or methyl,

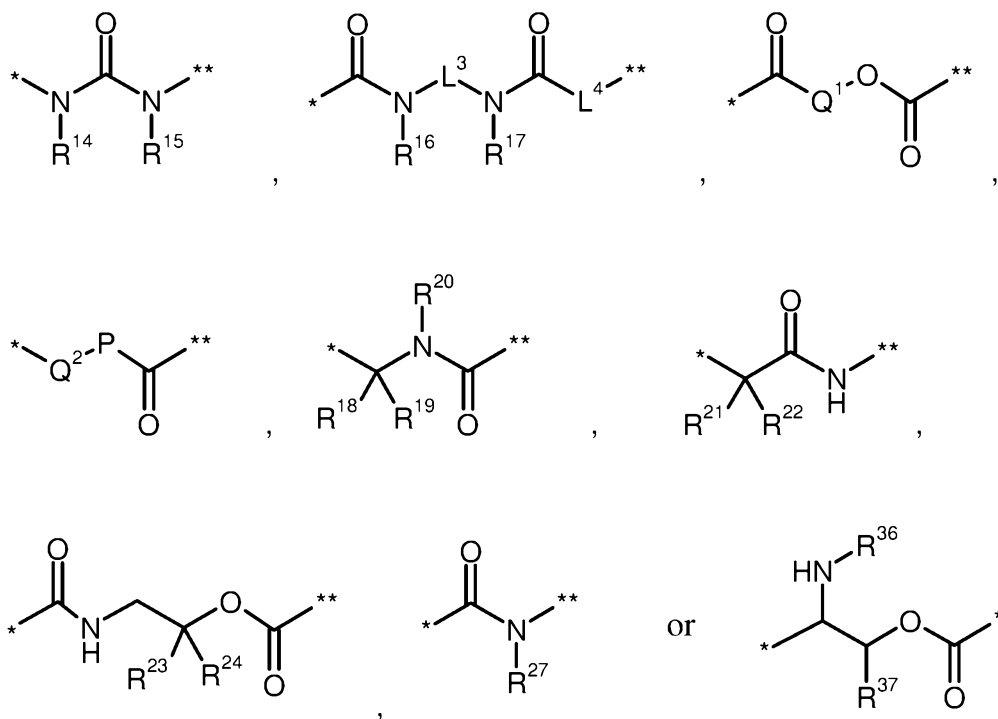
R³² stands for hydrogen or methyl,

L^{1B} stands for linear (C₂-C₆)-alkanediyl,

and

wherein (C₂-C₆)-alkanediyl may be substituted with one to two methyl substituents,

10 **B** stands for a bond or a group of the formula



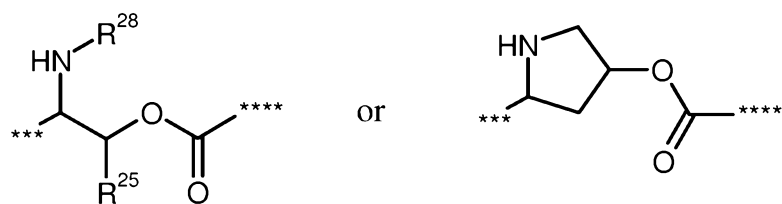
wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

L^3 stands for a bond or ethane-1,2-diyl,

L^4 stands for a bond or a group of the formula



wherein

5 *** denotes the linkage site to the carbonyl group,

 **** denotes the linkage site to L^2 ,

R^{25} stands for hydrogen or methyl,

R^{28} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

Q^1 stands for a four- to seven-membered heterocycle,

10 R^{14} stands for hydrogen,

R^{15} stands for hydrogen,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

or

15 R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

R^{18} stands for hydrogen,

R^{19} stands for hydrogen, methyl, propan-2-yl, 2-methylpropan-1-yl or 1-methylpropan-1-yl,

R^{20} stands for hydrogen or methyl,

20 or

R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidinyl ring,

R^{21} stands for hydrogen or methyl,

R^{22} stands for hydrogen or methyl,

5 or

R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

R^{23} stands for methyl,

R^{24} stands for hydrogen or methyl,

10 R^{27} stands for hydrogen,

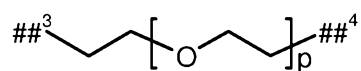
R^{36} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

R^{37} stands for hydrogen or methyl,

or

R^{36} and R^{37} together with the atoms to which they are bound form a pyrrolidine ring,

15 L^2 stands for linear (C_2 - C_6)-alkanediyl or for a group of the formula



wherein

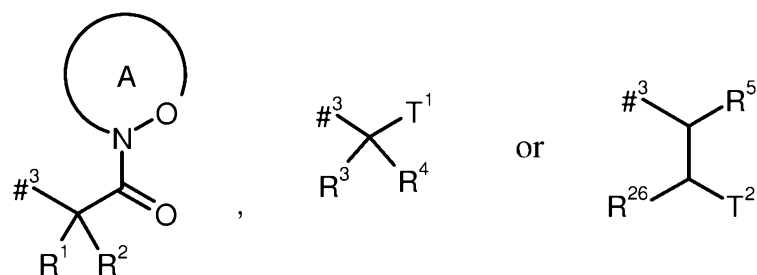
p stands for a number from 2 to 6,

##^3 denotes the linkage site to the group B,

20 ##^4 denotes the linkage site to the nitrogen atom,

wherein (C_2 - C_{10})-alkanediyl may be substituted with one or two methyl substituents.

D stands for a group of the formula



wherein

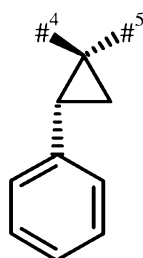
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached may form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



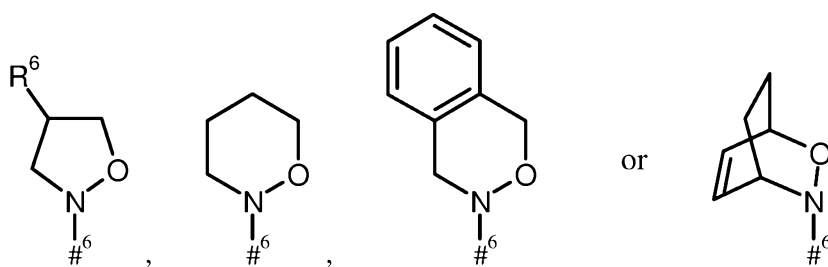
10

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

15 the ring A with the N-O group contained therein stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

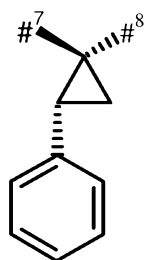
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen,

R⁴ stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or
1*H*-indol-3-ylmethyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached may form a
(1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

15 T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-
R¹⁰ or -CH₂-O-R¹¹,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

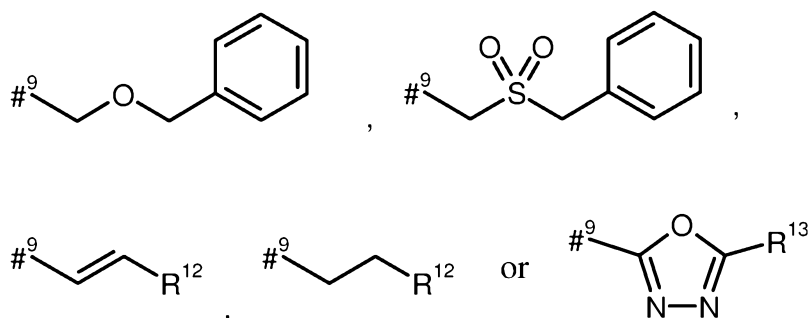
5 or

R^8 and R^9 together with the nitrogen atom which they are attached may form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl, which may be substituted with methoxycarbonyl or carboxyl in the phenyl group,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(\text{R}^{26})\text{-T}^2$,

15 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

20 T^2 stands for phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention relates to binder-drug conjugates of the general formula (Ia) as indicated above, wherein

n stands for a number from 1 to 20,

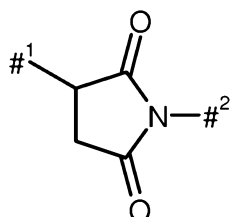
5 AK stands for AK₁ or AK₂

wherein

AK₁ stands for an antibody or an antigen binding antibody fragment which binds to EGFR and is bound to the group G by the sulfur atom of a cysteine radical of the binder,

10 AK₂ stands for an antibody or an antigen binding antibody fragment which binds to EGFR and is bound to the group G by the NH side group of a lysine radical of the binder,

G for the case when AK = AK₁ stands for the group of the formula



15 wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

or

for the case when AK = AK₂, G stands for carbonyl,

20 L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



wherein

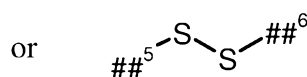
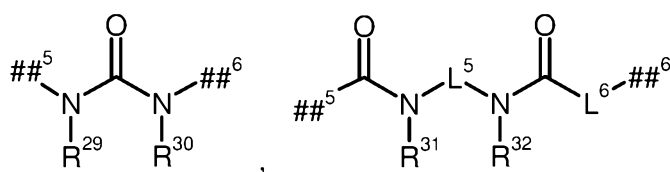
m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

5 L^{1A} stands for linear (C₂-C₆)-alkanediyl,

B¹ stands for a group with the formula



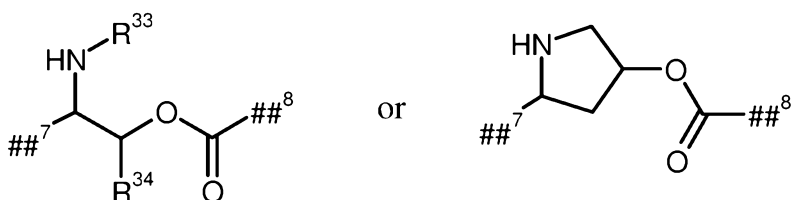
wherein

##⁵ denotes the linkage site to the group L^{1A},

10 ##⁶ denotes the linkage site to the group L^{1B},

L⁵ stands for a bond,

L⁶ stands for a bond or a group of the formula



wherein

15 ##⁷ denotes the linkage site to the carbonyl group,

##⁸ denotes the linkage site to L^{1B},

R^{33} stands for hydrogen, methylcarbonyl or *tert*-butoxycarbonyl,

R^{34} stands for hydrogen or methyl,

R^{29} stands for hydrogen,

R^{30} stands for hydrogen,

5 R^{31} stands for hydrogen or methyl,

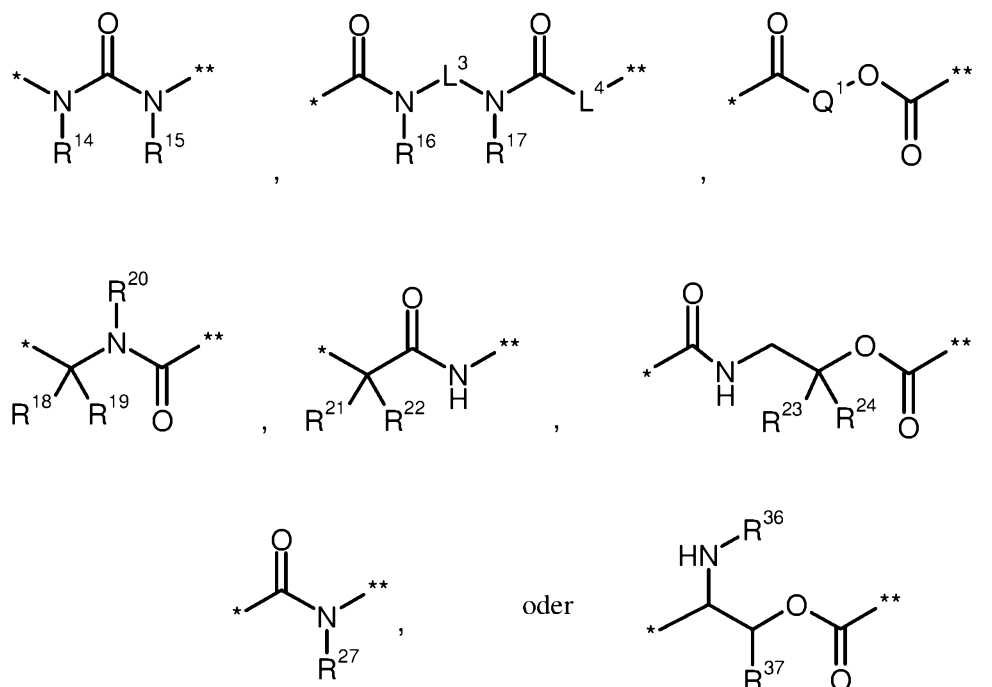
R^{32} stands for hydrogen or methyl,

L^{1B} stands for linear (C_2 - C_6)-alkanediyl,

and

wherein (C_2 - C_6)-alkanediyl may be substituted with one to two methyl substituents,

10 B stands for a bond or a group of the formula



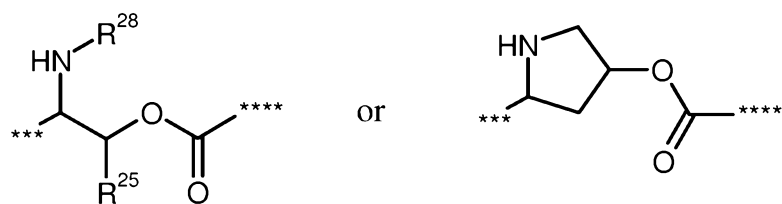
wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

L^3 stands for a bond or ethane-1,2-diyl,

L^4 stands for a bond or a group of the formula



wherein

5 *** denotes the linkage site to the carbonyl group,

 **** denotes the linkage site to L^2 ,

R^{25} stands for hydrogen or methyl,

R^{28} stands for hydrogen, methylcarbonyl *tert*-butoxycarbonyl,

Q^1 stands for a four- to seven-membered heterocycle,

10 R^{14} stands for hydrogen,

R^{15} stands for hydrogen,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

or

15 R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyling,

R^{18} stands for hydrogen,

R^{19} stands for hydrogen, methyl, propan-2-yl, 2-methylpropan-1-yl or 1-methylpropan-1-yl,

R^{20} stands for hydrogen or methyl,

20 or

R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidinyl ring,

R^{21} stands for hydrogen or methyl,

R^{22} stands for hydrogen or methyl,

5 or

R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

R^{23} stands for methyl,

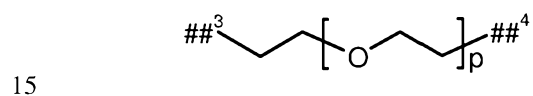
R^{24} stands for hydrogen or methyl,

10 R^{27} stands for hydrogen,

R^{36} stands for hydrogen, (C₁-C₄)-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

R^{37} stands for hydrogen or methyl,

L^2 stands for linear (C₂-C₆)-alkanediyl or for a group of the formula



wherein

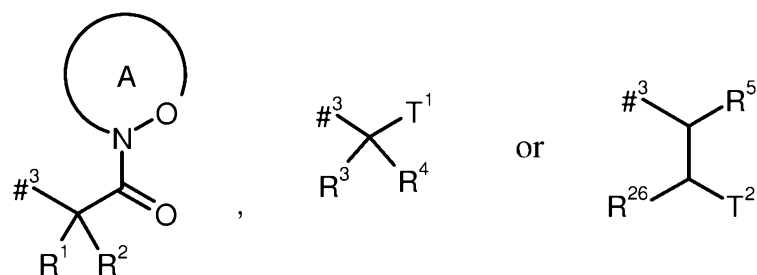
p stands for a number from 2 to 6,

$##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

20 wherein (C₂-C₁₀)-alkanediyl may be substituted with one or two methyl substituents,

D stands for a group of the formula



wherein

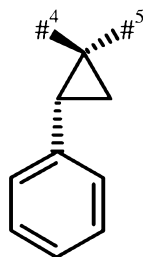
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



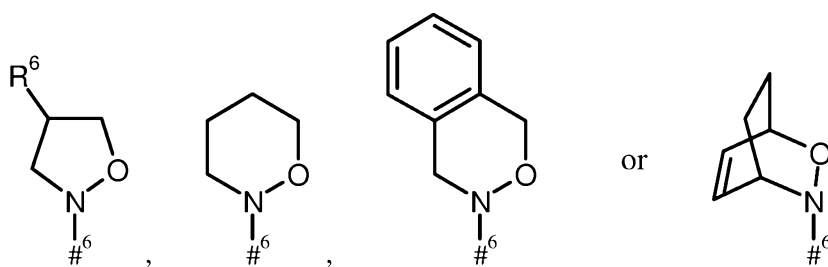
10

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

15 the ring A together with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

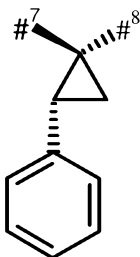
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen,

R⁴ stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached may form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

15 T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

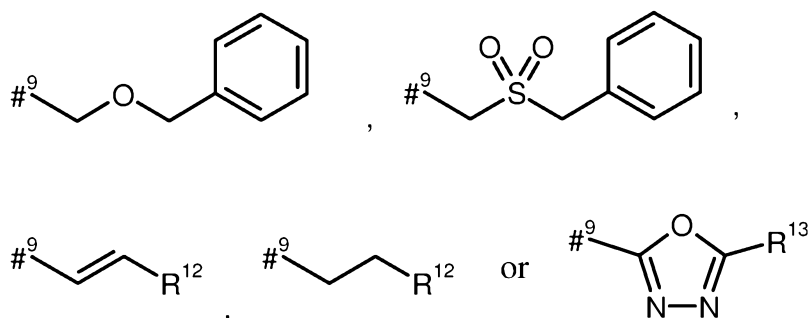
5 or

R^8 and R^9 together with the nitrogen atom to which they are bound may form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl, which may be substituted with methoxycarbonyl or carboxyl in the phenyl group,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(\text{R}^{26})\text{-T}^2$,

15 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

20 T^2 stands for phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the invention relates to binder-drug conjugates of the general formula (Ia), wherein

n stands for a number between 1 and 10,

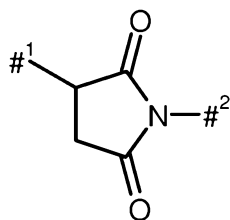
5 AK stands for AK₁ or AK₂

wherein

AK₁ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the sulfur atom of a cysteine radical of the binder,

10 AK₂ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G for the case when AK = AK₁ stands for a group of the formula



wherein

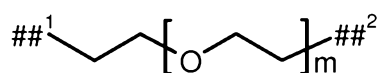
#¹ denotes the linkage site to the cysteine radical of the binder,

15 #² denotes the linkage site to the group L¹,

or

for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



20 wherein

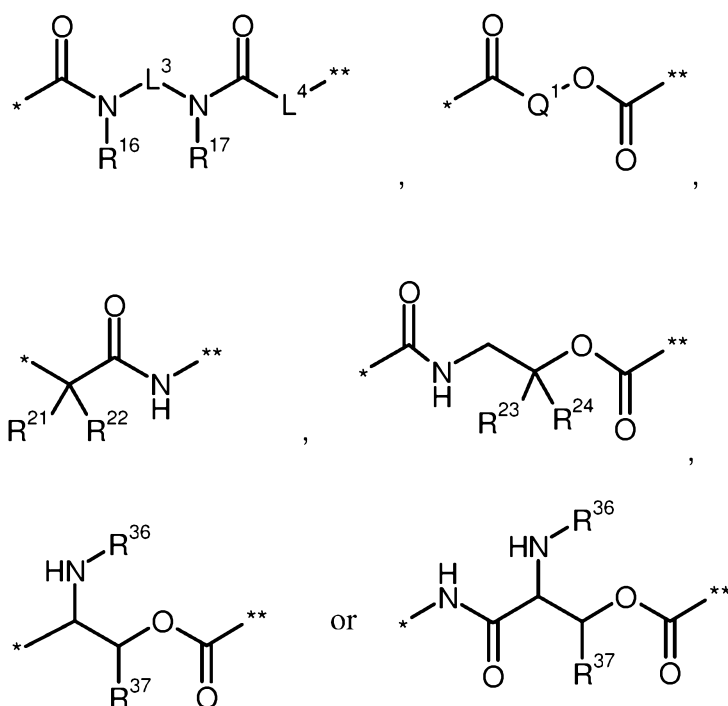
m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediiyl may be substituted with one or two methyl substituents,

5 B stands for a bond or a group of the formula



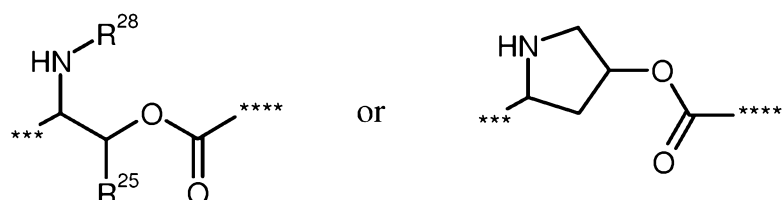
wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

10 L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

R^{25} stands for methyl,

5 R^{28} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

Q^1 stands for piperidine-1,4-diyl,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

or

10 R^{16} and R^{17} together with the atoms to which they are bound form a piperaziny ring,

R^{21} stands for hydrogen or methyl,

R^{22} stands for hydrogen or methyl,

or

15 R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

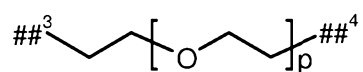
R^{23} stands for methyl,

R^{24} stands for hydrogen,

R^{36} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

R^{37} stands for hydrogen or methyl,

20 L^2 stands for linear (C_2 - C_6)-alkanediyl or for a group of the formula



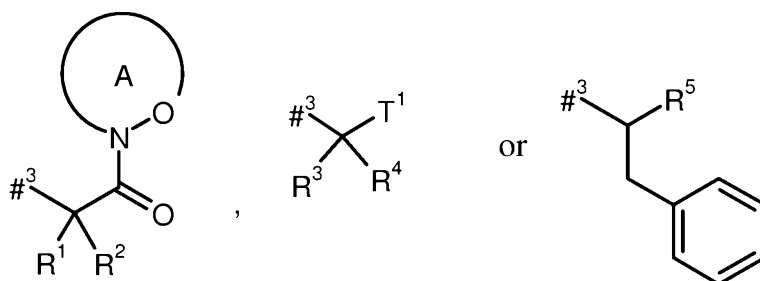
wherein

p stands for a number from 2 to 6,

$\#^3$ denotes the linkage site to the group B,

$\#^4$ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

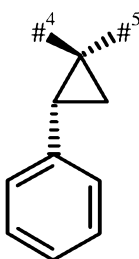
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

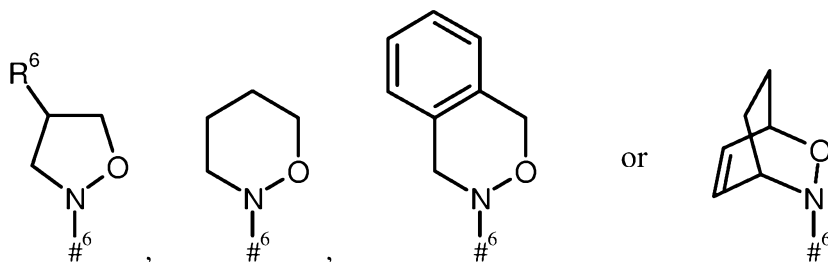


wherein

$\#^4$ denotes the linkage site to the vicinal nitrogen atom,

$\#^5$ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

5 $\#^6$ denotes the linkage site to the carbonyl group,

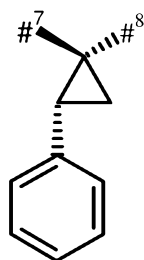
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

10 R^3 and R^4 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

$\#^7$ denotes the linkage site to the vicinal nitrogen atom,

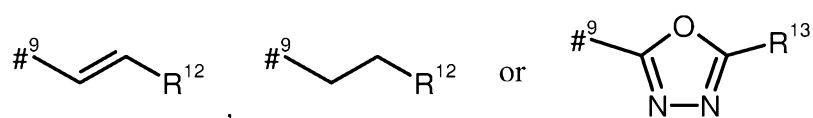
15 $\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$ or $-CH_2-O-R^{11}$,

wherein

- R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,
- R^8 stands for hydrogen or methyl,
- R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,
- 5 R^{11} stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

- 10 $\#^9$ denotes the linkage site to $\text{---CHCH}_2\text{---phenyl}$,
- R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $\text{---S(O)}_2\text{OH}$,
- R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

- 15 R^{35} stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention relates to binder-drug conjugates of the general formula (Ia) as indicated above, wherein

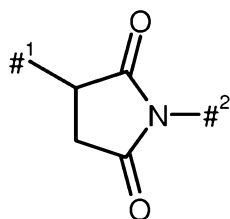
- n stands for a number from 1 to 10,
- 20 AK stands for AK_1 or AK_2

wherein

AK_1 stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the sulfur atom of a cysteine radical of the binder,

AK₂ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G for the case when AK = AK₁ stands for a group of the formula



5 wherein

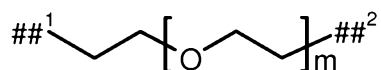
#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

or

for the case when AK = AK₂, G stands for carbonyl,

10 L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



wherein

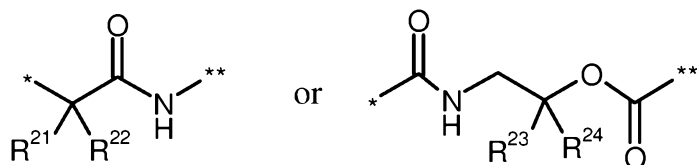
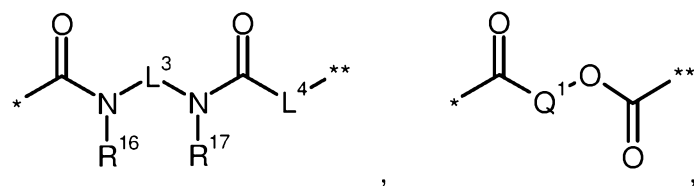
m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

15 ##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



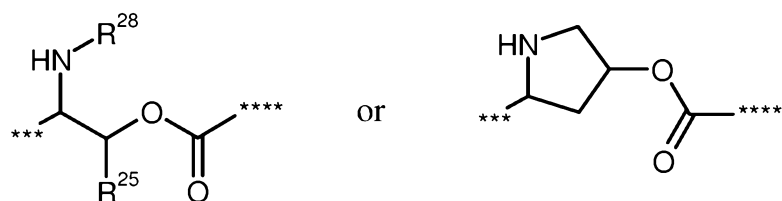
wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

5 L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

10 **** denotes the linkage site to L²,

R²⁵ stands for methyl,

R²⁸ stands for hydrogen, methylcarbonyl or *tert*-butoxycarbonyl,

Q¹ stands for piperidine-1,4-diyl,

R¹⁶ stands for hydrogen or methyl,

15 R¹⁷ stands for hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyling,

R^{21} stands for hydrogen or methyl,

R^{22} stands for hydrogen or methyl,

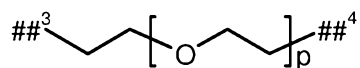
5 or

R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

R^{23} stands for methyl,

R^{24} stands for hydrogen,

10 L^2 stands for linear (C_2-C_6) -alkanediyl or for a group of the formula



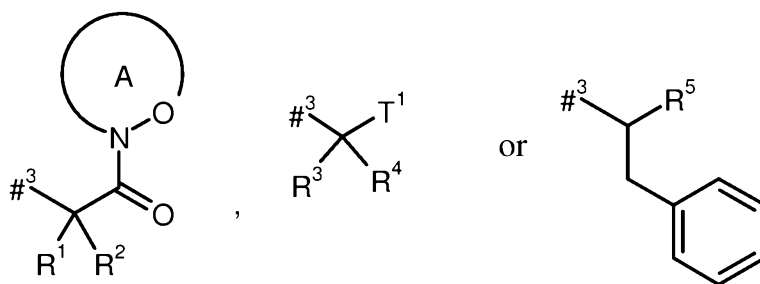
wherein

p stands for a number from 2 to 6,

$##^3$ denotes the linkage site to the group B,

15 $##^4$ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

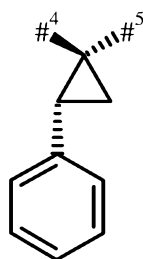
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

5 R^1 and R^2 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

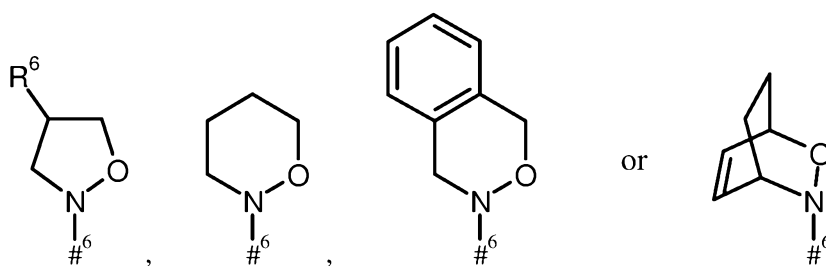


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

10 #⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

15 #⁶ denotes the linkage site to the carbonyl group,

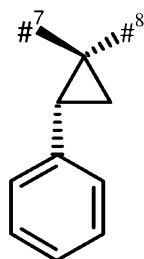
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for benzyl, 1-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



5 wherein

$\#^7$ denotes the linkage site to the vicinal nitrogen atom,

$\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$ or $-CH_2-O-R^{11}$,

wherein

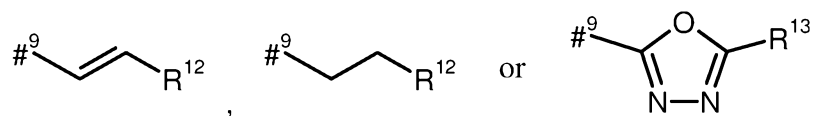
10 R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

15 R^{11} stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $-CHCH_2$ -phenyl,

R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

5 R^{35} stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention relates to binder-drug conjugates of the general formula (Ia) as indicated above, wherein

n stands for a number from 1 to 10,

10 AK stands for AK_2

wherein

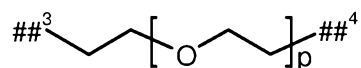
AK_2 stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G stands for carbonyl,

15 L^1 stands for a bond,

B stands for a bond,

L^2 stands for linear (C_3-C_6) -alkanediyl or for a group of the formula



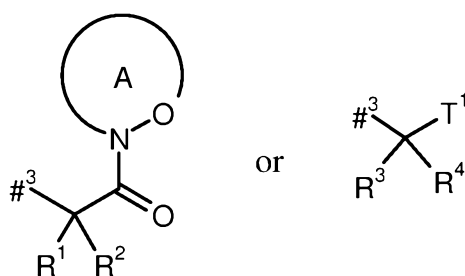
wherein

20 p stands for a number of 2 or 3,

$##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

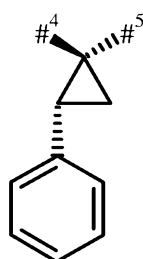
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for benzyl or 1*H*-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



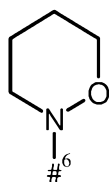
10

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



15

wherein

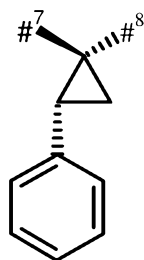
#⁶ denotes the linkage site to the carbonyl group,

R³ stands for hydrogen,

R⁴ stands for benzyl or 1*H*-indol-3-ylmethyl,

5 or

R³ and R⁴ together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

10 #⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷ or -C(=O)-NR⁸R⁹,

wherein

15 R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R⁸ stands for hydrogen,

R⁹ stands for hydrogen or benzyl,

R³⁵ stands for methyl,

as well as their salts and solvates as well as the solvates of the salts.

20 The preferred subject matter of the present invention relates to binder-drug conjugates of the general formula (Ia) as indicated above, wherein

n stands for a number from 1 to 10,

AK stands for AK₂,

wherein

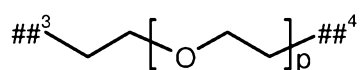
AK₂ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G stands for carbonyl,

L¹ stands for a bond,

B stands for a bond,

L² stands for linear (C₃-C₆)-alkanediyl or for a group of the formula



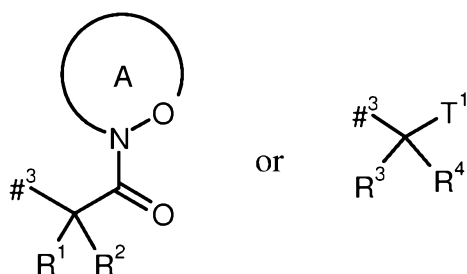
wherein

p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

##⁴ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

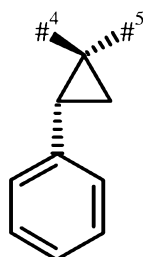
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

R^2 stands for benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



5

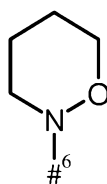
wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula

10



wherein

#⁶ denotes the linkage site to the carbonyl group,

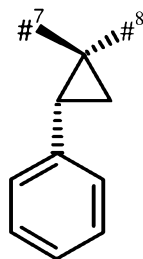
R^3 stands for hydrogen,

R^4 stands for benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

15

or

R^3 and R^4 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

5 T¹ stands for a group of the formula -C(=O)-OR⁷ or -C(=O)-NR⁸R⁹,

wherein

R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R⁸ stands for hydrogen,

10 R⁹ stands for hydrogen or benzyl,

R³⁵ stands for methyl,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention relates to binder-drug conjugates of the general formula (Ia) as indicated above, wherein

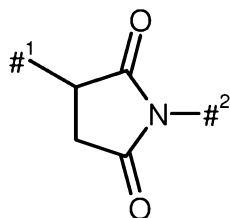
15 n stands for a number from 1 to 10,

AK stands for AK₁

wherein

AK₁ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the sulfur atom of a cysteine radical of the binder,

20 G stands for a group of the formula

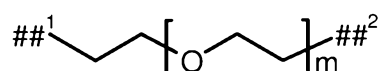


wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

5 L¹ stands for a bond, linear (C₃-C₅)-alkanediyl or a group of the formula



wherein

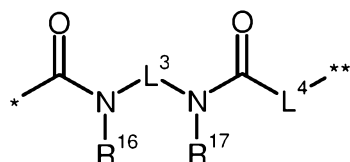
m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

10 ##² denotes the linkage site to the group B,

wherein (C₃-C₅)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



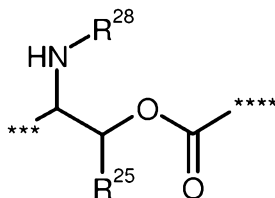
wherein

15 * denotes the linkage site to L¹,

** denotes the linkage site to L²,

L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

5 R^{25} stands for methyl,

R^{28} stands for hydrogen, methylcarbonyl or *tert*-butoxycarbonyl,

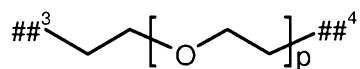
R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

or

10 R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

L^2 stands for linear (C_3 - C_5)-alkanediyl or for a group of the formula



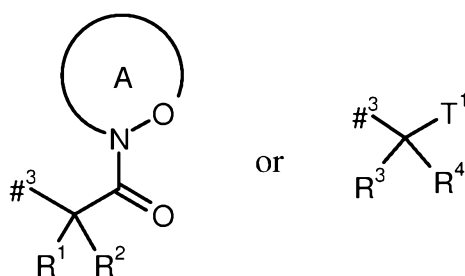
wherein

p stands for a number of 2 or 3,

15 $##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

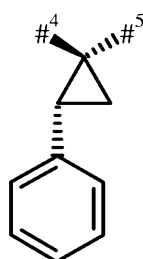
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for benzyl or 1*H*-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



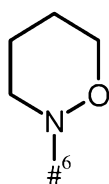
10

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



15

wherein

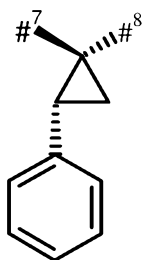
#⁶ denotes the linkage site to the carbonyl group,

R³ stands for hydrogen,

R⁴ stands for benzyl or 1*H*-indol-3-ylmethyl,

5 or

R³ and R⁴ together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

10 #⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷ or -C(=O)-NR⁸R⁹,

wherein

15 R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R⁸ stands for hydrogen,

R⁹ stands for hydrogen or benzyl,

R³⁵ stands for methyl,

as well as their salts and solvates as well as the solvates of the salts.

20 The preferred subject matter of the present invention relates to binder-drug conjugates of the general formula (Ia) as indicated above, wherein

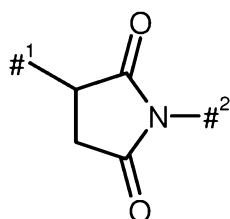
n stands for a number from 1 to 10,

AK stands for AK_1 ,

wherein

AK₁ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the
5 group G via the sulfur atom of a cysteine radical of the binder,

G stands for a group of the formula

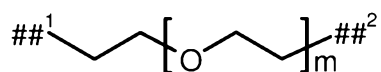


wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

10 $\#^2$ denotes the linkage site to the group L^1 ,

L¹ stands for a bond, linear (C₃-C₅)-alkanediyl or a group of the formula



wherein

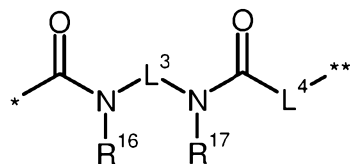
m stands for a number of 2 or 3,

15 ##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₃-C₅)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



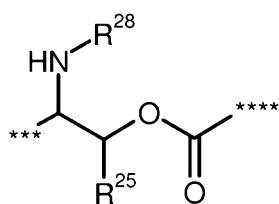
wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

5 L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

10 **** denotes the linkage site to L²,

R²⁵ stands for methyl,

R²⁸ stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

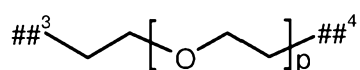
R¹⁶ stands for hydrogen or methyl,

R¹⁷ stands for hydrogen or methyl,

15 or

R¹⁶ and R¹⁷ together with the atoms to which they are bound form a piperazinyl ring,

L² stands for linear (C₃-C₅)-alkanediyl or for a group of the formula



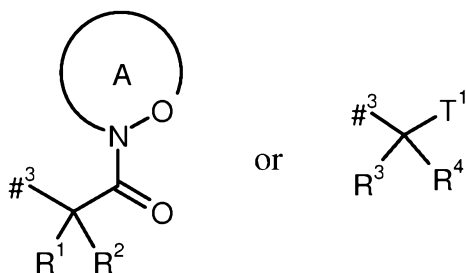
wherein

p stands for a number of 2 or 3,

$\#^3$ denotes the linkage site to the group B,

$\#^4$ denotes the linkage site to the nitrogen atom,

5 D stands for a group of the formula



wherein

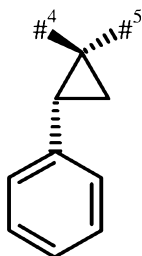
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

10 R^2 stands for benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

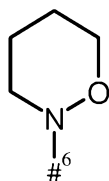


15 wherein

$\#^4$ denotes the linkage site to the vicinal nitrogen atom,

$\#^5$ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

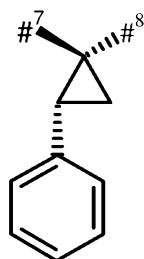
5 #⁶ denotes the linkage site to the carbonyl group,

R³ stands for hydrogen,

R⁴ stands for benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

15 T¹ stands for a group of the formula -C(=O)-OR⁷ or -C(=O)-NR⁸R⁹,

wherein

R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

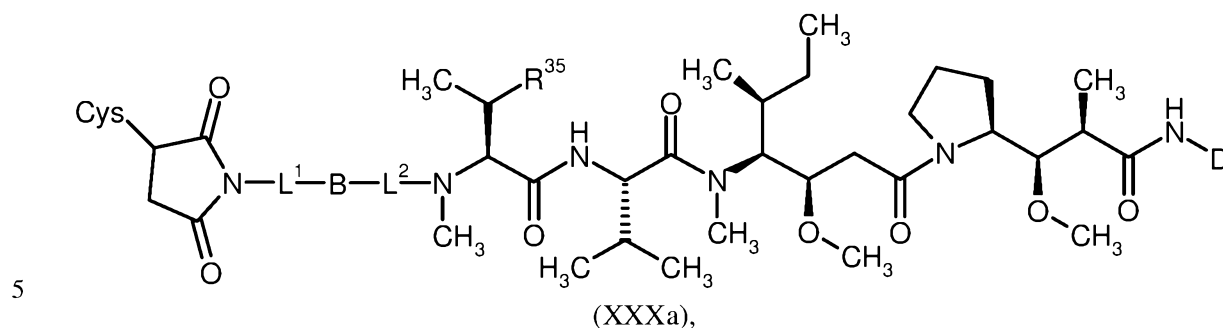
R⁸ stands for hydrogen,

R^9 stands for hydrogen or benzyl,

R^{35} stands for methyl,

as well as their salts and solvates as well as the solvates of the salts.

Another subject matter of the present invention relates to compounds of the formula (XXXa)



in which

Cys stands for a cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

10 L^1 stands for a bond, linear (C_1-C_{10}) -alkanediyl, a group of the formula



wherein

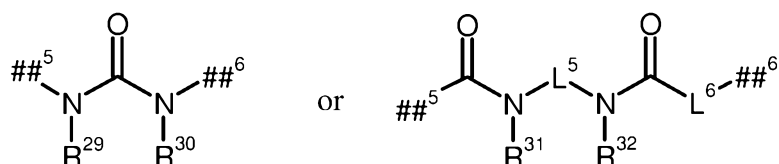
m stands for a number from 2 to 6,

$\# \#^1$ denotes the linkage site to the group G,

15 $\# \#^2$ denotes the linkage site to the group B,

L^{1A} stands for linear (C_2-C_{10}) -alkanediyl,

B^1 stands for a group of the formula



wherein

$\#\#^5$ denotes the linkage site to the group L^{1A} ,

$\#\#^6$ denotes the linkage site to the group L^{1B} ,

L^5 stands for a bond or (C₂-C₄)-alkanediyl,

5 L^6 stands for a bond,

R^{29} stands for hydrogen or (C₁-C₄)-alkyl,

R^{30} stands for hydrogen or (C₁-C₄)-alkyl,

or

10 R^{29} and R^{30} together with the atoms to which they are bound form a five- or six-membered heterocycle,

R^{31} stands for hydrogen or (C₁-C₄)-alkyl,

R^{32} stands for hydrogen or (C₁-C₄)-alkyl,

or

15 R^{31} and R^{32} together with the atoms to which they are bound form a five- or six-membered heterocycle,

L^{1B} stands for linear (C₂-C₁₀)-alkanediyl,

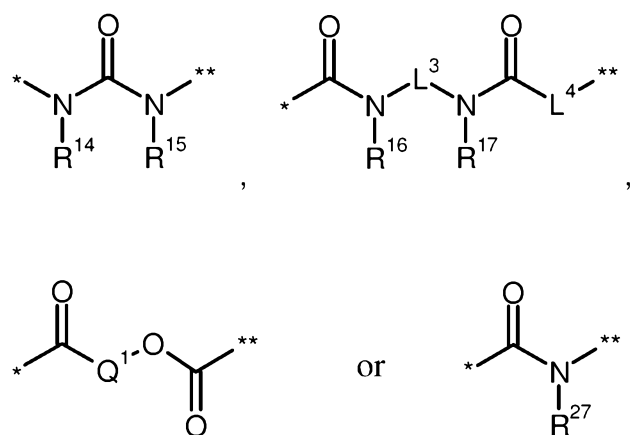
and

wherein (C₁-C₁₀)-alkanediyl may be substituted with one to four substituents selected independently of one another from the group comprising methyl, hydroxyl and benzyl

20 and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another to form a (C₃-C₆)-cycloalkyl ring or a phenyl ring by including the carbon atoms optionally situated between them,

B stands for a bond or a group of the formula



wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

5 P stands for O or NH,

L³ stands for a bond or (C₂-C₄)-alkanediyl,

L⁴ stands for a bond,

Q¹ stands for a four- to seven-membered heterocycle,

10 Q² stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,

R¹⁴ stands for hydrogen or (C₁-C₄)-alkyl,

R¹⁵ stands for hydrogen or (C₁-C₄)-alkyl,

or

15 R¹⁴ and R¹⁵ together with the atoms to which they are bound form a five- or six-membered heterocycle,

R¹⁶ stands for hydrogen or (C₁-C₄)-alkyl,

R¹⁷ stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{16} and R^{17} together with the atoms to which they are bound form a five- or six-membered heterocycle,

R^{18} stands for hydrogen or (C₁-C₄)-alkyl,

5 R^{19} stands for hydrogen or the side group of a natural α -amino acid or its homologs or isomers,

R^{20} stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidinyl ring,

10 R^{21} stands for hydrogen or (C₁-C₄)-alkyl,

R^{22} stands for hydrogen or (C₁-C₄)-alkyl,

or

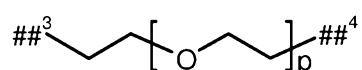
R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

15 R^{23} stands for (C₁-C₄)-alkyl,

R^{24} stands for hydrogen or (C₁-C₄)-alkyl,

R^{27} stands for hydrogen or (C₁-C₄)-alkyl,

L^2 stands for linear (C₂-C₁₀)-alkanediyl or for a group of the formula



20 wherein

p stands for a number from 2 to 6,

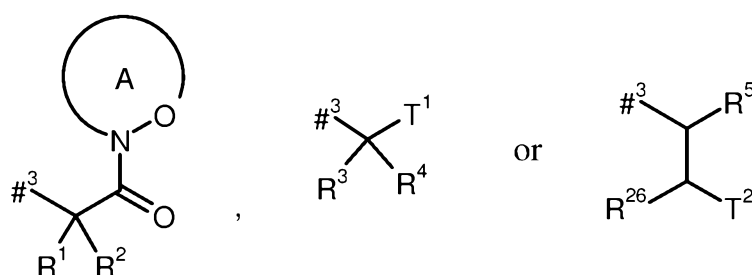
$##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

wherein (C₂-C₁₀)-alkanediyl may be substituted with 1 to 4 substituents selected independently of one another from the group comprised of methyl, hydroxyl and benzyl, and

5 wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another to form a (C₃-C₆) cycloalkyl ring or a phenyl ring, including the carbon atoms optionally situated between them,

D stands for a group of the formula



wherein

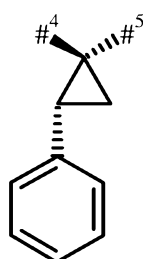
10 #³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen or methyl,

R² stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or
15 1*H*-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



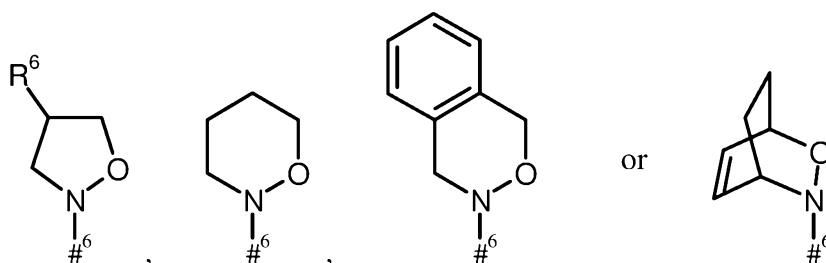
wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic,
optionally substituted heterocycle of the formula

5



wherein

#⁶ denotes the linkage site to the carbonyl group,

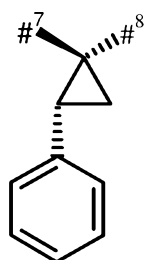
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

10 R³ stands for hydrogen or methyl,

R⁴ stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxy-ethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

15 R³ and R⁴ together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

5 wherein

R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R⁸ stands for hydrogen or methyl,

R⁹ stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

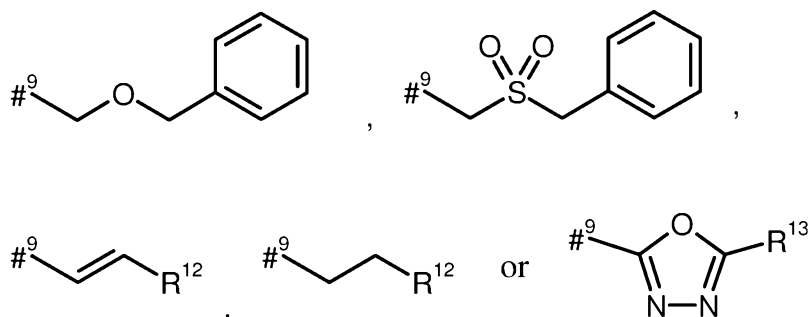
10 or

R⁸ and R⁹ together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R¹⁰ stands for benzoyl,

15 R¹¹ stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R⁵ stands for hydrogen, methyl or a group of the formula



wherein

#⁹ denotes the linkage site to -CHC(R²⁶)-T²,

20 R¹² stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula -S(O)₂OH,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

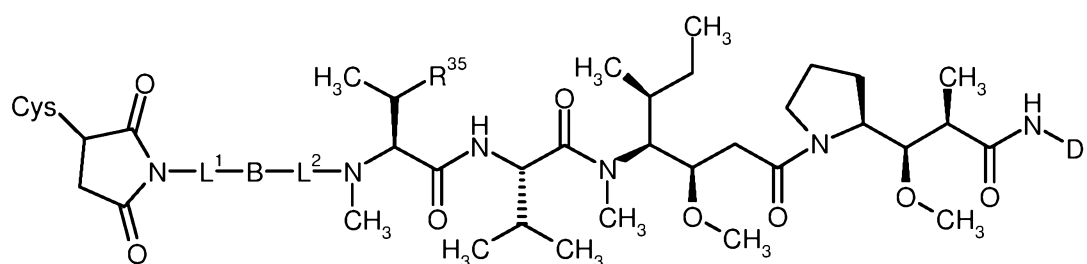
R^{26} stands for hydrogen or hydroxyl,

T^2 stands for phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

5 R^{35} stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention is compounds of formula (XXXa) as indicated above, wherein



10

(XXXa),

Cys stands for a cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

L^1 stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



15

wherein

m stands for a number of 2 or 3,

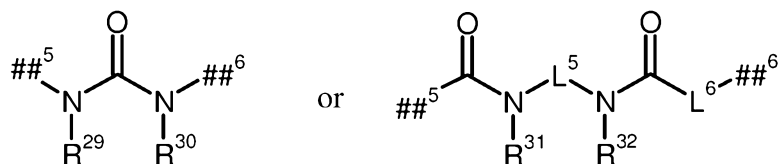
$##^1$ denotes the linkage site to the group G,

$##^2$ denotes the linkage site to the group B,

L^{1A} stands for linear (C₂-C₆)-alkanediyl,

20

B^1 stands for a group of the formula



wherein

##⁵ denotes the linkage site to the group L^{1A},

##⁶ denotes the linkage site to the group L^{1B},

5 L⁵ stands for a bond,

L⁶ stands for a bond,

R²⁹ stands for hydrogen,

R³⁰ stands for hydrogen,

R³¹ stands for hydrogen or methyl,

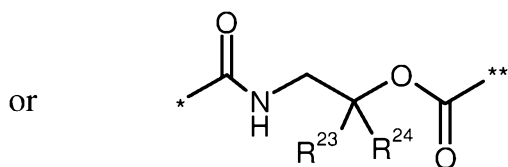
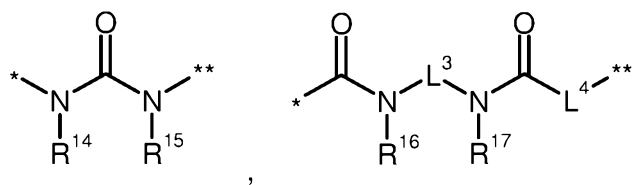
10 R³² stands for hydrogen or methyl,

L^{1B} stands for linear (C₂-C₆)-alkanediyl,

and

wherein (C₂-C₆)-alkanediyl may be substituted with one to two methyl substituents,

B stands for a bond or a group of the formula



15

wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

L^3 stands for a bond or ethane-1,2-diyl,

L^4 stands for a bond,

5 R^{14} stands for hydrogen,

R^{15} stands for hydrogen,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

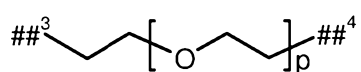
or

10 R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyrling,

R^{23} stands for methyl,

R^{24} stands for hydrogen or methyl,

L^2 stands for linear (C_2 - C_6)-alkanediyl or for a group of the formula



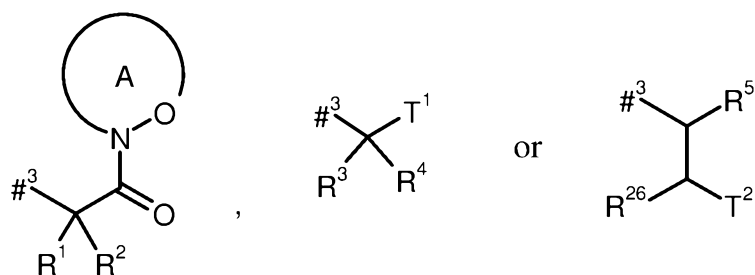
15 wherein

p stands for a number of 2 or 3,

$##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

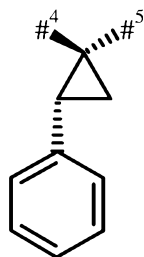
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



10

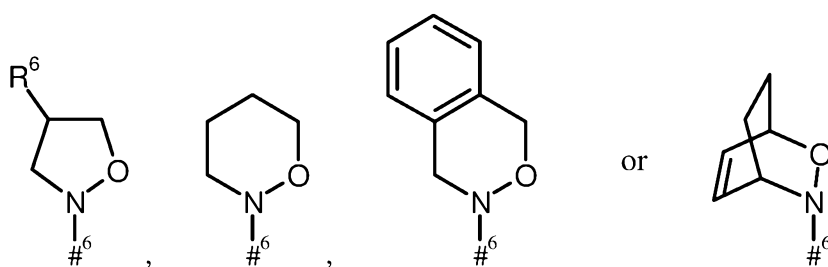
wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula

15



wherein

#⁶ denotes the linkage site to the carbonyl group,

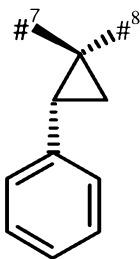
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen,

R⁴ stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

15 T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

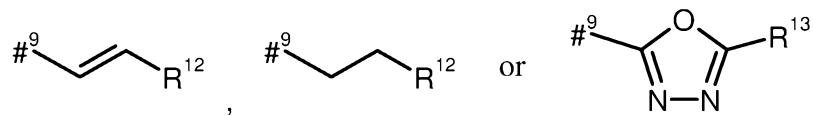
5 or

R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

#⁹ denotes the linkage site to $-\text{CHCH}_2\text{-phenyl}$,

15 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{35} stands for methyl or hydroxyl,

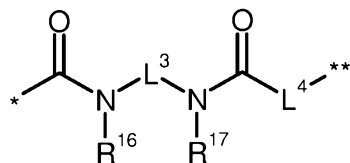
20 as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention is compounds of formula (XXXa) as indicated above, wherein

Cys stands for a cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

L^1 stands for a bond or linear (C₂-C₆)-alkanediyl,

B stands for a bond or a group of the formula



wherein

5 * denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

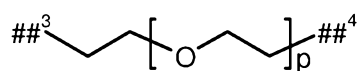
L^3 stands for a bond,

L^4 stands for a bond,

R^{16} stands for hydrogen or methyl,

10 R^{17} stands for hydrogen or methyl,

L^2 stands for linear (C₂-C₆)-alkanediyl or for a group of the formula



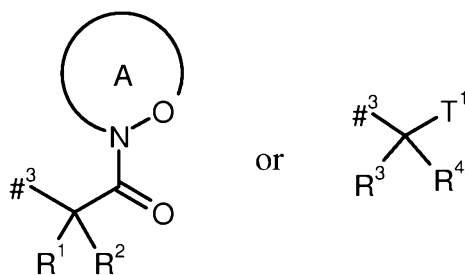
wherein

p stands for a number of 2 or 3,

15 $##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

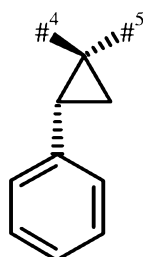
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for benzyl or 1*H*-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



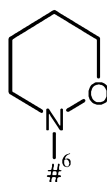
10

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



15

wherein

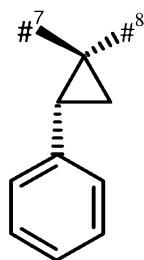
#⁶ denotes the linkage site to the carbonyl group,

R³ stands for hydrogen,

R⁴ stands for benzyl or 1*H*-indol-3-ylmethyl,

5 or

R³ and R⁴ together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

10 #⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷ or -C(=O)-NR⁸R⁹,

wherein

R⁷ stands for hydrogen,

15 R⁸ stands for hydrogen,

R⁹ stands for hydrogen,

R³⁵ stands for methyl,

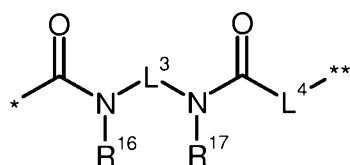
as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention is compounds of formula (XXXa) as
20 indicated above, wherein

Cys stands for a cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

L^1 stands for a bond or linear (C_2-C_6) -alkanediyl,

B stands for a bond or a group of the formula



5

wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

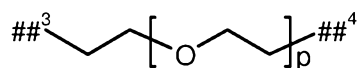
L^3 stands for a bond,

10 L^4 stands for a bond,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

L^2 stands for linear (C_2-C_6) -alkanediyl or for a group of the formula



15

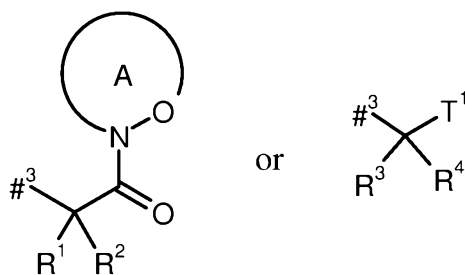
wherein

p stands for a number of 2 or 3,

$##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

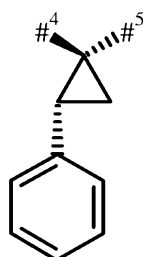
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



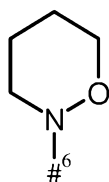
10

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



15

wherein

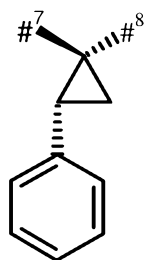
#6 denotes the linkage site to the carbonyl group,

R^3 stands for hydrogen,

R^4 stands for benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

5 or

R^3 and R^4 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

10 #⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$ or $-C(=O)-NR^8R^9$,

wherein

R^7 stands for hydrogen,

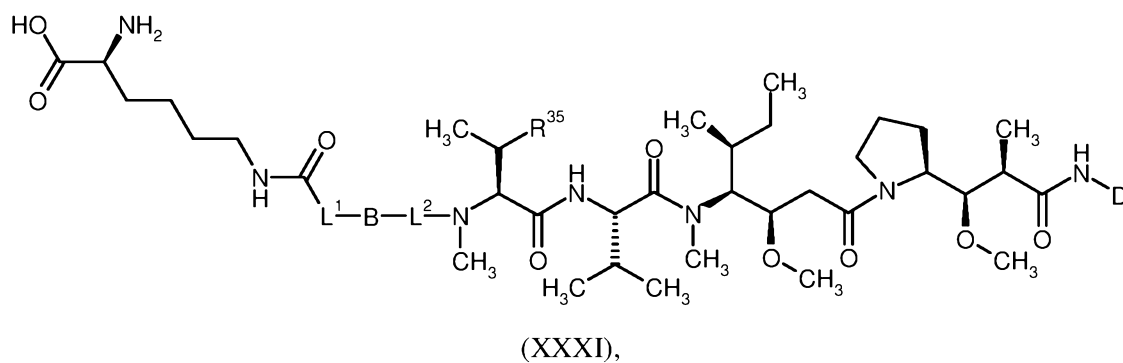
15 R^8 stands for hydrogen,

R^9 stands for hydrogen,

R^{35} stands for methyl,

as well as their salts and solvates as well as the solvates of the salts.

Another subject matter of the present invention relates to compounds of formula (XXXI)



in which

L¹ stands for a bond, linear (C₁-C₁₀)-alkanediyl, a group of the formula



wherein

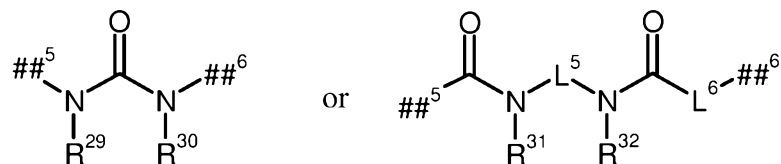
m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

10 L^{1A} stands for linear (C₂-C₁₀)-alkanediyl,

B^1 stands for a group of the formula



wherein

##⁵ denotes the linkage site to the group L^{1A},

15 $\#\#^6$ denotes the linkage site to the group L^{1B} ,

L⁵ stands for a bond or (C₂-C₄)-alkanediyl,

L^6 stands for a bond,

R^{29} stands for hydrogen or (C₁-C₄)-alkyl,

R^{30} stands for hydrogen or (C₁-C₄)-alkyl,

or

5 R^{29} and R^{30} together with the atoms to which they are bound form a five- or six-membered heterocycle,

R^{31} stands for hydrogen or (C₁-C₄)-alkyl,

R^{32} stands for hydrogen or (C₁-C₄)-alkyl,

or

10 R^{31} and R^{32} together with the atoms to which they are bound form a five- or six-membered heterocycle,

L^{1B} stands for linear (C₂-C₁₀)-alkanediyl,

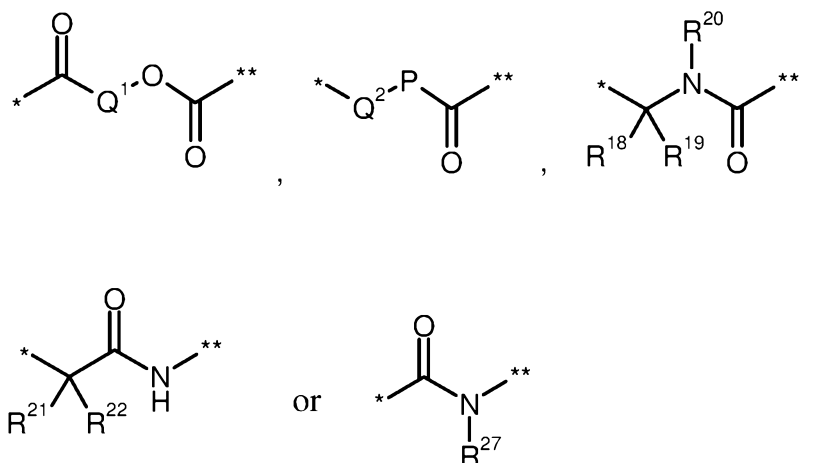
and

wherein (C₁-C₁₀)-alkanediyl may be substituted with one to four substituents selected independently from one another from the group comprising methyl, hydroxyl and benzyl

15 and

wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally situated between them, to form a (C₃-C₆)-cycloalkyl ring or a phenyl ring,

B stands for a bond or a group of the formula



wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

5 P stands for O or NH,

Q¹ stands for a four- to seven-membered heterocycle,

Q² stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,

R¹⁸ stands for hydrogen or (C₁-C₄)-alkyl,

10 R¹⁹ stands for hydrogen or the side group of a natural α-amino acid or its homologs or isomers,

R²⁰ stands for hydrogen or (C₁-C₄)-alkyl,

or

15 R¹⁹ and R²⁰ together with the atoms to which they are bound form a pyrrolidinyl ring,

R²¹ stands for hydrogen or (C₁-C₄)-alkyl,

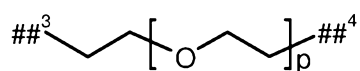
R²² stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{27} stands for hydrogen or (C_1-C_4) -alkyl,

L^2 stands for linear (C_2-C_{10}) -alkanediyl or for a group of the formula



5

wherein

p stands for a number from 2 to 6,

$\#^3$ denotes the linkage site to the group B,

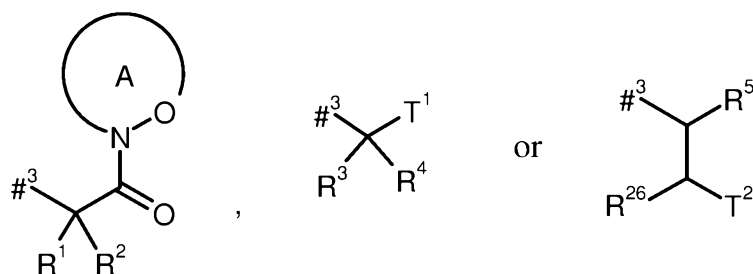
$\#^4$ denotes the linkage site to the nitrogen atom,

10 Wherein (C_2-C_{10}) -alkanediyl may be substituted with 1 to 4 substituents selected independently of one another from the group comprising methyl, hydroxyl and benzyl,

and

15 wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally present between them to form a (C_3-C_6) cycloalkyl ring or a phenyl ring,

D stands for a group of the formula



wherein

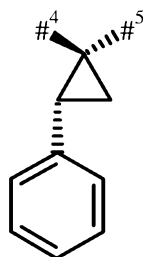
$\#^3$ denotes the linkage site to the nitrogen atom,

20 R^1 stands for hydrogen or methyl,

R^2 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

5 or

R^1 and R^2 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

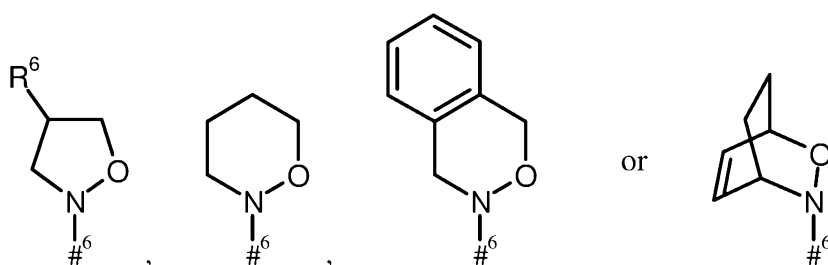


wherein

10 #⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



15 wherein

#⁶ denotes the linkage site to the carbonyl group,

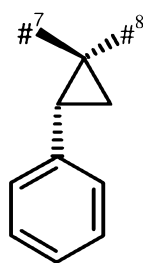
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxy-ethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

5 R^3 and R^4 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

$\#^7$ denotes the linkage site to the vicinal nitrogen atom,

10 $\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

wherein

15 R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

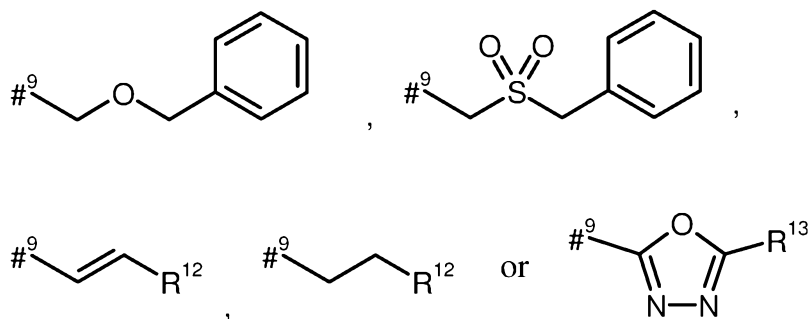
or

20 R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

R^{11} stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



5 wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(R^{26})-\text{T}^2$,

R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

10 R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

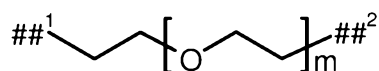
T^2 stands for phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

15 The preferred subject matter of the present invention is compounds of formula (XXXI) as indicated above, wherein

L^1 stands for a bond, linear (C_2-C_6) -alkanediyl, a group of the formula



wherein

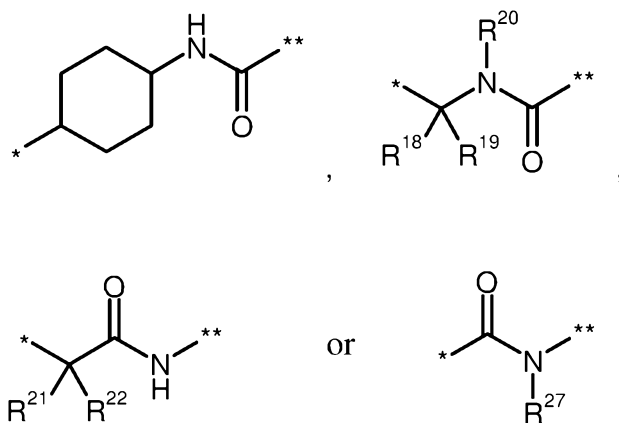
m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

5 B stands for a bond or a group of the formula



wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

10 R¹⁸ stands for hydrogen,

R¹⁹ stands for hydrogen, methyl, propan-2-yl, 2-methylpropan-1-yl or 1-methylpropan-1-yl,

R²⁰ stands for hydrogen or (C₁-C₄)-alkyl,

or

15 R¹⁹ and R²⁰ together with the atoms to which they are bound form a pyrrolidinyl ring,

R²¹ stands for hydrogen or methyl,

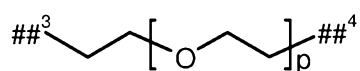
R²² stands for hydrogen or methyl,

or

R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

R^{27} stands for hydrogen or methyl,

5 L^2 stands for linear (C_2-C_6) -alkanediyl or for a group of the formula



wherein

p stands for a number of 2 or 3,

$##^3$ denotes the linkage site to the group B,

10 $##^4$ denotes the linkage site to the nitrogen atom,

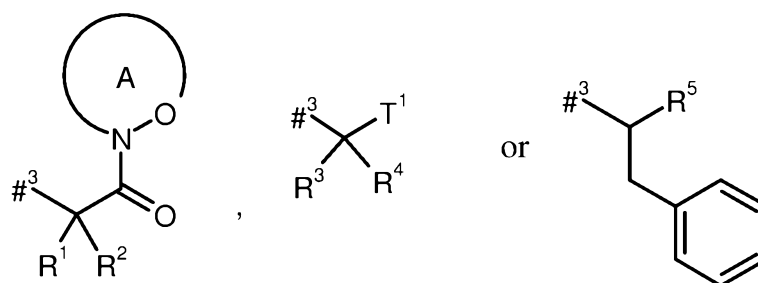
wherein (C_2-C_{10}) -alkanediyl may be substituted with one or two methyl substituents,

and

wherein two carbon atoms of the alkanediyl chain may be bridged in 1,4-relation to one another, including the carbon atoms optionally present between them to form a phenyl ring,

15

D stands for a group of the formula



wherein

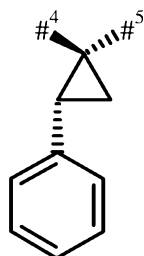
$##^3$ denotes the linkage site to the nitrogen atom,

20 R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

5 R^1 and R^2 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

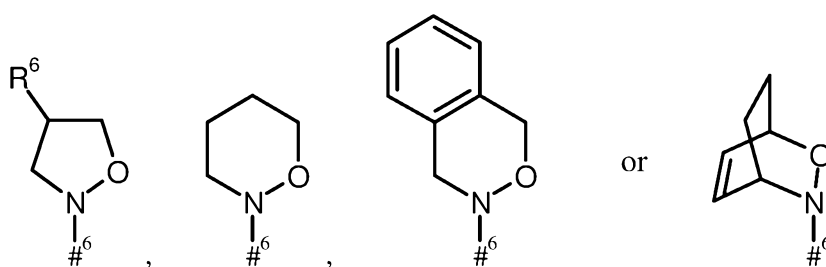


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

10 the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

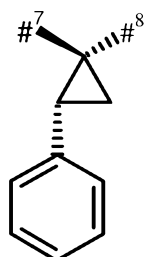
15 R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



5 wherein

$\#^7$ denotes the linkage site to the vicinal nitrogen atom,

$\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

10 wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

15 or

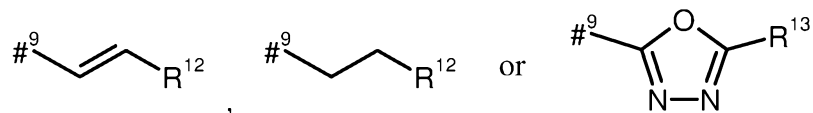
R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

R^{11} stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

20

R^5 stands for hydrogen, methyl or a group of the formula



wherein

#⁹ denotes the linkage site to –CHCH₂-phenyl,

R¹² stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula –S(O)₂OH,

R¹³ stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R³⁵ stands for methyl or hydroxyl,

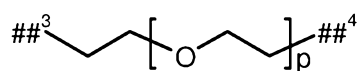
as well as their salts and solvates as well as the solvates of the salts.

10 The preferred subject matter of the present invention is compounds of formula (XXXI) as indicated above, wherein

L¹ stands for a bond,

B stands for a bond,

L² stands for linear (C₂-C₆)-alkanediyl or for a group of the formula



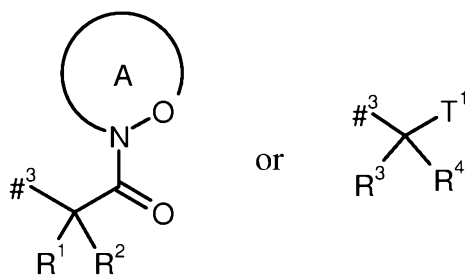
wherein

p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

##⁴ denotes the linkage site to the nitrogen atom,

20 D stands for a group of the formula



wherein

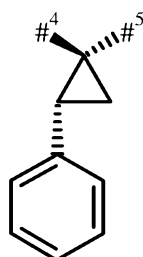
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for benzyl or 1*H*-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



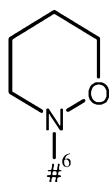
10

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



15

wherein

#⁶ denotes the linkage site to the carbonyl group,

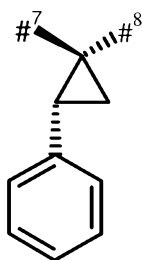
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

R³ stands for hydrogen,

5 R⁴ stands for benzyl or 1*H*-indol-3-ylmethyl,

or

R³ and R⁴ together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



10 wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷ or -C(=O)-NR⁸R⁹,

wherein

15 R⁷ stands for hydrogen,

R⁸ stands for hydrogen,

R⁹ stands for hydrogen,

R³⁵ stands for methyl,

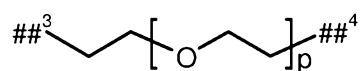
as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention is compounds of formula (XXXI) as indicated above, wherein

L^1 stands for a bond,

B stands for a bond,

5 L^2 stands for linear (C₂-C₆)-alkanediyl or for a group of the formula



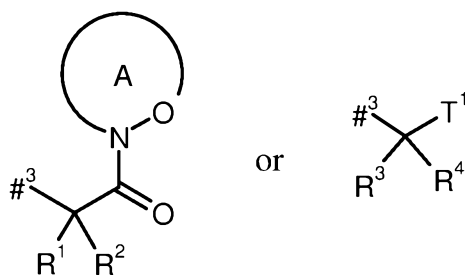
wherein

p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

10 ##⁴ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

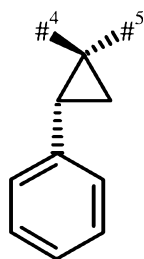
##³ denotes the linkage site to the nitrogen atom,

15 R¹ stands for hydrogen,

R² stands for benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

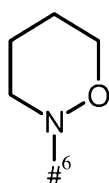


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

5 the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

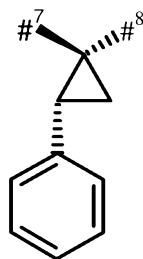
10 R⁶ stands for hydrogen, hydroxyl or benzyloxy,

R³ stands for hydrogen,

R⁴ stands for benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

15 R³ and R⁴ together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

5 T¹ stands for a group of the formula -C(=O)-OR⁷ or -C(=O)-NR⁸R⁹,

wherein

R⁷ stands for hydrogen,

R⁸ stands for hydrogen,

R⁹ stands for hydrogen,

10 R³⁵ stands for methyl,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention relates to compounds of formula (XXXa) and (XXXI) selected from the group:

15 *N*-[6-(3-{[(2*R*)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide,

20 *N*-[6-(3-{[(2*R*)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidine-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide,

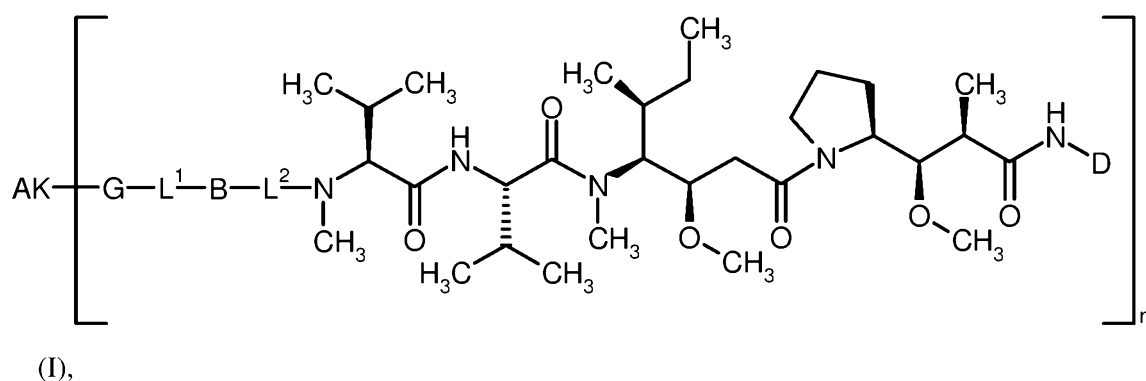
N-(6-{[(5*S*)-5-amino-5-carboxypentyl]amino}-6-oxohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-

oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate,

N-(6-{[(5*S*)-5-amino-5-carboxypentyl]amino}-6-oxohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide,

as well as their salts and solvates as well as the solvates of the salts.

Another preferred subject matter of the present invention relates to binder-drug conjugates of the general formula (I)



in which

n stands for a number from 1 to 50,

AK stands for a binder, preferably a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

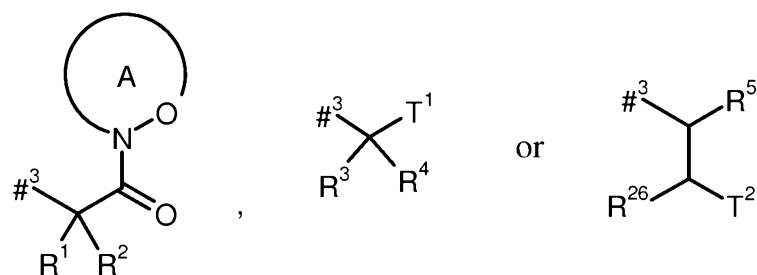
the group §-G-L¹-B-L²-§§ stands for a linker,

wherein

§ denotes the linkage site to the group AK and

§§ denotes the linkage site to the nitrogen atom,

20 D stands for a group of the formula



wherein

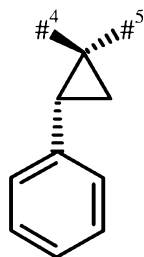
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for 1-hydroxyethyl, benzyl, 1-phenylethyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



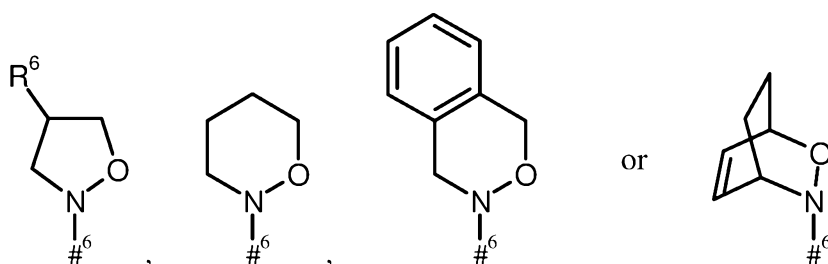
10

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

15 the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

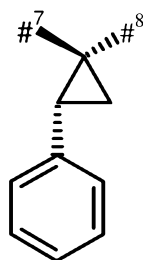
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen,

R⁴ stands for 1-hydroxyethyl, benzyl, 1-phenylethyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

15 T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

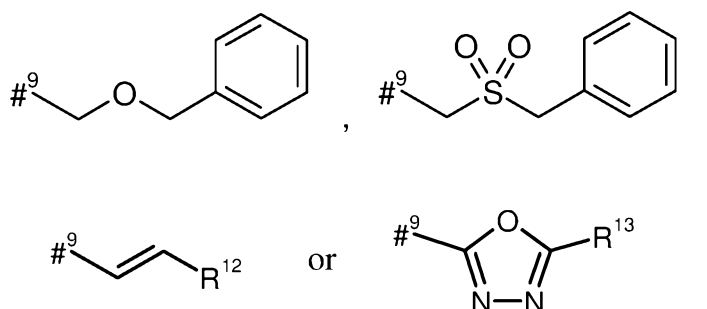
5 or

R^8 and R^9 together with the nitrogen atom to which they are attached form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $\text{-CHC(R}^{26}\text{)-T}^2$,

15 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $\text{-S(O)}_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

20 T^2 stands for phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the invention relates to binder-drug conjugates of the general formula (I), wherein

n stands for a number from 1 to 50,

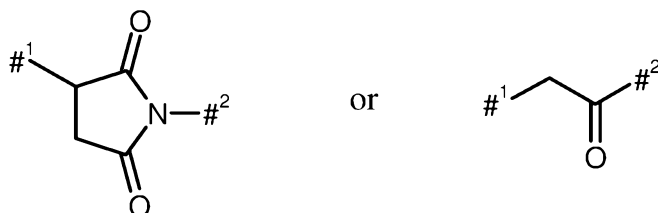
AK stands for AK₁ or AK₂

5 wherein

AK₁ denotes a binder which is bound by a nitrogen atom of the binder to the group G, preferably for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

10 AK₂ stands for a binder bound to group G by a nitrogen atom of the binder, preferably standing for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

G for the case when AK = AK₁ stands for a group of the formula



wherein

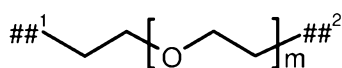
15 #¹ denotes the linkage site to the sulfur atom of the binder,

#² denotes the linkage site to the group L¹,

or

for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₁-C₁₀)-alkanediyl or for a group of the formula



20

wherein

m stands for a number from 2 to 6,

$\#^1$ denotes the linkage site to the group G,

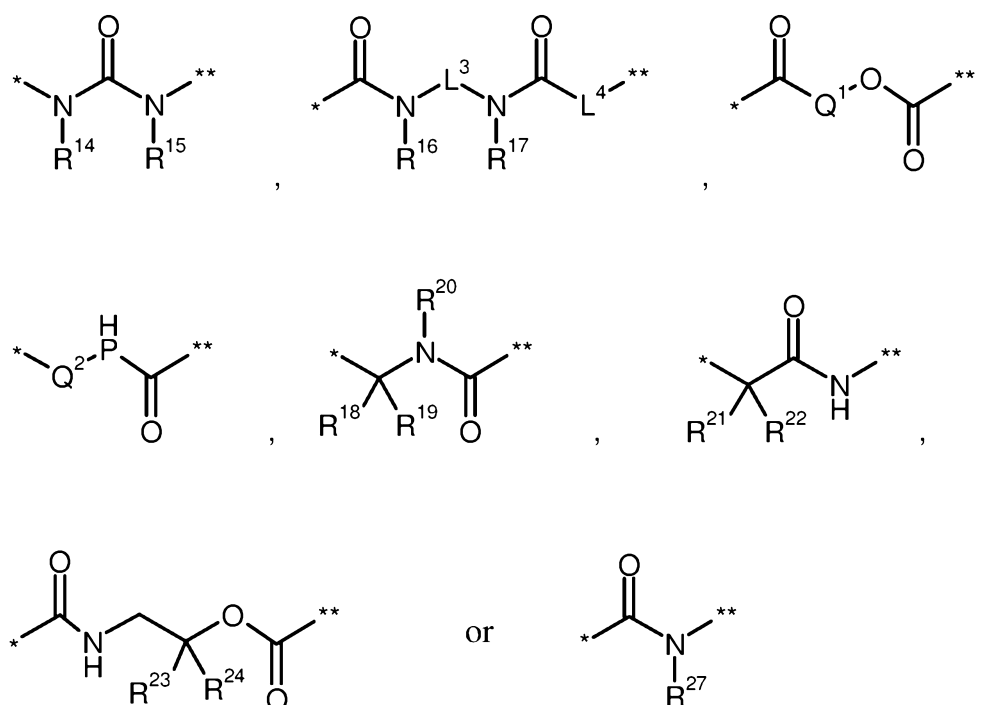
$\#^2$ denotes the linkage site to the group B,

wherein (C₁-C₁₀)-alkanediyl may be substituted with 1 to 4 methyl substituents,

5 and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another to form a (C₃-C₆)-cycloalkyl ring or a phenyl ring, including the carbon atoms optionally situated between them,

B stands for a bond or a group of the formula



10

wherein

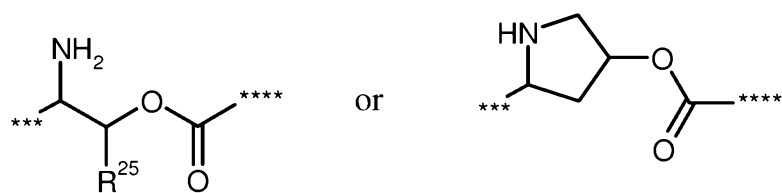
* denotes the linkage site to L¹,

** denotes the linkage site to L²,

P stands for O or NH,

15 L³ for a bond or (C₂-C₄)-alkanediyl,

L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

5 **** denotes the linkage site to L²,

R²⁵ stands for hydrogen or methyl,

Q¹ stands for a four- to seven-membered heterocycle,

Q² stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,

10 R¹⁴ stands for hydrogen or (C₁-C₄)-alkyl,

R¹⁵ stands for hydrogen or (C₁-C₄)-alkyl,

or

R¹⁴ and R¹⁵ together with the atoms to which they are bound form a five- or six-membered heterocycle,

15 R¹⁶ stands for hydrogen or (C₁-C₄)-alkyl,

R¹⁷ stands for hydrogen or (C₁-C₄)-alkyl,

or

R¹⁶ and R¹⁷ together with the atoms to which they are bound form a five- or six-membered heterocycle,

20 R¹⁸ stands for hydrogen or (C₁-C₄)-alkyl,

R¹⁹ stands for hydrogen or the side group of a natural α -amino acid or its homologs or isomers,

R^{20} stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidinyl ring,

5 R^{21} stands for hydrogen or (C₁-C₄)-alkyl,

R^{22} stands for hydrogen or (C₁-C₄)-alkyl,

or

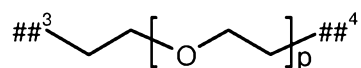
R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

10 R^{23} stands for (C₁-C₄)-alkyl,

R^{24} stands for hydrogen or (C₁-C₄)-alkyl,

R^{27} stands for hydrogen or (C₁-C₄)-alkyl,

L^2 stands for linear (C₂-C₁₀)-alkanediyl or for a group of the formula



15 wherein

p stands for a number from 2 to 6,

##^3 denotes the linkage site to the group B,

##^4 denotes the linkage site to the nitrogen atom,

wherein (C₂-C₁₀)-alkanediyl may be substituted with 1 to 4 methyl substituents,

20 and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another to form a (C₃-C₆)-cycloalkyl ring or a phenyl ring, including the carbon atoms optionally situated between them,

D has the meanings given above,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the invention is binder-drug conjugates of the general formula (I)

in which

5 n stands for a number from 1 to 50,

AK stands for AK₁ or AK₂

wherein

10 AK₁ stands for an antibody or an antigen binding antibody fragment and is bound to the group G via a sulfur atom, preferably standing for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

AK₂ stands for an antibody or an antigen binding antibody fragment and is bound to the group G via a nitrogen atom, preferably standing for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

G, L¹, B, L² and D have the meanings given above

15 as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention is binder-drug conjugates of general formula (I)

in which

n stands for a number from 1 to 20,

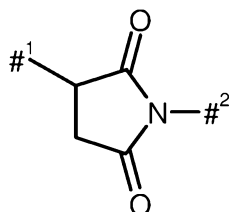
20 AK stands for AK₁ or AK₂

wherein

AK₁ stands for an antibody or an antigen binding antibody fragment which binds to EGFR and is bound to the group G via the sulfur atom of a cysteine radical of the binder,

AK₂ stands for an antibody or an antigen binding antibody fragment which binds to EGFR and is bound to the group G via the NH side group of a lysine radical of the binder,

G for the case when AK = AK₁ stands for a group of the formula



5

wherein

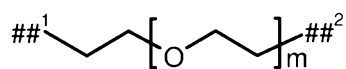
#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

or

10 for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₂-C₆)-alkanediyl or for a group of the formula



wherein

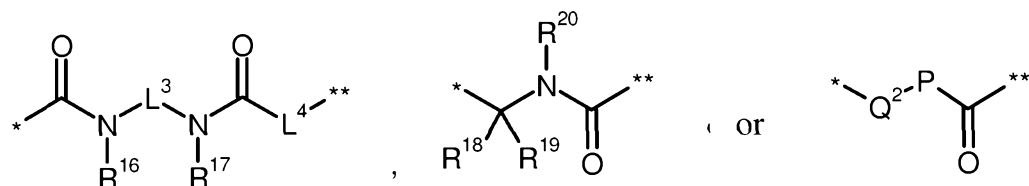
m stands for a number from 2 to 6,

15 ##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



wherein

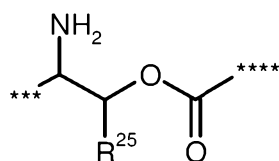
* denotes the linkage site to L¹,

** denotes the linkage site to L²,

5 P stands for O or NH,

L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



wherein

10 *** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L²,

R²⁵ stands for methyl,

Q² stands for cyclopentyl or cyclohexyl,

R¹⁶ stands for hydrogen or methyl,

15 R¹⁷ stands for hydrogen or methyl,

or

R¹⁶ and R¹⁷ together with the atoms to which they are bound form a piperazinyl ring,

R¹⁸ stands for hydrogen,

R^{19} stands for hydrogen, methyl, propan-2-yl, 2-methylpropan-1-yl or 1-methylpropan-1-yl,

R^{20} stands for hydrogen or methyl,

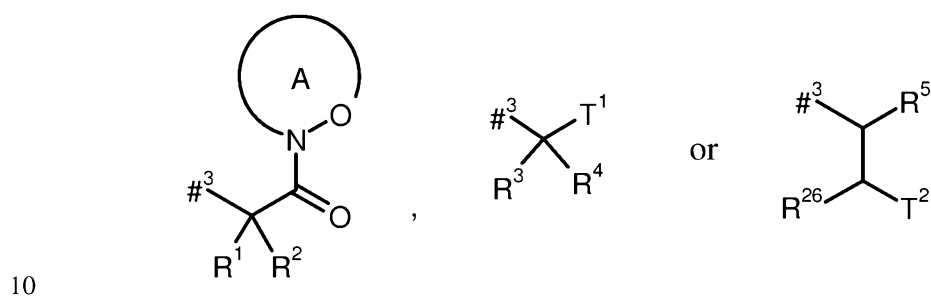
or

5 R^{19} and R^{20} together with the atoms to which they are attached form a pyrrolidinyl ring,

L^2 stands for linear (C_2 - C_6)-alkanediyl,

wherein (C_2 - C_6)-alkanediyl may be substituted with one or two methyl substituents,

D stands for a group of the formula



wherein

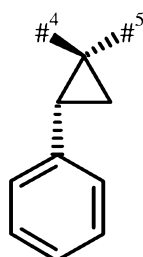
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

15 or

R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



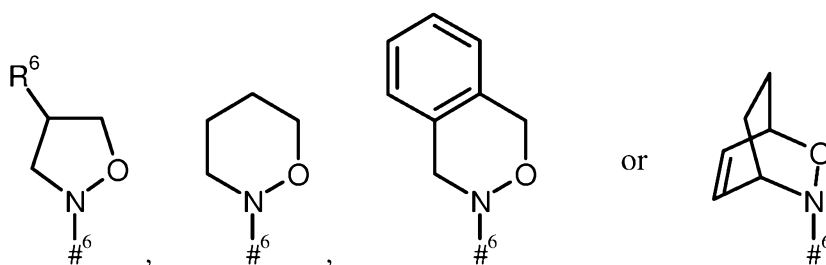
wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic,
optionally substituted heterocycle of the formula

5



wherein

#⁶ denotes the linkage site to the carbonyl group,

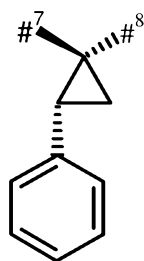
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

10 R³ stands for hydrogen,

R⁴ stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



15

wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

5 R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R⁸ stands for hydrogen or methyl,

R⁹ stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

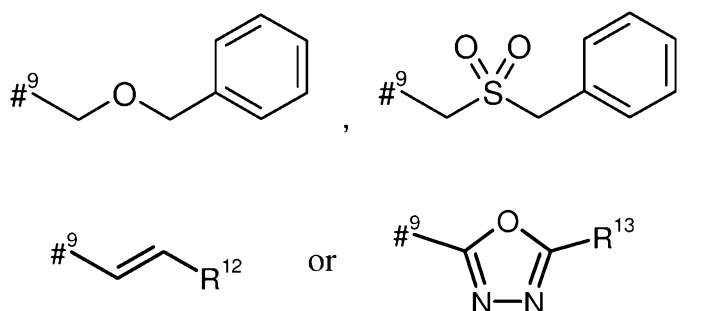
or

10 R⁸ and R⁹ together with the nitrogen atom to which they are attached form a four- to seven-membered heterocycle,

R¹⁰ stands for benzoyl,

R¹¹ stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

15 R⁵ stands for hydrogen, methyl or a group of the formula



wherein

#⁹ denotes the linkage site to -CHC(R²⁶)-T²,

20 R¹² stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula -S(O)₂OH,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

5 as well as their salts and solvates as well as the solvates of the salts.

Especially preferred as the subject matter of the present invention are binder-drug conjugates of general formula (I)

in which

n stands for a number from 1 to 10,

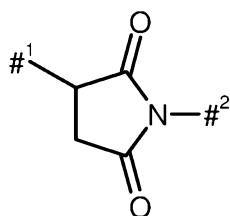
10 AK stands for AK_1 or AK_2

wherein

AK_1 stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the sulfur atom of a cysteine radical of the binder,

15 AK_2 stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G for the case when $AK = AK_1$ stands for a group of the formula



wherein

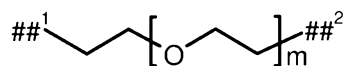
#¹ denotes the linkage site to the cysteine radical of the binder,

20 #² denotes the linkage site to the group L^1 ,

or

for the case when $AK = AK_2$, G stands for carbonyl,

L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



wherein

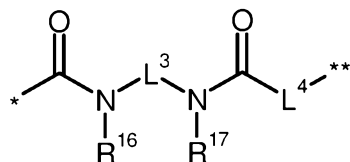
m stands for a number of 2 or 3,

5 ##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



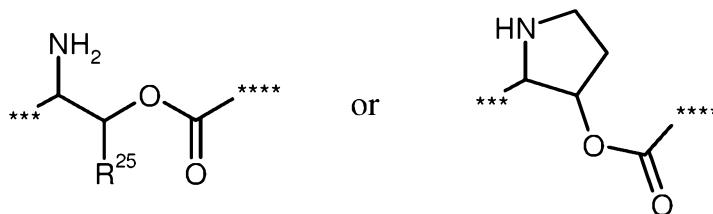
10 wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



15

wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L²,

R^{25} stands for methyl,

R^{16} stands for hydrogen or methyl,

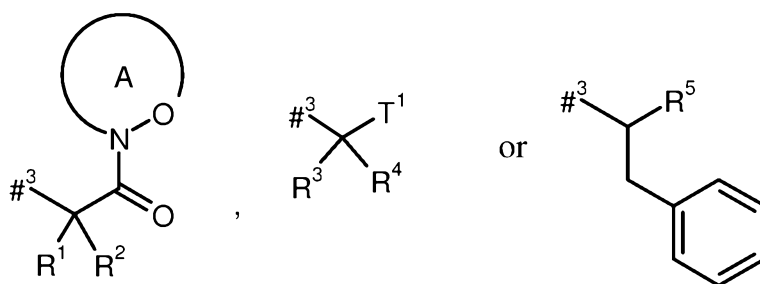
R^{17} stands for hydrogen or methyl,

or

5 R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

L^2 stands for linear (C_2 - C_6)-alkanediyl,

D stands for a group of the formula



wherein

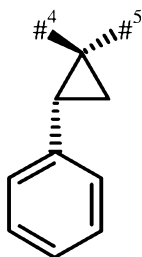
10 $\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

15 R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

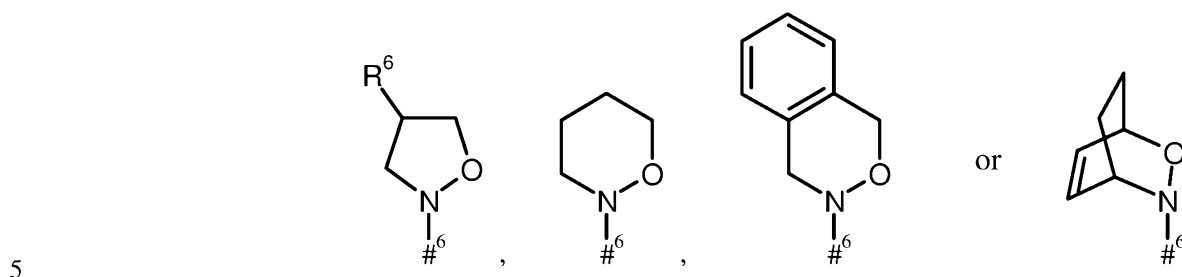


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

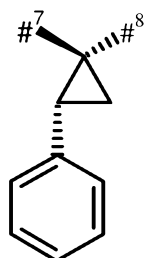
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

R³ stands for hydrogen,

10 R⁴ stands for benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



15 wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹ or -CH₂-O-R¹¹,

wherein

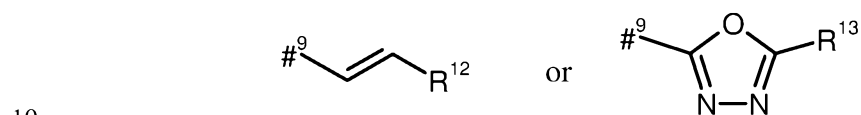
R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, benzyl or adamantylmethyl,

5 R⁸ stands for hydrogen or methyl,

R⁹ stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

R¹¹ stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R⁵ stands for hydrogen or a group of the formula



wherein

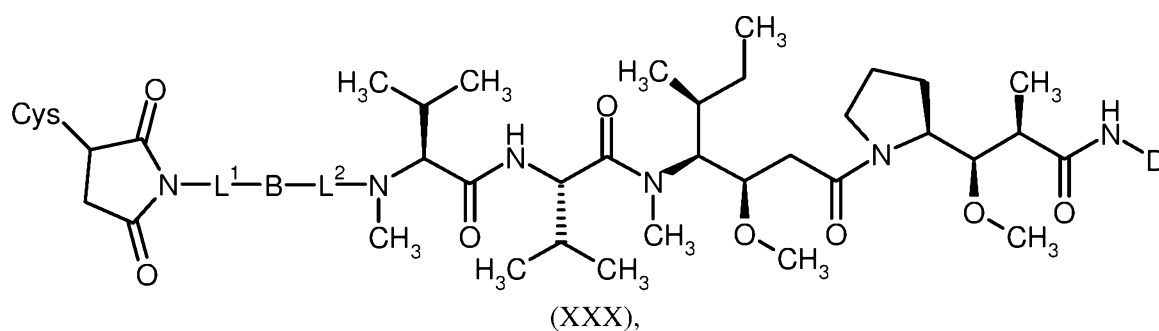
#⁹ denotes the linkage site to -CHC(R²⁶)phenyl,

R¹² stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula -S(O)₂OH,

15 R¹³ stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

as well as their salts and solvates as well as the solvates of the salts.

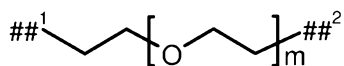
Another subject matter of the present invention relates to compounds of formula (XXX)



in which

Cys stands for a cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

L¹ stands for a bond, linear (C₁-C₁₀)-alkanediyl or for a group of the formula



5

wherein

m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

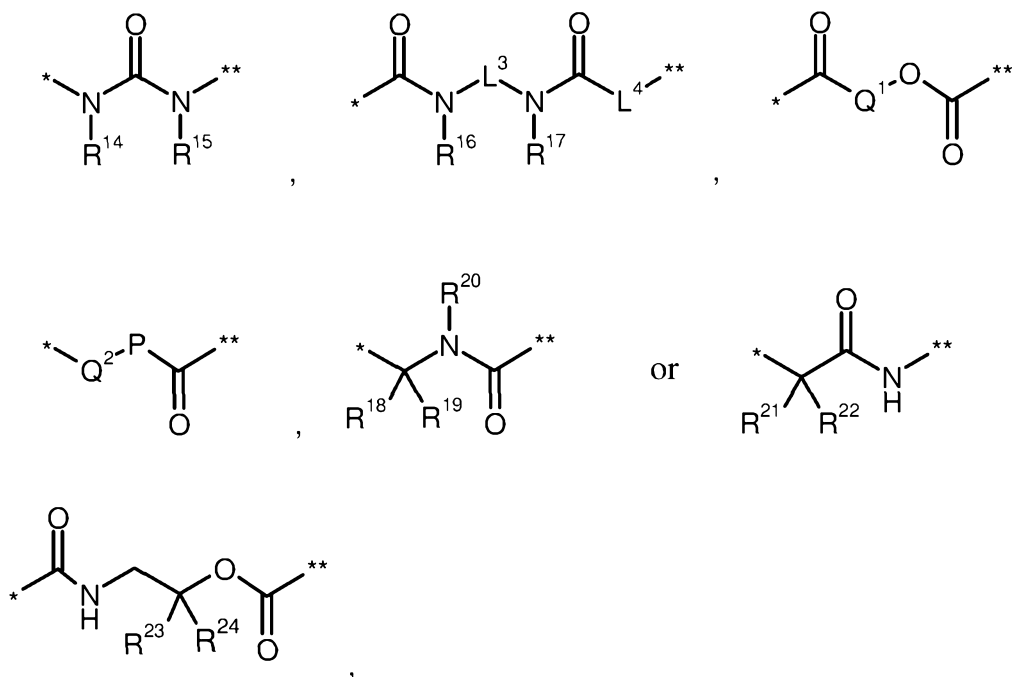
##² denotes the linkage site to the group B,

10 wherein (C₁-C₁₀)-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another to form a (C₃-C₆)-cycloalkyl ring or a phenyl ring, including the carbon atoms optionally situated between them,

15 B stands for a bond or a group of the formula



wherein

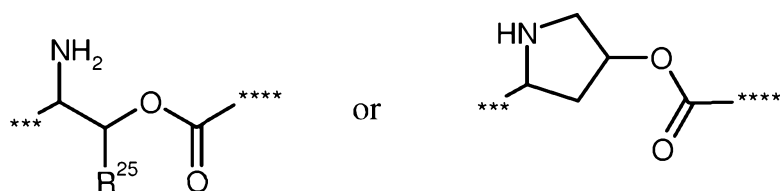
* denotes the linkage site to L¹,

** denotes the linkage site to L²,

5 P stands for O or NH,

L³ for a bond or (C₂-C₄)-alkanediyl,

L⁴ stands for a bond or a group of the formula



wherein

10 *** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L²,

R²⁵ stands for hydrogen or methyl,

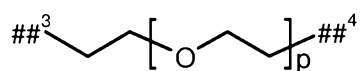
- Q¹ stands for a three- to seven-membered carbocycle or a four- to seven-membered azaheterocycle,
- Q² stands for a three- to seven-membered carbocycle or a four- to seven-membered azaheterocycle,
- 5 R¹⁴ stands for hydrogen or (C₁-C₄)-alkyl,
- R¹⁵ stands for hydrogen or (C₁-C₄)-alkyl,
- or
- R¹⁴ and R¹⁵ together with the atoms to which they are bound form a five- or six-membered heterocycle,
- 10 R¹⁶ stands for hydrogen or (C₁-C₄)-alkyl,
- R¹⁷ stands for hydrogen or (C₁-C₄)-alkyl,
- or
- R¹⁶ and R¹⁷ together with the atoms to which they are bound form a five- or six-membered heterocycle,
- 15 R¹⁸ stands for hydrogen or (C₁-C₄)-alkyl,
- R¹⁹ stands for hydrogen or the side group of a natural α -amino acid or its homologs or isomers,
- R²⁰ stands for hydrogen or (C₁-C₄)-alkyl,
- or
- 20 R¹⁹ and R²⁰ together with the atoms to which they are bound form a pyrrolidinyl ring,
- R²¹ stands for hydrogen or (C₁-C₄)-alkyl,
- R²² stands for hydrogen or (C₁-C₄)-alkyl,
- or

R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{23} stands for (C₁-C₄)-alkyl,

R^{24} stands for hydrogen or (C₁-C₄)-alkyl,

5 L^2 stands for linear (C₂-C₁₀)-alkanediyl or for a group of the formula



wherein

p stands for a number from 2 to 6,

$##^3$ denotes the linkage site to the group B,

10 $##^4$ denotes the linkage site to the nitrogen atom,

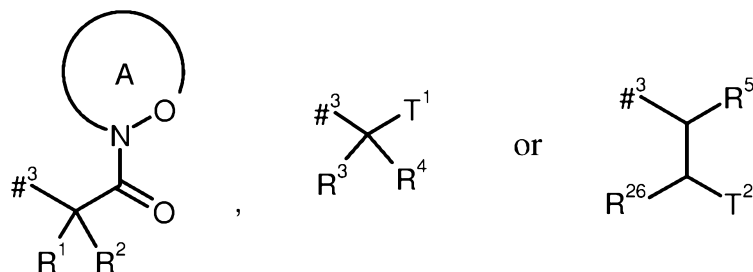
wherein (C₂-C₁₀)-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally present between them to form a (C₃-C₆) cycloalkyl ring or a phenyl ring,

15

D stands for a group of the formula



wherein

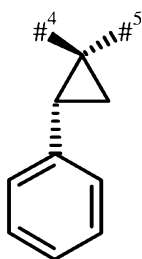
$##^3$ denotes the linkage site to the nitrogen atom,

20 R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



5

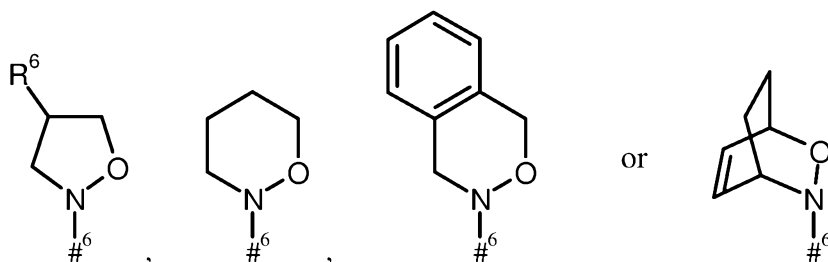
wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula

10



wherein

#⁶ denotes the linkage site to the carbonyl group,

R^6 stands for hydrogen, hydroxyl or benzyloxy,

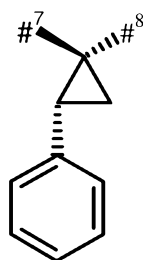
15

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

5 $\#^7$ denotes the linkage site to the vicinal nitrogen atom,

$\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

wherein

10 R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

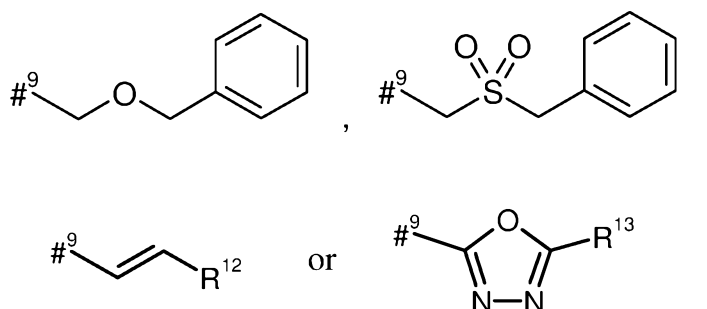
or

15 R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

R^{11} stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

20 R^5 stands for hydrogen, methyl or a group of the formula



wherein

#⁹ denotes the linkage site to $-\text{CHC}(\text{R}^{26})-\text{T}^2$,

5 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

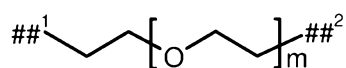
T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

10 as well as their salts and solvates as well as the solvates of the salts.

In addition, compounds of formula (XXX) that are especially preferred within the scope of the present invention are those in which

Cys stands for a cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

15 L^1 stands for a bond, linear ($\text{C}_2\text{-C}_6$)-alkanediyl, a group of the formula



wherein

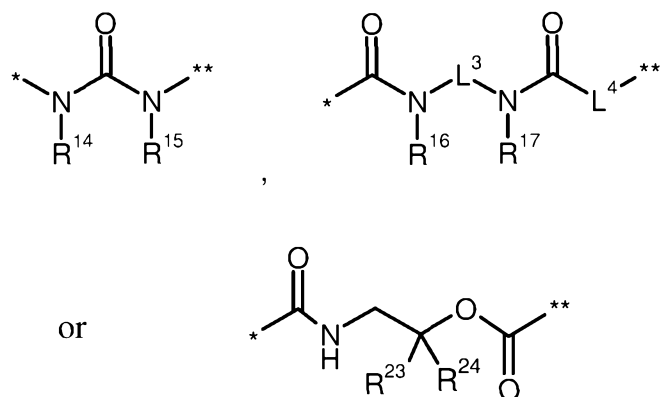
m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

20 ##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



wherein

5 * denotes the linkage site to L¹,

 ** denotes the linkage site to L²,

 L³ stands for a bond or ethane-1,2-diyl,

 L⁴ stands for a bond,

 R¹⁴ stands for hydrogen,

10 R¹⁵ stands for hydrogen,

 R¹⁶ stands for hydrogen or methyl,

 R¹⁷ stands for hydrogen or methyl,

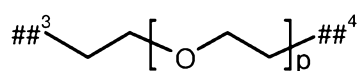
or

 R¹⁶ and R¹⁷ together with the atoms to which they are bound form a piperazinyl ring,

15 R²³ stands for methyl,

 R²⁴ stands for hydrogen or methyl,

L² stands for linear (C₂-C₆)-alkanediyl or for a group of the formula



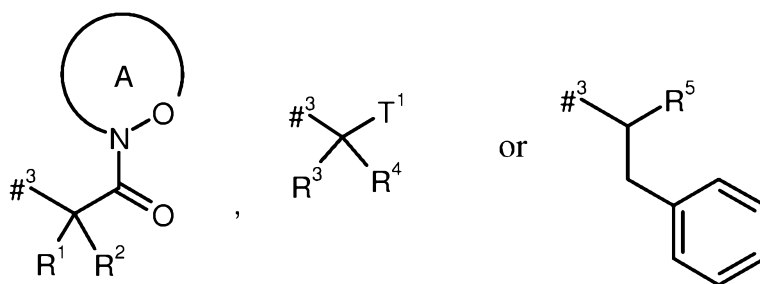
wherein

p stands for a number of 2 or 3,

##^3 denotes the linkage site to the group B,

5 ##^4 denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

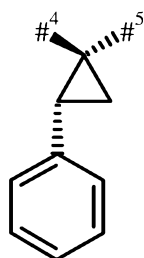
##^3 denotes the linkage site to the nitrogen atom,

10 R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



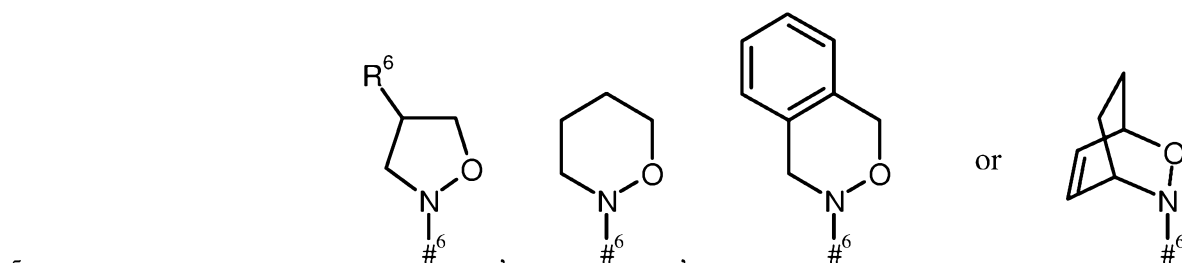
15

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

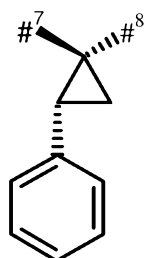
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

R³ stands for hydrogen,

10 R⁴ stands for benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



15 wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R⁷ stands for hydrogen, methyl ethyl, *n*-propyl, benzyl or adamantylmethyl,

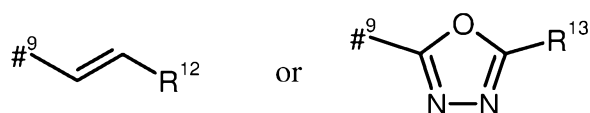
5 R⁸ stands for hydrogen or methyl,

R⁹ stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

R¹⁰ stands for benzoyl,

R¹¹ stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

10 R⁵ stands for hydrogen or a group of the formula



wherein

#⁹ denotes the linkage site to -CHC(R²⁶)phenyl,

15 R¹² stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula -S(O)₂OH,

R¹³ stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

as well as their salts and solvates as well as the solvates of the salts.

Within the scope of the present invention, compounds of formula (Ia) are also preferred, in which

20 $n = 1-20$, especially preferably $n = 1-10$ and most especially preferably $n = 2-8$.

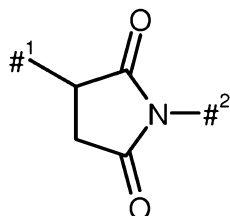
Within the scope of the present invention, compounds of formula (Ia) are preferred, in which

AK stands for AK₁

wherein

AK₁ stands for an antibody or an antigen binding antibody fragment which binds to EGFR and is bound to the group G by the sulfur atom of a cysteine radical of the binder,

G stands for a group of the formula



5

wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

and

10 n, L¹, B, L², D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), wherein

AK stands for AK₂

wherein

15 AK₂ stands for an antibody or an antigen binding antibody fragment which binds to EGFR and is bound to the group G via the NH side group of a lysine radical of the binder,

G stands for carbonyl,

and

20 n, L¹, B, L², D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

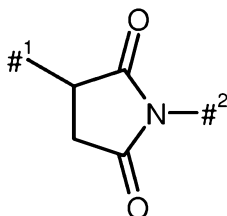
Also preferred within the scope of the present invention are compounds of formula (Ia), in which

AK stands for AK₁

wherein

AK₁ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the sulfur atom of a cysteine radical of the binder,

5 G stands for a group of the formula



wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

10 and

n, L¹, B, L², D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), in which

AK stands for AK₂

15 wherein

AK₂ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G stands for carbonyl,

and

20 n, L¹, B, L², D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of general formula (Ia), in which

AK stands for AK₂

wherein

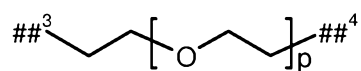
5 AK₂ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G stands for carbonyl,

L¹ stands for a bond,

B stands for a bond,

10 L² stands for linear (C₃-C₆)-alkanediyl or for a group of the formula



wherein

p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

15 ##⁴ denotes the linkage site to the nitrogen atom,

n, D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

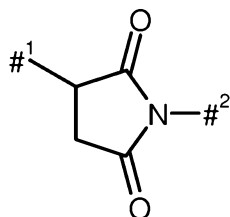
Also preferred within the scope of the present invention are compounds of general formula (Ia), in which

20 AK stands for AK₁

wherein

AK₁ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the sulfur atom of a cysteine radical of the binder,

G stands for a group of the formula

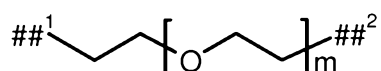


wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

5 #² denotes the linkage site to the group L¹,

L¹ stands for a bond, linear (C₃-C₅)-alkanediyl, a group of the formula



wherein

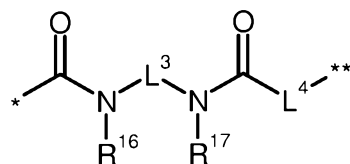
m stands for a number of 2 or 3,

10 ##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₃-C₅)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



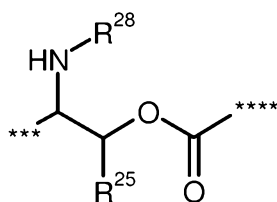
15 wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

L³ stands for a bond or ethane-1,2-diyl,

L^4 stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

5 **** denotes the linkage site to L^2 ,

R^{25} stands for methyl,

R^{28} stands for hydrogen, methylcarbonyl or *tert*-butoxycarbonyl,

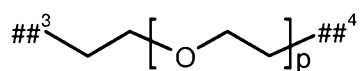
R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

10 or

R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

L^2 stands for linear (C₃-C₅)-alkanediyl or for a group of the formula



wherein

15 p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

##⁴ denotes the linkage site to the nitrogen atom,

and

n, D and R^{35} have the meanings given above

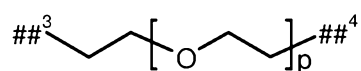
20 as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

L¹ stands for a bond,

B stands for a bond,

5 L² stands for linear (C₃-C₆)-alkanediyl or for a group of the formula



wherein

p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

10 ##⁴ denotes the linkage site to the nitrogen atom,

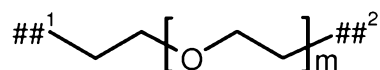
and

n, AK, Cys, G, D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), in which

15 L¹ stands for linear (C₁-C₁₀)-alkanediyl or a group of the formula



wherein

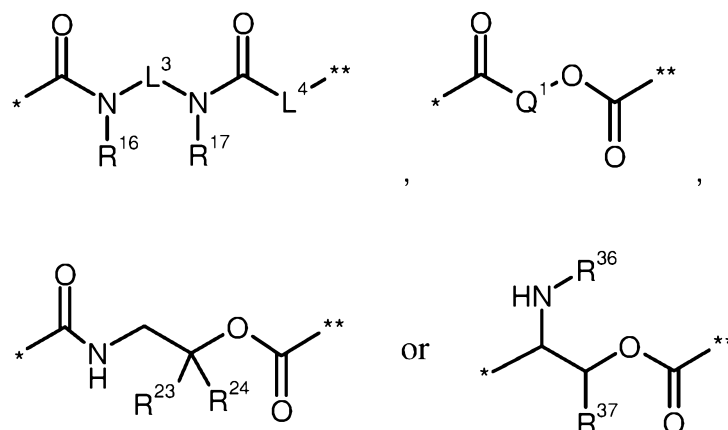
m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

20 ##² denotes the linkage site to the group B,

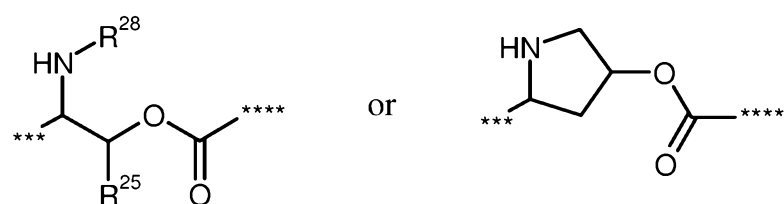
wherein (C₁-C₁₀)-alkanediyl may be substituted with 1 to 4 substituents selected independently of one another from the group methyl, hydroxyl and benzyl,

B stands for a bond or a group of the formula



wherein

- * denotes the linkage site to L^1 ,
- 5 ** denotes the linkage site to L^2 ,
- L^3 for a bond or (C_2-C_4) -alkanediyl,
- L^4 stands for a group of the formula



wherein

- 10 *** denotes the linkage site to the carbonyl group,
- **** denotes the linkage site to L^2 ,
- R^{25} stands for hydrogen or methyl,
- R^{28} stands for hydrogen, (C_1-C_4) -alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,
- 15 Q^1 stands for a four- to seven-membered heterocycle,
- R^{16} stands for hydrogen or (C_1-C_4) -alkyl,

R^{17} stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{16} and R^{17} together with the atoms to which they are bound form a five- or six-membered heterocycle,

5 R^{23} stands for (C₁-C₄)-alkyl,

R^{24} stands for hydrogen or (C₁-C₄)-alkyl,

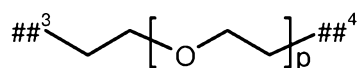
R^{36} stands for hydrogen, (C₁-C₄)-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

R^{37} stands for hydrogen or methyl,

10 or

R^{36} and R^{37} together with the atoms to which they are bound form a pyrrolidine ring,

L^2 stands for linear (C₂-C₁₀)-alkanediyl or for a group of the formula



wherein

15 p stands for a number from 2 to 6,

##^3 denotes the linkage site to the group B,

##^4 denotes the linkage site to the nitrogen atom,

wherein (C₂-C₁₀)-alkanediyl may be substituted with 1 to 4 substituents selected independently of one another from the group comprising methyl, hydroxyl and benzyl,

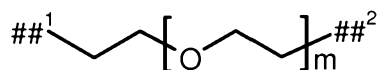
20 and

n, AK, G, D and R^{35} have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), in which

L^1 stands for linear (C_2 - C_6)-alkanediyl or for a group of the formula



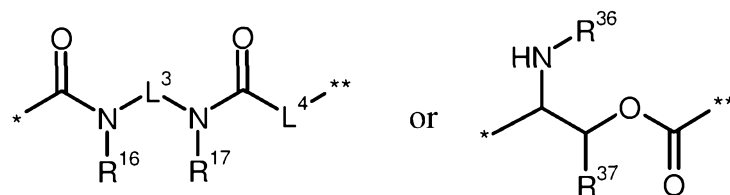
wherein

m stands for a number of 2 or 3,

5 $\#\#^1$ denotes the linkage site to the group G,

$\#\#^2$ denotes the linkage site to the group B,

B stands for a bond or a group of the formula



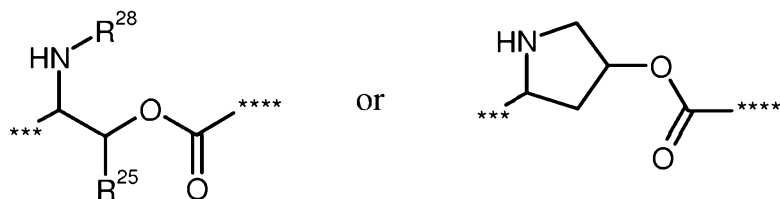
wherein

10 $*$ denotes the linkage site to L^1 ,

$**$ denotes the linkage site to L^2 ,

L^3 stands for a bond or ethane-1,2-diyl,

L^4 stands for a group of the formula



15 wherein

$***$ denotes the linkage site to the carbonyl group,

$****$ denotes the linkage site to L^2 ,

R²⁵ stands for hydrogen or methyl,

R²⁸ stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

R¹⁶ stands for hydrogen or methyl,

R¹⁷ stands for hydrogen or methyl,

5 or

R¹⁶ and R¹⁷ together with the atoms to which they are bound form a piperazinyling,

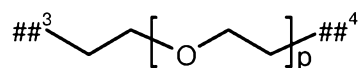
R³⁶ stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

R³⁷ stands for hydrogen or methyl,

or

10 R³⁶ and R³⁷ together with the atoms to which they are bound form a pyrrolidine ring,

L² stands for linear (C₂-C₆)-alkanediyl or for a group of the formula



wherein

p stands for a number of 2 or 3,

15 ##³ denotes the linkage site to the group B,

##⁴ denotes the linkage site to the nitrogen atom,

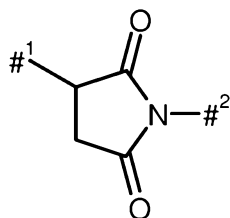
and

n, AK, G, D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

20 Also preferred within the scope of the present invention are compounds of formula (Ia) and (XXXa), in which

G stands for a group of the formula

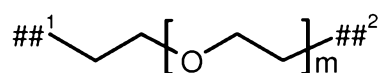


wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

5 L¹ stands for linear (C₃-C₅)-alkanediyl or for a group of the formula



wherein

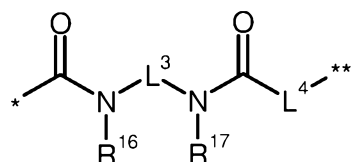
m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

10 ##² denotes the linkage site to the group B,

wherein (C₃-C₅)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



wherein

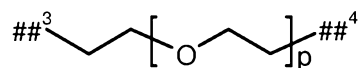
15 * denotes the linkage site to L¹,

** denotes the linkage site to L²,

L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond,

L^2 for linear (C₃-C₅)-alkanediyl or for a group of the formula



wherein

p stands for a number of 2 or 3,

5 $##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

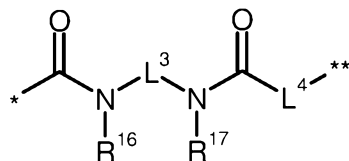
and

n , AK_1 , Cys, D, R^{16} and R^{17} have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

10 Also preferred within the scope of the present invention are compounds of formula (Ia) and (XXXa), in which

B stands for a bond or a group of the formula



wherein

15 * denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

L^3 stands for a bond or ethane-1,2-diyl,

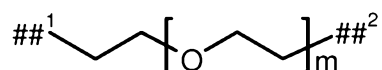
L^4 stands for a bond,

n , AK, Cys, G, L^1 , L^2 , D, R^{16} , R^{17} and R^{35} have the meanings given above

20 as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

L^1 stands for a bond, linear (C₃-C₅)-alkanediyl, a group of the formula



5 wherein

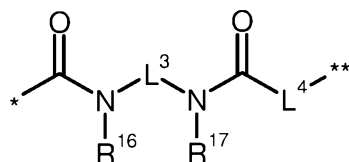
m stands for a number of 2 or 3,

$##^1$ denotes the linkage site to the group G,

$##^2$ denotes the linkage site to the group B,

wherein (C₃-C₅)-alkanediyl may be substituted with one or two methyl substituents,

10 B stands for a bond or a group of the formula



wherein

*

denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

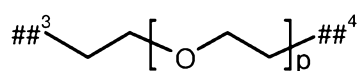
15 L^3 stands for a bond,

L^4 stands for a bond

R^{16} stands for hydrogen,

R^{17} stands for hydrogen,

L^2 stands for linear (C₃-C₆)-alkanediyl or for a group of the formula



wherein

p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

5 ##⁴ denotes the linkage site to the nitrogen atom,

n, AK, Cys, G, D and R³⁵ have the meanings given above

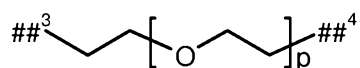
as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

10 L¹ stands for a bond,

B stands for a bond,

L² stands for linear (C₃-C₆)-alkanediyl or for a group of the formula



wherein

15 p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

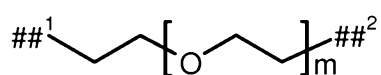
##⁴ denotes the linkage site to the nitrogen atom,

n, AK, Cys, G, D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

20 Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

L¹ stands for linear (C₃-C₅)-alkanediyl or for a group of the formula



wherein

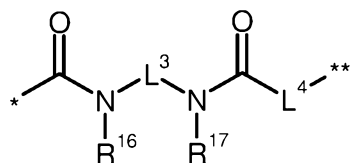
m stands for a number of 2 or 3,

$\#\#^1$ denotes the linkage site to the group G,

5 $\#\#^2$ denotes the linkage site to the group B,

wherein (C₃-C₅)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a group of the formula



wherein

10 * denotes the linkage site to L¹,

** denotes the linkage site to L²,

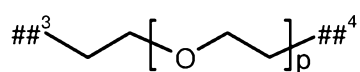
L³ stands for a bond,

L⁴ stands for a bond

R¹⁶ stands for hydrogen,

15 R¹⁷ stands for hydrogen,

L² stands for linear (C₃-C₆)-alkanediyl or for a group of the formula



wherein

p stands for a number of 2 or 3,

$\#^3$ denotes the linkage site to the group B,

$\#^4$ denotes the linkage site to the nitrogen atom,

n, AK, Cys, G, D and R^{35} have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

5 Also preferred within the scope of the present invention are compounds of formula (Ia), in which

n stands for a number from 2 to 8, preferably 2 to 5,

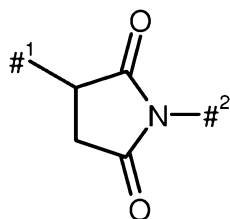
AK stands for AK_1 or AK_2

wherein

10 AK_1 stands for an antibody or an antigen binding antibody fragment which binds to C4.4a and is bound to the group G via the sulfur atom of a cysteine radical of the binder,

AK_2 stands for an antibody or an antigen binding antibody fragment which binds to C4.4a and is bound to the group G via the NH side group of a lysine radical of the binder,

15 G for the case when $AK = AK_1$ stands for a group of the formula



wherein

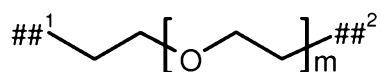
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ denotes the linkage site to the group L^1 ,

20 or

for the case when $AK = AK_2$, G stands for carbonyl,

L^1 stands for a bond, linear (C_3 - C_5)-alkanediyl, a group of the formula



wherein

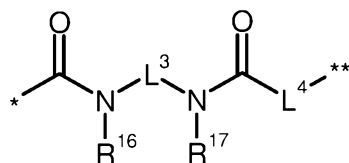
m stands for a number of 2 or 3,

$\#\#^1$ denotes the linkage site to the group G,

5 $\#\#^2$ denotes the linkage site to the group B,

wherein (C₃-C₅)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



wherein

10 * denotes the linkage site to L¹,

** denotes the linkage site to L²,

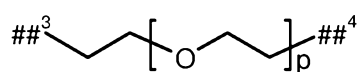
L³ stands for a bond,

L⁴ stands for a bond

R¹⁶ stands for hydrogen,

15 R¹⁷ stands for hydrogen,

L² stands for linear (C₃-C₆)-alkanediyl or for a group of the formula



wherein

p stands for a number of 2 or 3,

$\#^3$ denotes the linkage site to the group B,

$\#^4$ denotes the linkage site to the nitrogen atom,

and

D and R³⁵ have the meanings given above

5 as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), in which

n stands for a number from 2 to 8, preferably 2 to 5,

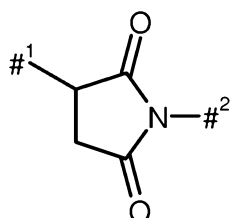
AK stands for AK₁ or AK₂

wherein

10 AK₁ stands for an antibody or an antigen binding antibody fragment which binds to C4.4a and is bound to the group G via the sulfur atom of a cysteine radical of the binder,

AK₂ stands for an antibody or an antigen binding antibody fragment which binds to C4.4a and is bound to the group G via the NH side group of a lysine radical of
15 the binder,

G for the case when AK = AK₁ stands for a group of the formula



wherein

$\#^1$ denotes the linkage site to the cysteine radical of the binder,

20 $\#^2$ denotes the linkage site to the group L¹,

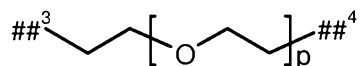
or

for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond,

B stands for a bond,

L² stands for linear (C₃-C₆)-alkanediyl or for a group of the formula



5 wherein

p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

##⁴ denotes the linkage site to the nitrogen atom,

D and R³⁵ have the meanings given above

10 as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), in which

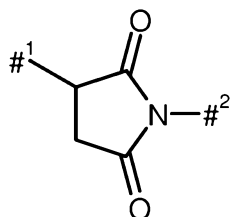
n stands for a number from 2 to 8, preferably 2 to 5,

AK stands for AK₁,

wherein

15 AK₁ stands for an antibody or an antigen binding antibody fragment which binds to C4.4a and is bound to the group G via the sulfur atom of a cysteine radical of the binder,

G stands for a group of the formula

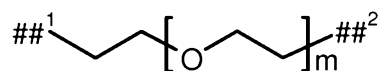


20 wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

L¹ stands for linear (C₃-C₅)-alkanediyl or for a group of the formula



5 wherein

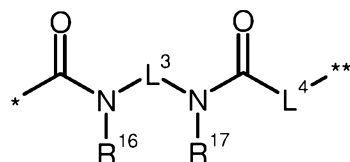
m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₃-C₅)-alkanediyl may be substituted with one or two methyl substituents,

10 B stands for a group of the formula



wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

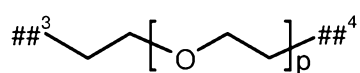
15 L³ stands for a bond,

L⁴ stands for a bond

R¹⁶ stands for hydrogen,

R¹⁷ stands for hydrogen,

L² stands for linear (C₃-C₆)-alkanediyl or for a group of the formula



wherein

p stands for a number of 2 or 3,

##^3 denotes the linkage site to the group B,

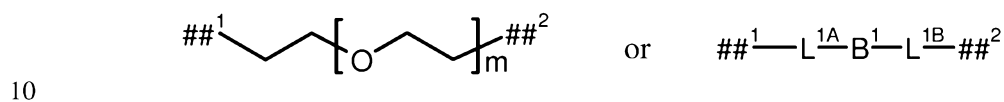
5 ##^4 denotes the linkage site to the nitrogen atom,

D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), in which

L¹ stands for a bond, linear (C₃-C₅)-alkanediyl, a group of the formula



wherein

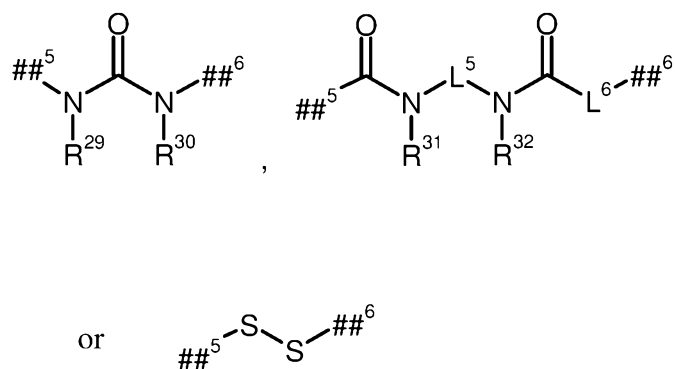
m stands for a number of 2 or 3,

##^1 denotes the linkage site to the group G,

##^2 denotes the linkage site to the group B,

15 L^{1A} stands for linear (C₂-C₆)-alkanediyl,

B¹ stands for a group of the formula



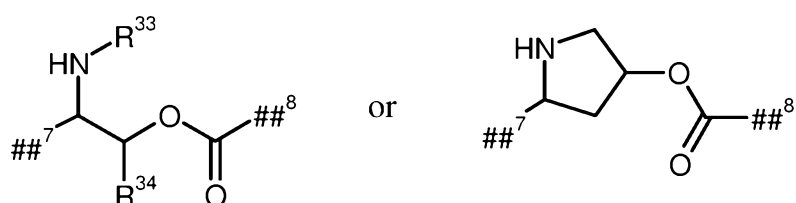
wherein

##⁵ denotes the linkage site to the group L^{1A},

##⁶ denotes the linkage site to the group L^{1B},

L⁵ stands for a bond or ethane-1,2-diyl,

5 L⁶ stands for a bond or a group of the formula



wherein

##⁷ denotes the linkage site to the carbonyl group,

##⁸ denotes the linkage site to L^{1B},

10 R³³ stands for hydrogen, (C₁-C₄)-alkylcarbonyl or *tert*-butoxy-carbonyl,

R³⁴ stands for hydrogen or methyl,

R²⁹ stands for hydrogen or methyl,

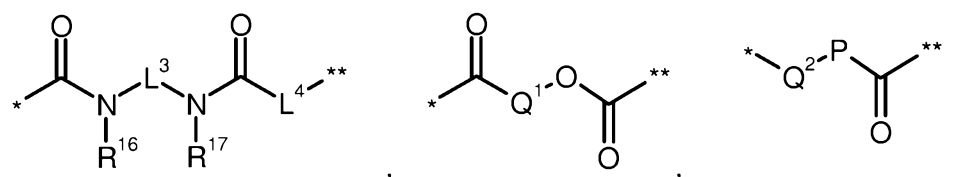
R³⁰ stands for hydrogen or methyl,

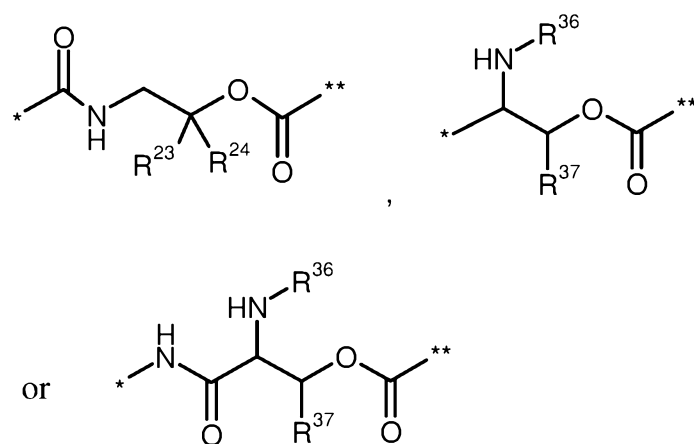
15 R³¹ stands for hydrogen or methyl,

R³² stands for hydrogen or methyl,

L^{1B} stands for linear (C₃-C₆)-alkanediyl,

B stands for a bond or a group of the formula





wherein

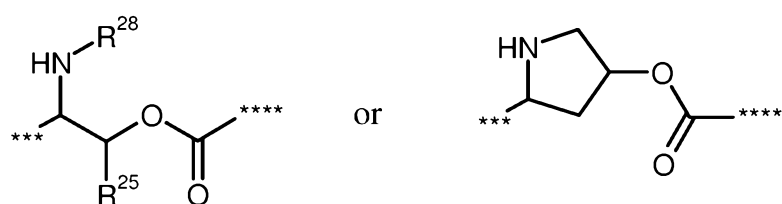
* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

5 P stands for O,

L^3 stands for a bond or ethane-1,2-diyl,

L^4 stands for a group of the formula



wherein

10 *** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

R^{25} stands for hydrogen or methyl,

R^{28} stands for hydrogen, (C₁-C₄)-alkylcarbonyl or *tert*-butoxycarbonyl,

Q^1 stands for a four- to seven-membered heterocycle,

15 Q^2 stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

R^{23} stands for (C₁-C₄)-alkyl,

R^{24} stands for hydrogen or (C₁-C₄)-alkyl,

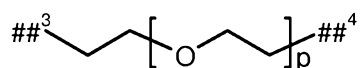
5 R^{36} stands for hydrogen, (C₁-C₄)-alkylcarbonyl or *tert*-butyloxycarbonyl,

R^{37} stands for hydrogen or methyl,

or

R^{36} and R^{37} together with the atoms to which they are bound form a pyrrolidine ring,

L^2 stands for linear (C₂-C₆)-alkanediyl or for a group of the formula



10

wherein

p stands for a number of 2 or 3,

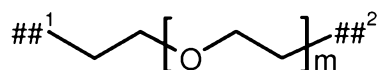
##^3 denotes the linkage site to the group B,

##^4 denotes the linkage site to the nitrogen atom,

15 as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), in which

L^1 stands for linear (C₃-C₅)-alkanediyl or for a group of the formula



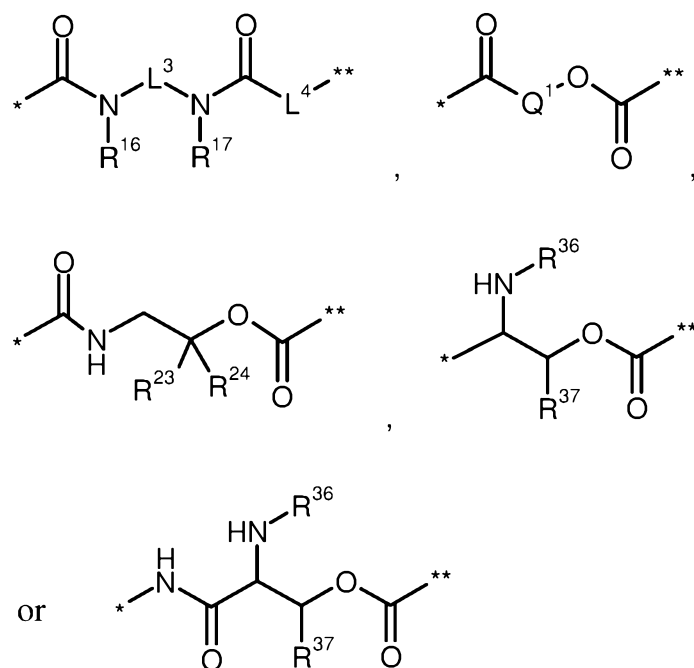
wherein

20 m stands for a number of 2 or 3,

##^1 denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

B stands for a group of the formula



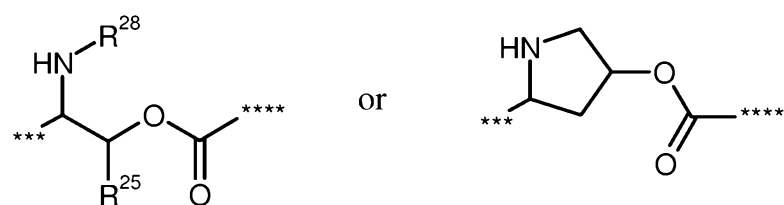
5 wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

L^3 stands for a bond or ethane-1,2-diyl,

L^4 stands for a group of the formula



10

wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

R^{25} stands for methyl,

R^{28} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

Q^1 stands for piperidine-1,4-diyl,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

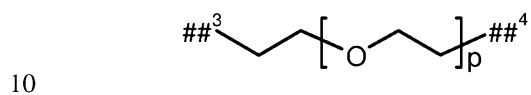
5 R^{23} stands for methyl,

R^{24} stands for hydrogen,

R^{36} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

R^{37} stands for hydrogen or methyl,

L^2 stands for linear (C_2 - C_6)-alkanediyl or for a group of the formula



wherein

p stands for a number of 2 or 3,

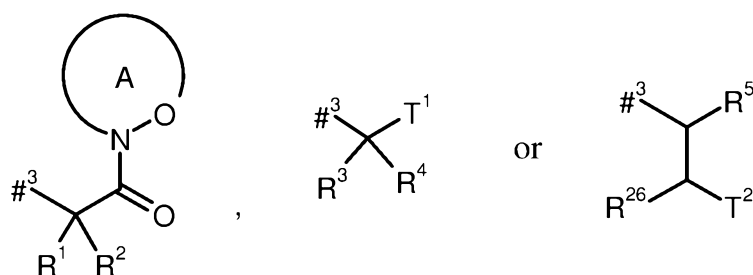
$##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

15 as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

D stands for a group of the formula



wherein

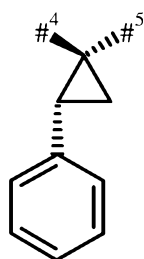
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen or methyl,

5 R² stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

or

10 R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

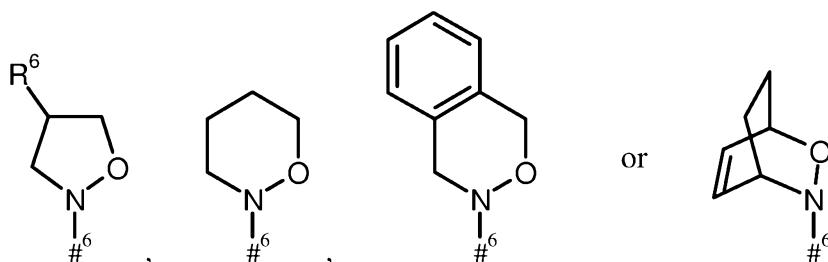


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

15 the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

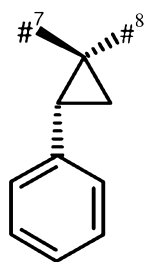
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxy-ethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

$\#^7$ denotes the linkage site to the vicinal nitrogen atom,

$\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

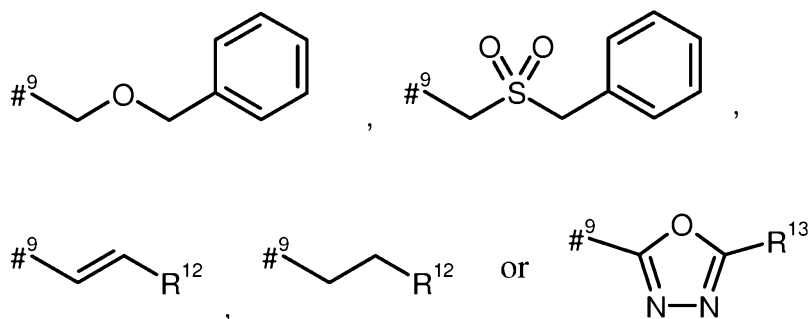
or

R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

R^{11} stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



5

wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(R^{26})-\text{T}^2$,

R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

10 R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen,

T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

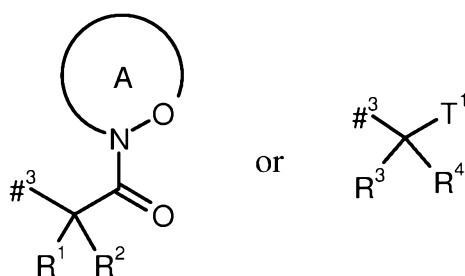
and

15 n, AK, Cys, G, L^1 , B, L^2 , D and R^{35} have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

D stands for a group of the formula



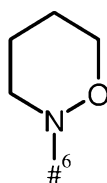
wherein

#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

the ring A with the N-O group contained in it stands for a heterocycle of the formula



wherein

10 #⁶ denotes the linkage site to the carbonyl group,

R³ stands for hydrogen,

R⁴ stands for benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

T¹ stands for a group of the formula -C(=O)-OR⁷ or -C(=O)-NR⁸R⁹,

wherein

15 R⁷ stands for hydrogen,

R⁸ stands for hydrogen,

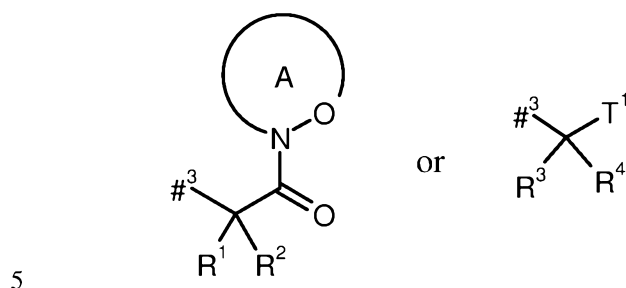
R⁹ stands for hydrogen,

n, AK, Cys, G, L¹, B, L², D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

D stands for a group of the formula



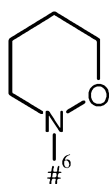
wherein

#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

R² stands for benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

10 the ring A with the N-O group contained in it stands for a heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

15 R³ stands for hydrogen,

R⁴ stands for benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

T¹ stands for a group of the formula -C(=O)-NR⁸R⁹,

wherein

R^8 stands for hydrogen,

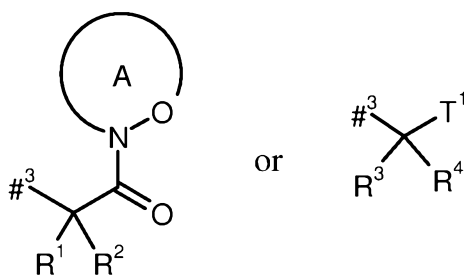
R^9 stands for hydrogen,

n, AK, Cys, G, L^1 , B, L^2 , D and R^{35} have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

- 5 Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

D stands for a group of the formula



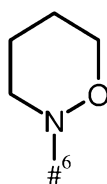
wherein

- 10 $\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

the ring A with the N-O group contained in it stands for a heterocycle of the formula



15

wherein

$\#^6$ denotes the linkage site to the carbonyl group,

R^3 stands for hydrogen,

R^4 stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

T^1 stands for a group of the formula $-C(=O)-NR^8R^9$,

wherein

R^8 stands for hydrogen,

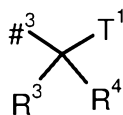
5 R^9 stands for hydrogen,

n, AK, Cys, G, L^1 , B, L^2 , D and R^{35} have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

10 D stands for a group of the formula



wherein

$\#^3$ denotes the linkage site to the nitrogen atom,

R^3 stands for hydrogen,

15 R^4 stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

T^1 stands for a group of the formula $-C(=O)-OR^7$ or $-C(=O)-NR^8R^9$,

wherein

R^7 stands for hydrogen,

R^8 stands for hydrogen,

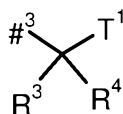
20 R^9 stands for hydrogen,

n, AK, Cys, G, L^1 , B, L^2 , D and R^{35} have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

D stands for a group of the formula



5 wherein

#³ denotes the linkage site to the nitrogen atom,

R³ stands for hydrogen,

R⁴ stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

T¹ stands for a group of the formula -C(=O)-NR⁸R⁹,

10 wherein

R⁸ stands for hydrogen,

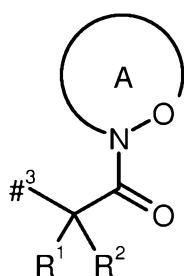
R⁹ stands for hydrogen,

n, AK, Cys, G, L¹, B, L², D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

15 Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

D stands for a group of the formula



wherein

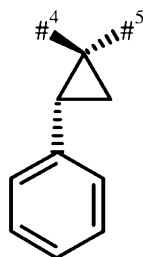
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen or methyl,

R² stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

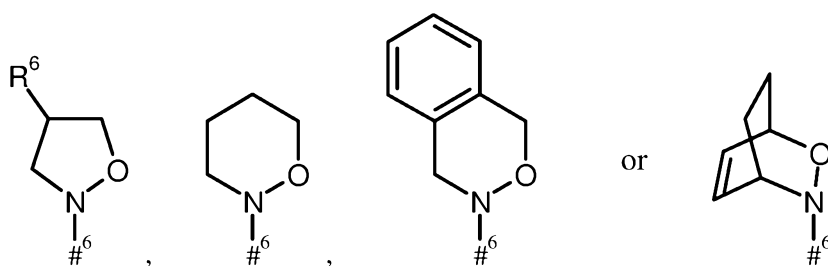


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

R⁶ stands for hydrogen, hydroxyl or benzyloxy,

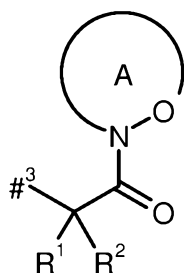
and

n, AK, Cys, G, L¹, B, L² and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa)
5 and (XXXI), in which

D stands for a group of the formula



wherein

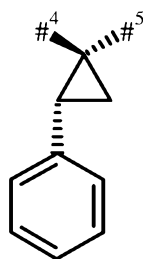
#³ denotes the linkage site to the nitrogen atom,

10 R¹ stands for hydrogen,

R² stands for benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a
(1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



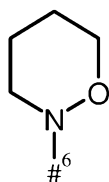
15

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



5 wherein

#⁶ denotes the linkage site to the carbonyl group,

and

n, AK, Cys, G, L¹, B, L² and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

10 Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

R³⁵ stands for hydroxyl,

and

n, AK, Cys, G, L¹, B, L², D and R³⁵ have the meanings given above

15 as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

R³⁵ stands for methyl,

and

20 n, AK, Cys, G, L¹, B, L², D and R³⁵ have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

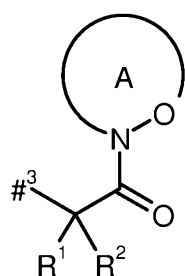
Also especially preferred within the scope of the present invention are compounds of formula (XXXa), in which

Cys stands for a L-cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

5 as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (I) and (XXX), in which

D stands for a group of the formula



10 wherein

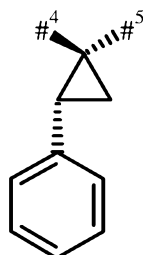
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

R² stands for benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

15 R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

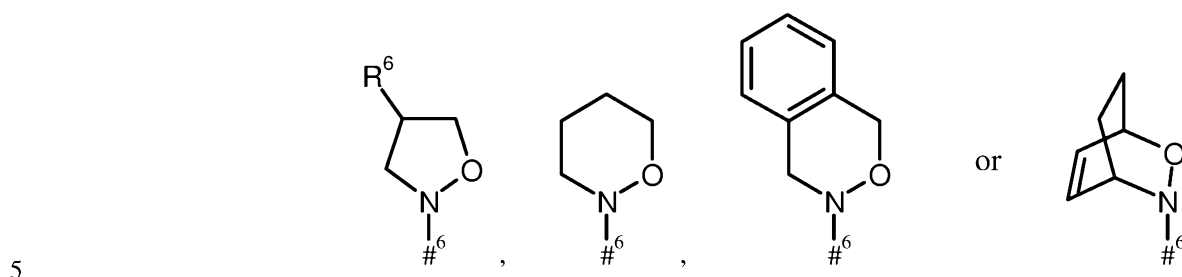


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

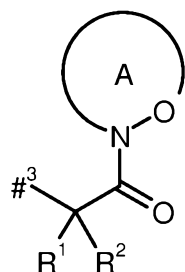
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

n, AK, Cys, G, L¹, L² and B have the meanings given above

10 as well as their salts and solvates as well as the solvates of the salts.

Also especially preferred within the scope of the present invention are compounds of formula (Ia) and (XXXa), in which

D stands for a group of the formula



15 wherein

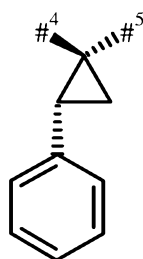
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

R^2 stands for benzyl or 1H-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2- phenylcyclopropane-1,1-diyl group of the formula



5

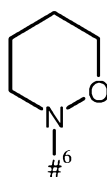
wherein

$\#^4$ denotes the linkage site to the vicinal nitrogen atom,

$\#^5$ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a heterocycle of the formula

10



wherein

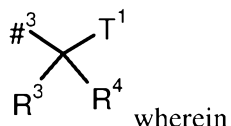
$\#^6$ denotes the linkage site to the carbonyl group,

n, AK, Cys, G, L^1 , L^2 and B have the meanings given above

15 as well as their salts and solvates as well as the solvates of the salts.

Another especially preferred subject matter of the present invention is compounds of formula (I), in which

D stands for a group of the formula



T^1 stands for $-C(=O)-OH$ or $-C(=O)-NH_2$ and

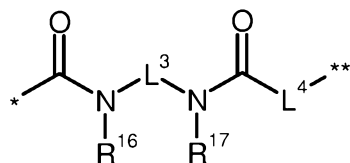
n , AK , G , L^1 , B , L^2 , $\#^3$, R^3 and R^4 have the meanings given above.

Also preferred within the scope of the present invention are compounds of formula (I), in which

5 $n = 1-20$, especially preferably $n = 1-10$ and most especially preferably $n = 2-8$.

Also preferred within the scope of the present invention are compounds of formula (Ia) and (XXX), in which

B stands for a bond or a group of the formula



10 wherein

$*$ denotes the linkage site to L^1 ,

$**$ denotes the linkage site to L^2 ,

L^3 stands for a bond or ethane-1,2-diyl,

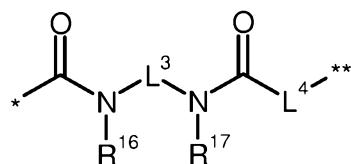
L^4 stands for a bond,

15 n , AK , Cys , G , L^1 , L^2 , D , R^{16} and R^{17} have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

Especially preferred within the scope of the present invention are compounds of formula (I) and (XXX), in which

B stands for a bond or a group of the formula



wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

5 L^3 and L^4 stands for a bond,

n, AK, Cys, G, L^1 , L^2 , D, R^{16} and R^{17} have the meanings given above

as well as their salts and solvates as well as the solvates of the salts.

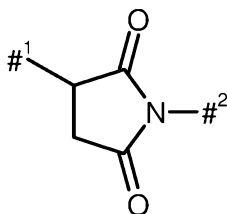
Also preferred within the scope of the present invention are binder-drug conjugates of general formula (I), in which

10 AK stands for AK_1

wherein

AK_1 stands for a binder that is bound to the group G via a sulfur atom of the binder, preferably a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

15 G stands for a group of the formula

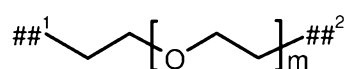


wherein

$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ denotes the linkage site to the group L^1 ,

20 L^1 stands for a bond, linear (C_1 - C_{10})-alkanediyl or for a group of the formula



wherein

m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

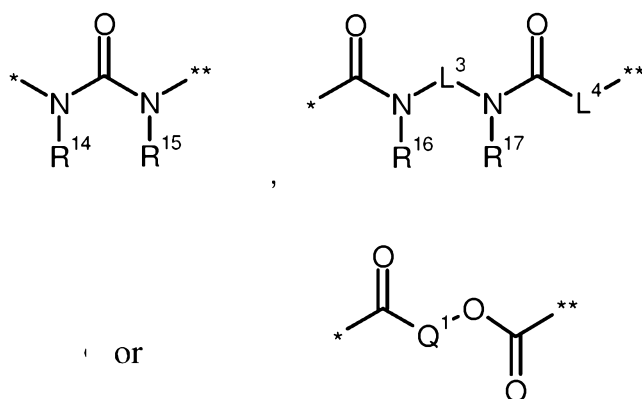
5 $\#\#^2$ denotes the linkage site to the group B,

wherein (C₁-C₁₀)-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

10 optionally situated between them,

B stands for a bond or a group of the formula



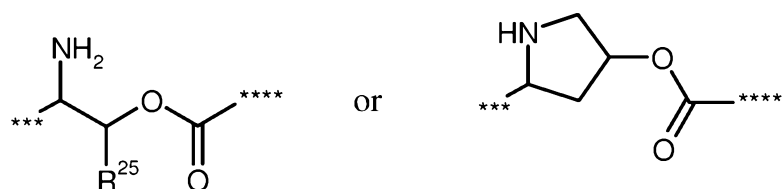
wherein

* denotes the linkage site to L^1 ,

15 ** denotes the linkage site to L^2 ,

L³ for a bond or (C₂-C₄)-alkanediyl,

L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

5 R^{25} stands for hydrogen or methyl,

Q^1 stands for a four- to seven-membered heterocycle,

R^{14} stands for hydrogen or (C_1-C_4) -alkyl,

R^{15} stands for hydrogen or (C_1-C_4) -alkyl,

or

10 R^{14} and R^{15} together with the atoms to which they are bound form a five- or six-membered heterocycle,

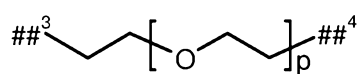
R^{16} stands for hydrogen or (C_1-C_4) -alkyl,

R^{17} stands for hydrogen or (C_1-C_4) -alkyl,

or

15 R^{16} and R^{17} together with the atoms to which they are bound form a five- or six-membered heterocycle,

L^2 stands for linear (C_2-C_{10}) -alkanediyl or for a group of the formula



wherein

20 p stands for a number from 2 to 6,

##³ denotes the linkage site to the group B,

##⁴ denotes the linkage site to the nitrogen atom,

wherein (C₂-C₁₀)-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

5 wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally present between them to form a (C₃-C₆) cycloalkyl ring or a phenyl ring,

as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are binder-drug conjugates of general
10 formula (I), in which

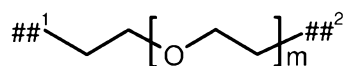
AK stands for AK₂

wherein

AK₂ stands for a binder that is bound to the group G via the NH side group of a lysine radical of the binder,

15 G stands for carbonyl,

L¹ stands for a bond, linear (C₁-C₁₀)-alkanediyl, a group of the formula



wherein

m stands for a number from 2 to 6,

20 ##¹ denotes the linkage site to the group G,

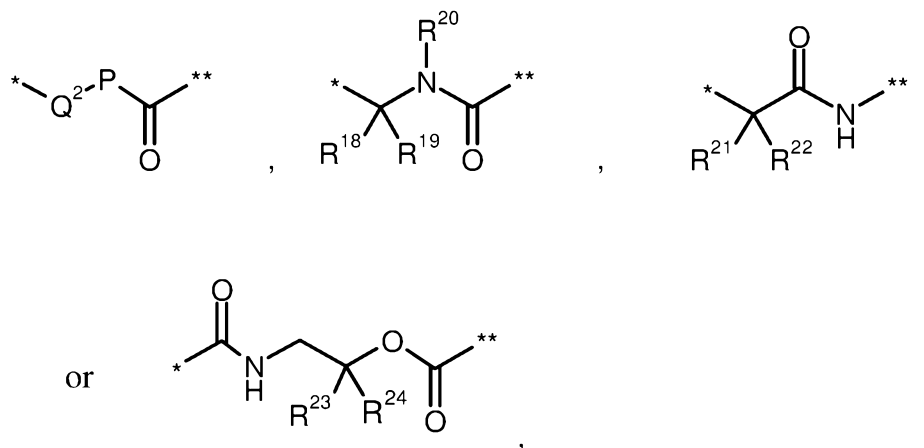
##² denotes the linkage site to the group B,

wherein (C₁-C₁₀)-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another to form a (C₃-C₆)-cycloalkyl ring or a phenyl ring, including the carbon atoms optionally situated between them,

B stands for a bond or a group of the formula



wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

P stands for O or NH,

10 Q² stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,

R¹⁸ stands for hydrogen or (C₁-C₄)-alkyl,

R¹⁹ stands for hydrogen or the side group of a natural α-amino acid or its homologs or isomers,

15 R²⁰ stands for hydrogen or (C₁-C₄)-alkyl,

or

R¹⁹ and R²⁰ together with the atoms to which they are bound form a pyrrolidinyl ring,

R²¹ stands for hydrogen or (C₁-C₄)-alkyl,

R^{22} stands for hydrogen or (C_1-C_4) -alkyl,

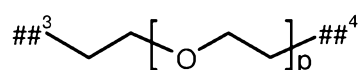
or

R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

5 R^{23} stands for (C_1-C_4) -alkyl,

R^{24} stands for hydrogen or (C_1-C_4) -alkyl,

L^2 stands for linear (C_2-C_{10}) -alkanediyl or for a group of the formula



wherein

10 p stands for a number from 2 to 6,

##^3 denotes the linkage site to the group B,

##^4 denotes the linkage site to the nitrogen atom,

wherein (C_2-C_{10}) -alkanediyl may be substituted with 1 to 4 methyl substituents,

and

15 wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally present between them to form a (C_3-C_6) cycloalkyl ring or a phenyl ring,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention are binder-drug conjugates of general
20 formula (Ia) as indicated above, in which

n stands for a number from 1 to 20,

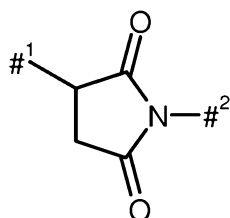
AK stands for AK_1 or AK_2

wherein

AK₁ stands for a binder that is bound to the group G via a sulfur atom of the binder, preferably a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

5 AK₂ stands for a binder that is bound to the group G via a nitrogen atom of the binder, preferably a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

G for the case when AK = AK₁ stands for a group of the formula



wherein

10 #¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

or

for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



15

wherein

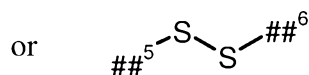
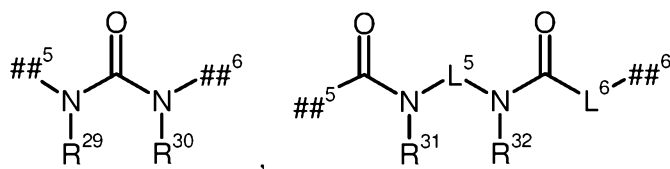
m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

20 L^{1A} stands for linear (C₂-C₆)-alkanediyl,

B¹ stands for a group of the formula



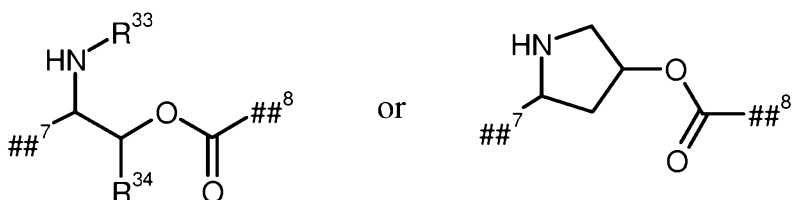
wherein

##⁵ denotes the linkage site to the group L^{1A},

##⁶ denotes the linkage site to the group L^{1B},

5 L⁵ stands for a bond,

L⁶ stands for a bond or a group with the formula



wherein

##⁷ denotes the linkage site to the carbonyl group,

10 ##⁸ denotes the linkage site to L^{1B},

R³³ stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

R³⁴ stands for hydrogen or methyl,

R²⁹ stands for hydrogen,

R³⁰ stands for hydrogen,

15 R³¹ stands for hydrogen or methyl,

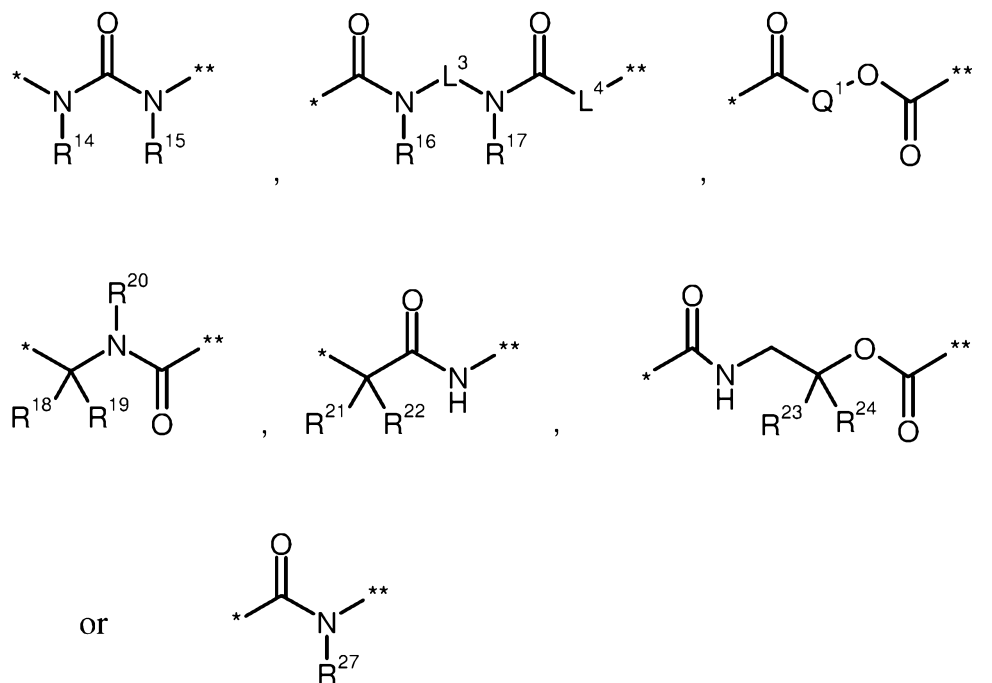
R³² stands for hydrogen or methyl,

L^{1B} stands for linear (C₂-C₆)-alkanediyl,

and

wherein (C₂-C₆)-alkanediyl may be substituted with one to two methyl substituents,

B stands for a bond or a group of the formula



5

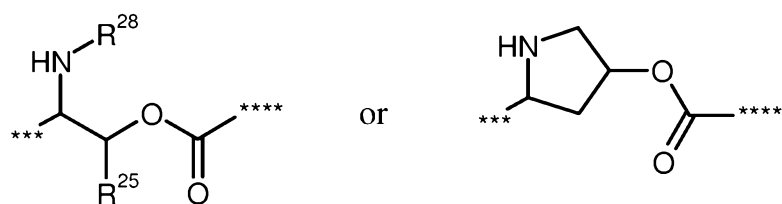
wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

L³ stands for a bond or ethane-1,2-diyl,

10 L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

R^{25} stands for hydrogen or methyl,

R^{28} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

5 Q^1 stands for a four- to seven-membered heterocycle,

R^{14} stands for hydrogen,

R^{15} stands for hydrogen,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

10 or

R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyrning,

R^{18} stands for hydrogen,

R^{19} stands for hydrogen, methyl, propan-2-yl, 2-methylpropan-1-yl or 1-methylpropan-1-yl,

15 R^{20} stands for hydrogen or methyl,

or

R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidinyl ring,

R^{21} stands for hydrogen or methyl,

20 R^{22} stands for hydrogen or methyl,

or

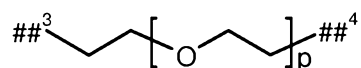
R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

R^{23} stands for methyl,

R^{24} stands for hydrogen or methyl,

R^{27} stands for hydrogen,

L^2 stands for linear (C_2 - C_6)-alkanediyl or for a group of the formula



5 wherein

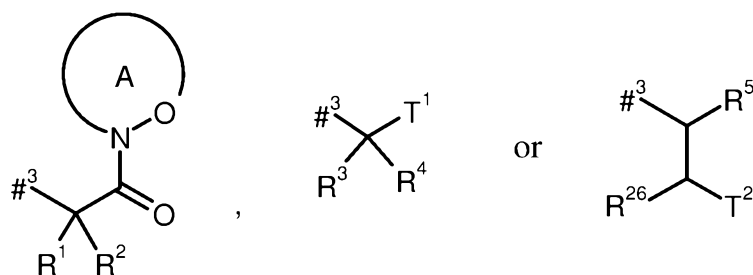
p stands for a number from 2 to 6,

$##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

wherein (C_2 - C_{10})-alkanediyl may be substituted with one or two methyl substituents,

10 D stands for a group of the formula



wherein

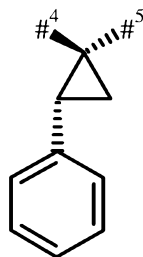
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

15 R^2 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

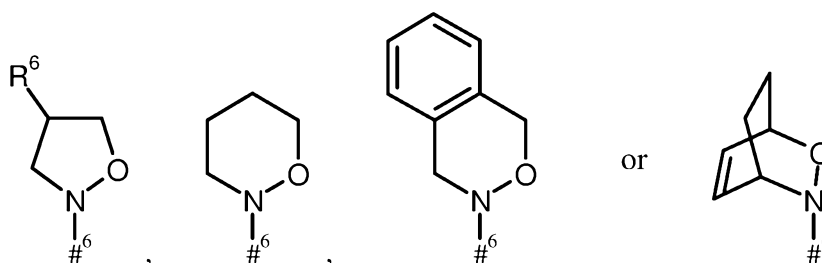


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

5 the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

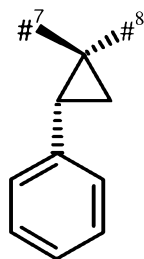
10 R⁶ stands for hydrogen, hydroxyl or benzyloxy,

R³ stands for hydrogen,

R⁴ stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

15 R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

- 5 T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

- 10 R⁸ stands for hydrogen or methyl,

R⁹ stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

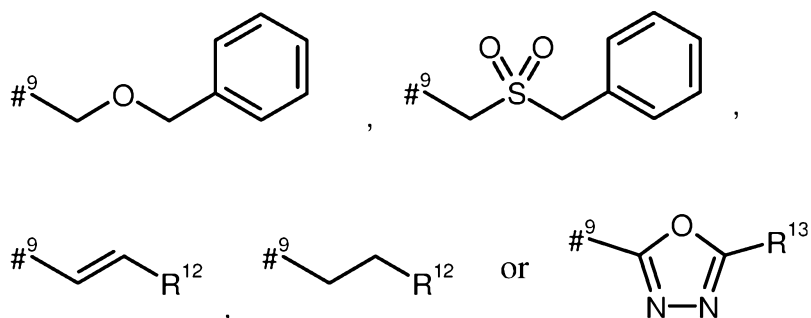
or

R⁸ and R⁹ together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

- 15 R¹⁰ stands for benzoyl,

R¹¹ stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R⁵ stands for hydrogen, methyl or a group of the formula



wherein

#⁹ denotes the linkage site to -CHC(R²⁶)-T²,

5 R¹² stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula -S(O)₂OH,

R¹³ stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R²⁶ stands for hydrogen or hydroxyl,

T² stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

10 R³⁵ stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

The preferred subject matter of the present invention is binder-drug conjugates of general formula (Ia) as indicated above, in which

n stands for a number from 1 to 10,

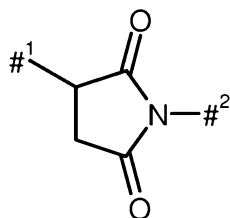
15 AK stands for AK₁ or AK₂

wherein

AK₁ stands for a binder bound to the group G via a sulfur atom of the binder,

AK₂ stands for a binder bound to the group G via a nitrogen atom of the binder,

G for the case when AK = AK₁ stands for a group of the formula



wherein

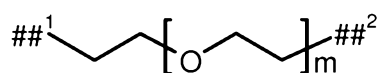
#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

5 or

for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



wherein

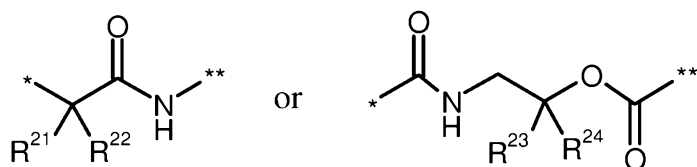
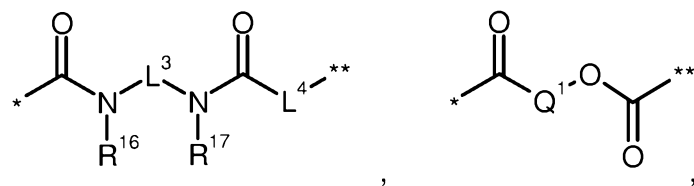
10 m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



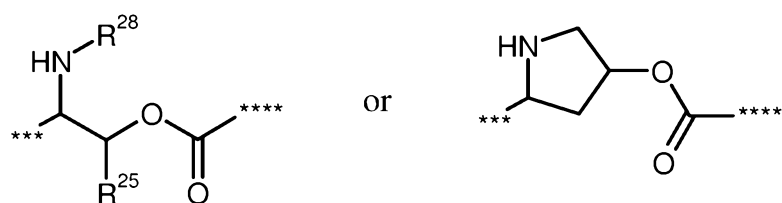
wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

5 L^3 stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

10 **** denotes the linkage site to L^2 ,

R²⁵ stands for methyl,

R²⁸ stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

Q¹ stands for piperidine-1,4-diyl,

R¹⁶ stands for hydrogen or methyl,

15 R¹⁷ stands for hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

R^{21} stands for hydrogen or methyl,

R^{22} stands for hydrogen or methyl,

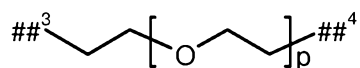
5 or

R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

R^{23} stands for methyl,

R^{24} stands for hydrogen,

10 L^2 stands for linear (C_2-C_6) -alkanediyl or for a group of the formula



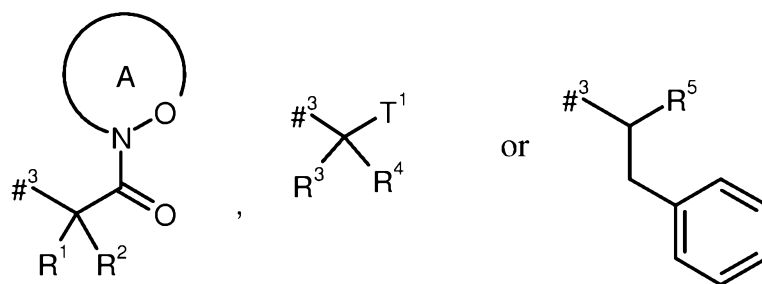
wherein

p stands for a number from 2 to 6,

$##^3$ denotes the linkage site to the group B,

15 $##^4$ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

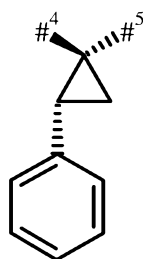
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-hydroxybenzyl, 1-phenylethyl, or 1H-indol-3-ylmethyl,

or

- 5 R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

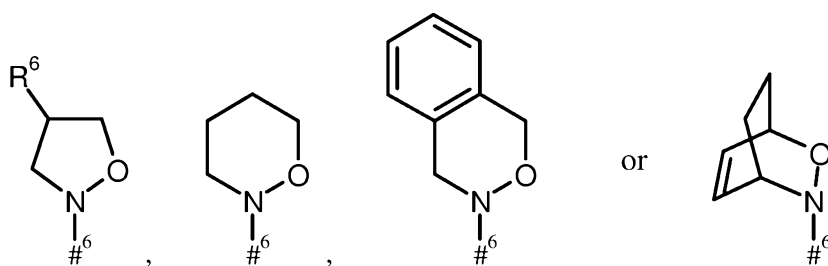


wherein

#4 denotes the linkage site to the vicinal nitrogen atom,

- 10 #5 denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

- 15 #6 denotes the linkage site to the carbonyl group,

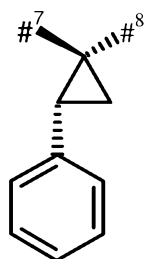
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for benzyl, 1-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



5 wherein

$\#^7$ denotes the linkage site to the vicinal nitrogen atom,

$\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$ or $-CH_2-O-R^{11}$,

wherein

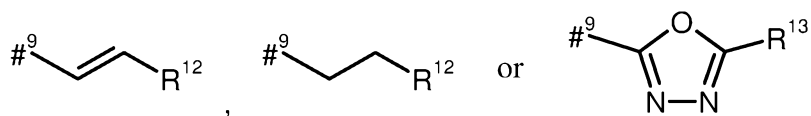
10 R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

15 R^{11} stands for benzyl, which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $-CHCH_2$ -phenyl,

R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

5 R^{35} stands for methyl or hydroxyl,

as well as their salts and solvates as well as the solvates of the salts.

Especially preferred within the scope of the present invention are binder-drug conjugates of formula (Ia), in which

n stands for a number from 2 to 8,

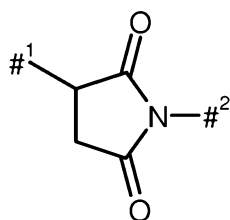
10 AK stands for AK_1 or AK_2 ,

wherein

AK_1 stands for a binder that is bound to the group G via a sulfur atom of the cysteine radical of the binder, preferably for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

15 AK_2 stands for a binder that is bound to the group G via a nitrogen atom of the lysine radical of the binder, preferably a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

G for the case when $AK = AK_1$ stands for a group of the formula



20 wherein

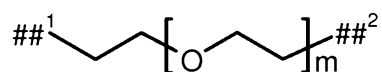
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ denotes the linkage site to the group L^1 ,

or

for the case when $AK = AK_2$, G stands for carbonyl,

L^1 stands for a bond, linear (C_2 - C_6)-alkanediyl, a group of the formula



wherein

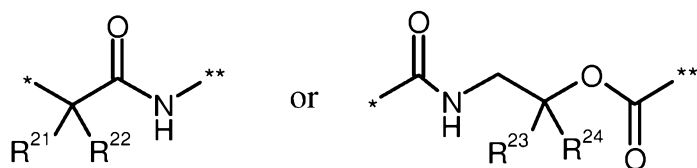
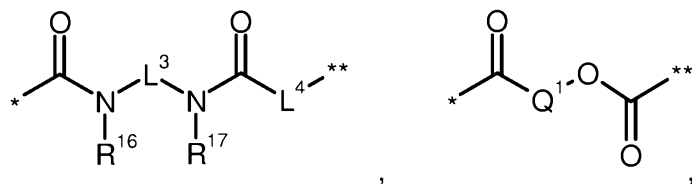
5 m stands for a number of 2 or 3,

##^1 denotes the linkage site to the group G,

##^2 denotes the linkage site to the group B,

wherein (C_2 - C_6)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



10

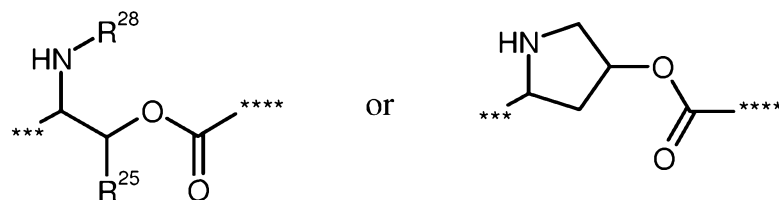
wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

L^3 stands for a bond or ethane-1,2-diyl,

15 L^4 stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

5 R^{25} stands for methyl,

R^{28} stands for hydrogen, methylcarbonyl or *tert*-butoxycarbonyl,

Q^1 stands for piperidine-1,4-diyl,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

10 or

R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

R^{21} stands for hydrogen or methyl,

R^{22} stands for hydrogen or methyl,

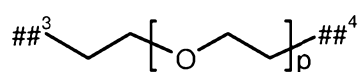
or

15 R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

R^{23} stands for methyl,

R^{24} stands for hydrogen,

L^2 stands for linear (C_2-C_6) -alkanediyl or for a group of the formula



20

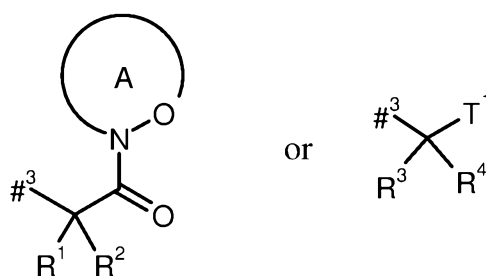
wherein

p stands for a number from 2 to 6,

$\#^3$ denotes the linkage site to the group B,

$\#^4$ denotes the linkage site to the nitrogen atom,

5 D stands for a group of the formula



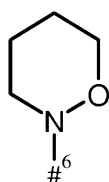
wherein

$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

10 R^2 stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

the ring A with the N-O group contained therein stands for



wherein

$\#^6$ denotes the linkage site to the carbonyl group,

15 R^3 stands for hydrogen,

R^4 stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

T^1 stands for a group of the formula $-C(=O)-NR^8R^9$,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl or ethyl,

R^{35} stands for methyl,

as well as their salts and solvates as well as the solvates of the salts.

- 5 Especially preferred within the scope of the present invention are binder-drug conjugates of formula (Ia), in which

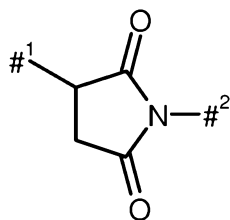
n stands for a number from 2 to 8, preferably 2 to 5,

AK stands for AK_1 ,

wherein

- 10 AK_1 stands for a binder that is bound to the group G via a sulfur atom of the cysteine radical of the binder, preferably for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

G stands for a group of the formula



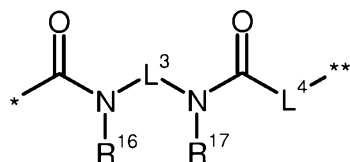
- 15 wherein

$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ denotes the linkage site to the group L^1 ,

L^1 stands for pentane-1,5-diyl,

B stands for a group of the formula



wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

L^3 stands for a bond,

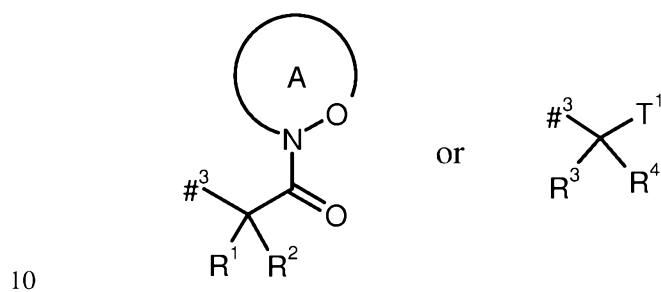
5 L^4 stands for a bond,

R^{16} stands for hydrogen,

R^{17} stands for hydrogen,

L^2 stands for propane-1,3-diyl,

D stands for a group of the formula



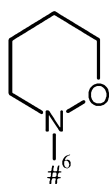
wherein

$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

15 the ring A with the N-O group contained therein stands for



wherein

#⁶ denotes the linkage site to the carbonyl group,

R³ stands for hydrogen,

R⁴ stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

T¹ stands for a group of the formula -C(=O)-NR⁸R⁹,

5 R⁸ stands for hydrogen,

R⁹ stands for hydrogen,

R³⁵ stands for methyl,

as well as their salts and solvates as well as the solvates of the salts.

Especially preferred within the scope of the present invention are binder-drug conjugates of
10 formula (Ia), in which

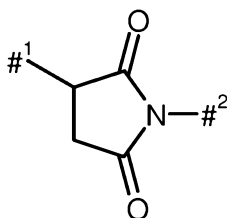
n stands for a number from 2 to 8, preferably 2 to 5,

AK stands for AK₁,

wherein

AK₁ stands for a binder that is bound to the group G via a sulfur atom of the cysteine
15 radical of the binder, preferably for a chimeric, humanized or human antibody,
especially preferably an anti-EGFR antibody,

G stands for a group of the formula



wherein

20 #¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

L¹ stands for a bond,

B stands for a bond,

L² stands for hexane-1,6-diyl,

and D has the meaning given above,

5 as well as their salts and solvates as well as the solvates of the salts.

Especially preferred within the scope of the present invention are binder-drug conjugates of formula (Ia), in which

n stands for a number from 2 to 8, preferably from 2 to 5,

AK stands for AK₂,

10 wherein

AK₂ stands for a binder that is bound to the group G via a nitrogen atom of the binder, preferably a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

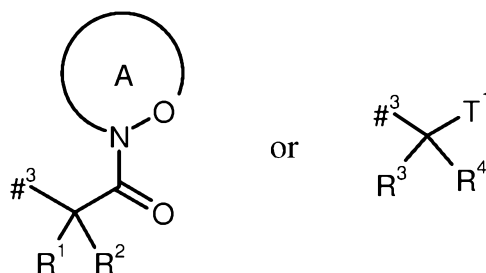
G stands for carbonyl,

15 L¹ stands for a bond,

B stands for a bond,

L² stands for pentane-1,5-diyl,

D stands for a group of the formula



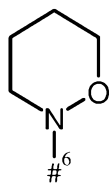
20 wherein

#³ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

the ring A with the N-O group contained therein stands for



5 wherein

#⁶ denotes the linkage site to the carbonyl group,

R^3 stands for hydrogen,

R^4 stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

T^1 stands for a group of the formula $-C(=O)-NR^8R^9$,

10 R^8 stands for hydrogen,

R^9 stands for hydrogen,

R^{35} stands for methyl,

as well as their salts and solvates as well as the solvates of the salts.

Especially preferred within the scope of the present invention are binder-drug conjugates of
15 formula (Ia), in which

n stands for a number from 2 to 8, preferably 2 to 5,

AK stands for AK_2 ,

wherein

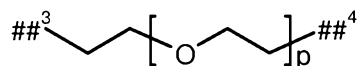
AK_2 stands for a binder that is bound to the group G via a nitrogen atom of the lysine
20 radical of the binder, preferably a chimeric, humanized or human antibody,
especially preferably an anti-EGFR antibody,

G stands for carbonyl,

L¹ stands for a bond,

B stands for a bond,

L² stands for a group of the formula



5 wherein

p stands for the number 3,

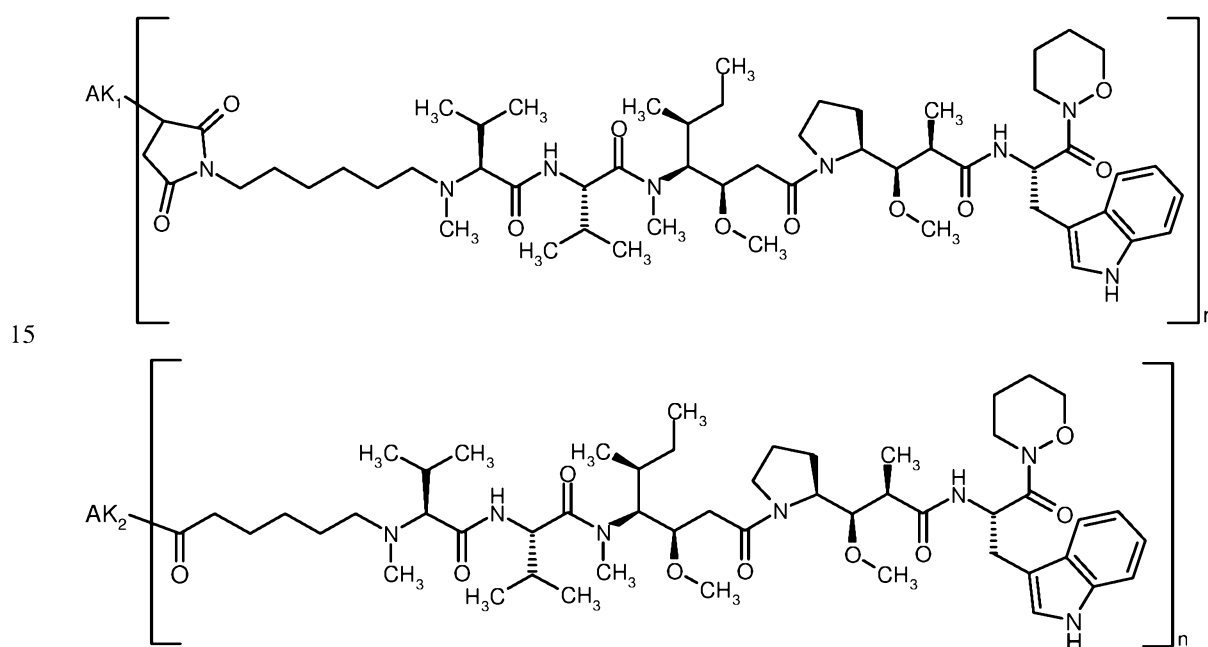
##³ denotes the linkage site to the group B,

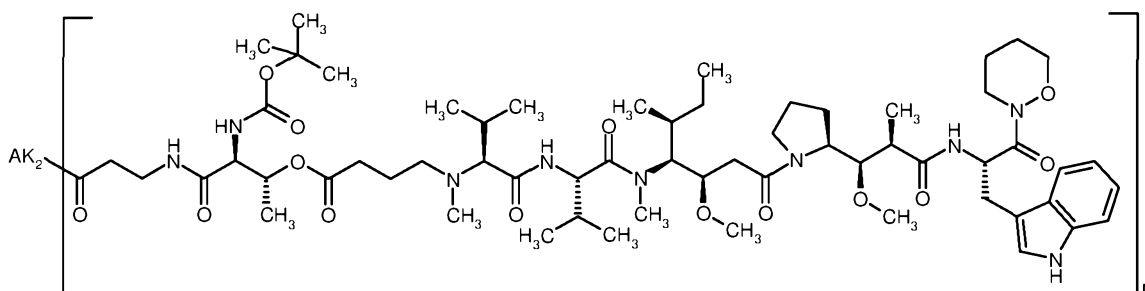
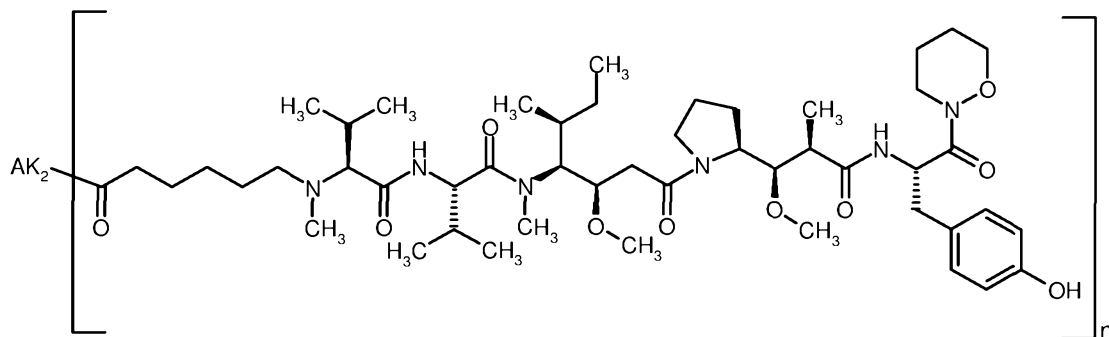
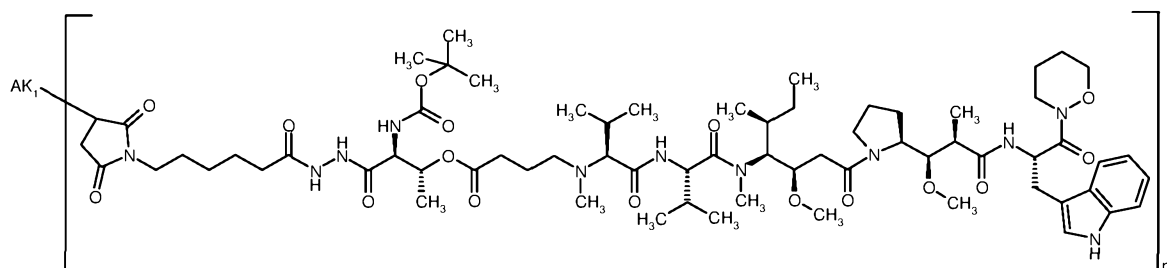
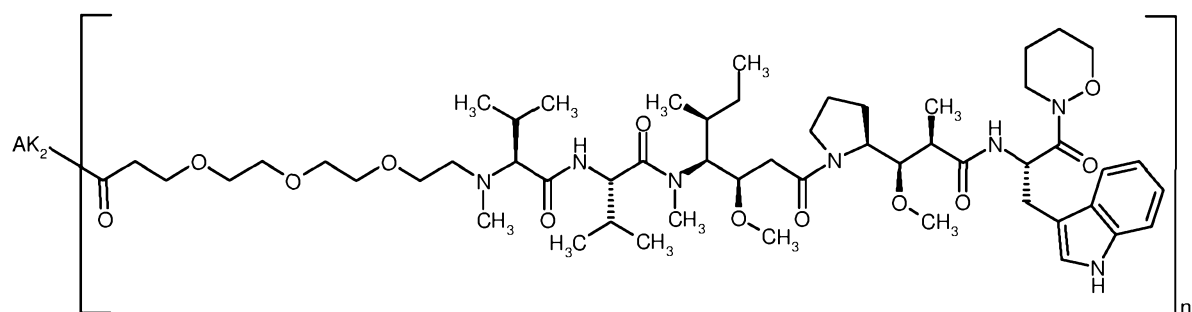
##⁴ denotes the linkage site to the nitrogen atom,

and D has the meaning given above,

10 as well as their salts and solvates as well as the solvates of the salts.

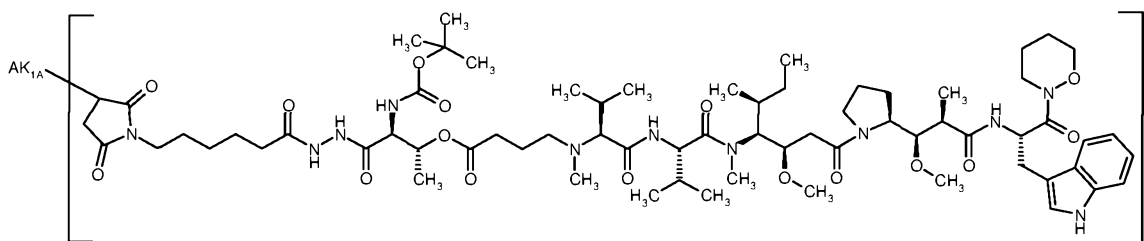
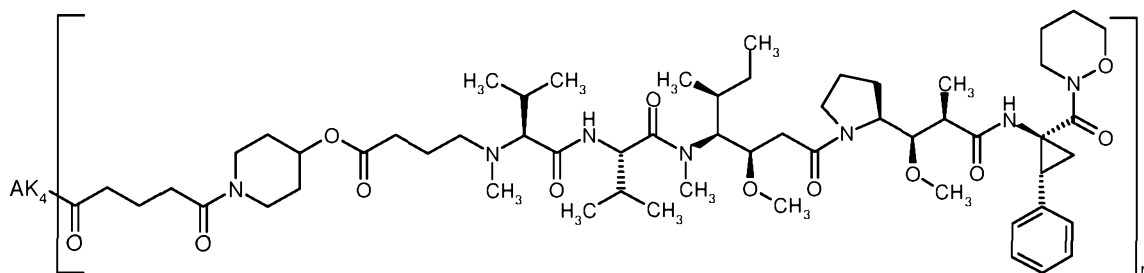
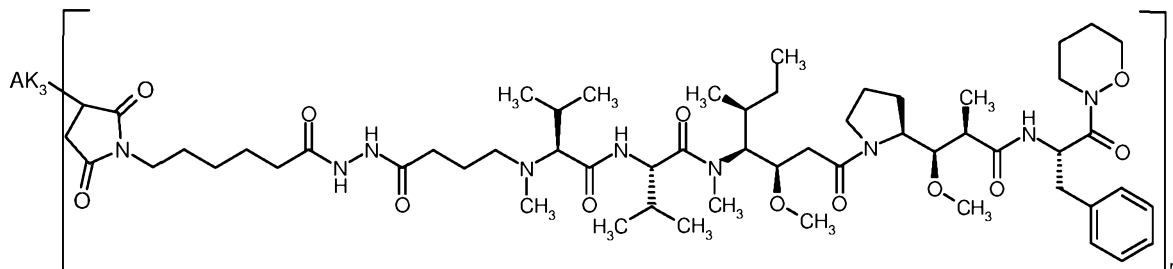
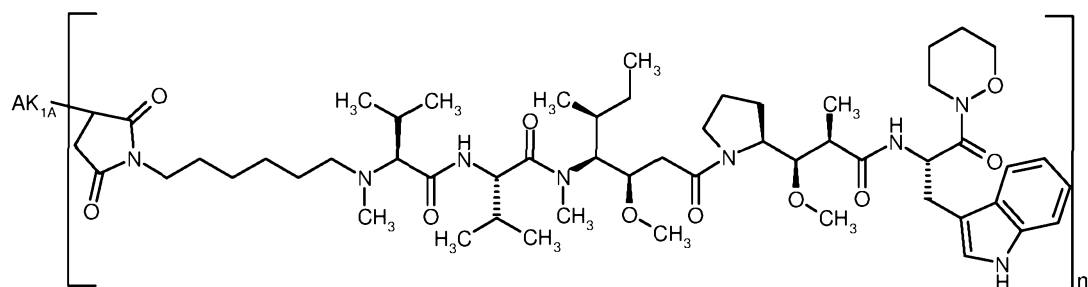
According to the invention, the drug-binder conjugate preferably comprises the following compounds in particular, where n stands for a number from 2 to 8, preferably 2 to 8, and AK stands for a chimeric, human or humanized antibody or an antigen binding antibody fragment which binds to mesothelin, C4.4a or EGFR:



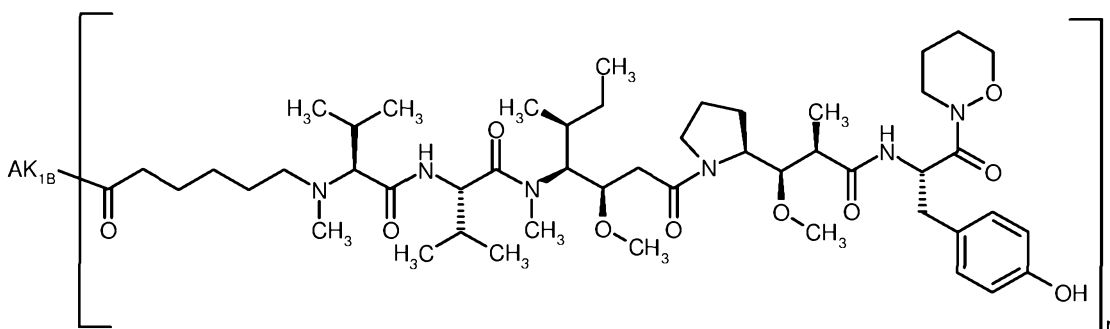


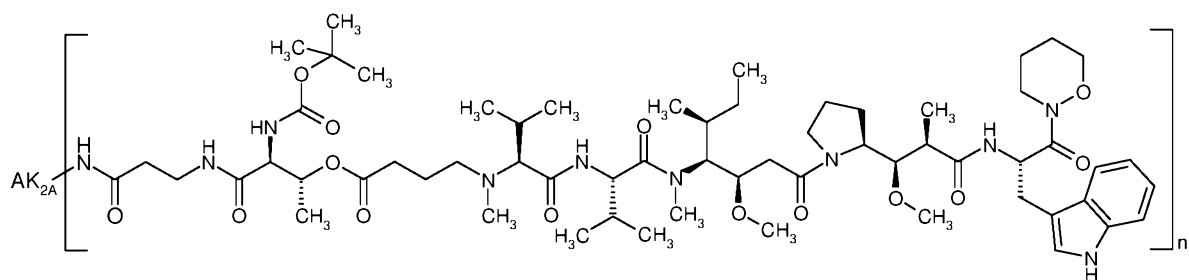
5

In addition, according to the invention the drug-binder conjugate is especially preferably selected from the following compounds:



5





in which

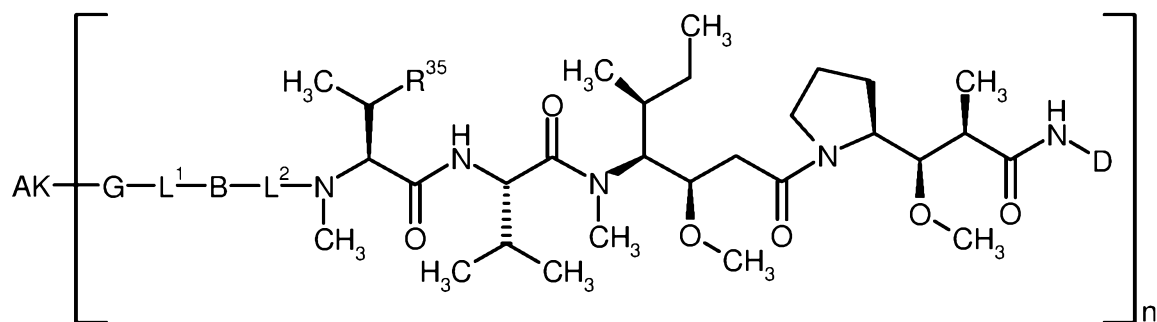
n stands for a number from 2 to 8, preferably 2 to 5,

and

5 AK_{1A}, AK_{1B}, AK_{2A}, AK₃ and AK₄ stand for the antibodies indicated.

AK

binder-drug conjugate of the following formula Ia



10 wherein

n stands for a number from 2 to 8;

AK stands for AK₁ or AK₂

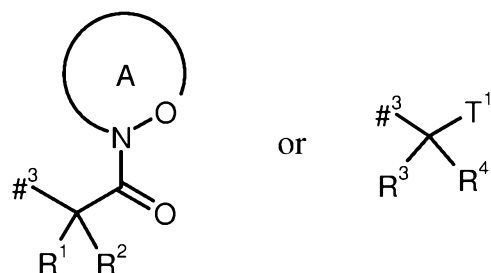
wherein

15 AK₁ stands for a chimeric, human or humanized antibody or an antigen binding antibody fragment which is bound to mesothelin, EGFR or C4.4a and is bound to the group G via the sulfur atom of a cysteine radical of the binder,

AK₂ stands for a chimeric, human or humanized antibody or an antigen binding antibody fragment which is bound to mesothelin, EGFR or C4.4a and is bound to the group G via the NH side group of a lysine radical of the binder,

R³⁵ stands for methyl;

D stands for a group of the formula



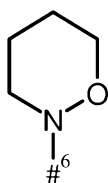
wherein

5 #³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

R² stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

the ring A with the N-O group contained therein stands for



10 wherein

#⁶ denotes the linkage site to the carbonyl group,

R³ stands for hydrogen,

R⁴ stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

T¹ stands for a group of the formula -C(=O)-NR⁸R⁹,

15 R⁸ stands for hydrogen or methyl,

R⁹ stands for hydrogen, methyl or ethyl,

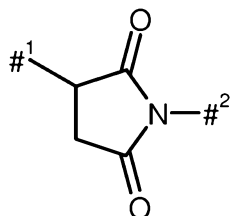
the group §-G-L1-B-L2-§§ stands for a linker,

wherein

§ denotes the linkage site to the group AK and

§§ denotes the linkage site to the nitrogen atom,

G for the case when $AK = AK_1$ stands for a group of the formula



5

wherein

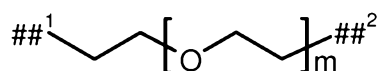
#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

or

10 for the case when $AK = AK_2$, G stands for carbonyl,

L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



wherein

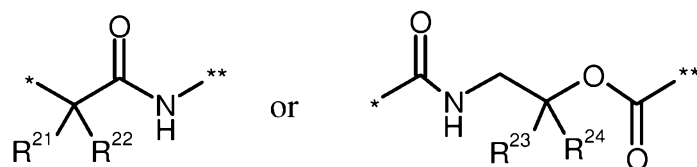
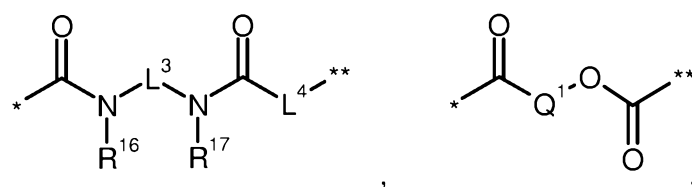
m stands for a number of 2 or 3,

15 ##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



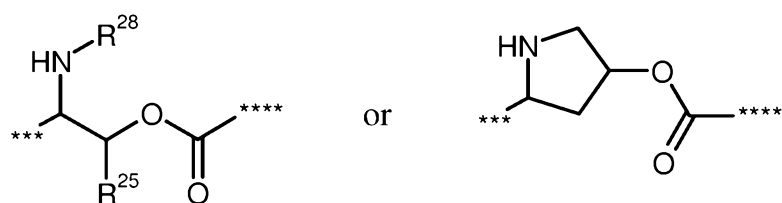
wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

5 L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

10 **** denotes the linkage site to L²,

R²⁵ stands for methyl,

R²⁸ stands for hydrogen, methylcarbonyl or *tert*-butoxycarbonyl,

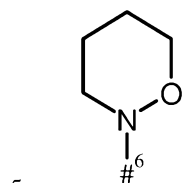
Q¹ stands for piperidine-1,4-diyl,

R¹⁶ stands for hydrogen or methyl,

15 R¹⁷ stands for hydrogen or methyl,

AK stands for a human or humanized antibody or an antigen binding antibody fragment which is bound to mesothelin, EGFR or C4.4a and is bound to the group G via the sulfur atom of a cysteine radical of the binder,

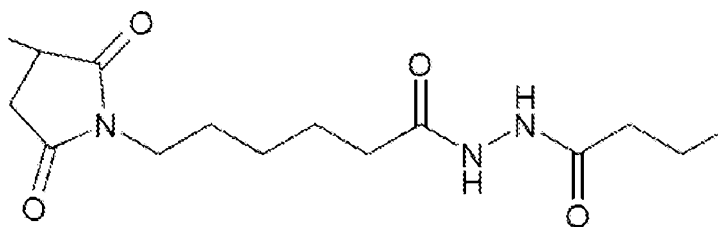
X₁ stands for NH₂ or



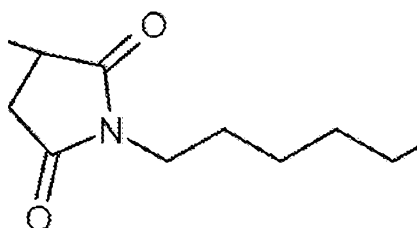
and

X₂ stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl.

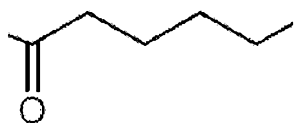
When the toxophore is bound to a cysteine radical of the antibody, the linker

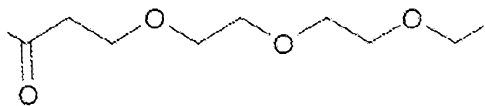


10 may be replaced by the following linker, for example:



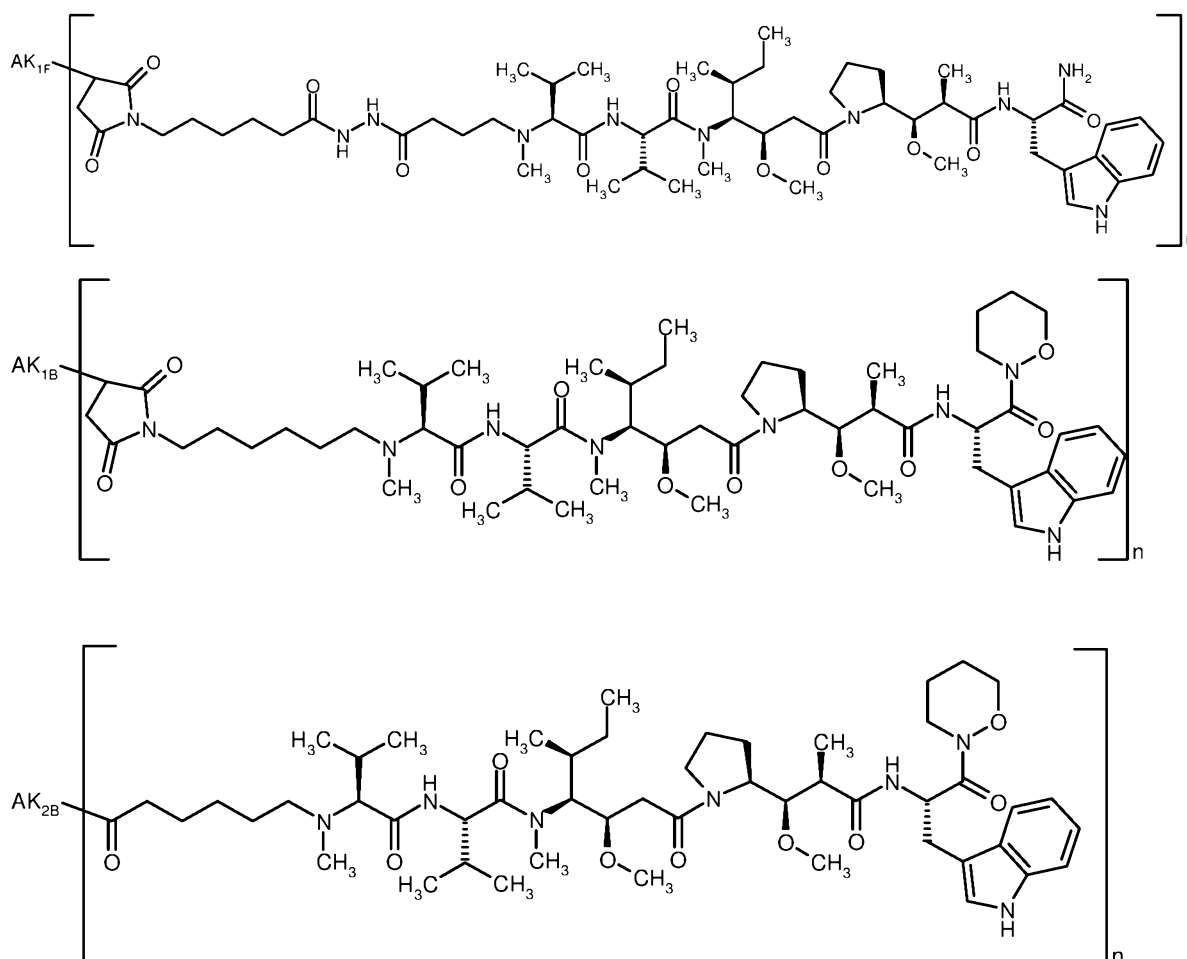
When the toxophore is bound to an NH group of the lysine radical of the antibody, the linker may be replaced by the following:

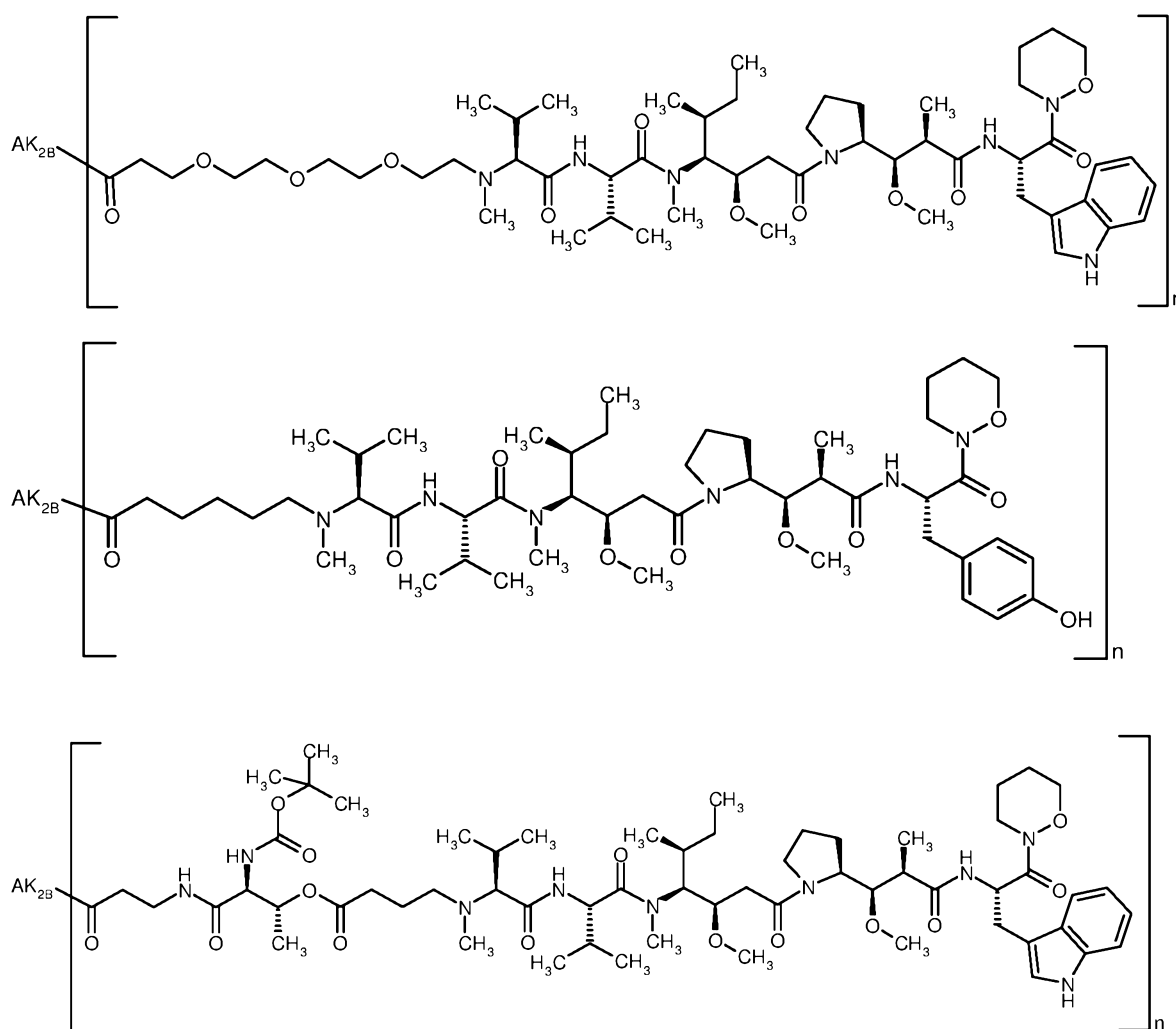




According to the invention the drug-binder conjugate is especially comprised of the following compounds, where n stands for a number from 2 to 8, preferably 2 to 5, and AK stands for a chimeric, human or humanized antibody or an antigen binding antibody fragment which binds to mesothelin, EGFR or C4.4a:

5





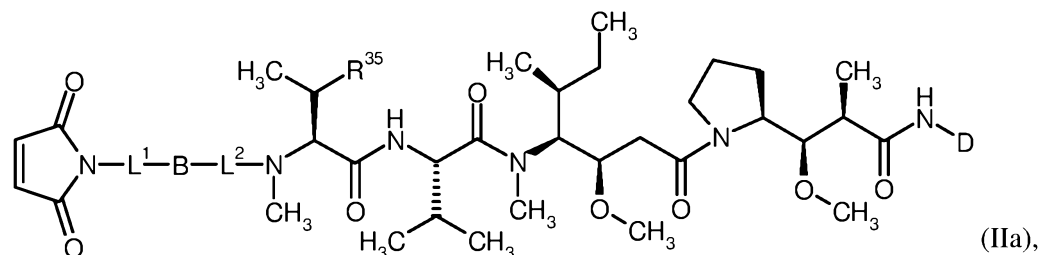
- 5 In these formulas, AK_{1F} , AK_{1B} and AK_{2B} may be replaced by other chimeric, human or humanized anti-C4.4a antibodies, anti-EGFR antibodies or anti-mesothelin antibodies.

The definitions of radicals given in the respective combinations and/or preferred combinations of radicals in detail can also be replaced by definitions of radicals of other combinations independently of the respective combinations of radicals given.

- 10 Combinations of two or more of the aforementioned preferred ranges are most especially preferred.

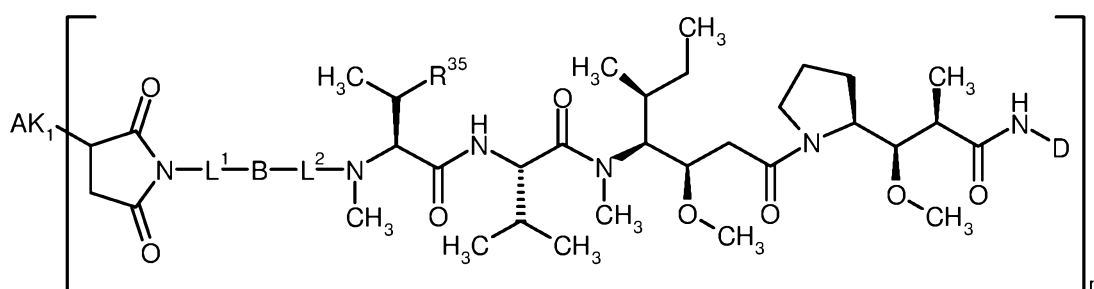
An additional subject matter of the present invention is a method for synthesis of the compounds of formula (Ia) according to the invention, which is characterized in that a solution of the binder in PBS buffer

[A] is mixed with a suitable reducing agent such as, for example, dithiothreitol or *tris*-(2-carboxyethyl)phosphine hydrochloride and then is reacted with a compound of formula (IIa)



5 in which D, L¹, B, L² and R³⁵ each have the meanings given above,

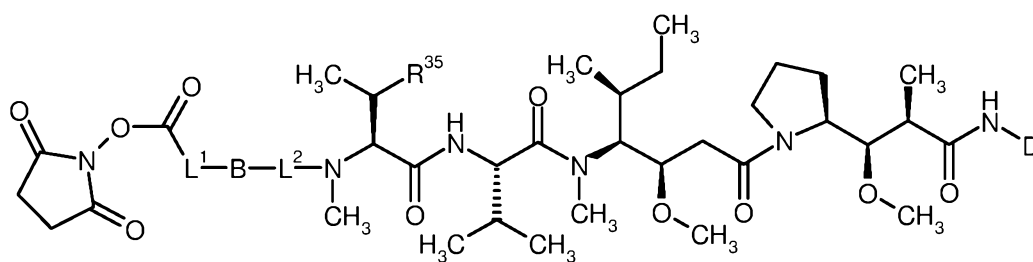
to form a compound of formula (I-A)



in which n, AK₁, D, L¹, B, L² and R³⁵ each have the meanings given above,

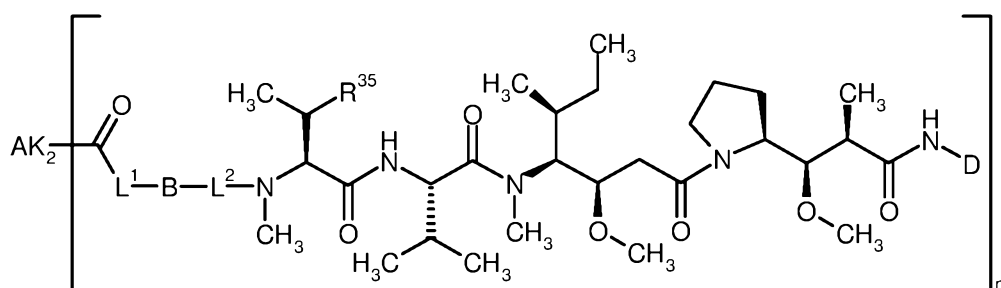
10 or

[B] reacting it with a compound of formula (IIIa)



in which D, L¹, B, L² and R³⁵ each have the meanings given above,

15 to form a compound of formula (Ia-B)

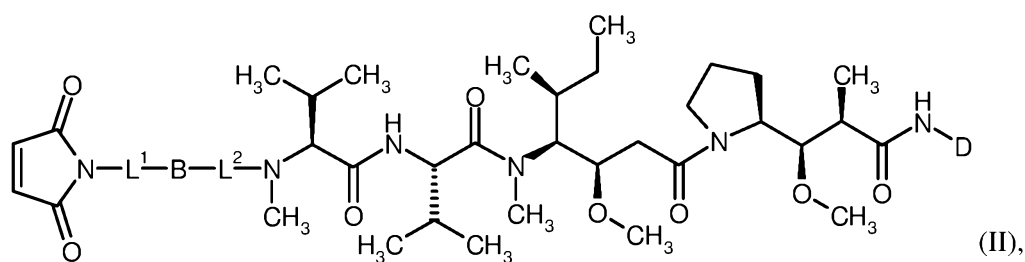


(Ia-B),

in which n , AK_2 , D , L^1 , B , L^2 and R^{35} each have the meanings given above.

An additional subject matter of the present invention is a method for synthesis of the compounds of formula (I) according to the invention, which is characterized in that a solution of the binder in PBS buffer

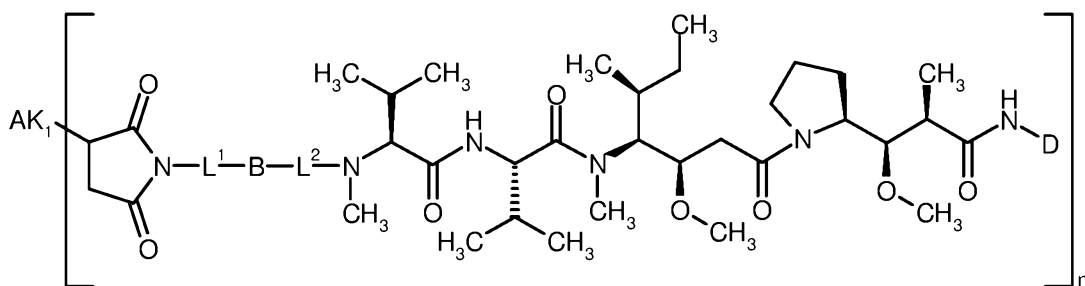
[A] is mixed with a suitable reducing agent such as, for example, dithiothreitol or *tris*-(2-carboxyethyl)phosphine hydrochloride and then is reacted with a compound of formula (II)



10

in which D , L^1 , B and L^2 each have the meanings given above,

to form a compound of formula (I-A)



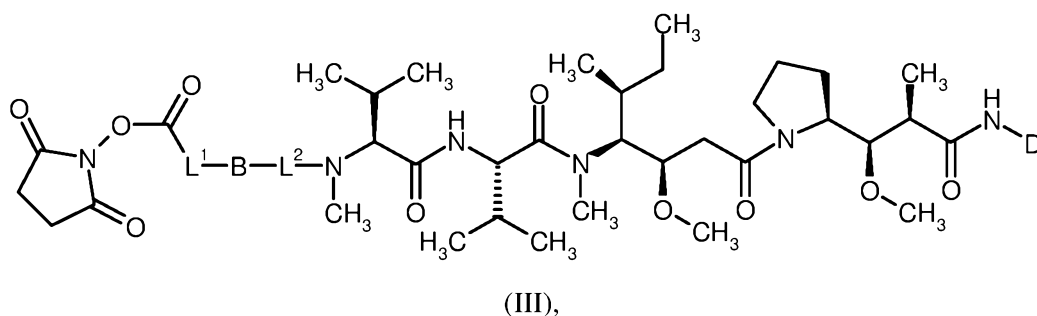
(I-A),

15

in which n , AK_1 , D , L^1 , B and L^2 each have the meanings given above,

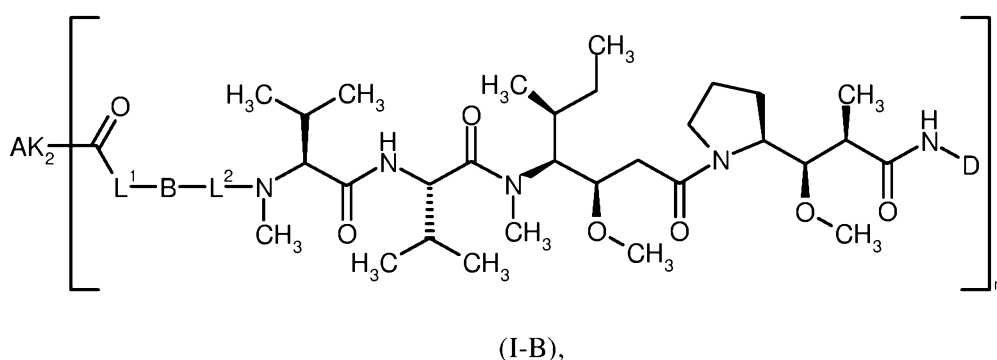
or

[B] reacting it with a compound of formula (III)



5 in which D, L¹, B and L² each have the meanings given above,

to form a compound of formula (I-B)



in which n, AK₂, D, L¹, B and L² each have the meanings given above.

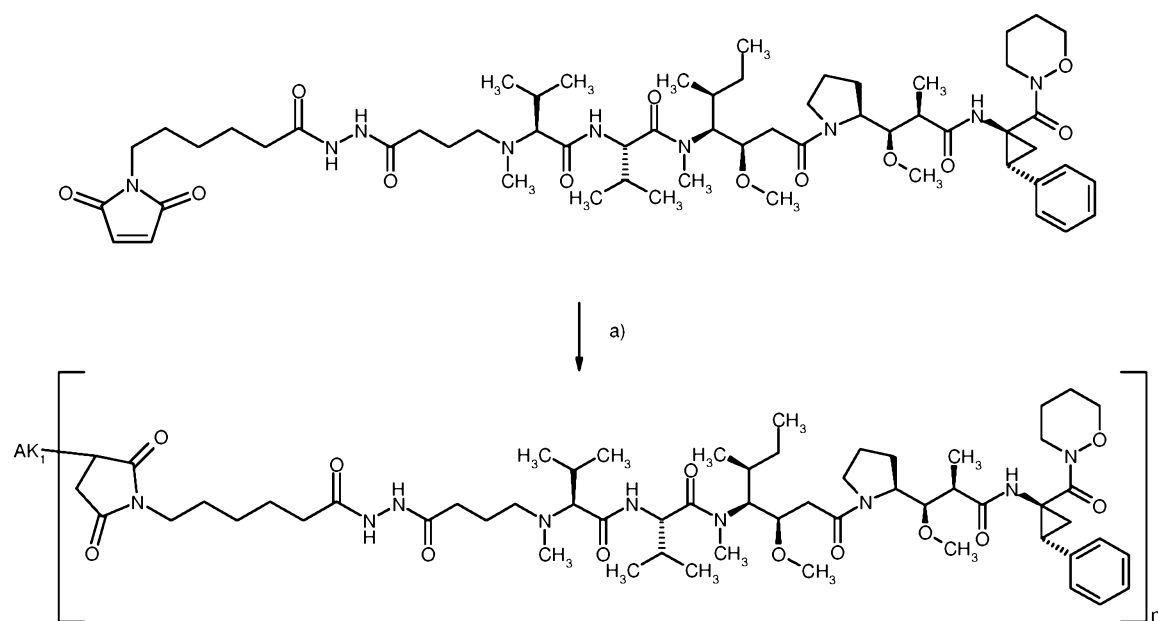
10 Cysteine coupling:

Partial reduction of the antibody and subsequent conjugation of the (partially) reduced antibody with a compound of formula (II) and/or (IIa) takes place according to methods with which those skilled in the art are familiar; see, for example, Ducry et. al., *Bioconj. Chem.* 2010, **21**, 5 and references therein, Klussman et. al., *Bioconj. Chem.* 2004, **15**(4), 765-773. The mild reduction of the antibody by adding 2-6 equivalence of TCEP to the antibody present in a suitable buffer solution, preferably phosphate buffer, and stirring for 30-180 minutes at temperatures between 15°C and 40°C, preferably at room temperature. Next the conjugation is performed by adding a solution of a compound of formula (II) and/or (IIa) in DMSO, acetonitrile or DMF to the solution of the (partially) reduced antibody in PBS buffer and then reacting them at a temperature of 0°C to +40°C, in particular from +10°C to +30°C for a period of 30 minutes to 6 hours, in particular one to two hours.

Lysine coupling:

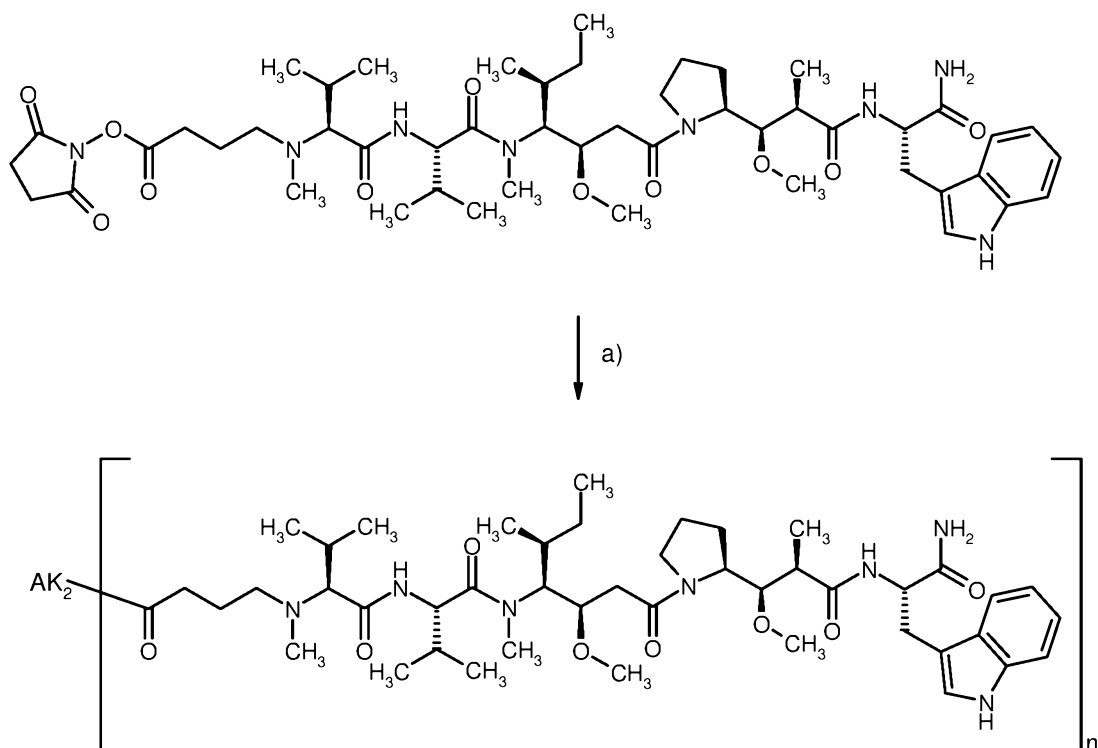
First the compounds of formula (III) and/or (IIa) or comparable activated carboxyl components are synthesized by traditional methods of peptide chemistry. These compounds are then dissolved in inert solvents such as DMSO or DMF and added to the antibody, which is preferably present in phosphate buffer at a neutral pH. The solution is stirred for 1-16 hours at a temperature between 15°C and 40°C, preferably at RT.

The synthesis processes described above are then illustrated as an example on the basis of the following schemes (schemes 1 and 2):

Scheme 1

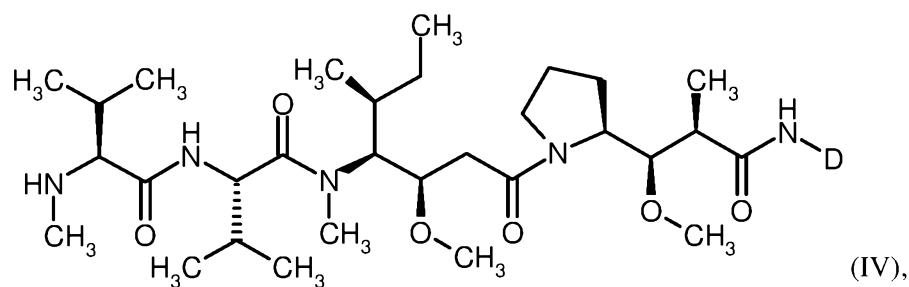
[a): 1. AK, TCEP, PBS buffer, RT; 2. Addition of maleimide derivative in DMSO, RT].

Scheme 2



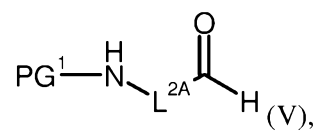
[a): AK, PBS buffer, RT mixed with activated carboxyl derivative of the linker-drug components].

- 5 The compounds of formula (II) in which L^1 and B stand for a bond can be synthesized by reductive amination of a compound of formula (IV)



in which D has the meaning given above

in an inert solvent with a compound of formula (V)

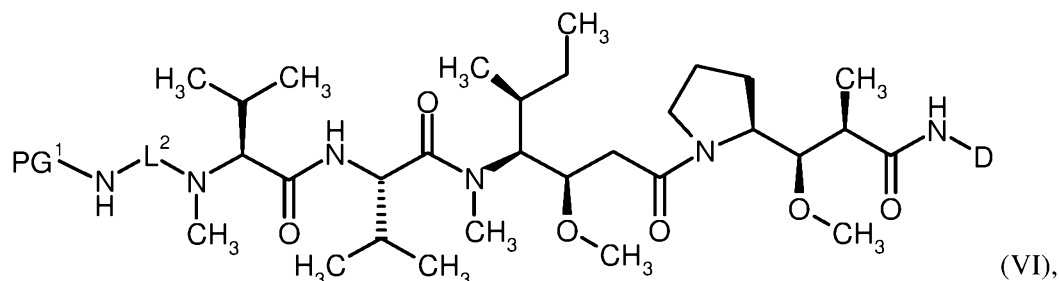


in which

L^{2A} has the meaning of L^2 as defined above but is shortened in the alkyl chain length by one carbon atom,

PG^1 stands for an amino protective group such as, for example, (9H-fluorene-9-ylmethoxy) carbonyl, *tert*-butoxycarbonyl or benzyloxycarbonyl,

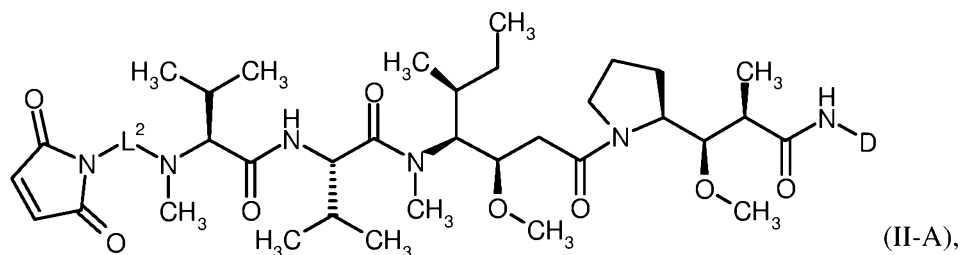
5 to form a compound of formula (VI)



in which D, L^2 and PG^1 have the meanings given above,

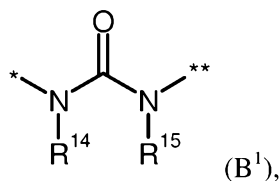
splitting off the protective group PG^1 from this compound by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable base with methyl-2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate to form a compound of formula (II-A)

10



in which D and L^2 each have the meanings given above.

The compounds of formula (II) in which B stands for a group of the formula (B^1)

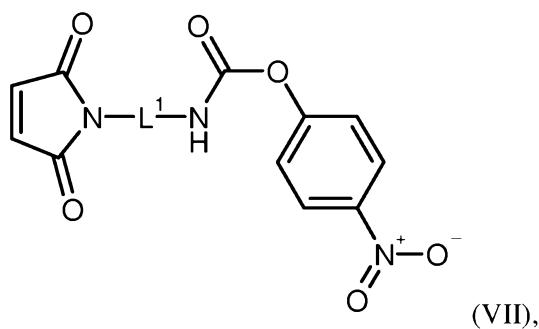


15

in which *, **, R^{14} and R^{15} each have the meanings given above,

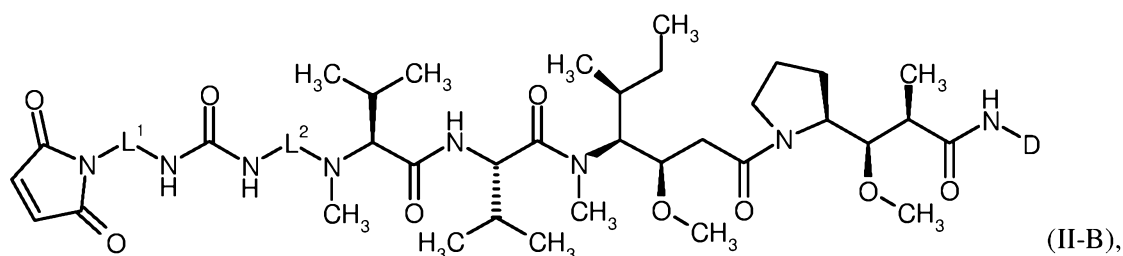
can be synthesized by splitting off the protective group PG¹ from a compound of formula (VI) by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable base with a compound of formula (VII)

5



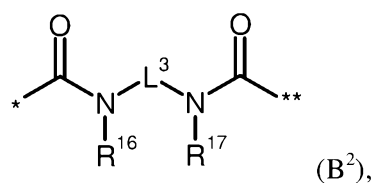
in which L¹ has the meaning given above

to form a compound of formula (II-B)



in which D, L¹ and L² each have the meanings given above.

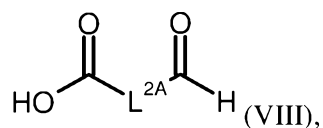
10 The compounds of formula (II) in which B stands for a group of the formula (B²)



in which *, **, L³, R¹⁶ and R¹⁷ each have the meanings given above,

can be synthesized by reductive amination of a compound of formula (IV) in an inert solvent with a compound of formula (VIII)

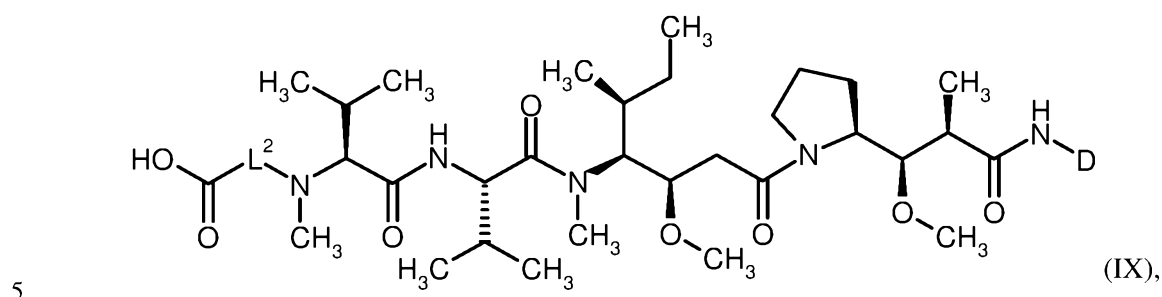
15



in which

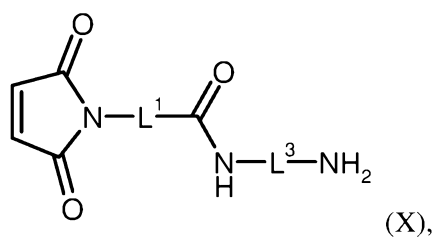
L^{2A} has the meaning given above for L^2 but the alkyl chain length has been shortened by one carbon atom,

to form a compound of formula (IX)



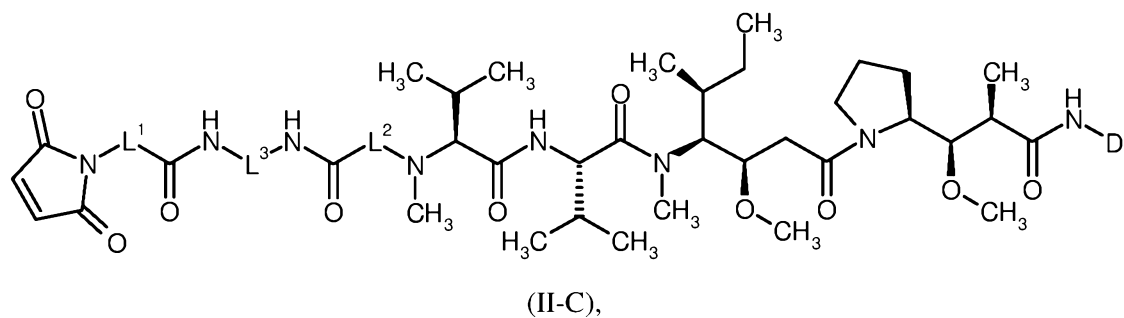
in which D and L^2 have the meanings given above

and this compound is reacted in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of formula (X)



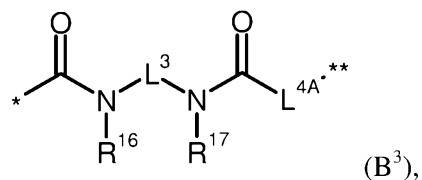
10 in which L^1 and L^3 each have the meanings given above,

to form a compound of formula (II-C)



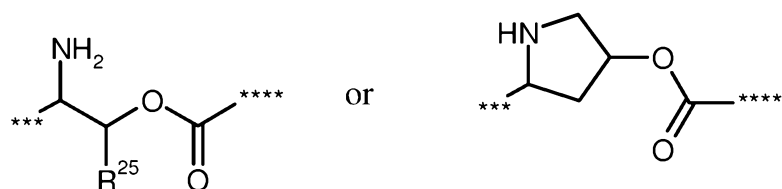
in which D, L^1 , L^2 and L^3 each have the meanings given above.

15 A compound of formula (II), in which B stands for a group of the formula (B^3)



in which *, **, L³, R¹⁶ and R¹⁷ each have the meanings given above and

L^{4A} stands for a group of the formula



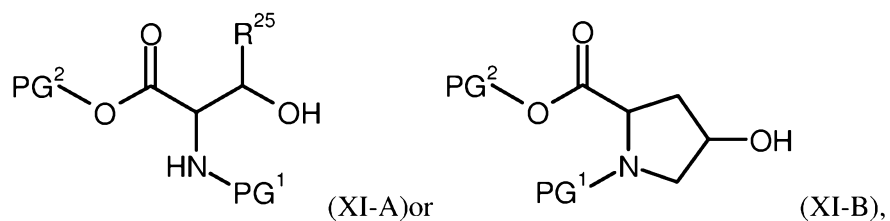
5 wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L²,

R²⁵ stands for hydrogen or methyl,

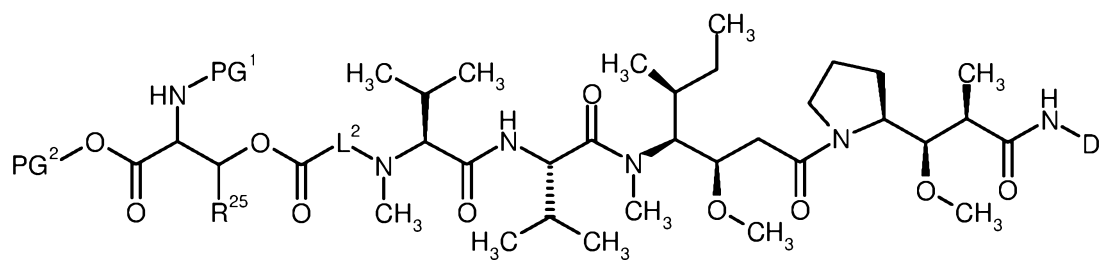
10 can be synthesized by reacting a compound a compound of formula (IX) in an inert solvent in the presence of a suitable base and a suitable coupling reagent with a compound of formula (XI-A) or (XI-B)



in which R²⁵ and PG¹ each have the meanings given above and

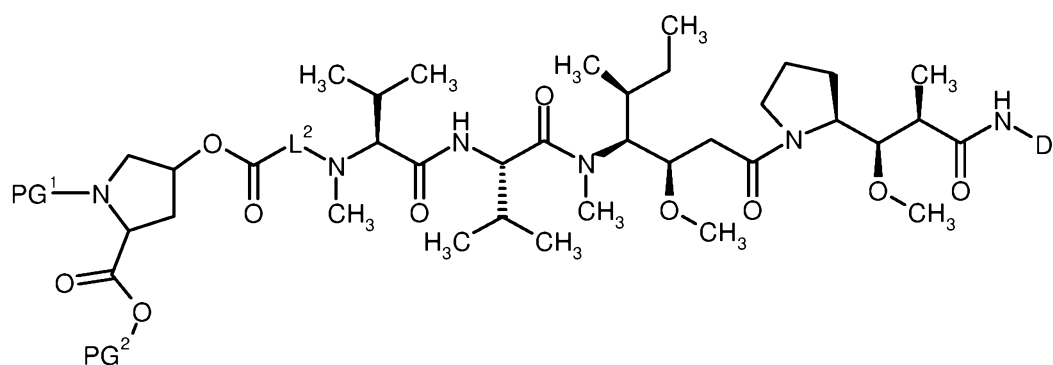
PG² stands for a suitable carboxyl protective group, in particular benzyl,

15 to form a compound (XII-A) and/or (XII-B)



(XII-A)

or

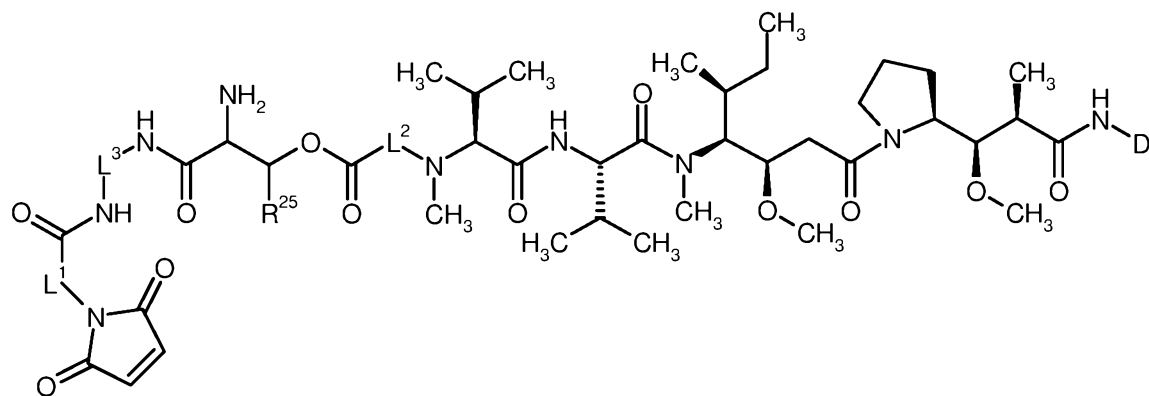


(XII-B),

5

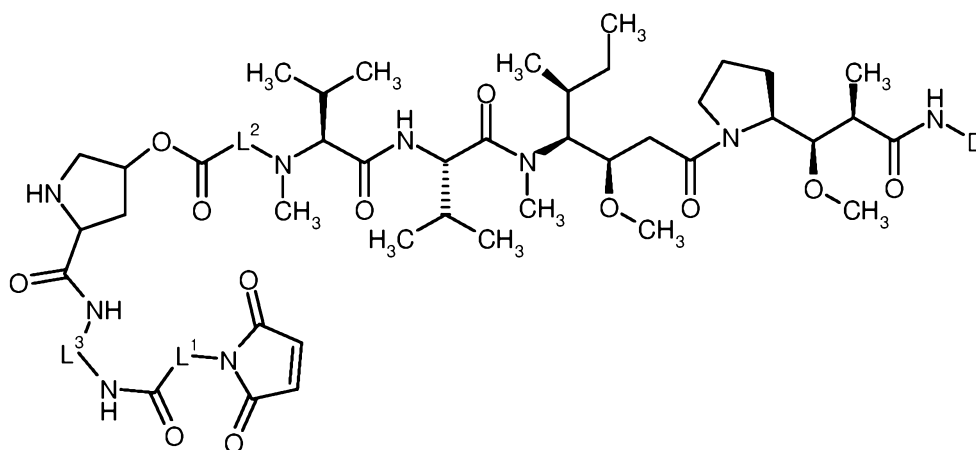
in which D, PG¹, PG² and L² have the meanings given above,

then splitting off the protective group PG² from this compound using methods known to those skilled in the art and reacting the deprotected compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of formula (X), and then splitting
 10 off the protective group PG¹ by methods with which those skilled in the art are familiar to form a compound of formula (II-D-A) and/or (II-D-B)



(II-D-A)

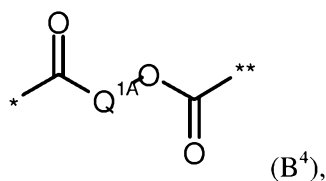
or



(II-D-B),

in which D, L¹, L² and L³ have the meanings given above.

A compound of formula (II) in which B stands for a group of the formula (B⁴)

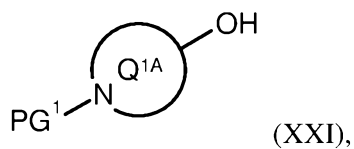


5

in which *, ** each have the meanings given above and

Q^{1A} stands for an N-linked four- to seven-membered heterocycle,

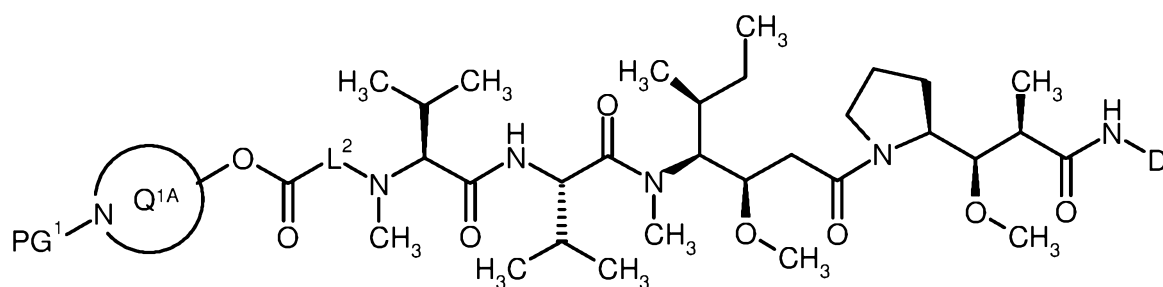
can be synthesized by reacting a compound of formula (IX) in an inert solvent in the presence of a suitable base and a suitable coupling reagent with a compound of formula (XXI)



10

in which PG¹ and Q^{1A} each have the meanings given above,

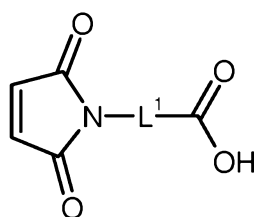
to form a compound of formula (XXII)



(XXII),

in which PG¹, Q^{1A}, D and L² have the meanings given above

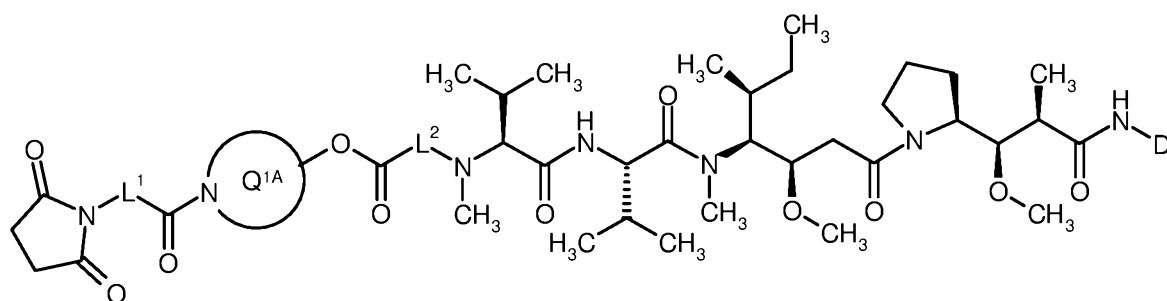
- 5 then splitting off the protective group PG¹ from this compound by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of formula (XXIII)



(XXIII),

- 10 in which L¹ has the meaning given above

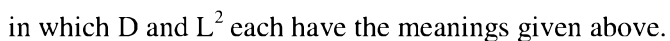
to form a compound of formula (II-D)



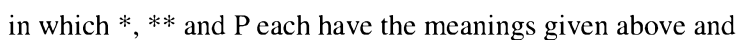
(II-D),

in which Q^{1A}, D, L¹ and L² have the meanings given above.

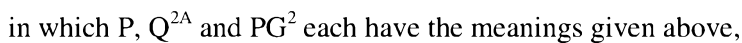
- 15 The compounds of formula (III), in which L¹ and B stand for a bond can be synthesized by reacting a compound of formula (IX) with N-hydroxysuccinimide in an inert solvent in the presence of a suitable coupling reagent and a suitable base to form a compound of formula (III-A):



5



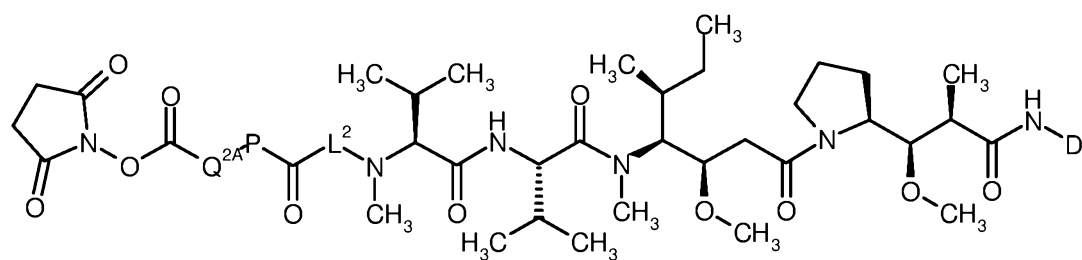
10



15

in which D , P , Q^{2A} , L^2 and PG^2 each have the meanings given above,

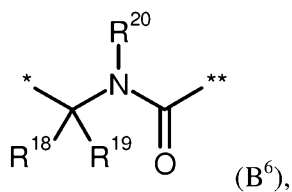
splitting off the protective group PG² from this compound by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable base with N-hydroxysuccinimide to form a compound of formula (III-B)



(III-B),

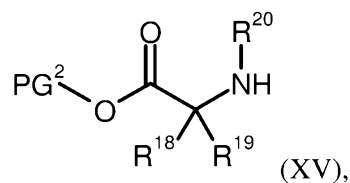
in which D, P, Q^{2A} and L² each have the meanings given above.

The compounds of formula (III), in which L¹ stands for a bond and B stands for a group of the
5 formula (B⁶)



in which *, **, R¹⁸, R¹⁹ and R²⁰ each have the meanings given above,

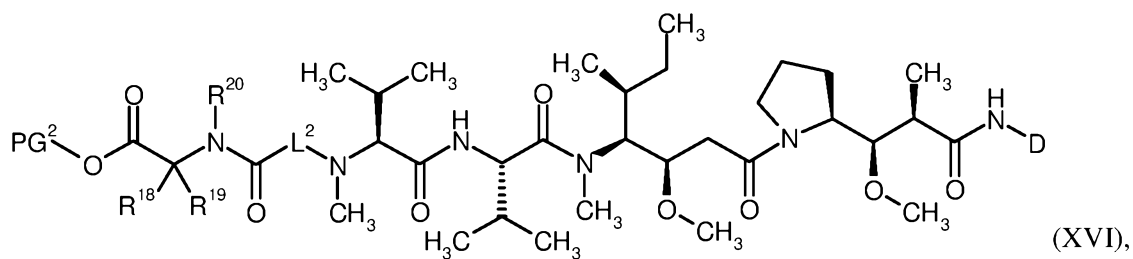
can be synthesized by reacting a compound of formula (IX) in an inert solvent in the presence of
a suitable coupling reagent and a suitable base with a compound of formula (XV)



10

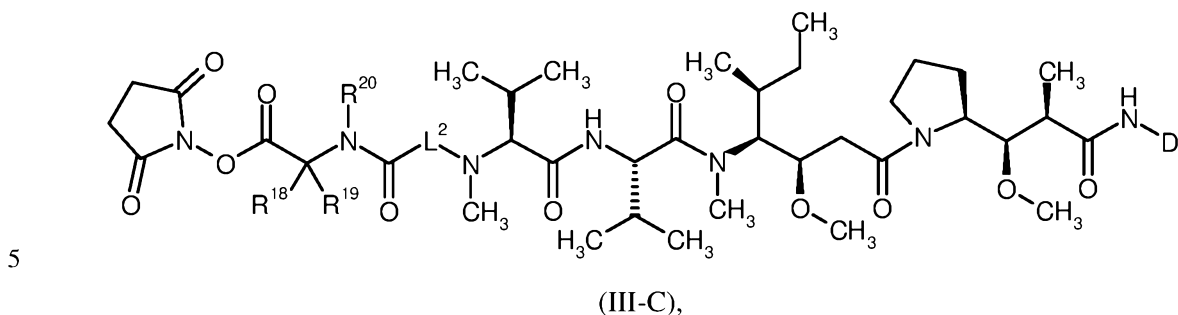
in which R¹⁸, R¹⁹, R²⁰ and PG² each have the meanings given above,

to form a compound of formula (XVI)



in which D, R¹⁸, R¹⁹, R²⁰, L² and PG² each have the meanings given above,

then splitting off the protective group PG² from this compound by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with N-hydroxysuccinimide to form a compound of formula (III-C)



in which D, R¹⁸, R¹⁹, R²⁰ and L² each have the meanings given above.

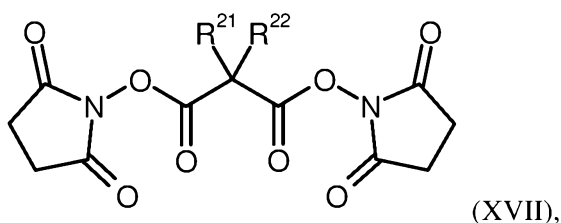
The compounds of formula (III), in which L¹ stands for a bond and B stands for a group of formula (B⁷)



in which *, **, R²¹ and R²² each have the meanings given above,

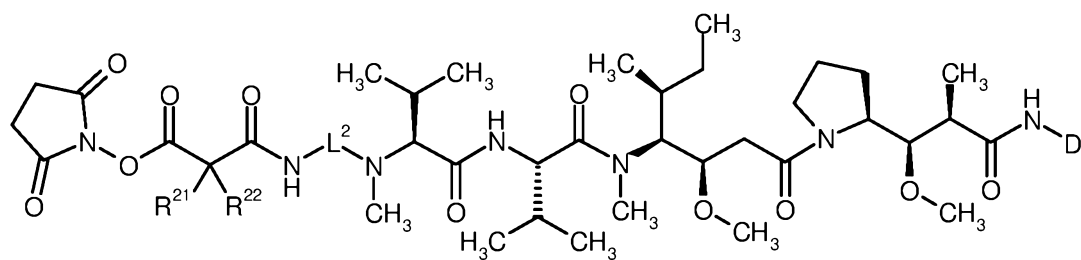
may be synthesized by splitting off the protective group PG¹ from a compound of formula (VI) by methods with which those skilled in the art are familiar, and then reacting the resulting deprotected compound in an inert solvent in the presence of a suitable base with a compound of formula (XVII)

15



in which R²¹ and R²² each have the meanings given above,

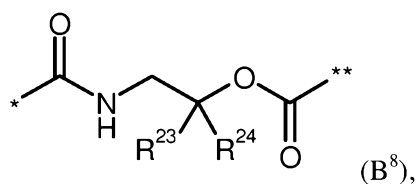
to form a compound of formula (III-D)



(III-D),

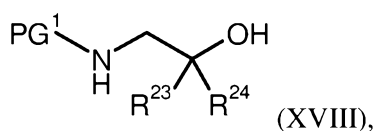
in which D, R^{21} , R^{22} and L^2 each have the meanings given above.

The compounds of formula (III) in which B stands for a group of the formula (B^8)

(B^8),

in which *, **, R^{23} and R^{24} each have the meanings given above,

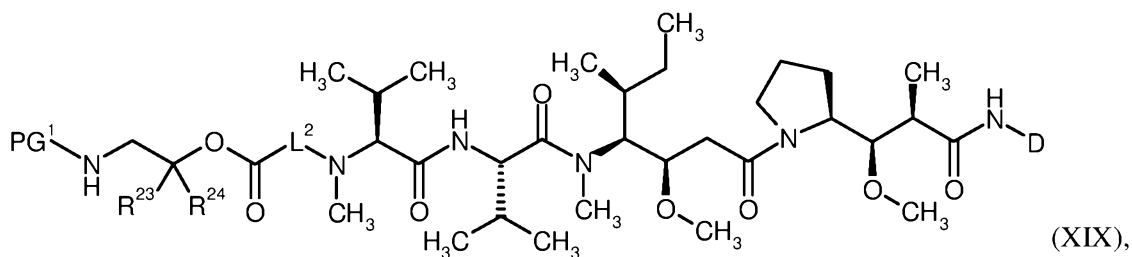
can be synthesized by reacting a compound of formula (IX) in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of formula (XVIII)



(XVIII),

10 in which R^{23} , R^{24} and PG^1 each have the meanings given above

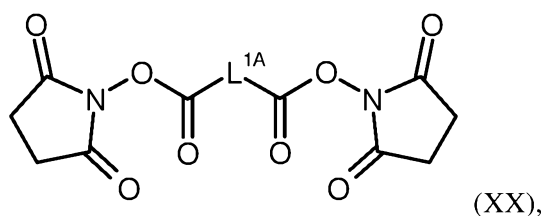
to form a compound of formula (XIX)



(XIX),

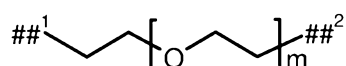
in which D, R^{23} , R^{24} , L^2 and PG^1 each have the meanings given above,

15 splitting off the protective group PG^1 from this compound by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of formula (XX)



in which

L^{1A} stands for linear (C_1-C_{10}) -alkanediyl or for a group of the formula



5 wherein

m stands for a number from 2 to 6,

$##^1$ denotes the linkage site to the group G,

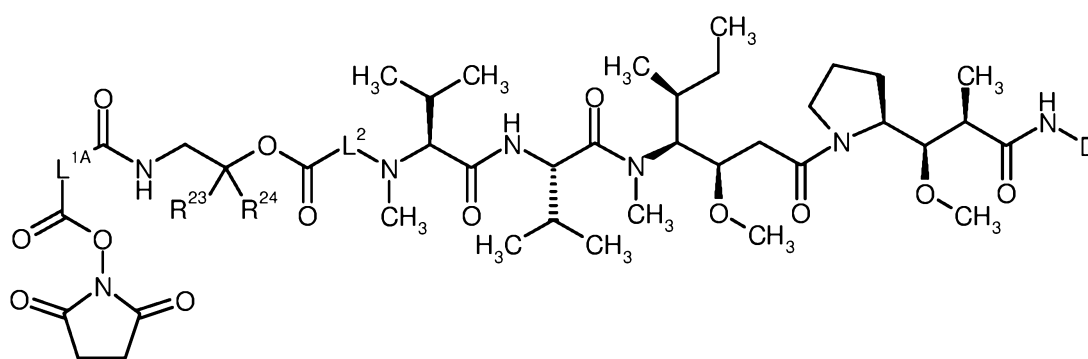
$##^2$ denotes the linkage site to the group B,

wherein (C_1-C_{10}) -alkanediyl may be substituted with 1 to 4 methyl substituents,

10 and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another may be bridged to form a (C_3-C_6) -cycloalkyl ring or a phenyl ring including the carbon atoms optionally situated between them,

to form a compound of formula (III-E)

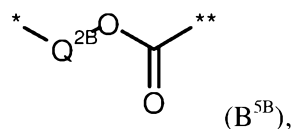


15

(III-E),

in which D, R^{23} , R^{24} , L^{1A} and L^2 each have the meanings given above.

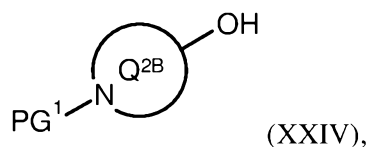
The compounds of formula (III), in which B stands for a group of the formula (B^{5B})



in which * and ** each have the meanings given above and

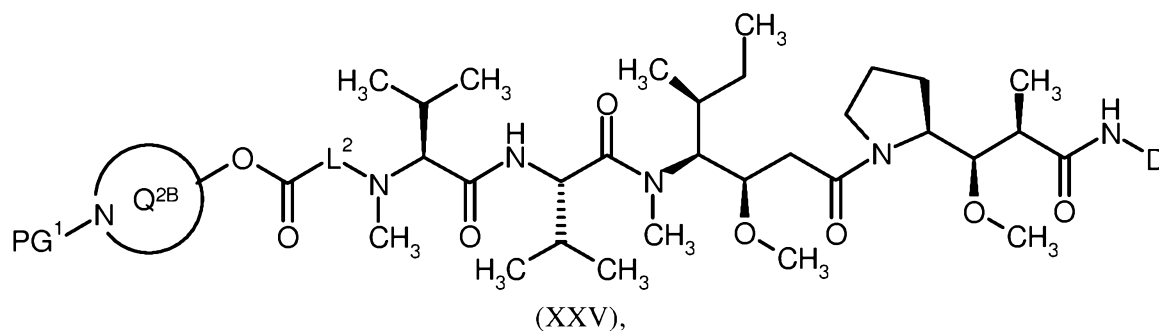
Q^{2B} stands for a N-linked four- to seven-membered heterocycle,

- 5 can be synthesized by reacting a compound of formula (IX) in an inert solvent in the presence of a suitable base and a suitable coupling reagent with a compound of formula (XXIV



in which PG^1 and $\text{Q}^{2\text{B}}$ each have the meanings given above,

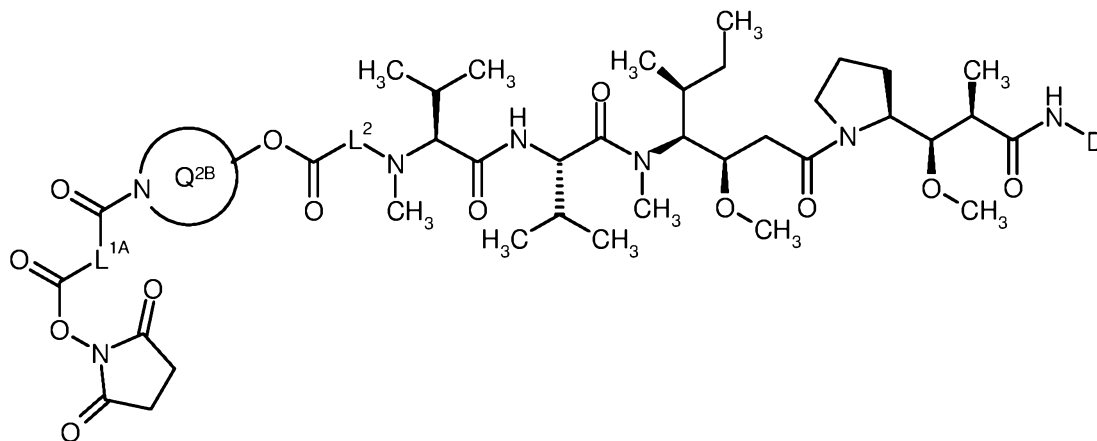
to form a compound of formula (XXV)



in which PG^1 , $\text{Q}^{2\text{B}}$, D and L^2 have the meanings given above,

splitting off the protective group PG¹ from this compound by methods with which those skilled in the art are familiar

- 15 and then reacting the deprotected compound in an inert solvent in the presence of a suitable base with a compound of formula (XX) to yield a compound of formula (III-F)



(III-F),

in which Q^{2B} , D, L^{1A} and L^2 have the meanings given above.

The reactions (IV) + (V) $\rightarrow$ (VI) and (IV) + (VIII) $\rightarrow$ (IX) take place in the usual solvents that are typically used for reductive amination and are inert under the reaction conditions, optionally in the presence of an acid and/or a water extracting agent as the catalyst. Such solvents include, for example, alcohols such as methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol or *tert*-butanol, ethers such as tetrahydrofuran, 1,4-dioxane, 1,2-dimethoxyethane or bis-(2-methoxyethyl)ether or other solvents such as dichloromethane, 1,2-dichloroethane, *N,N*-dimethyl formamide and water. It is also possible to use mixtures of these solvents. The preferred solvent is a 1,4-dioxane/water mixture that is used with the addition of acetic acid or dilute hydrochloric acid as the catalyst.

Complex borohydrides in particular are suitable reducing agents for this reaction, such as, for example, sodium borohydride, sodium cyanoborohydride, sodium triacetoxymethylborohydride, tetra-*n*-butylammonium borohydride or borane-pyridine complex. Sodium cyanoborohydride or borane pyridine complex is preferably used.

The reactions (IV) + (V) $\rightarrow$ (VI) and (IV) + (VIII) $\rightarrow$ (IX) usually take place in a temperature range from 0°C to +120°C, preferably at +50°C to +100°C. The reactions may be performed at normal, elevated or reduced pressure (e.g., from 0.5 to 5 bar). It is customary to work under normal pressure.

The coupling reactions described above (IX) + (X) $\rightarrow$ (II-C), (XII-A) and/or (XII-B) + (X) $\rightarrow$ (II-D-A) and/or (II-D-B), (IX) + (XIII) $\rightarrow$ (XIV), (IX) + (XV) $\rightarrow$ (XVI) and (XXII) + (XXIII) $\rightarrow$ (II-D) (amide formed from the respective amine and carboxylic acid components) are performed according to the standard methods of peptide chemistry (see, for example, M. Bodanszky, *Principles of Peptide Synthesis*, Springer Verlag, Berlin, 1993; M. Bodanszky and A. Bodanszky,

The Practice of Peptide Synthesis, Springer Verlag, Berlin, 1984; H.-D. Jakubke and H. Jeschkeit, *Aminosäuren, Peptide, Proteine [Amino Acids, Peptides, Proteins]*, Verlag Chemie, Weinheim, 1982).

Inert solvents for these coupling reactions include, for example, ethers like diethyl ether, diisopropyl ether, *tert*-butylmethyl ether, tetrahydrofuran, 1,4-dioxane, 1,2-dimethoxyethane or bis-(2-methoxyethyl)ether, hydrocarbons such as benzene, toluene, xylene, pentane, hexane, heptane, cyclohexane or petroleum fractions, halohydrocarbons such as dichloromethane, trichloromethane, tetrachloromethane, 1,2-dichloroethane, trichloroethylene or chlorobenzene or dipolar aprotic solvents such as acetone, methyl ethyl ketone, acetonitrile, ethyl acetate, pyridine, dimethyl sulfoxide (DMSO), *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), *N,N'*-dimethylpropylene urea (DMPU) or *N*-methylpyrrolidinone (NMP). It is likewise possible to use mixtures of such solvents. *N,N*-Dimethylformamide is preferred.

Suitable activation/condensation agents for these coupling reactions include, for example, carbodiimides such as *N,N'*-diethyl, *N,N'*-dipropyl, *N,N'*-diisopropyl, *N,N'*-dicyclohexylcarbodiimide (DCC) or *N*-(3-dimethylaminoisopropyl)-*N'*-ethylcarbodiimide hydrochloride (EC), phosgene derivatives such as *N,N'*-carbonyldiimidazole (CDI) or isobutyl chloroformate, 1,2-oxazolium compounds such as 2-ethyl-5-phenyl-1,2-oxazolium 3-sulfate or 2-*tert*-butyl-5-methylisoxazolium perchlorate, acylamino compounds such as 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, phosphorus compounds such as propane phosphonic acid anhydride, cyanophosphonic acid diethyl ester, bis-(2-oxo-3-oxazolidinyl)phosphoryl chloride, benzotriazole-1-yloxy-*tris*-(dimethylamino)phosphonium hexafluorophosphate or benzotriazole-1-yloxy-*tris*-(pyrrolidino)phosphonium hexafluorophosphate (PyBOP), or uronium compounds such as *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU), *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), 2-(2-oxo-1-(2*H*)-pyridyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TPTU), *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) or *O*-(1*H*-6-chlorobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TCTU), optionally in combination with additional excipients such as 1-hydroxybenzotriazole (HOBt) or *N*-hydroxysuccinimide (HOSu) as well as bases such as alkali carbonates, e.g., sodium or potassium carbonate or tertiary amine bases such as triethylamine, *N*-methylmorpholine, *N*-methylpiperidine, *N,N*-diisopropylethylamine, pyridine or 4-*N,N*-dimethylaminopyridine.

Within the context of the present invention, the preferred activation/condensation agents for such coupling reactions include *N*-(3-dimethylaminoisopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) in combination with 1-hydroxybenzotriazole (HOBt) and *N,N*-diisopropylethylamine or

O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) likewise in combination with *N,N*-diisopropylethylamine.

The coupling reactions (IX) + (X) → (II-C), (XII-A) and/or (XII-B) + (X) → (II-D-A) and/or (II-D-B), (IX) + (XIII) → (XIV), (IX) + (XV) → (XVI) and (XXII) + (XXIII) → (II-D) are usually performed in a temperature range from -20°C to +60°C, preferably at 0°C to +40°C. The reactions may be performed under normal, elevated or reduced pressure (e.g., from 0.5 to 5 bar). It is customary to work under normal pressure.

The ester-forming reactions (IX) + (XVIII) → (XII) and (IX) + (XI-A) and/or (XI-B) → (XII-A) and/or (XII-B), (IX) + (XXIV) → (XXV) as well as (IX) + (XXI) → (XXII) take place like the amide coupling reactions described above. These reactions preferably take place in dichloromethane using *N*-(3-dimethylaminoisopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) and 4-dimethylaminopyridine at a temperature of +50°C to 100°C under normal pressure.

The functional groups optionally present in the compounds – such as amino, hydroxyl and carboxyl groups in particular – may also be present in a temporarily protected form in the process steps described above, if this is expedient or necessary. Such protective groups are introduced and removed according to conventional methods known in peptide chemistry (see, for example, T.W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*, Wiley, New York, 1999; M. Bodanszky and A. Bodanszky, *The Practice of Peptide Synthesis*, Springer Verlag, Berlin, 1984). In the presence of multiple protected groups, their re-release may optionally be performed simultaneously in a one-pot reaction or also in separate reaction steps.

The preferred amino protective groups PG¹ include *tert*-butoxycarbonyl (Boc), benzyloxycarbonyl (Z) or (9*H*-fluorene-9-ylmethoxy)carbonyl (Fmoc); *tert*-butyl or benzyl is preferably used as the protective group PG² for a hydroxyl or carboxyl function. A *tert*-butyl or *tert*-butoxycarbonyl group is usually split off by treatment with a strong acid such as hydrochloric acid, hydrobromic acid or trifluoroacetic acid in an inert solvent such as diethyl ether, 1,4-dioxane, dichloromethane or acetic acid. This reaction may optionally also take place without the addition of an inert solvent. In the case of benzyl or benzyloxycarbonyl as the protective group, such a protective group is preferably removed by hydrogenolysis in the presence of a suitable palladium catalyst such as, for example, palladium on activated carbon. The (9*H*-fluorene-9-ylmethoxy)carbonyl group is generally split off with the help of a secondary amine base such as diethylamine or piperidine.

The reaction (VI) → (II-A) takes place in a solvent that is inert under the reaction conditions, such as, for example, ethers, e.g., tetrahydrofuran, 1,4-dioxane, 1,2-dimethoxyethane or

bis-(2-methoxyethyl)ether, alcohols such as methanol, ethanol, isopropanol, n-butanol or *tert*-butanol or dipolar aprotic solvents such as acetone, methyl ethyl ketone, acetonitrile, ethyl acetate, pyridine, dimethyl sulfoxide (DMSO), *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), *N,N'*-dimethylpropylene urea (DMPU) or *N*-methylpyrrolidinone (NMP) or water. It is likewise possible to use mixtures of such solvents. A mixture of 1,4-dioxane and water is preferably used.

Suitable bases for the reaction (VI) $\rightarrow$ (II-A) include, for example, alkali carbonates such as potassium carbonate, sodium carbonate or lithium carbonate, alkali bicarbonates such as sodium or potassium bicarbonate or alkali alcoholates such as sodium methanolate, sodium ethanolate or potassium *tert*-butylate. Sodium bicarbonate is preferred.

The reaction (VI) $\rightarrow$ (II-A) takes place in a temperature range from 0°C to +50°C, preferably at +10°C to +30°C. The reaction may be performed under normal, elevated or reduced pressure (e.g., from 0.5 to 5 bar). It is customary to work under normal pressure.

The reaction (VI) + (VII) $\rightarrow$ (II-B) takes place in a solvent that is inert under the reaction conditions such as, for example, ethers, e.g., tetrahydrofuran, 1,4-dioxane, 1,2-dimethoxyethane or bis-(2-methoxyethyl) ether, alcohols such as methanol, ethanol, isopropanol, *n*-butanol or *tert*-butanol or dipolar aprotic solvents like acetone, methyl ethyl ketone, acetonitrile, ethyl acetate, pyridine, dimethyl sulfoxide (DMSO), *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), *N,N'*-dimethylpropylene urea (DMPU) or *N*-methylpyrrolidinone (NMP) or water. It is also possible to use mixtures of such solvents. DMF is preferred.

Suitable bases for the reaction (VI) + (VII) $\rightarrow$ (II-B) include, for example, tertiary amine bases such as triethylamine, *N*-methylmorpholine, *N*-methylpiperidine, *N,N*-diisopropylethylamine, pyridine or 4-*N,N*-dimethylaminopyridine. *N,N*-Diisopropylethylamine is preferred.

The reaction (VI) + (VII) $\rightarrow$ (II-B) takes place in a temperature range from 0°C to +50°C, preferably at +10°C to +30°C. The reaction may take place under normal, elevated or reduced pressure (e.g., from 0.5 to 5 bar). It is customary to work under normal pressure.

The reactions (IX) $\rightarrow$ (III-A), (XIV) $\rightarrow$ (III-B) and (XVI) $\rightarrow$ (III-C) as well as (VI) + (XVII) $\rightarrow$ (III-D), (XIX) + (XX) $\rightarrow$ (III-E) and (XXV) + (XX) $\rightarrow$ (III-F) take place in a solvent that is inert under the reaction conditions. Suitable solvents include, for example, ethers such as diethyl ether, diisopropyl ether, *tert*-butylmethyl ether, tetrahydrofuran, 1,4-dioxane, 1,2-dimethoxyethane or bis-(2-methoxyethyl)ether, hydrocarbons such as benzene, toluene, xylene, pentane, hexane, heptane, cyclohexane or petroleum fractions, halohydrocarbons such as dichloromethane,

trichloromethane, tetrachloromethane, 1,2-dichloroethane, trichloroethylene or chlorobenzene or dipolar aprotic solvents such as acetone, methyl ethyl ketone, acetonitrile, ethyl acetate, pyridine, dimethyl sulfoxide (DMSO), *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), *N,N'*-dimethylpropylene urea (DMPU) or *N*-methylpyrrolidinone (NMP). It is likewise possible
5 to use mixtures of such solvents. *N,N*-Dimethylformamide is preferred.

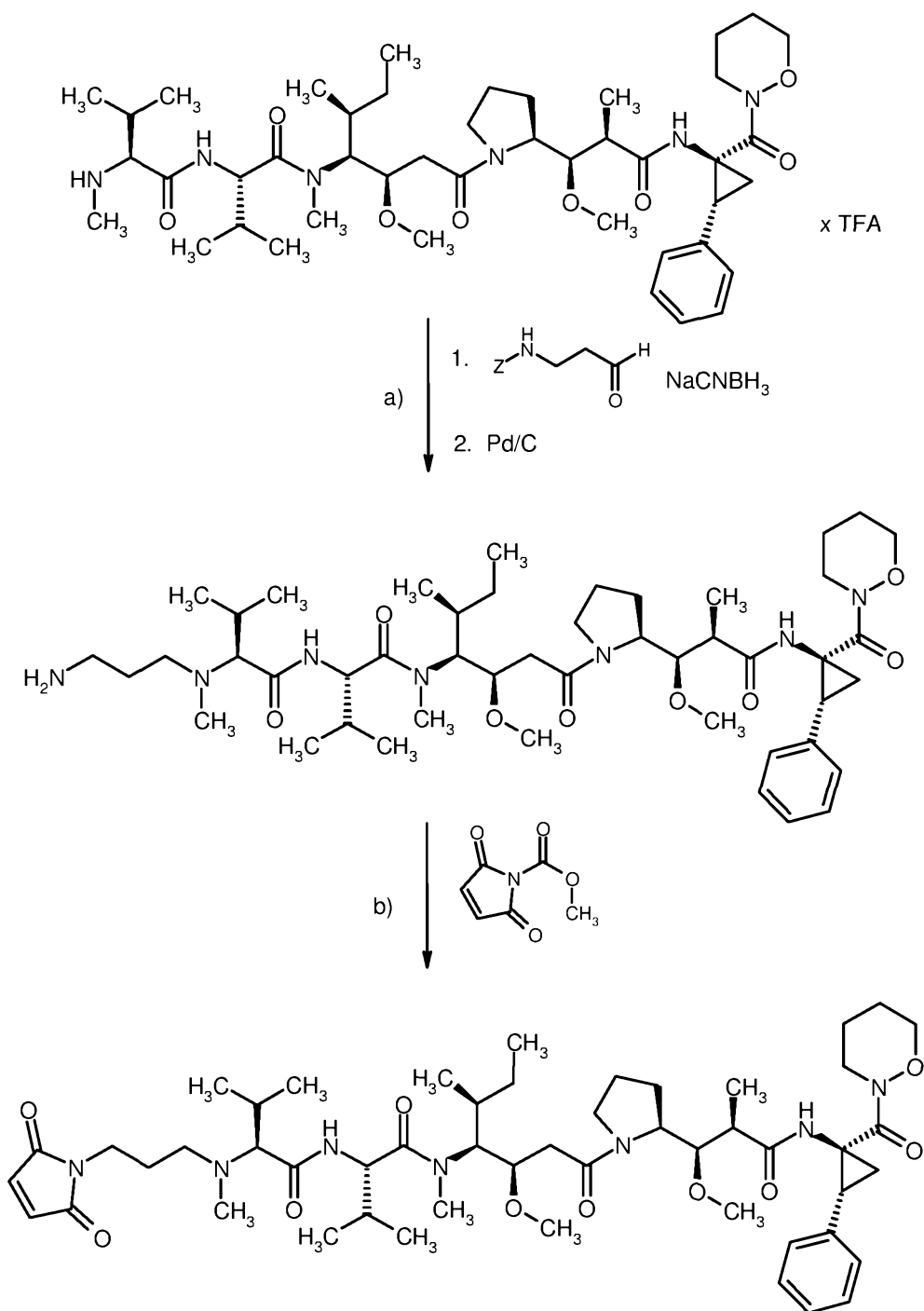
Suitable bases for these reactions include, for example, tertiary amines like triethylamine, *N*-methyldmorpholine, *N*-methylpiperidine, *N,N*-diisopropylethylamine, pyridine or 4-*N,N*-dimethylaminopyridine. *N,N*-Diisopropylethylamine is preferred, optionally with the addition of 4-*N,N*-dimethylaminopyridine.

10 The reactions (IX) $\rightarrow$ (III-A), (XIV) $\rightarrow$ (III-B) and (XVI) $\rightarrow$ (III-C) as well as (VI) + (XVII) $\rightarrow$ (III-D) and (XIX) + (XX) $\rightarrow$ (III-E) take place in a temperature range from 0°C to +50°C, preferably at +10°C to +30°C. The reaction may be carried out under normal, elevated or reduced pressure (e.g., from 0.5 to 5 bar). It is customary to work under normal pressure.

The compounds of formulas (II), (III), (I-A) and/or (I-B) are subsets of the compounds of
15 formulas (IIa), (IIIa), (Ia-A) and/or (Ia-B), where R³⁵ stands for methyl. Compounds (IIa) and (IIIa) are synthesized as in the synthesis of the compound of formula (II) and (III) as described above.

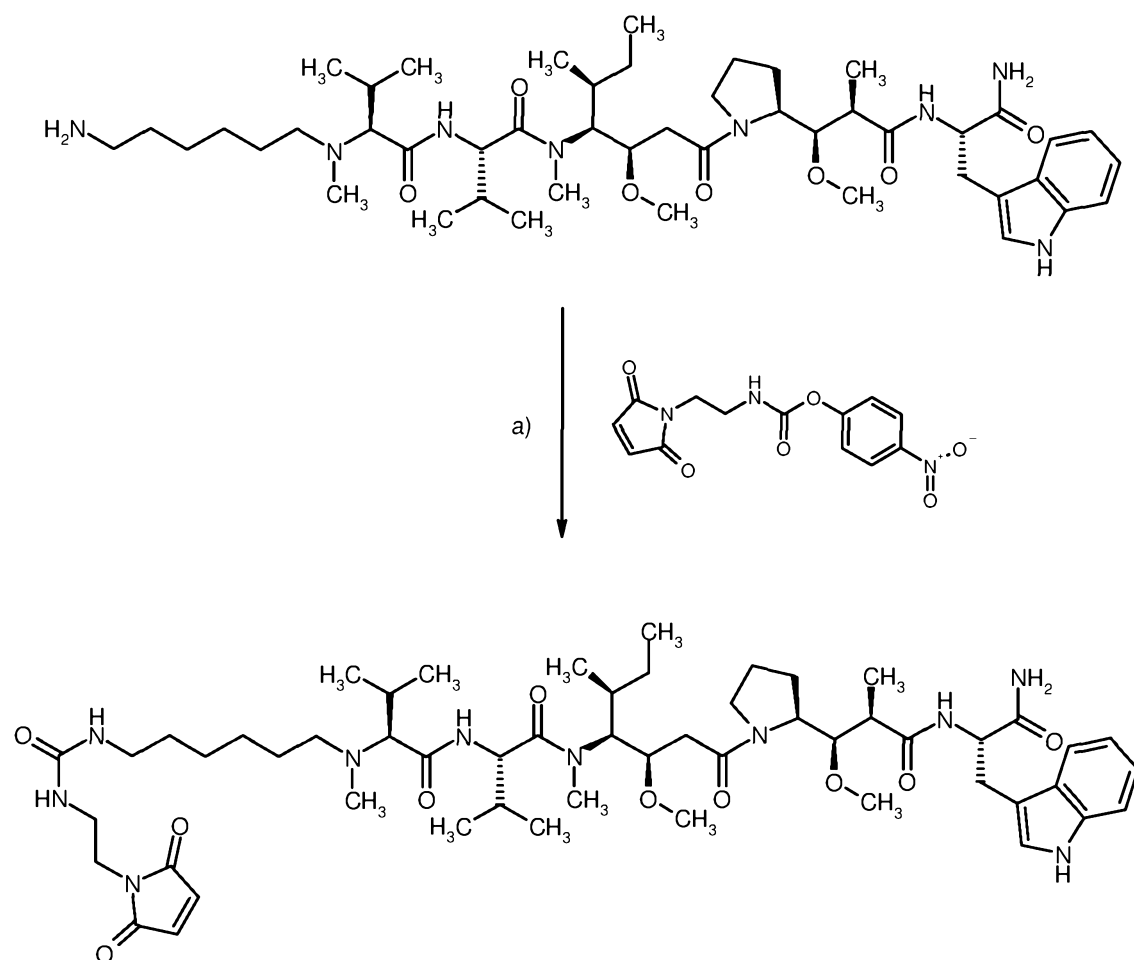
The methods described above are illustrated by the following synthesis schemes (schemes 3 through 13, 18) as examples:

Scheme 3

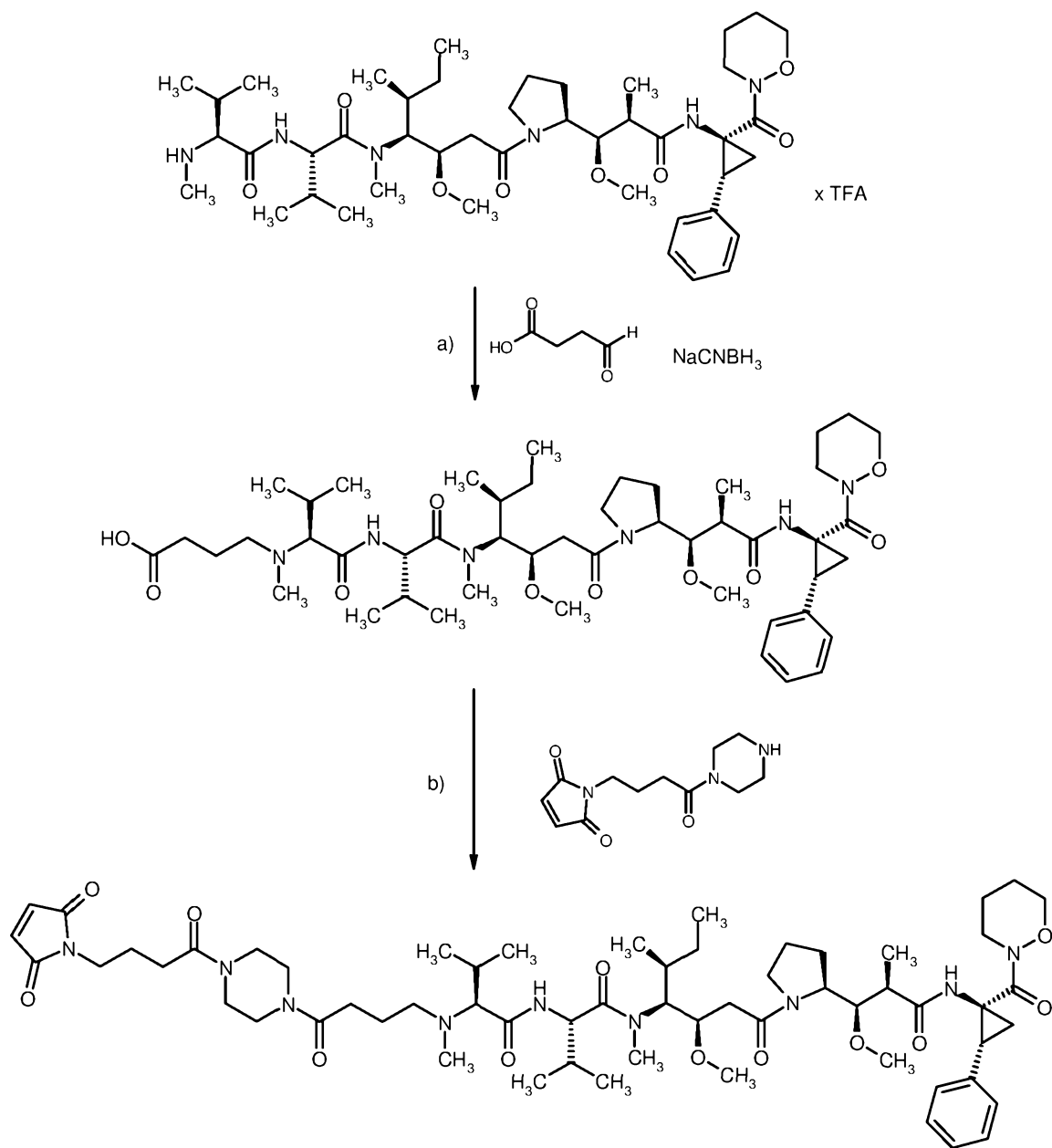


[a): 1. Water/dioxane, 1N HCl, 100°C; 2. H_2 , Pd/C, methanol, RT; b): $NaHCO_3$, H_2O , dioxane, RT].

Scheme 4



Scheme 5



[a): Water/dioxane, 1N HCl, 100°C; b): HATU, diisopropylamine, DMF, RT].

Chemical reaction scheme showing the synthesis of compound 10 from a starting material.

Starting Material: A complex peptide derivative with a carboxylic acid group.

Reaction a):

1. $\text{Boc-NH-CH(CH}_3\text{)-CH}_2\text{-OH}$ (with Bn-O-CO- group)
2. Pd/C

Intermediate 9a: The starting material reacts with the above reagents to form intermediate 9a, which contains a $\text{Boc-NH-CH(CH}_3\text{)-CH}_2\text{-O-CO-}$ group.

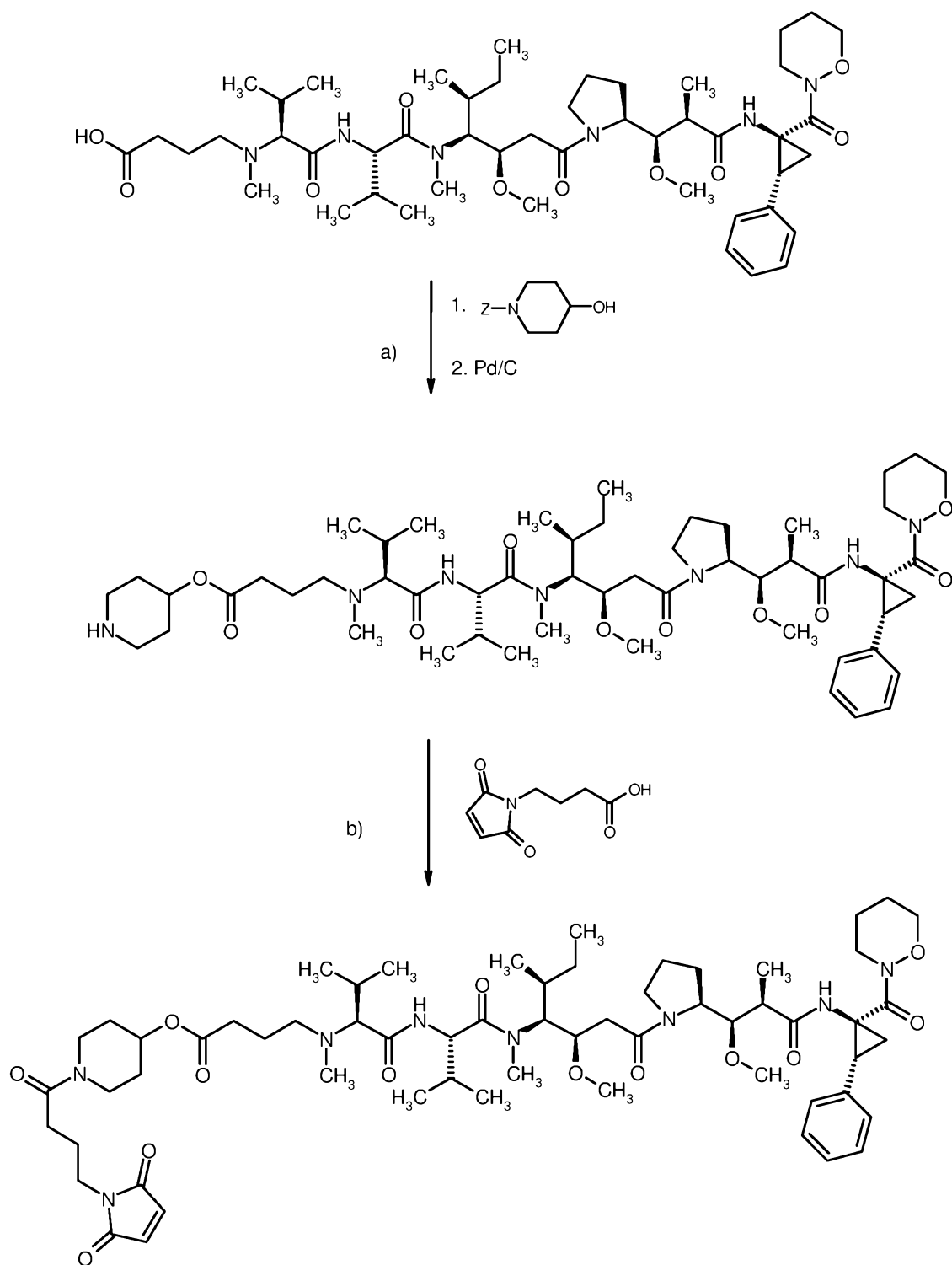
Reaction b):

1. $\text{6-(2,5-dioxo-2,5-dihydro-1H-pyridin-1-yl)hexan-1-ylhydrazide} \cdot \text{x HCl}$
2. TFA

Product 10: The intermediate 9a reacts with the above reagents to form the final product 10, which contains a $\text{6-(2,5-dioxo-2,5-dihydro-1H-pyridin-1-yl)hexan-1-ylhydrazide}$ group.

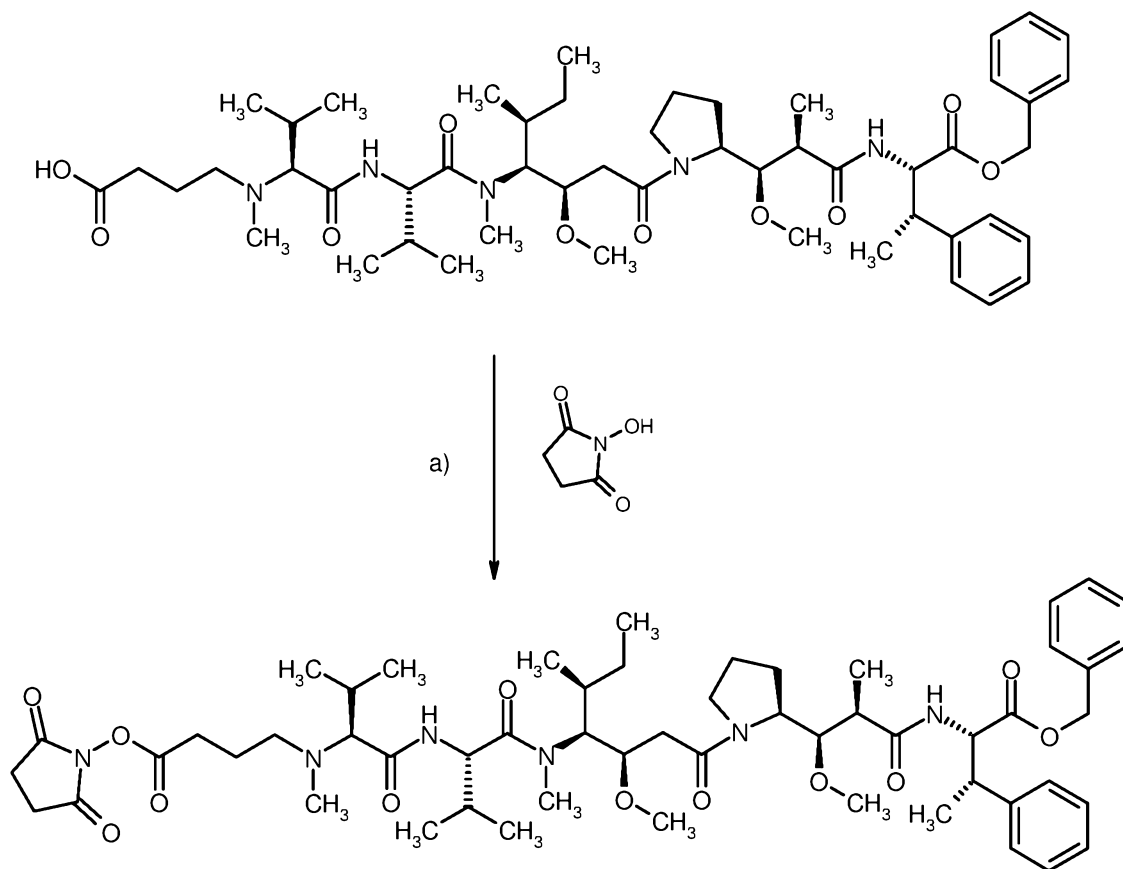
[a): 1. EDCI, DMAP, dichloromethane, RT; 2. H₂, methanol, RT ; b): 1. EDCI, HOBT, diisopropylamine, DMF, RT; 2. dichloromethane, RT].

Scheme 7



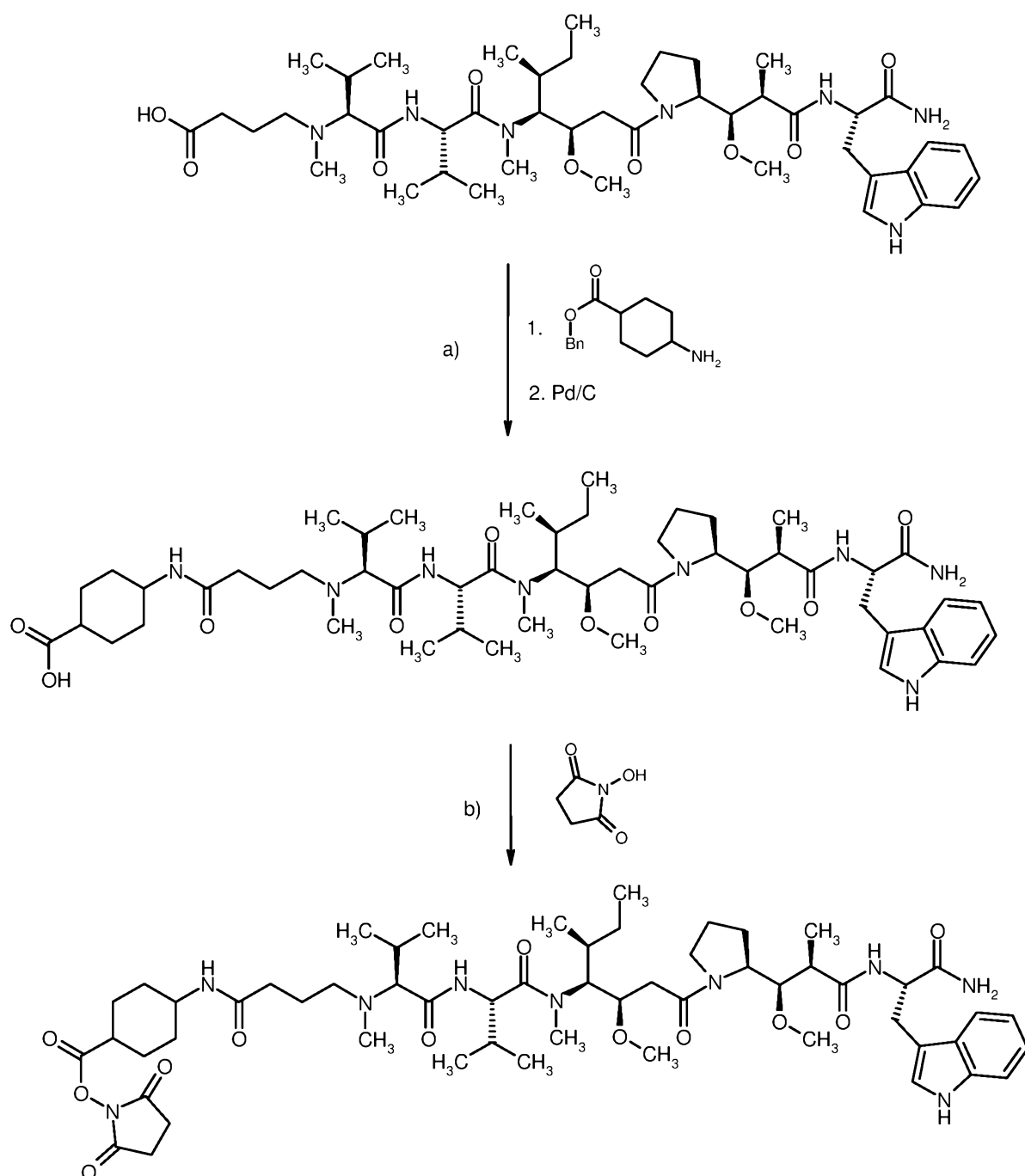
[a): 1. EDCI, DMAP, dichloromethane, RT; 2. H₂, methanol, RT ; b): HATU, diisopropylamine, DMF, RT].

Scheme 8



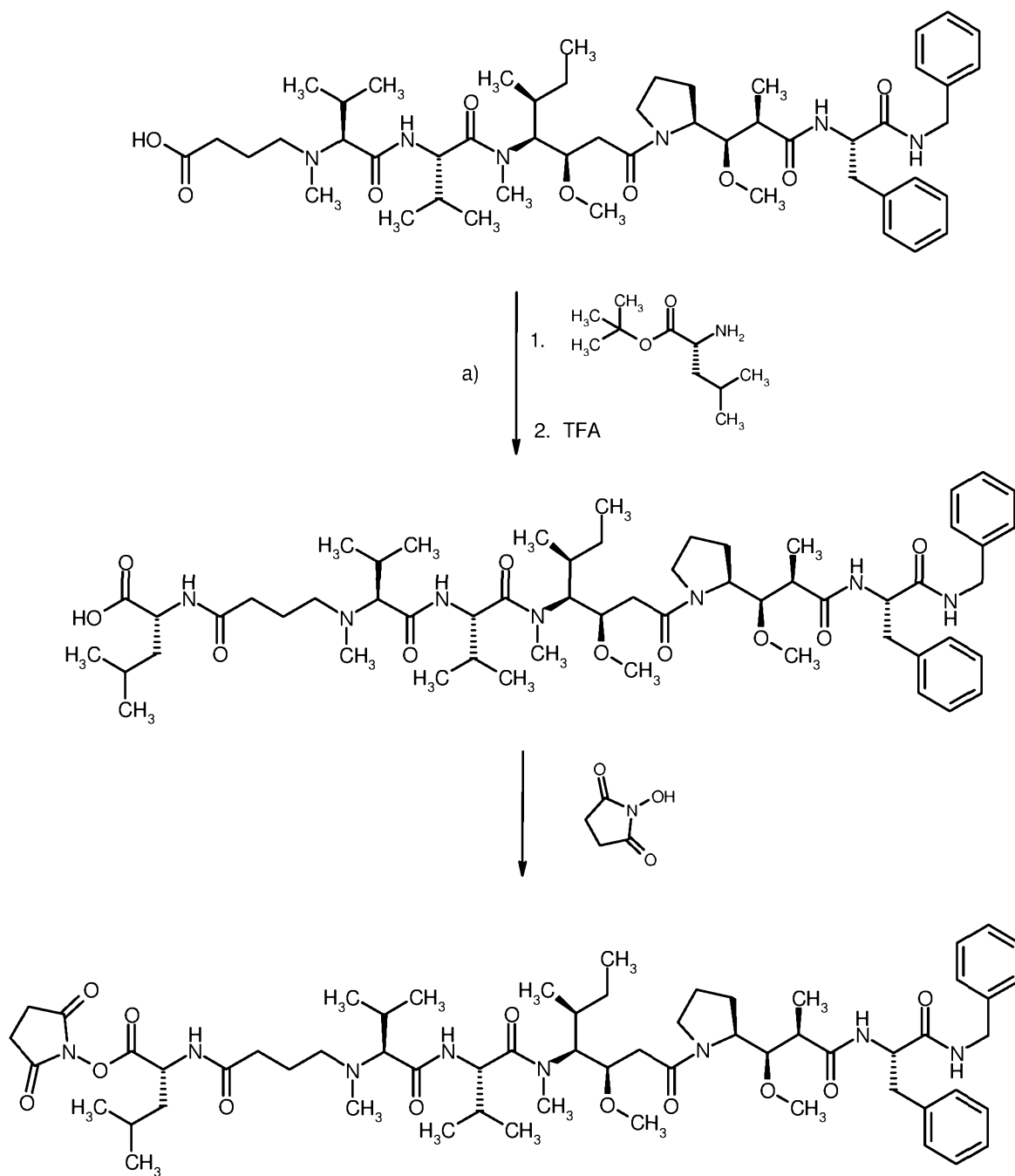
[a): EDCI, dichloromethane, RT].

Scheme 9



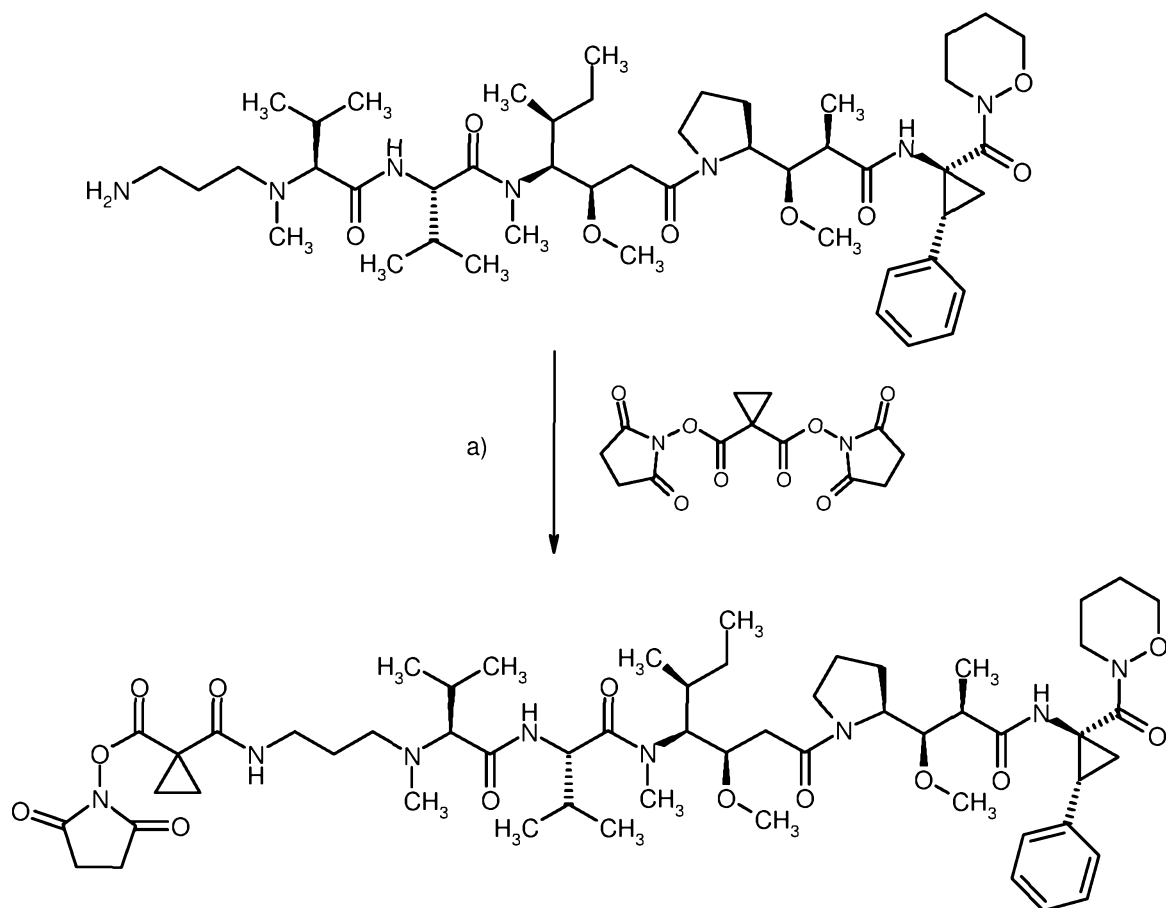
[a): HATU, diisopropylethylamine, DMF, RT; 2. H₂, methanol, RT; b): EDCI, DMAP, dichloro-
5 methane, RT].

Scheme 10



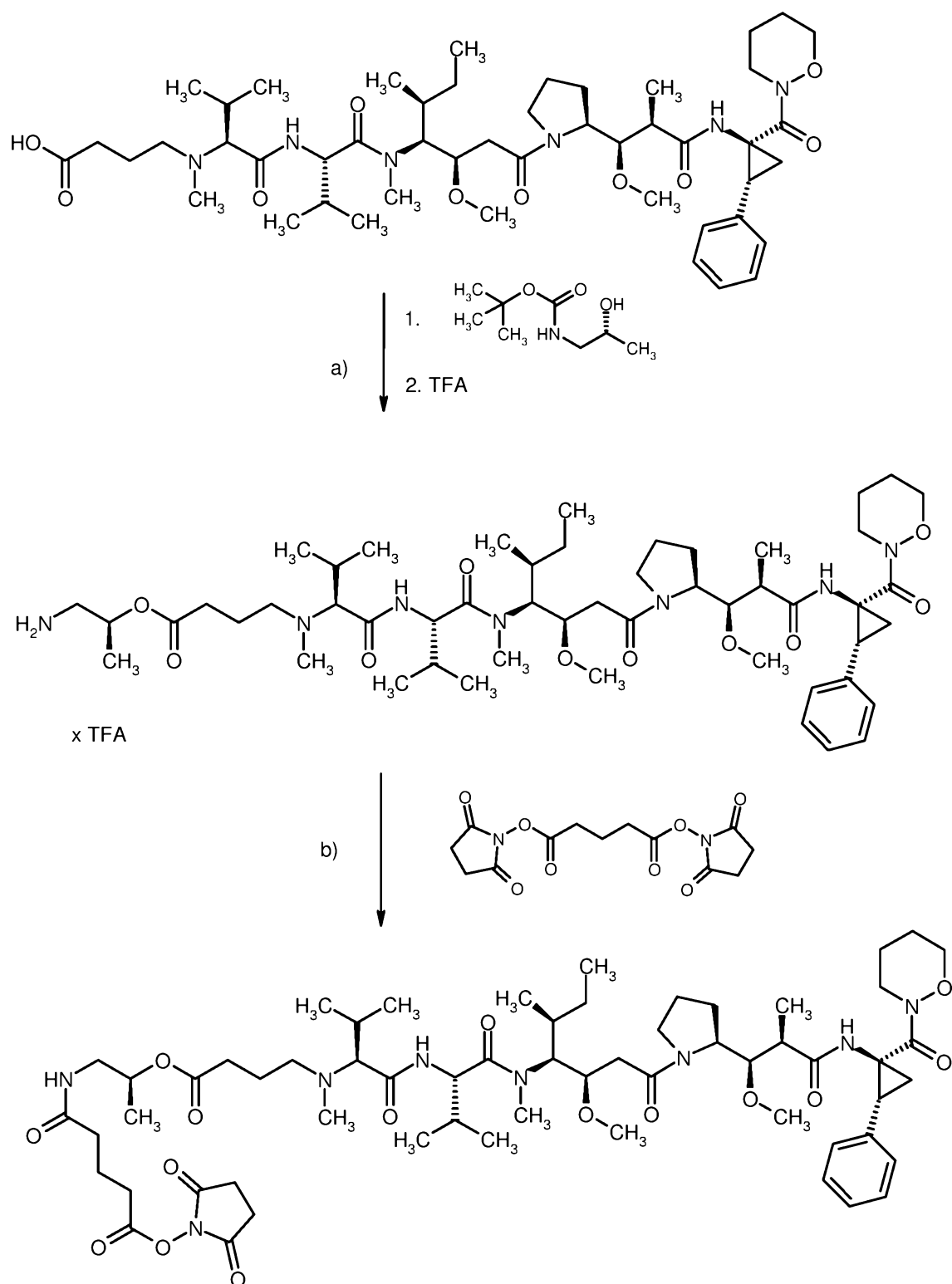
[a): 1. HATU, diisopropylethylamine, DMF, RT; 2. dichloromethane, RT; b): EDCI, DMAP, dichloromethane, RT].

Scheme 11



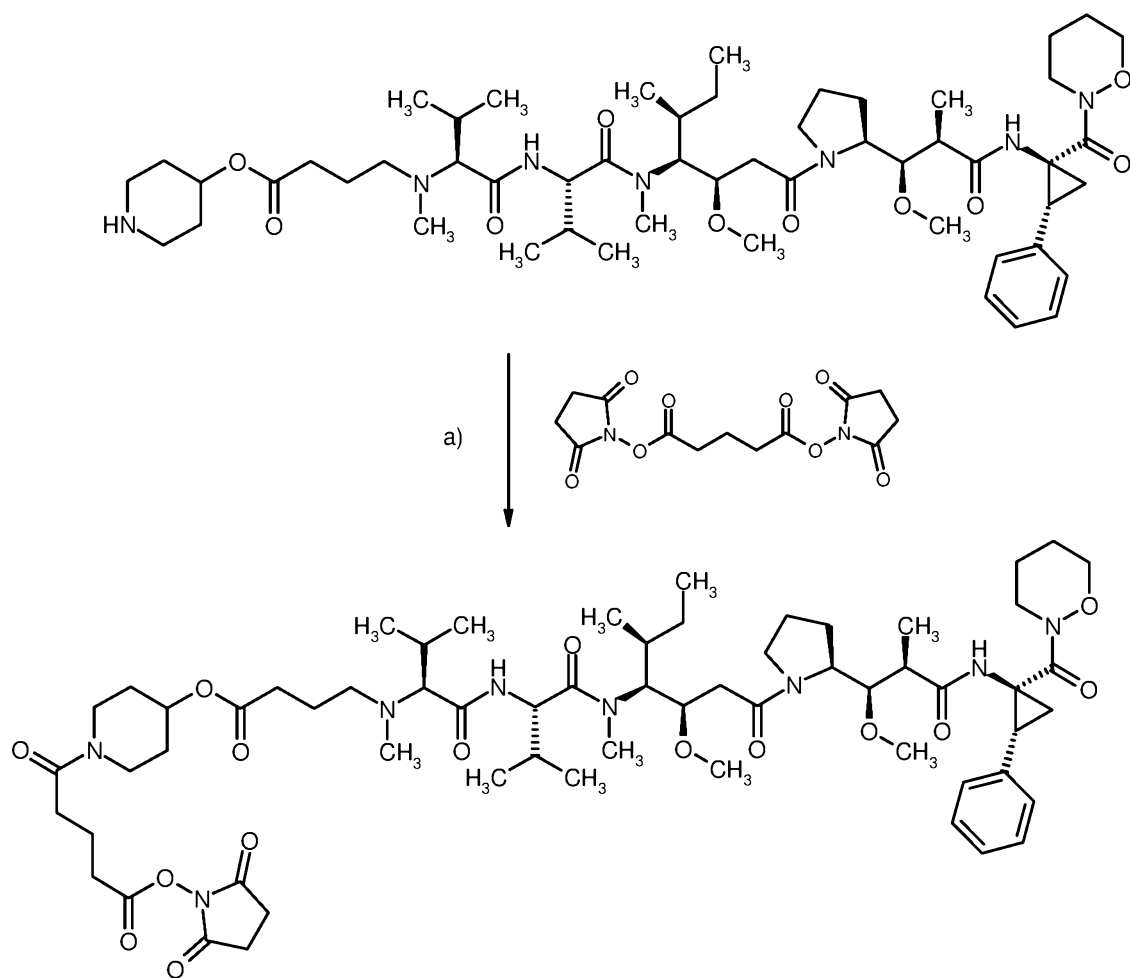
[a): Diisopropylethylamine, DMF, RT].

Scheme 12



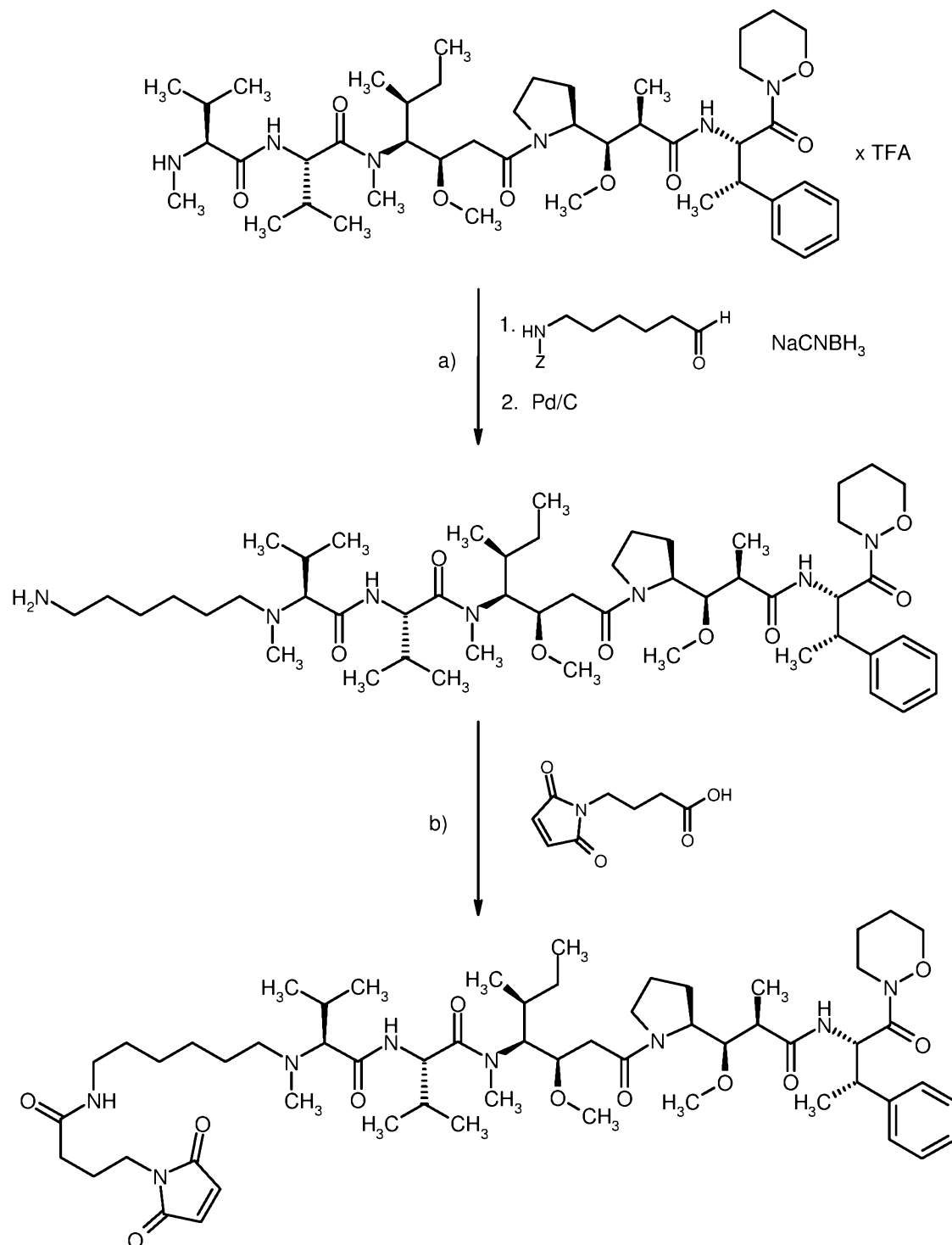
[a): 1. EDCI, DMAP, dichloromethane, RT; 2. Dichloromethane, RT ; b): Diisopropylamine, DMAP, dichloromethane, RT].

Scheme 13



[a): DMAP, diisopropylamine, dichloromethane, RT].

Scheme 18

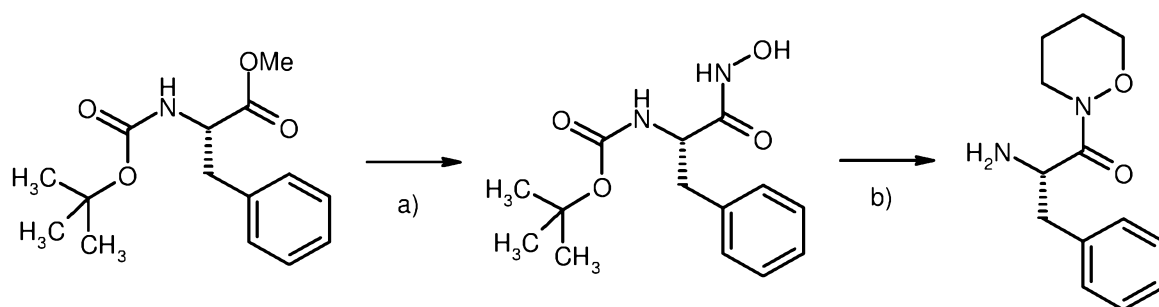


[a): 1. Water/dioxane, 1N HCl, 100°C; 2. H₂, Pd/C, methanol, RT; b): HATU, diisopropylethylamine, RT].

- 5 The compounds of formula (IV) may be synthesized from commercially available amino acid building blocks or those known from the literature (see, for example, Pettit et al., *Synthesis* 1996,

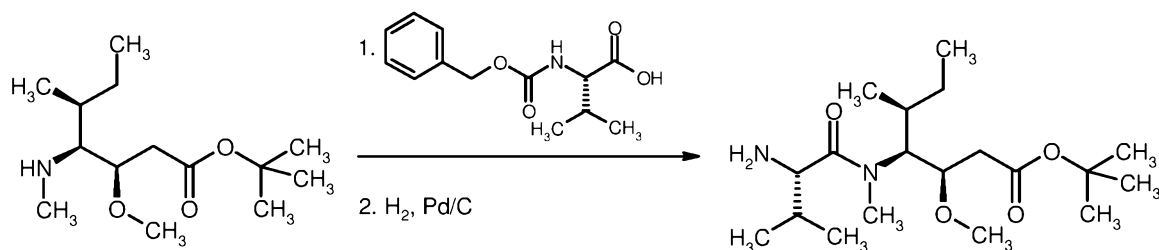
719; Shioiri et al., *Tetrahedron Lett.* 1991, 32, 931; Shioiri et al., *Tetrahedron* 1993, 49, 1913; Koga et al., *Tetrahedron Lett.* 1991, 32, 2395; Vidal et al., *Tetrahedron* 2004, 60, 9715; Poncet et al., *Tetrahedron* 1994, 50, 5345. Pettit et al., *J. Org. Chem.* 1994, 59, 1796) as with the processes known from the literature, by using the standard methods of peptide chemistry and as described in the present experimental part. The following synthesis schemes (schemes 14 through 16) illustrate this synthesis process as an example.

Scheme 14



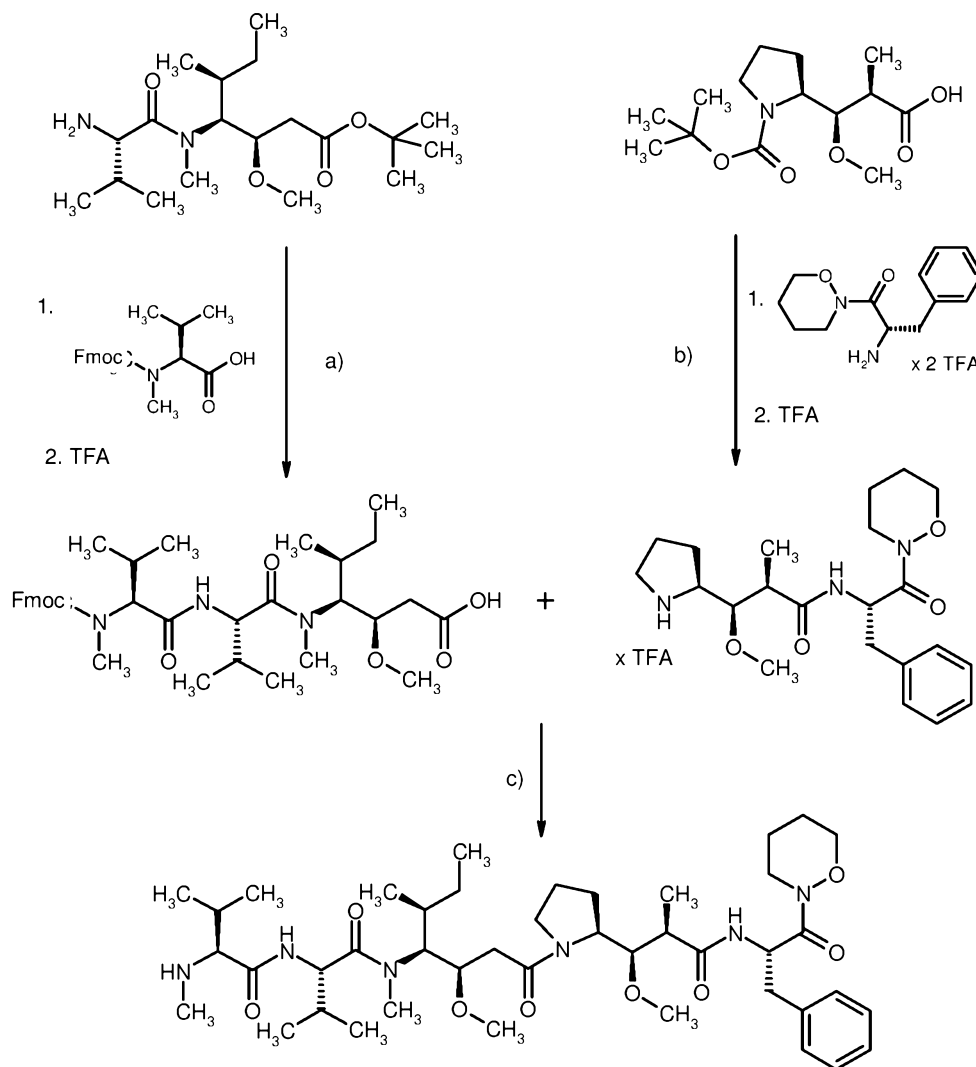
10 [a): Hydroxylamine hydrochloride, KOH, MeOH, 0°C → RT; b): BrCH₂(CH₂)₂CH₂Br, K₂CO₃, acetone, reflux].

Scheme 15



15 [a): 1. Diisopropylethylamine, BEP, dichloromethane, -10°C → RT; 2. MeOH].

Scheme 16



- [a): 1. Diisopropylethylamine, BEP, DMF, RT; 2. Dichloromethane; b): 1. HATU, diisopropylethylamine, DMF, RT; 2. Dichloromethane, RT; c): 1. HATU, diisopropylethylamine, DMF, RT; 2. Piperidine, DMF, RT].

The compounds of formulas (XI), (XIII), (XV), (XVII) and (XXI), including where applicable chiral or diastereomeric forms thereof are commercially available or have been described as such in the literature or can be synthesized by methods like those published in the literature in a manner that would be self-evident to those skilled in the art. Several detailed publications and specifications in the literature regarding the synthesis of the starting materials can also be found in the experimental part in the section for synthesis of the starting compounds and intermediates.

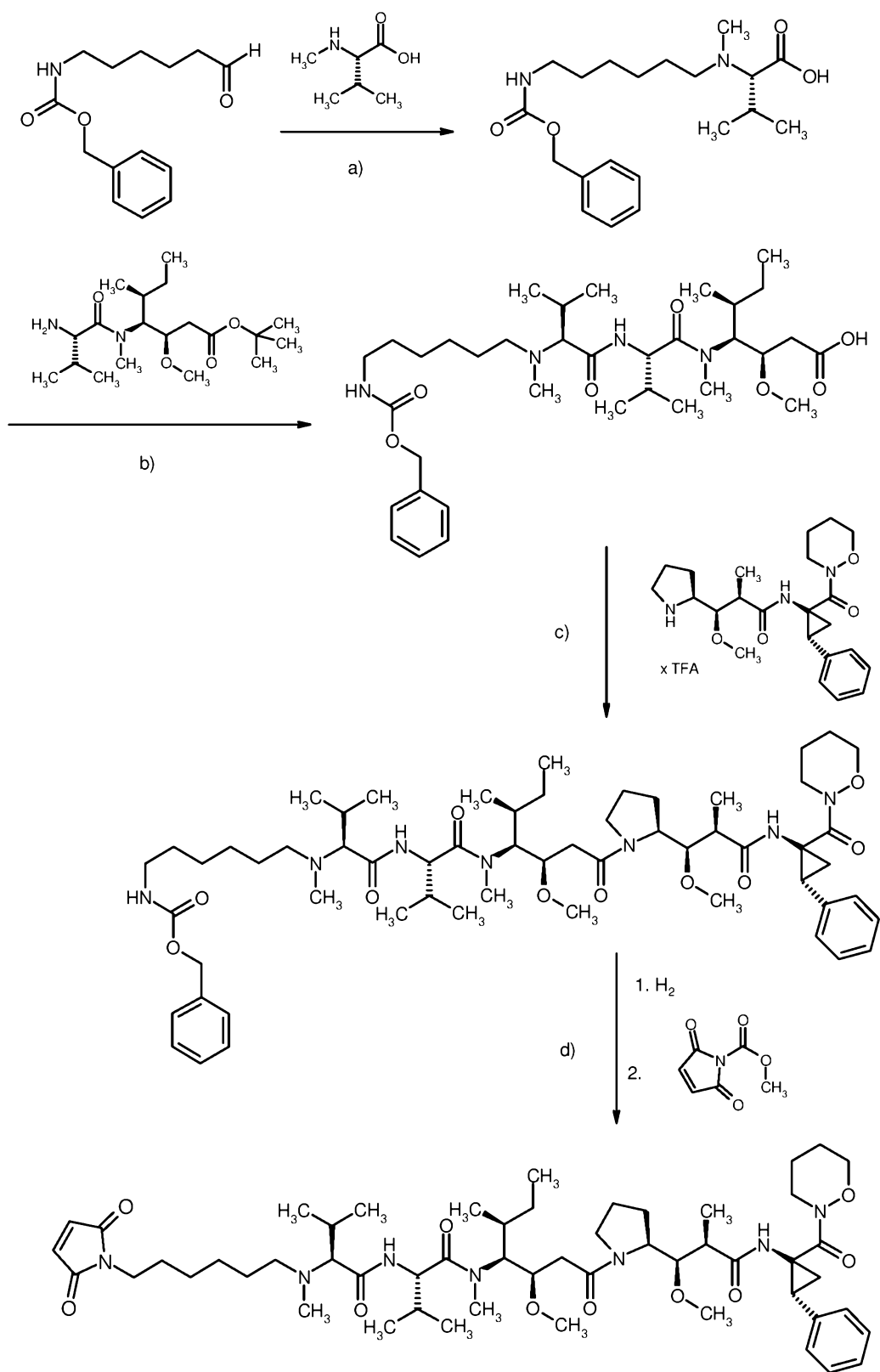
The compounds of formulas (V), (VII), (VIII), (X), (XVIII), (XX) and (XXIII) including where appropriate chiral or diastereomeric forms thereof are known in the literature or they can be synthesized by methods like those described in the literature in a manner obvious to those skilled

in the art. Numerous detailed specifications as well as references from the literature regarding the synthesis of the starting materials can be found in the experimental part in the section on synthesis of the starting compounds and intermediates.

Alternatively individually steps of the synthesis sequence may be performed in a different order.

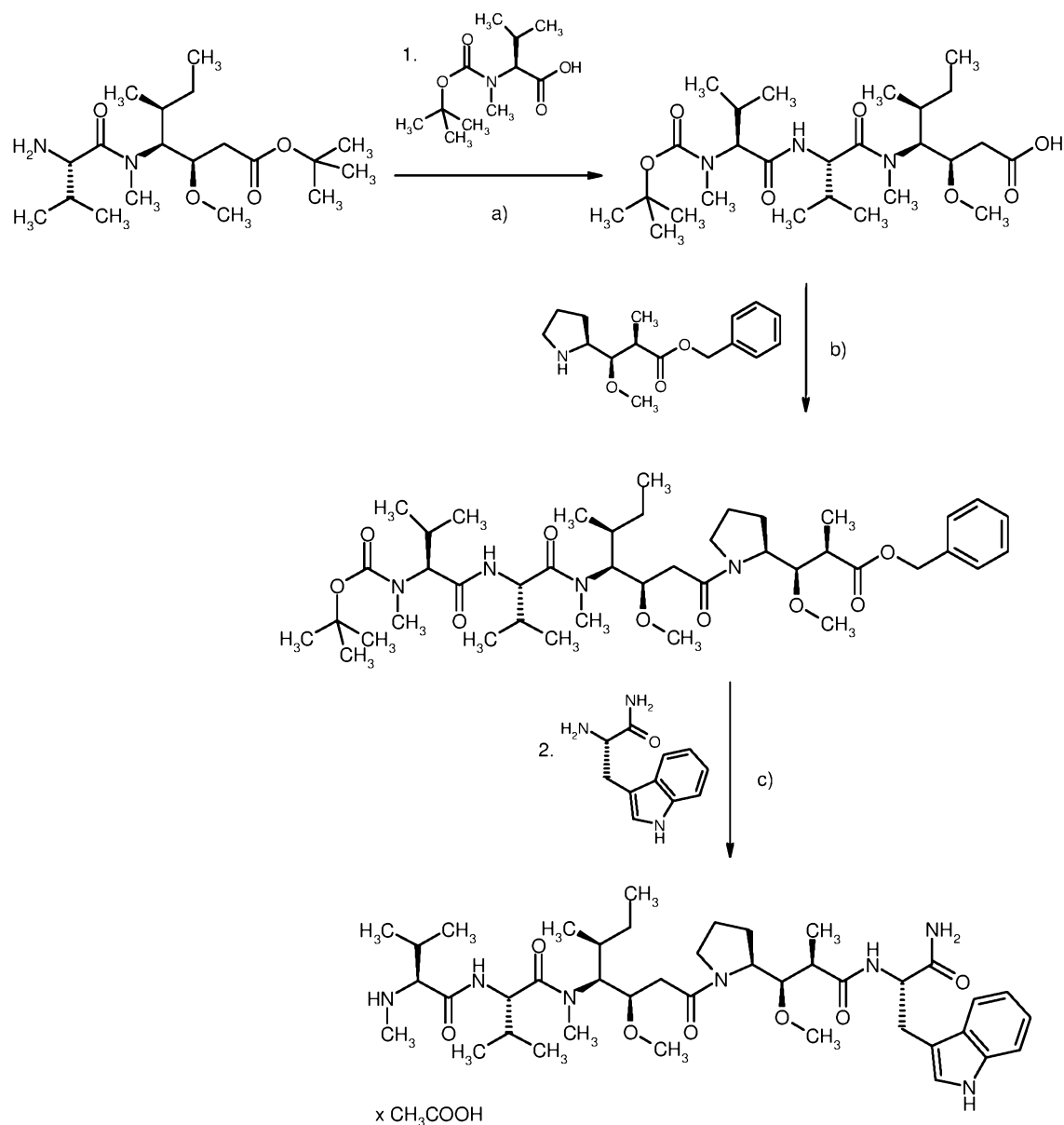
- 5 This procedure is illustrated in the following synthesis schemes (schemes 17, 19 and 20) as an example.

Scheme 17



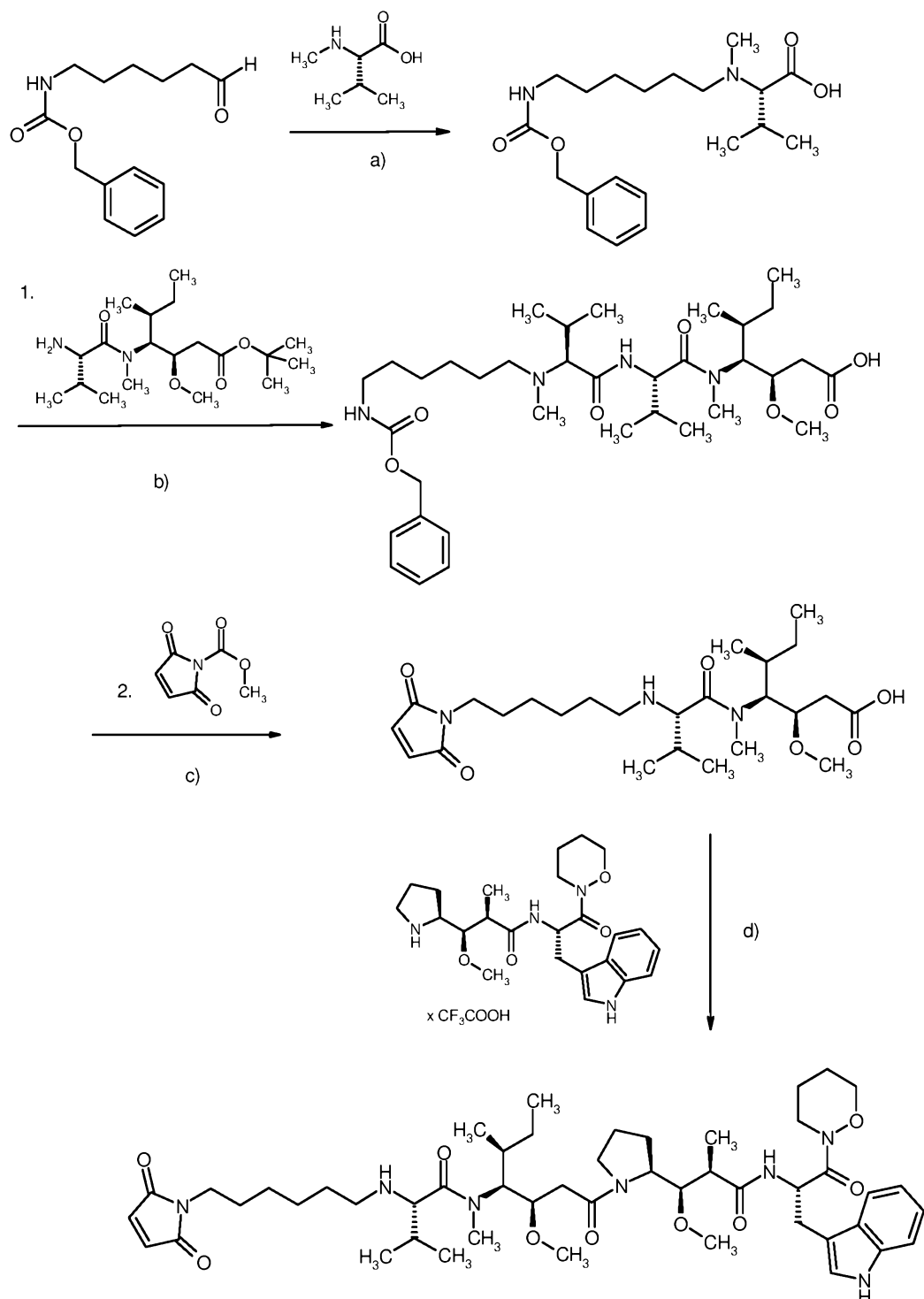
[a): Borane-pyridine complex, acetic acid, MeOH; b): 1. HOBt, EDCI, diisopropylethylamine, DMF, RT; 2. TFA, dichloromethane, RT; c): HATU, diisopropylethylamine, DMF, RT; d)1. Pd/C, MeOH, RT; 2. NaHCO₃, dioxane, water].

Scheme 19



[a): 1. HATU, diisopropylethylamine, DMF, RT; 2. TFA, dichloromethane, RT ; 3. ((H₃C)₃. C(C=O))₂O, DMF, diisopropylethylamine; b): diisopropylethylamine, BEP, DMF, RT; c): 1. H₂, Pd/C (10%). methanol, RT ; 2. HATU, diisopropylethylamine, DMF, RT; 3. TFA, dichloromethane, RT].

Scheme 20



[a): Borane-pyridine complex, acetic acid, MeOH; b): 1. HOBt, EDCI, diisopropylethylamine, DMF, RT; 2. TFA, dichloromethane, RT; c): 1. H₂, Pd/C, MeOH, RT; 2. NaHCO₃, dioxane, water; d): HATU, diisopropylethylamine, DMF, RT].

In one embodiment, the binder is bound to a target molecule that is present on a cancer cell. In a preferred embodiment, the binder binds to a cancer target molecule.

In another preferred embodiment, the target molecule is a selected cancer target molecule.

In an especially preferred embodiment, the target molecule is a protein.

- 5 In one embodiment, the target molecule is an extracellular target molecule. In a preferred embodiment, the extracellular target molecule is a protein.

Cancer target molecules are known to those skilled in the art. Examples of these are given below.

Examples of cancer target molecules include:

(1) EGF receptor (NCBI reference sequence NP_005219.2)

- 10 Sequence (1210 amino acids):

>gil29725609|reflNP_005219.2| epidermal growth factor receptor isoform a precursor
[*Homo sapiens*]

MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLSLQR
MFNNCEVVLGNLEITYVQRNYDLSFLKTIQEVAGYVLIALNTVERIPLNLQIIRG
15 NMYYENSYALAVLSNYDANKTGLKELPMRNLQEILHGAVRFSNNPALCNVESI
QWRDIVSSDFLSNMSMDFQNHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICA
QQCSGRRCRGKSPSDCCHNOCAAGCTGPRESDECLVCRKFRDEATCKDTCPLML
YNPTTYQMDVNPEGKYSFGATCVKKCPRNYVVTDHGSCVRACGADSYEMEED
GVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFR
20 GDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGR TKQH
GQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTK
IISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLE
GEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAG
VMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCP TNGPKIPSIATGMV
25 GALLLLL VVALGIGLFMRRRHIVRKRTLRRLLQERELVEPLTPSGEAPNQALLRIL
KETEFKKIKVLGSGAFGT VYKGLWIPEGEKV KIPVAIKELREATSPKANKEILDE
AYVMASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLDYVREHKDNIGSQYLLN
WCVQIAKGMNYLED RRLVHRDLAARNVLVKTPQHVKITDFGLAKLLGAEKEE
YHAEGGKVPKWMALLESILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIPASE
30 ISSILEKGERLPQPPICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYL
VIQGDERMHLPSP TDSNFYRALMDEEDMDDVVD ADEYLIPQQGFFSSPSTSRTPL

LSSLSATSNNSTVACIDRNLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPE
YINQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVGNPEYLNTVQPT
CVNSTFDSPAHWAAQKGSHQISLDNPDYQQDFFPKEAKPNGIFKGSTAENAEYLR
VAPQSSEFIGA

- 5 The extracellular domain is underlined for emphasis.

(2) Mesothelin (SwissProt reference Q13421-3)

Sequence (622 amino acids):

>sp|Q13421-3|MSLN_HUMAN isoform 2 of mesothelin OS=*Homo sapiens* GN=MSLN

10 MALPTARPLLGSCGTPALGSLFLFSLGWVQPSRTLGETGQEAAPLDGVLAN
PPNISS
LSRQLLGFPCAEVSGLSTERVRELAVALAQKNVKLSTEQLRCLAHRLSEPPEDL
DALPL
DLLLFLNPDAFSGPQACTRFFSRITKANVDLLPRGAPERQRLPAALACWGVGRS
LLSEA
15 DVRALGGLACDLPGRFVAESA EVLLPRLVSCPGPLDQDQQAARAALQGGGPP
YGGPSTW
SVSTMDALRGLLPVLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPETILRPRFRE
VEKT
ACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLDVLKH
20 KLDELY
PQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETALKALLEVNKGHEMSPQVATLI
DRFVK
GRGQLDKDTLDTLTAFYPGYLCSLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDV
LYPKA
25 RLA FQNMNGSEYFVKIQSFLGGAPTEDLKALSQQNVSM DLATFMKLRTDAVLP
LTVAEVQ
KLLGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLSM
QEALSGT
PCLLGPGPVLTVLALLLASTLA

- 30 where mesothelin is coded by amino acids 296-598. Amino acids 37-286 code for "megakaryocyte potentiating factor." Mesothelin is anchored in the cell membrane by a GPI anchor and is localized extracellularly.

(3) Carboanhydrase IX (SwissProt reference Q16790)

Sequence (459 amino acids):

>sp|Q16790|CAH9_HUMAN Carbonic anhydrase 9 OS=*Homo sapiens* GN=CA9 PE=1
SV=2

5 MAPLCSPWLPLLIPAPAPGLTVQLLLSLLLVPVHPQRLPRMQEDSPLGGGSSG
 EDDPL
 GEEDLPSEEDSPREEDPPGEEDLPGEEDLPGEEDLPEVKPKSEEEGSLKLEDLPTV
 EAPG
 DPQEPQONNAHRDKEGDDQSHWRYGGDPPWPRVSPACAGRFQSPVDIRPQLAAF
 10 CPALRPL
 ELLGFQLPPLPELRLRNNGHSVQLTLPPGLEMALGPGREYRALQLHLHWGAAGR
 PGSEHT
 VEGHRFP AEIHVVHLSTAFARVDEALGRPGLAVLAAFLEEGPEENSAYEQLLS
 RLEEIA
 15 EEGSETQVPGLDISALLPSDFSR YFQYEGSLTTPPCAQGVIVTVFNQTVMLSAKQ
 LHTLS
 DTLWGP GDSRLQLNFRATQPLNGRVIEASFPAGVDSSPRAAEPVQLNSCLAAGDI
 LALVF
 GLLFAVTSVAFLVQMRRQHRRGTKGGVS YRPAEVAETGA

20 The extracellular domain is underlined for emphasis.

(4) C4.4a (NCBI reference sequence NP_055215.2; synonym LYPD3)

Sequence (346 amino acids):

>gil93004088|ref|NP_055215.2| ly6/PLAUR domain-containing protein 3 precursor
[*Homo sapiens*]

25 MDPARKAGAQAMIWTAGWLLLLLLRGGAQALECYSCVQKADDGCSPNKMKT
 VKCAPGVDVCTEAVGAVETIHGQFSLAVRGCGSGLPGKNDRGLDLHGLLAFIQL
 QQCAQDRCNAKLNLTSRALDPAGNESAYPPNGVECYSCVGLSREACQGTSPPV
 VSCYNASDHVYKGCDFGNVTLTAANVTVSLPVRGCVQDEFCTRDGVTGP GFTL
 SGSCCQGSRCNSDLRNKTYFSPRIPLVRLPPPEPTTVASTTSVTTST SAPVRPTST
 30 TKPMPAPTSQTPRQGV EHEASRDEEPRLTGGAAGHQDRSNSGQYPAKGGPQQP
 HNKGCVAPTAGLAALLVAAGVLL

The matured extracellular domain is underlined for emphasis (SEQ ID NO: 1).

(5) CD52 (NCBI reference sequence NP_001794.2)

>gil68342030|reflNP_001794.2| CAMPATH-1 antigen precursor [*Homo sapiens*]

5 MKRFLFLLLTISLLVMVQIQTGLSGQNDTSQTSSPSASSNISGGIFLFFVANAIHHLF
CFS

(6) HER2 (NCBI reference sequence NP_004439.2)

>gil54792096|reflNP_004439.2| receptor tyrosine-protein kinase erbB-2 isoform a
[*Homo sapiens*]

10 MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMRLHLYQGC
QVVQGNLELTYLPTNASLSFLQDIQEVQGYVLIHNPVRQVPLQRLRIVRGTLQF
EDNYALAVLDNGDPLNNTTPVTGASPGGLRELQRLSLTEILKGGVLIQRNPQLC
YQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGESSEDCQSLT
RTVCAGGCARCKGPLPTDCHEQCAAGCTGPKHSDCLACLFHNSGICELHCPA
15 LVTYNTDTFESMPNPEGRYTFGASCVTACPYNLSTDVGSCTLCPLHNQEVTA
EDGTQRCEKCSKPCARVCYGLGMEHLREVRVTSANIQEFAGCKKIFGSLAFLP
ESFDGDPASNTAPLQPEQLQVFETLEEITGYLYISAWPDSLPLDSVFQNLQVIRGR
ILHNGAYSLTLQGLGISWLGLRSLRELGSGLALIHNNHLCFVHTVPWDQLFRNP
HQALLHTANRPEDECVGEGLACHQLCARGHCWGPGPTQCVNCSQFLRGQECVE
ECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPF
20 CVARCPSGVKPDLSPYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGCPAEQRAS
PLTSIISAVVGILLVVVLGVVFGILIKRRQQKIRKYTMRRLLQETELVEPLTPSGA
MPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRENT
SPKANKEILDEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLMPYGCLLDHVREN
RGRLLGSQDLLNWCMIQAKGMSYLEDVRLVHRDLAARNVLVKSPNHVKITDFGL
25 ARLLDIDETEHADGGKVPIKWMALLESILRRRFTHQSDVWSYGVTVWELMTFG
AKPYDGIPAREIPDLLEKGERLPQPPICTIDVYMIMVKCWMIDSECRPRFRELVSE
FSRMARDPQRFVVIQNEGLGPASPLDSTFYRSLLEDDDMGDLVDAEEYLVPQQG
FFCPDPAPGAGGMVHHRHRSSTRSGGGDLTLGLEPSEEEAPRSPLAPSEGAGSD
VFDGDLGMGAAGLQSLPTHDPSPQLQRYSEDPTVPLPSETDGYVAPLTCSPQPE
30 YVNQPDVRPQPPSPREGPLPAARPAGATLERPKTLSPGKNGVVKDVFAFGGAVE
NPEYLTPQGGGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPE
YLGLDVPV

(7) CD20 (NCBI reference sequence NP_068769.2)

>gil23110987|reflNP_068769.2| B-lymphocyte antigen CD20 [*Homo sapiens*]

MTTPRNSVNGTFPAEPMKGPIAMQSGPKPLFRRMSSLVGPTQSFFMRESKTLGA
VQIMNGLFHIALGGLLMIPAGIYAPICVTWYPLWGGIMYIISGSLLAATEKNSR
5 KCLVKGKMIMNSLSLFAAISGMILSIMDILNIKISHFLKMESLNFIRAHTPYINIYN
CEPANPSEKNSPSTQYCYSIQSLFLGILSVMLIFAFFQELVIAGIVENEWKRTCSRP
KSNIVLLSAEEKKEQTIEIKEEVVGLTETSSQPKNEEDIEIPIQEEEEETETNFPEP
PQDQESSPIENDSSP

(8) The lymphocyte activating antigen CD30 (SwissProt ID P28908)

10 >gil68348711|reflNP_001234.2| tumor necrosis factor receptor superfamily member 8
isoform 1 precursor [*Homo sapiens*]

MRVLLAALGLLFLGALRAFPQDRPFEDTCHGNPSHYDCAVRRCCYRCPMGLF
PTQQCPQRPTDCRKQCEPDYYLDEADRCTACVTCRDDLVEKTPCAWNSSRVC
ECRPGMFCSTSAVNSCARCFHVSVPAGMIVKFPGTAQKNTVCEPASPGVSPAC
15 ASPENCKEPSSGTIPQAKPTPVSPATSSASTMPVRGGTRLAQEAASKLTRAPDSPS
SVGRPSSDPGLSPTQPCPEGSGDCRKQCEPDYYLDEAGRCTACVSCSRDDLVEK
TPCAWNSSRTCECRPGMICATSATNSRARCVPYPICAAETVTKPQDMAEKDTTF
EAPPLGTQPD CNTPENGEAPASTSPTQSLLVDSQASKTLPIPTSAPVALSSTGKP
VLDAGPVLFWVILVLVVVVGSSAFLCHRRACRKRIKQLHLCYPVQTSQPKLE
20 LVDSRPRRSSTQLRSGASVTEPVAEERGLMSQPLMETCHSVGAAYLESPLQDA
SPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVGTVKAELPEGRGLAGPA
EPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPTAASGK

(9) The lymphocyte adhesion molecule CD22 (SwissProt ID P20273)

25 >gil157168355|reflNP_001762.2| B-cell receptor CD22 isoform 1 precursor [*Homo sapiens*]

MHLLGPWLLLLVLEYLAFSDSSKWVFEHPETLYAWEGACVWIPCTYRALDGD
ESFILFHNPEYNKNTSKFDGTRL YESTKD GKVPSEQKR VQFLGDKNKNCTLSIHP
VHLNDSGQLGLRMESKTEKWMERIHNVSERFPFPHIQLPPEIQESQEVTLTCLL
NFSCYGYPIQLQWLLEGVPMRQAAVTSTSLTIKSVFTRSELKFSPQWSHHGKIVT
30 CQLQDADGKFLSNDTVQLNVKHTPKLEIKVTPSDAIVREGDSVTMTCEVSSSNP
EYTTVSWLKDGTSLKKQNTFTLNLREVTKDQSGKYCCQVSNDVGPGRSEEVFL

QVQYAPEPSTVQILHSPA VEGSQVEFLCMSLANPLPTNYTWYHNGKEMQGRTE
 EKVHIPKILPWHAGTYSCVAENILGTGQRGPGAELDVQYPPKKVTTVIQNPMPPIR
 EGDVTLSHCNYNSSNPSVTRYEWKPHGAWEEPSLGV LKIQNVGWDNTTIACAA
 CNSWCSWASPVALNVQYAPRDVVRVKIKPLSEIHSGNSVSLQCDFSSSHPKQEVQ
 5 FFWEKNGRLLGKESQLNFD SISPEDAGSYSCWVNNSIGQTASKAWTLEVLYAPR
 RLRVSMSPGDQVMEGKSATLTCESDANPPVSHYTWFDWNNQSLPYHSQKLRLE
 PVKVQHSGAYWCQGTNSVGKGRSPLSTLTVYYSPETIGRRVAVGLGSC LAILILA
 ICGLKLQRRWKRTQSQQGLQENSSGQSFFVRNKKVRRAPLSEGPLHSLGCYNPM
 MEDGISYTTLRFPENIPRTGDAESSEMQRPPDCDDTVTYSALHQRQVGDYEN
 10 VIPDFPEDEGIHYSELIQFGVGERPQAQENV D YVILKH

(10) The myeloid cell surface antigen CD33 (SwissProt ID **P20138**)

>gil130979981|reflNP_001763.3| myeloid cell surface antigen CD33 isoform 1 precursor
[*Homo sapiens*]

MPLLLLLPLLWAGALAMDPNFWLQVQESVTVQEGLCVLPCTFFHPIPYDKN S
 15 PVHGYWFREGAIISRDS PVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDAR
 RRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRPKILIPGTLEPGH SKNL TCS
 VSWACEQGTPIFSWLSAAPTSLGPRTHSSVLIITPRPDHGTNLTCQVKFAGA
 GVTTERTIQLNVTYVPQNPTTGIFPGDGS GKQETRAGVVHGAIGGAGVTALLAL
 CLCLIFFIVKTHRRKAARTAVGRNDTHPTTGSASPKHQKKSKLHGPTETSSCSGA
 20 APTVEMDEELHYASLNFHGMNPSKDTSTEYSEVRTQ

(11) The transmembrane glycoprotein NMB (SwissProt ID Q14956)

>gil52694752|reflNP_001005340.1| transmembrane glycoprotein NMB isoform a
precursor [*Homo sapiens*]

MECLYYFLGFLLLAARLPLDAAKRFHDVLGNERPSAYMREHNQLNGWSSDEND
 25 WNEKLYPVWKRGMRWKNSWKGGRVQAVLTSDSPALVGSNITFAVNLI FPRC
 QKEDANGNIVYEKNCRNEAGLSADPYVYNWTAWSESDSGENGTGQSHHN VFP
 DGKPFPHHPGWRRWNFIYVFHTLGQYFQKLGRCSVRVSVNTANVT LGPQLMEV
 TVYRRHGRAYVP I AQVKDVYVVT DQIPVFVTMFQKNDRNSSDETFLKDL PIMFD
 VLIHDP SHFLNYSTINYKWSFGDNTGLFVSTNHTVNHTYVLNGTFSLNLTVKAA
 30 APGPCPPPPPPRPSKPTPSLATTLKSYDSNTPGPAGDNPLELSRIPDENCQINRYG
 HFQATITIVEGILEVNIIQMTDVLMPVPWPPESSLIDFVVTCQGSIPTEVCTIISDPTC
 EITQNTVCSPVDVDEMCLLTVRRTFNGSGTYCVNLTLGDDTSLALTSTLISVPDR

DPASPLRMANSALISVGCLAIFVTVISLLVYKKHKEYNPIENSPGNVVRSGKLSVF
LNRAKAVFFPGNQEKDPLLKNQEFKGV

(12) The adhesion molecule CD56 (SwissProt ID P13591)

5 >gil94420689|reflNP_000606.3| neural cell adhesion molecule 1 isoform 1 [*Homo sapiens*]

MLQTKDLIWTLFFLGTA VSLQVDIVPSQGEISVGESKFFLCQVAGDAKDKDISWF
SPNGEKLTPNQQRISVVWNDDSSSTLTIYNANIDDA GIYKCVVTGEDGSESEATV
NVKIFQKLMFKNAPTPQEFREGEDAVIVCDVSSLPPTIIWKHKGRDVILKKDVR
FIVLSNNYLQIRGIKKTDEGTYRCEGRILARGEINFKDIQVIVNVPPTIQRQNIVN
10 ATANLGQSVTLVCDAEGFPEPTMSWTKDGEQIEQEEDDEKYIFSDDSSQLTIKKV
DKNDEAEYICIAENKAGEQDATIHLKVFAKPKITYVENQTAMELEEQVTLTCEA
SGDPIPSITWRTSTRNISSEKTLDGHMVVRSHARVSSLTLKSIQYTDAGEYICTA
SNTIGQDSQSMYLEVQYAPKLQGPVAVYTWEGN

QVNITCEVFAYPSATISWFRDGQLLPSSNYSNIKIYNTPSASYLEVTPDSEND FGN
15 YNCTAVNRIGQESLEFILVQADTPSSPSIDQVEPYSSSTAQVQFDEPEATGGVPILK
YKAEWRAVGEEVWHSKWYDAKEASMEGIVTIVGLKPETTYAVRLAALNGKGL
GEISAASEFKTQPVQGEPSAPKLEGQMGEDGNSIKVNLIKQDDGGSPIRHYLVRY
RALSSSEWKPEIRLP SGSDHVMLKSLDWNAEYEVYVVAENQQGKSAAHFVVRT
SAQPTAIPANGSPTSGLSTGAIVGILIVIFVLLL VVDITCYFLNKCGLFMCIAVNL
20 CGKAGPGAKGKDMEEGKA AFSKDESKEPIVEVRTEEERTPNHDGGKHTEPNETT
PLTEPEKGPVEAKPECQETETKPAPAEVKTVPN DATQTKENESKA

(13) The surface molecule CD70 (SwissProt ID P32970)

>gil4507605|reflNP_001243.1| CD70 antigen [*Homo sapiens*]
MPEEGSGCSVRRRPYGCVLRAALVPLVAGLVICLVVCIQRFAQAQQQLPLESLG
25 WDVAELQLNHTGPQQDPRLYWQGGPALGRSFLHGP ELDKGQLRIHRDGIYMVH
IQVTLAICSSTTASRHHPTTLAVGICSPASRSISLLRLSFHQGCTIASQRLTPLARGD
TLCTNLTGTLLPSRNTDETFFGVQWVRP

(14) The surface molecule CD74 (SwissProt ID P04233)

30 >gil10835071|reflNP_004346.1| HLA class II histocompatibility antigen gamma chain
isoform b [*Homo sapiens*]

MHRRRSRSCREDQKPVMDQDRDLISNNEQLPMLGRRPGAPESKCSRGALYTGF
ILVTLLLAGQATTAYFLYQQQGRDLKLTVTSQNLQLENLRMKLPKPPKPVSKMR
MATPLLMQALPMGALPQGPMQNATKYGNMTEDHVMHLLQNADPLKVYPPLK
GSFPENLRHLKNTMETIDWKVFESWMHHWLLFEMSRHSLEQKPTDAPPKESLEL
5 EDPSSGLGVTKQDLGPVPM

(15) The B-lymphocyte antigen CD19 (SwissProt ID P15391)

>gil296010921|reflNP_001171569.1| B-lymphocyte antigen CD19 isoform 1 precursor
[*Homo sapiens*]

MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSR
10 ESPLKPFLKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQP
GWTVNVEGSGELFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAK
DRPEIWEGEPPCLPPRDSL NQSLSQDLTMAPGSTLWLSGCVPPDSVSRGPLSWTH
VHPKGPKSLLSLELKDDRPARDMWV METGLLLPRATAQDAGKY YCHRGNLTM
SFHLEITARPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRK
15 RKRMTDPTRRRFFKVTPPPGSGPQNQYGNVLSLPTPTSGLGRAQRWAAGLGGTAP
SYGNPSSDVQADGALGSRSPPGVGPEEEEEGEGYEEPDSEEDSEFYENDSNLGQD
QLSQDGS GYENPEDEPLGPEDEDSFSNAESYENEDEELTQPVARTMDFLSPHGSA
WDPSREATSLAGSQSYEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENMDNPD
GPDPAWGGGGGRMG TWSTR

20 (16) The surface protein mucin 1 (SwissProt ID P15941)

>gil65301117|reflNP_002447.4| mucin-1 isoform 1 precursor [*Homo sapiens*]

MTPGTQSPFFLLLLLLTVLTVVTGSGHASSTPGGEKETSATQRSSVPSSTEKNALST
GVSFFFLSFHISNLQFNSSLED PSTDY YQELQRDISEMFLQIYKQGGFLGLSNIKFR
PGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISDVS VSDVPFPFSA
25 QSGAGVPGWGIALLVLCVLVALAIVYLI ALAVCQCRRKNYGLDIFPARDTYH
PMSEYPTYHTHGRYVPPSSTD RSPYEKVSAGNGGSSLSYTNPAVAATSANL

(17) The surface protein CD138 (SwissProt ID P18827)

>gil29568086|reflNP_002988.3| syndecan-1 precursor [*Homo sapiens*]

MRRAALWLWLCALALSLQPALPQIVATNLPPEDQDGS GDDSDNFSGSGAGALQ
30 DITLSQQTPSTWKDTQLLTA IPTSPEPTGLEATAASTSTLPAGEGPKEGEAVVLPE
VEPGLTAREQEATPRPRETTQLPTTHQASTTTATTAQEPATSHPHRDMQPGHHE

TSTPAGPSQADLHTPHTEDGGPSATERAAEDGASSQLPAAEGSGEQDFTFETSGE
 NTAVVAVEPDRRNQSPVDQGATGASQGLLDRKEVLGGVIAGGLVGLIFAVCLV
 GFMLYRMKKKDEGSYSLEEPKQANGGAYQKPTKQEEFYA

(18) Integrin alphaV (GenBank Accession No. NP_002201.1)

5 >gil4504763|reflNP_002201.1| integrin alpha-V isoform 1 precursor [*Homo sapiens*]
 MAFPPRRRLRLGPRGLPLLLSGLLLPLCRAFNLVDSPA EYSGPEG SYFGFAVDF
 FVPSASSRMFLLVGAPKANTTQPGIVEGGQVLKCDWSSTRRCQPIEFDATGNRD
 YAKDDPLEFKSHQWFGASVRSKQDKILACAPLYHWRTEMKQEREPVGT CFLQD
 GTKTVEYAPCRSQDIDADGQGFCQGGFSIDFTKADR VLLGGPGSFYWQQLISD
 10 QVAEIVSKYDPNVYSIKYNNQLATRTAQAI FDDSYLGYSVAVGDFNGDGIDDFV
 SGVPRAARTLGMVYIYD GKNMSSLYNFTGEQMAAYFGFSVAATDINGDDYAD
 VFIGAPLFMDRGSDGKLQEVGQVS VSLQRASGDFQTTKLNGFEVFARFGSAIAPL
 GDLDQDGFNDIAIAAPYGGEDKKGIVYIFNGRSTGLNAVPSQILEGQWAARSMP
 PSFGYSMKGATDIDKNGYPDLIVGAFGVDRAILYRARPVITVNAGLEVYPSILNQ
 15 DNKTCSLPGTALKVSCFNVR FCLKADGKGVLPRKLN FQVELLLDKLKQKGAIRR
 ALFLYSRSPSHSKNMTISR GGLMQCEELIAYLRDESEFRDKLTPITIFMEYRLDYR
 TAADTTGLQPILNQFTPANISRQA HILLDCGEDNVCKPKLEVSVDS DQKKIYIGD
 DNPLTLIVKAQNQGEGAYEAELIVSIPLQADFIGVVRNNEALARLS CAFKTENQT
 RQVVCDLGNPMKAGTQLLAGLRF SVHQSEMDTSVKFDLQIQSSNLFDK VSPV
 20 VSHKVDLAVLAAVEIRGVSSPDHIFLPIPNWEHKENPETEEDVGPV VQHIYELRN
 NGPSSFSKAMLHLQWPYKYNNNTLLYILHYDIDGPMNCTSDMEINPLRIKISSLQ
 TTEKNDTVAGQGERDHLITKRDLALSEGDIHTLGCGVAQCLKIVCQVGR LDRGK
 SAILYVKSLLTWTFMKNKENQNHSYSLKSSASFNVIEFPYKNLPIEDITNSTL VTT
 NVTWGIQPAPMPVPVWV IILAVLAGLLLLAVLVFVMYRMGFFKRV RPPQEEQE
 25 REQLQPHENGEGNSET

(19) The teratocarcinoma-derived growth factor 1 protein TDGF1 (GenBank Accession No.: NP_003203.1)

>gil4507425|reflNP_003203.1| teratocarcinoma-derived growth factor 1 isoform 1 precursor [*Homo sapiens*]
 30 MDCRKMARFSYSVIWIMAISKVFELGLVAGLGHQEFARPSRGYLA FRDDSIWPQ
 EEP AIRPRSSQRVPPMGIQHSKELNRTCCLNGGTCMLGSFCACPPSFYGRNCEHD

VRKENCGSVPHDTWLPKKCSLCKCWHGQLRCFPQAFLPGCDGLVMDEHLVAS
RTPELPPSARTTTFMLVGICLSIQSYY

(20) The prostate-specific membrane antigen PSMA (SwissProt ID: Q04609)

>gil4758398|reflNP_004467.1| glutamate carboxypeptidase 2 isoform 1 [*Homo sapiens*]

5 MWNLLHETDSAVATARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEATNITP
KHNMKAFDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKEFGLDSV
ELAHYDVLLSYPNKTHPNYISIINEDGNEIFNTSLFEPPPPGYENVSDIVPPFSAFSP
QGMPEGDLVYVNYARTEDFFKLERDMKINCSGKIVIARYGKVFRGNKVKNAQL
AGAKGVILYSDPADYFAPGVKSYPDGWNLPGGGVQGRNINLNGAGDPLTPGY
10 PANEYAYRRGIAEAVGLPSIPVHPIGYDAQKLEKMGGSAAPPDSSWRGSLKVP
YNVGPFGFTGNFSTQKVKMHIHSTNEVTRIYNVIGTLRGAVEPDYVILGGHRDS
WVFGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTILFASWDAEEFGLLGSTEW
AEENSRLQERGVAYINADSSIEGNYTLRVDCTPLMYSLVHNLTKEKSPDEGFE
GKSLYESWTKKSPSPEFSGMPRISKLGSGNDFEVFFQRLGIASGRARYTKNWETN
15 KFSGYPLYHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVFELANSIVLPFDCR
DYAVVLRKYADKIYSISMKHPQEMKTYSVSFDLSLFSVKNFTEIASKFSERLQDF
DKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGESFPGI
YDALFDIESKVDPSKAWGEVQRQIYVAAFTVQAAAETLSEVA

(21) Tyrosine protein kinase EPHA2 (SwissProt ID: P29317)

20 >gil3296731|reflNP_004422.2| ephrin type-A receptor 2 precursor [*Homo sapiens*]

MELQAARACFALLWGCALAAAAAAQGKEVVLLDFAAAGGELGWLTHPYGKG
WDLMQNIMNDMPIYMYSVCNVMSGDQDNWLRTNWWYRGEAERIFIELKFTVR
DCNSFPGGASSCKETFNLYYAESDLDYGTNFQKRLFTKIDTIAPDEITVSSDFEAR
HVKLNVEERSVGPLTRKGFYLAQDIGACVALLSVRVYYKKCPELLQGLAHFPE
25 TIAGSDAPSLATVAGTCVDHAVVPPGGEEPRMHCAVDGEWLVPICQLCQAGY
EKVEDACQACSPGFFKFEASESPCLECPEHTLPSPEGATSCCEEGFFRAPQDPAS
MPCTRPPSAPHYLTAVGMGAKVELRWTPPQDSGGREDIVYSVTCEQCWPESGE
CGPCEASVRYSEPPHGLTRTSVTVSDLEPHMNYTFTVEARNGVSGLVTSRSFRTA
SVSINQTEPPKVRLEGRSTTSLSVSWSIPPPQQSRVWKYEVTYRKKGDSNSYNVR
30 RTEGFSVTLDDLAPDTTYLVQVQALTQEGQGAGSKVHEFQTLSPEGSGNLAVIG
GVAVGVVLLLVLAVGVGFFIHRRRKNQRARQSPEDVYFSKSEQLKPLKTYVDPHT
YEDPNQAVLKFTTEIHPSCVTRQKVIGAGEFGEVYKGMLKTSSGKKEVPVAIKT

5 LKAGYTEKQRVDFLGEAGIMGQFSHHNIIRLEGVISKYKPMMIITEYMENGALD
KFLREKDGESVLQLVGMLRGIAAGMKYLANMNYVHRDLAARNILVNSNLVC
KVSDFGLSRVLEDDPEATYTTSGGKIPIRWTAPEAISYRKFTSASDVWSFGIVMW
EVMTYGERPYWELSNHEVMKAINDGFRLPTPMDCPSAIYQLMMQCWQQERAR
RPKFADIVSILDKLIRAPDSLKTLADFDPRVSIRLPSTSGSEGVPFRTVSEWLESIK
MQQYTEHFMAAGYTAIEKVVQMTNDDIKRIGVRLPGHQKRIAYSLLGLKDQVN
TVGIPI

(22) The surface protein SLC44A4 (GenBank Accession No. NP_001171515)

10 >gil295849282|reflNP_001171515.1| choline transporter-like protein 4 isoform 2 [*Homo sapiens*]

15 MGGKQRDEDEAYGKPVKYDPSFRGPIKNRSCTDVICCVLFLLFILGYIVVGIVA
WLYGDPRQVLYPRNSTGAYCGMGENKDKPYLLYFNIFSCILSSNIISVAENGLQC
PTPQTVITSLQQELCPSFLLPSAPALGRCFPWTNVTTPPALPGITNDTTIQQGISGLID
SLNARDISVKIFEDFAQSWYWILVALGVALVLSLLFILLRLVAGPLVLVLILGVL
GVLAYGIYYCWEEYRVLDRDKGASISQLGFTTNLSAYQSVQETWLAALIVLAVLE
AILLMLIFLRQIRIAIALLEASKAVGQMMSTMFYPLVTFVLLLCIAYWAMT
ALYLATSGQPQYVLWASNISPGCEKVPINTSCNPTAHLVNSSCPGLMCFVFGYS
SKGLIQRSVFNLQIYGVGLGLFWTLNWVLALGQCVLAGAFASFYWAFHKPQDIPT
FPLISAFIRTLRYHTGSLAFGALILTLVQIARVILEYIDHKLRGVQNPVARCIMCCF
20 KCCLWCLEKFIKFLNRNAYIMIAIYGKNFCVSAKNAFMLLMRNIVRVVLDKVT
DLLLFFGKLLVVGGVGVLSFFFFSGRIPGLGKDFKSPHLNYYWLPIMTSILGAYVI
ASGFFSVFGMCVDTLFLCFLEDLERNNGSLDRPYMSKSLLKILGKKNEAPPDN
KKRKK

(23) The surface protein BMPR1B (SwissProt: O00238)

25 (24) The transport protein SLC7A5 (SwissProt: Q01650)

(25) The epithelial antigen of the prostate STEAP1 (SwissProt: Q9UHE8)

(26) The ovarian carcinoma antigen MUC16 (SwissProt: Q8WXI7)

(27) The transport protein SLC34A2 (SwissProt: O95436)

(28) The surface protein SEMA5b (SwissProt: Q9P283)

30 (29) The surface protein LYPD1 (SwissProt: Q8N2G4)

- (30) The endothelin receptor type B EDNRB (SwissProt: P24530)
- (31) The ring finger protein RNF43 (SwissProt: Q68DV7)
- (32) The prostate carcinoma associated protein STEAP2 (SwissProt: Q8NFT2)
- (33) The cation channel TRPM4 (SwissProt: Q8TD43)
- 5 (34) The complement receptor CD21 (SwissProt: P20023)
- (35) The B-cell antigen receptor complex associated protein CD79b (SwissProt: P40259)
- (36) The cell adhesion antigen CEACAM6 (SwissProt: P40199)
- (37) The dipeptidase DPEP1 (SwissProt: P16444)
- (38) The interleukin receptor IL20Ralpha (SwissProt: Q9UHF4)
- 10 (39) The proteoglycan BCAN (SwissProt: Q96GW7)
- (40) The ephrine receptor EPHB2 (SwissProt: P29323)
- (41) The prostatic stem cell associated protein PSCA (GenBank Accession No. NP_005663.2)
- (42) The surface protein LHFPL3 (SwissProt: Q86UP9)
- (43) The receptor protein TNFRSF13C (SwissProt: Q96RJ3)
- 15 (44) The B-cell antigen receptor complex associated protein CD79a (SwissProt: P11912)
- (45) The receptor protein CXCR5 (SwissProt: P32302)
- (46) The ion channel P2X5 (SwissProt: Q93086)
- (47) The lymphocyte antigen CD180 (SwissProt: Q99467)
- (48) The receptor protein FCRL1 (SwissProt: Q96LA6)
- 20 (49) The receptor protein FCRL5 (SwissProt: Q96RD9)
- (50) The MHC class II molecule Ia antigen HLA-DOB (GenBank Accession No: NP_002111.1)
- (51) The T-cell protein VTCN1 (SwissProt: Q7Z7D3).

(52) Single-pass type-I membrane protein "Programmed cell death 1 ligand 1"

(synonyms: CD274, B7H1, PDCD1L1, PDCD1LG1, PDL1) (SwissProt: Q9NZQ7) – both are isoforms

5 >sp|Q9NZQ7|PDL1L1_HUMAN Programmed cell death 1 ligand 1 OS=*Homo sapiens*
GN=CD274 PE=1 SV=1

MRIFAVFIFMTYWHLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALI
VYWEMEDKNIIQFVHGEEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQ
DAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPTSEHELTCQAEGYP
KAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
10 ENHTAELVIPPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKC
GIQDTNSKKQSDTHLEET

(53) Single-pass type I membrane protein "ICOSLG" (synonyms: B7H2, B7RP1, ICOSL, KIAA0653, CD275) – (SwissProt: O75144), both are isoforms

15 >sp|O75144|ICOSL_HUMAN ICOS ligand OS=*Homo sapiens* GN=ICOSLG PE=1
SV=2

MRLGSPGLLFLLFSSLRADTQEKEVRAMVGSDVELSCACPEGSRFDLNDVYVY
WQTSESKTVVITYHIPQNSSLENVDSRYRNRALMSPAGMLRGDFSLRLFNVT PQD
EQKFHCLVLSQSLGFQEVLSVEVTLHVAANFSVPVVSAPHSPSQDELTFCTCT SIN
GYPRPNVYWINKTDNSLLDQALQNDTVFLNMRGLYDVVSVLRIARTPSVNIGCC
20 IENVLLQQNLTVGSQTGNDIGERDKITENPVSTGEKNAATWSILAVLCLLVVVA
VAIGWVCRDRCLQHSYAGAWAVSPETELTGHV

(54) Tyrosine kinase "Fibroblast growth factor receptor 3" (FGFR-3, EC=2.7.10.1, CD333, JTK4), (SwissProt: P22607) - four isoforms (alternative splicing)

25 >sp|P22607|FGFR3_HUMAN Fibroblast growth factor receptor 3 OS=*Homo sapiens*
GN=FGFR3 PE=1 SV=1

MGAPACALALCVAVAIVAGASSES LGTEQRVVGRAAEVPGPEPGQEQQLVFGS
GDAVELSCPPPGGGPMGPTVWVKDGTGLVPSERVLVGPQRLQVLNASHEDSGA
YSCRQRLTQRVLCHF SVRVTDAPSSGDDDEGEDEAEDTGVD TGAPYWTRPERM
DKKLLAVPAANTVRFRCPAAGNPTPSISWLKNGREFRGEHRIGGIKLRHQQWSL
30 VMESVVP SDRGN YTCVVENKFGSIRQTYTLDV LERSPHRPILQAGLPANQTAVL
GSDVEFHCKVYSDAQPHIQWLKHVEVNGSKVGPDPGTPYVTVLKTAGANTTDKE

LEVLSLHNVTFEDAGEYTCLAGNSIGFSHSAWLVVLPAAEEELVEADEAGSVYA
 GILSYGVGFFLFILVVAAVTLCRLRSPPKKGLGSPTVHKISRFLKRQVSLESNAS
 MSSNTPLVRIARLSSGEGPTLANVSELELPADPKWELSRARLTGKPLGEGCFGQ
 VVMAEAIGIDKDRAAKPVTVAVKMLKDDATDKDLSDLVSEMEMMKMIGKHK
 5 NIINLLGACTQGGPLYVLVEYAAKGNLREFLRARRPPGLDYSFDTCKPPEEQLTF
 KDLVSCAYQVARGMEYLASQKCIHRDLAARNVLVTEDNVMKIADFGGLARDVH
 NLDYYKKTNGRLPVKWMapeALFDRVYTHQSDVWSFGVLLWEIFTLGGSPYP
 GIPVEELFKLLKEGHRMDKPANCTHDLYMIMRECWAAPSQRPTFKQLVEDLD
 RVLTVTSTDEYLDLSAPFEQYSPGGQDTPSSSSSGDDSVFAHDLPPAPPSSGGSR
 10 T

(55) Single-pass type-I membrane protein "TYRP1" (CAS2, TYRP, TYRRP, DHICA oxidase, 5,6-dihydroxyindole-2-carboxylic acid oxidase, catalase B, glycoprotein 75, melanoma antigen gp75, tyrosinase-related protein 1), (SwissProt: P17643)

>sp|P17643|TYRP1_HUMAN 5,6-dihydroxyindole-2-carboxylic acid oxidase
 15 OS=*Homo sapiens* GN=TYRP1 PE=1 SV=2

MSAPKLLSLGCIFFPLLLFQQARAQFPRQCATVEALRSGMCCPDLSPVSGPGTDR
 CGSSSGRGRCEAVTADSRPHSPQYPHDGRDDREWPLRFFNRTCHCNGNFSGH
 NCGTCRPGWRGAACDQRVLIVRRNLLDLSKEEKNHFVRALDMAKRTTHPLFVI
 ATRRSEEILGPDGNTQPQFENISIYNYFVWTHYYSVKKTFLGVGQESFGEVDFSHE
 20 GPAFLTWHRYHLLRLEKDMQEMLQEPSFSLPYWNFATGKNVCDICTDDLMGSR
 SNFDSTLISPNSVFSQWRVVCDSLEDYDTLGTLCNSTEDGPIRRNPAGNVARPMV
 QRLPEPQDVAQCLEVGFLDTPPFYSNSTNSFRNTVEGYSDPTGKYDPAVRSLHN
 LAHLFLNGTGGQTHLSPNDPIFVLLHTFTDAVFDEWLRRYNADISTFPLENAPIG
 HNRQYNMVPFWPPVTNTEMFVTAPDNLGYTYEIQWPSREFSVPEIIAIAVVGALL
 25 LVALIFGTASYLIRARRSMDEANQPLLTDQYQCYAEEYEKLQNPQNQSVV

(56) Cell membrane protein, cleaved into secreted glypican-3 (GPC3, OCI5, GTR2-2, intestinal protein OCI-5, MXR7), (SwissProt: P51654)

>sp|P51654|GPC3_HUMAN Glypican-3 OS=*Homo sapiens* GN=GPC3 PE=1 SV=1
 MAGTVRTACLVVAMLLSLDFPGQAQPPPPPDATCHQVRSFFQRLQPGLKWVP
 30 ETPVPGSDLQVCLPKGPTCCSRKMEEKYQLTARLNMEQLLQSASMELKFLIIQN
 AAVFQEAFEIVVRHAKNYTNAMFKNNYPSLTPQAFEFVGEFFTDVSLYILGSDIN
 VDDMVNELFDSLFPVIYTQLMNPGLPDSALDINECLRGARRDLKVFGNFPKLIM

TQVSKSLQVTRIFLQALNLGIEVINTTDHLKFSKDCGRMLTRMWYCSYCQGLM
MVKPCGGYCNVVMQGCMAGVVEIDKYWREYILSLEELVNGMYRIYDMENVLL
GLFSTIHDSIQYVQKNAGKLTTTIGKLCAHSQQRQYRSAYYPEDLFIDKKVLKVA
HVEHEETLSSRRRELIQKLKSFISFYALPGYICSHSPVAENDTLCWNGQELVERY
5 SQAARNGMKNQFNLHELKMKGPPEPVVSQIIDKLKHINQLLRTMSMPKGRVLD
KNLDEEGFESGDCGDDDEDECIGSGDGMKVKVKNQLRFLAELAYDLDDVDDAPGN
SQQATPKDNEISTFHNLGNVHSPLKLLTSMAISVVCFFFLVH

In a preferred subject matter of the invention, the cancer target molecule is selected from the group consisting of the cancer target molecules (1) through (56).

- 10 In another preferred subject matter of the invention, the binder binds to an extracellular cancer target molecule, which is selected from the group consisting of the cancer target molecules (1) through (56).

In another preferred subject matter of the invention, the binder binds specifically to an extracellular cancer target molecule, which is selected from the group consisting of the cancer
15 target molecules (1) through (56).

In an especially preferred subject matter of the invention, the cancer target molecule is selected from the group consisting of EGF receptor (NP_005219.2), mesothelin (Q13421-3), C4.4a (NP_055215.2), carboanhydrase IX (CA IX; Q16790, NP_001207.2), HER2, glypican-3, TYRP1, fibroblast growth factor receptor 3, single-pass type I membrane protein ICOSLG and
20 programmed cell death 1 ligand 1.

In another especially preferred subject matter of the invention, the binder binds to an extracellular cancer target molecule, which is selected from the group consisting of EGF receptor (NP_005219.2), mesothelin (Q13421-3), C4.4a (NP_055215.2), carboanhydrase IX (CA IX; Q16790, NP_001207.2), HER2, glypican-3, TYRP1, fibroblast growth factor receptor 3, single-
25 pass type I membrane protein ICOSLG and programmed cell death 1 ligand 1.

In one preferred embodiment, the binder is internalized by the target cell after binding to its extracellular target molecule on the target cell by the binding. The result of this is that the binder-drug conjugate which may be an immunoconjugate or an ADC, is absorbed by the target cell.

In one embodiment, the binder is a binding protein. In a preferred embodiment, the binder is an
30 antibody, an antigen-binding antibody fragment, a multispecific antibody or an antibody mimetic.

Preferred antibody mimetics include affibodies, adnectins, anticalins, DARPins, avimers or nanobodies. Preferred multispecific antibodies include bi-specific and tri-specific antibodies.

In a preferred embodiment, the binder is an antibody or an antigen-binding antibody fragment; more preferably it is an isolated antibody or an isolated antigen-binding antibody fragment.

- 5 Preferred antigen binding antibody fragments include Fab, Fab', F(ab')₂ and Fv fragments, diabodies, Dabs, linear antibodies and scFv, Fab, diabodies and scFv are especially preferred.

In an especially preferred embodiment, the binder is an antibody. Especially preferred are monoclonal antibodies or antigen-binding antibody fragments thereof. Additionally especially preferred are human, humanized or chimeric antibodies or antigen-binding antibody fragments
10 thereof.

Antibodies or antigen-binding antibody fragments that bind cancer target molecules can be synthesized by the average person skilled in the art using known methods, for example, recombinant synthesis or recombinant expression. Binders for cancer target molecules can be purchased commercially or can be synthesized by an average person skilled in the art by using known
15 methods, e.g., chemical synthesis or recombinant expression. Additional methods of synthesis of antibodies or antigen-binding antibody fragments are described in WO 2007070538 (see page 22 "Antibodies"). Those skilled in the art are familiar with methods such as the so-called phage display technique which creates libraries (e.g., Morphosys HuCAL Gold) and can be used to discover antibodies or antigen-binding antibody fragments (see WO 200707058, pages 24 ff.,
20 Example 1 on page 70 and Example 2 on page 72). Additional methods of synthesis of antibodies using DNA libraries from B cells are described on page 26 of WO 2007070538, for example. Methods of humanizing antibodies are described on pages 30-32 of WO 2007070538 and in detail in Queen *et al.*, *Proc. Natl. Acad. Sci. USA* **86**:10029-10033, 1989 or in WO 90/0786. In addition, those skilled in the art are familiar with methods of recombinant expression of proteins
25 in general and in specific by antibodies (see, e.g., in Berger and Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology*, vol. 152, Academic Press, Inc.; Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, vol. 1-3; *Current Protocols in Molecular Biology*, F. M. Ausabel *et al.* (eds.), *Current Protocols*, Green Publishing Associates, Inc., John Wiley & Sons,
30 Inc.; Harlow *et al.*, *Monoclonal Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 19881, Paul (ed.); *Fundamental Immunology*, (Lippincott Williams & Wilkins, 1998; and Harlow, *et al.*, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1998. Those skilled in the art are familiar with the corresponding vectors, promoters and signal peptides, which are necessary for expression of a protein/antibody.

Conventional methods are also described on pages 41-45 of WO 2007070538. Methods of synthesis of an IgG1 antibody are described in WO 2007070538, e.g., on pages 74 ff. of Example 6; these methods, described on page 80 of WO 2007070538, for example, make it possible to internalize an antibody after binding it to its antigen. Similarly, those skilled in the art can utilize the methods described in WO 2007070538 for synthesis of carboanhydrase IX (Mn) antibodies to synthesize antibodies having other target molecule specificities.

Especially preferred binders according to the invention are antibodies in particular human or humanized antibodies. The antibodies preferably have an affinity of at least 10^{-7} M (as a Kd value; i.e., preferably those with smaller Kd values than 10^{-7} M), preferably of at least 10^{-8} M, especially preferably in the range of 10^{-9} M to 10^{-11} M. These Kd values can be determined, for example, by surface plasmon resonance spectroscopy.

The antibody-drug conjugates according to the invention also have affinities in these ranges. Through conjugation of the active ingredients, the affinity is preferably not influenced significantly (the affinity is usually reduced by less than one order of magnitude, e.g., max. from 10^{-8} M to 10^{-7} M).

The antibodies used according to the invention are also preferably characterized by a high selectivity. Selectivity is high when the antibody according to the invention has a better affinity for the target protein than for another independent antigen, e.g., human serum albumin, said affinity being better by a factor of 2, a factor of 5, a factor of 10 or in particular preferably a factor of 100 (the affinity can be determined, for example, by surface plasmon resonance spectroscopy).

Furthermore, the antibodies used according to the invention are preferably cross-reactive. To facilitate preclinical trials, e.g., toxicological studies or efficacy studies (e.g., in xenograft mice) and to be able to interpret them better, it is advantageous if the antibody to be used according to the invention not only binds the human target protein but also binds the species target protein in the species used for these studies. In one embodiment, the antibody used according to the invention, which is cross-reactive with the antibody used according to the invention but is also cross-reactive with the human target protein of at least one additional species. For toxicological studies and efficacy studies, species of rodent, dog and non-human primate families are especially preferred. Preferred rodent species include the mouse and the rat. Preferred non-human primates include Rhesus monkeys, chimpanzees and long-tailed macaques.

In one embodiment, the antibody used according to the invention is also cross-reactive with the target protein of at least one additional species in addition to being cross-reactive with the human

target protein, said additional species being selected from the group of species consisting of the mouse, the rat and the long-tailed macaque (*Macaca fascicularis*). Antibodies that are used according to the invention and are cross-reactive at least with the mouse target protein in addition to being cross-reactive with the human target protein are preferred in particular. Cross-reactive antibodies whose affinity for the target protein of the additional non-human species does not differ from the affinity for the human target protein by more than a factor of 50, in particular not more than a factor of 10 are preferred.

EGFR antibodies

Examples of antibodies that bind the cancer target molecule EGFR include cetuximab (INN No. 7906), panitumumab (INN No. 8499) and nimotuzumab (INN No. 8545). Cetuximab (Drug Bank Accession No. DB00002) is a chimeric anti-EGFR1 antibody that is produced in SP2/0 mouse myeloma cells and is distributed by ImClone Systems Inc., Merck KGaA / Bristol Myers Squibb Co. Cetuximab is indicated for treatment of metastatic EGFR-expressing colorectal carcinoma with the wild-type K-Ras gene. It has an affinity of 10^{-10} M.

Sequence:

Cetuximab light chain (kappa):

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQR TNGSPRL LIKYASESISGIPSRFSGS
 GSGTDFTLSINSVESEDIADYYCQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKS
 GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE
 KHKVYACEVTHQGLSSPVTKSFNRGEC

Cetuximab heavy chain:

QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGGNTDYN
 TPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT LTVSAA
 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
 YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF
 LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
 NQVSLTCLVKGFPYSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHEALHNHYTQKSLSLSPGK

Panitumumab (INN No. 8499) (Drug Bank Accession No. DB01269) is a recombinant monoclonal human IgG2 antibody that binds specifically to human EGF receptor 1 and is

distributed by Abgenix / Amgen. Panitumumab originates from the immunization of transgenic mice (XenoMouse). These mice are capable of producing human immunoglobulins (light and heavy chains). A special B-cell clone that produces antibodies to EGFR was selected and was immortalized with CHO cells (Chinese hamster ovary cells). These cells are now being used for the production of a 100% human antibody. Panitumumab is indicated for the treatment of EGFR-expressing, metastatic colorectal carcinoma, which is refractory to chemotherapeutic treatment with fluoropyrimidine, oxaliplatin and irinotecan. It has an affinity of 10^{-11} M.

Sequence:

Panitumumab light chain (kappa):

10 DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPS
RFSGSGSGTDFTFTISSLPEDATYFCQHFHDLPLAFGGGKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSLTTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Panitumumab heavy chain:

15 QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKGLEWIGHIYYSGNTN
YNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTMTVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVTVTPSSNFGTQTYTCNVDPKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS
20 LTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFC
SVMHEALHNHYTQKSLSLSPG

Nimotuzumab (INN No. 8545) (EP 00586002, EP 00712863) is a humanized monoclonal IgG1 antibody that binds specifically to the human EGF receptor 1 and is distributed by YM
25 BioSciences Inc. (Mississauga, Canada). It is produced in non-secreting NSO cells (mammalian cell line). Nimotuzumab has been approved for treatment of head and neck tumors, highly malignant astrocytomas and glioblastoma multiforme (not in EU or US) and pancreatic cancer (orphan drug, EMA). It has an affinity of 10^{-8} M.

Additional embodiments of EGFR antibodies include:

- 30
- Zalutumumab / 2F8 / HuMax-EGFr, Genmab Co. A/S (WO 02100348, WO 2004-056847, INN No. 8605)

- Necitumumab / 11F8, ImClone / IMC-11F8, ImClone Systems Inc. (Eli Lilly & Co.) (WO 2005090407, (EP 01735348-A1, US 20070264253-A1, US 07598350, WO 2005-090407 A1), INN No. 9083)
- 5 • Matuzumab / anti-EGFR mAb, Merck KGaA / anti-EGFR mAb, Takeda / EMD 72000 / EMD-6200 / EMD-72000 and EMD-55900 / mAb 425 / monoclonal antibody 425, Merck KGaA / Takeda (WO 09215683, INN No. 8103 (matuzumab))
- RG-7160 / GA-201 / GA201 / R-7160 / R7160 / RG7160 / RO-4858696 / RO-5083945 / RO4858696 / RO5083945, Glycart Biotechnology AG (Roche Holding AG) (WO 2010-112413 A1, WO 2010115554)
- 10 • GT-MAB 5.2-GEX / CetuGEX, Glycotope GmbH (WO 2008028686 A2, EP 01900750 A1, EP 01911766 A1, EP 02073842 A2, US 20100028947 A1)
- ISU-101, Isu Abxis Inc. (ISU Chemical Co. Ltd.) / Scancell (patent: WO 2008-004834 A1)
- 15 • ABT-806 / mAb 806 / ch-806 / anti-EGFR monoclonal antibody 806, Ludwig Institute for Cancer Research / Abbott / Life Science Pharmaceuticals (WO02092771, WO2005-081854 and WO-2009023265)
- SYM-004 (consists of two chimeric IgG1 antibodies (992 and 1024)), Symphogen A/S (WO 2010022736 A2)
- 20 • MR1-1 /MR1-1KDEL, IVAX Corp (Teva Pharmaceutical Industries Ltd.) (Duke University), (patent: WO 2001062931 A2)
- Antibodies to the deletion mutant, EGFRvIII, Amgen/Abgenix (WO 2005010151, US 07628986)
- SC-100, Scancell Ltd. (WO-2001088138-A1)
- 25 • MDX-447 / EMD 82633 / BAB-447 / H 447 / MAb, EGFR, Medarex/Merck KGaA, Bristol-Myers Squibb (US) / Merck KGaA (DE) / Takeda (JP), (WO 09105871, WO 09215683)
- Anti-EGFR mAb, Xencor (WO 2005056606)

- DXL-1218 / anti-EGFR monoclonal antibody (cancer), InNexus, InNexus Biotechnology Inc., pharmaceutical projects PH048638

In a preferred embodiment, the anti-EGFR antibodies are selected from the group consisting of cetuximab, panitumumab, nimotuzumab, zalutumumab, necitumumab, matuzumab, RG-716,
5 GT-MAB 5.2-GEX, ISU-101, ABT-806, SYM-004, MR1-1, SC-100, MDX-447 and DXL-1218.

In an especially preferred embodiment, the anti-EGFR antibodies are selected from the group consisting of cetuximab, panitumumab, nimotuzumab, zalutumumab, necitumumab and matuzumab.

Those skilled in the art will be familiar with methods with which additional antibodies having a
10 similar or better affinity and/or specificity for the target molecule can be synthesized from the CDR regions of the aforementioned antibodies by sequence variations.

In another embodiment, the anti-EGFR antibodies or antigen-binding antibody fragments are selected from the group consisting of

antibodies or antigen-binding antibody fragments comprising the three CDR regions of the light
15 chain and the three CDR regions of the heavy chain of one of the following antibodies: cetuximab, panitumumab, nimotuzumab, zalutumumab, necitumumab, matuzumab, RG-716, GT-MAB 5.2-GEX, ISU-101, ABT-806, SYM-004, MR1-1, SC-100, MDX-447, and DXL-1218.

In another preferred embodiment, the anti-EGFR antibodies or antigen-binding antibody fragments are selected from the group consisting of

20 antibodies or antigen-binding antibody fragments comprising the three CDR regions of the light chain and the three CDR regions of the heavy chain of one of the following antibodies: cetuximab, panitumumab, nimotuzumab, zalutumumab, necitumumab, matuzumab.

Carboanhydrase IX antibodies

Especially preferred binders according to the invention include anti-CAIX antibodies, in
25 particular human or humanized anti-CAIX antibodies. The antibodies preferably have an affinity of at least 10^{-7} M (as the K_d value, i.e., preferably those with K_d values of less than 10^{-7} M), preferably of at least 10^{-8} M, especially preferably in the range of 10^{-9} M to 10^{-11} M. The K_d values can be determined, for example, by surface plasmon resonance spectroscopy.

The antibody-drug conjugates according to the invention also have affinities in these ranges. The
30 affinity is preferably not influenced significantly by conjugation of the active ingredients (the

affinity is usually reduced less than one order of magnitude, i.e., from max. 10^{-8} M to 10^{-7} M, for example).

The antibodies used according to the invention are also characterized preferably by a high selectivity. A high selectivity occurs when the antibodies according to the invention have a better
5 affinity for the target protein by a factor of at least 2, a factor of 5, a factor of 10 or especially preferably a factor of 100 than the affinity for another independent antigen, e.g., human serum albumin (the affinity can be determined, for example, by surface plasmon resonance spectroscopy).

Furthermore, the antibodies used according to the invention are preferably cross-reactive. To
10 facilitate preclinical trials, e.g., toxicological or efficacy studies (e.g., in xenograft mice), and to be better able to interpret them, it is advantageous if the antibodies used according to the invention bind not only the human target protein but also bind the species target protein in the species used for the studies. In one embodiment, the antibody used according to the invention is cross-reactive with the target protein of at least one species in addition to the human target
15 protein. For toxicological studies and efficacy studies, the preferred species for use are those of the rodent, dog and non-human primate families. Preferred rodent species include the mouse and the rat. Preferred non-human primates include Rhesus monkeys, chimpanzees and long-tailed macaques.

In one embodiment, the antibody used according to the invention is cross-reactive with the target
20 protein of at least one additional species selected from the group of species consisting of mouse, rat and long-tailed macaque (*Macaca fascicularis*) in addition to being cross-reactive with the human target protein. Especially preferred are antibodies that can be used according to the invention and are at least cross-reactive with the mouse target protein in addition to being cross-reactive with the human target protein. The preferred cross-reactive antibodies are those whose
25 affinity for the target protein of the additional non-human species does not differ from the affinity for the human target protein by a factor of more than 50, in particular no more than a factor of 10. Anti-CAIX antibodies include those described, for example, in WO 2007/070538 A2. These antibodies may be used according to the invention.

Examples of antibodies that bind the cancer target molecule carboanhydrase IX are described in
30 WO 2007/070538 A2 (e.g., Claims 1-16).

In a preferred embodiment, the anti-carboanhydrase IX antibodies or antigen-binding antibody fragments are selected from the group consisting of anti-carboanhydrase IX antibodies or antigen-binding antibody fragments 3ee9 (Claim 4 (a) in WO 2007070538 A2), 3ef2 (Claim 4

(b) in WO 2007070538 A2), 1e4 (Claim 4 (c) in WO 2007070538 A2), 3a4 (Claim 4 (d) in WO 2007070538 A2), 3ab4 (Claim 4 (e) in WO 2007070538 A2), 3ah10 (Claim 4 (f) in WO 2007070538 A2), 3bb2 (Claim 4 (g) in WO 2007070538 A2), 1aa1 (Claim 4 (h) in WO 2007070538 A2), 5a6 (Claim 4 (i) in WO 2007070538 A2) and 5aa3 (Claim 4 (j) in WO 2007070538 A2).

In a preferred embodiment, the anti-carboanhydrase IX antibodies or antigen-binding antibody fragments are selected from the group consisting of:

anti-carboanhydrase IX antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody 3ee9 (from WO 2007070538 A2),

anti-carboanhydrase IX antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody 3ef2 (from WO 2007070538 A2),

anti-carboanhydrase IX antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody 1e4 (from WO 2007070538 A2),

anti-carboanhydrase IX antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody 3a4 (from WO 2007070538 A2),

anti-carboanhydrase IX antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody 3ab4 (from WO 2007070538 A2),

anti-carboanhydrase IX antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody 3ah10 (from WO 2007070538 A2),

anti-carboanhydrase IX antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody 3bb2 (from WO 2007070538 A2),

anti-carboanhydrase IX antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody 1aa1 (from WO 2007070538 A2),

anti-carboanhydrase IX antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody 5a6 (from WO 2007070538 A2) and

5 anti-carboanhydrase IX antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody 5aa3 (from WO 2007070538 A2).

The given sequences of the CDR regions are shown in Figures 2a-2c, pages 128-130 in WO 2007070538 A2.

10 In a preferred embodiment, the anti-carboanhydrase IX antibodies or antigen-binding antibody fragments are selected from the group consisting of:

an antibody or antigen-binding fragment consisting of the amino acid sequence of the variable light and variable heavy chains of the antibody 3ee9, as defined in WO 2007070538 A2 in Figure 4b on page 137,

15 an antibody or antigen-binding fragment consisting of the amino acid sequence of the variable light and variable heavy chains of the antibody 3ef2, as defined in WO 2007070538 A2 in Figure 4c on page 138 and/or in Figure 4b on page 137,

an antibody or antigen-binding fragment consisting of the amino acid sequence of the variable light and variable heavy chains of the antibody 1e4, as defined in WO 2007070538 A2 in Figure 4a on page 136,

20 an antibody or antigen-binding fragment consisting of the amino acid sequence of the variable light and variable heavy chains of the antibody 3a4, as defined in WO 2007070538 A2 in Figure 4a on page 136,

25 an antibody or antigen-binding fragment consisting of the amino acid sequence of the variable light and variable heavy chains of the antibody 3ab4, as defined in WO 2007070538 A2 in Figure 4a on page 136,

an antibody or antigen-binding fragment consisting of the amino acid sequence of the variable light and variable heavy chains of the antibody 3ah10, as defined in WO 2007070538 A2 in Figure 4a on page 136,

an antibody or antigen-binding fragment consisting of the amino acid sequence of the variable light and variable heavy chains of the antibody 3bb2, as defined in WO 2007070538 A2 in Figure 4b on page 137,

an antibody or antigen-binding fragment consisting of the amino acid sequence of the variable light and variable heavy chains of the antibody 1aa1, as defined in WO 2007070538 A2 in Figure 4a on page 136,

an antibody or antigen-binding fragment consisting of the amino acid sequence of the variable light and variable heavy chains of the antibody 5a6, as defined in WO 2007070538 A2 in Figure 4b on page 137, and

an antibody or antigen-binding fragment consisting of the amino acid sequence of the variable light and variable heavy chains of the antibody 5aa3, as defined in WO 2007070538 A2 in Figure 4b on page 137.

In an especially preferred embodiment, the anti-carboanhydrase IX antibody is the antibody 3ee9 from WO 2007070538 A2.

In an especially preferred embodiment, the anti-carboanhydrase IX antibody or the antigen-binding antibody fragment comprises the amino acid sequences of the CDR regions of the variable heavy chain of the antibody 3ee9 (VH3-CDR1: GFTFSSYGMS; VH3-CDR2: GISSLGSTTTYADSVK; VH3-CDR3: TGSPGTFMHGDH, see Figure 2a, page 128 in WO 2007070538 A2) and the amino acid sequences of the CDR regions of the variable light chain of the antibody 3ee9 (VLk1-CDR1: RASQDINNYLS; VLk1-CDR2: YGASNLQS; VLk1-CDR3: QQYYGRPT, see Figure 2b, page 129 in WO 2007070538 A2).

In an especially preferred embodiment, the anti-carboanhydrase IX antibody or the antigen-binding antibody fragment comprises the amino acid sequences of the variable heavy chain of the antibody 3ee9

(VH3:ELVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVSGISSLGST TYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGSPGTFMHGDHWGQ GTLVTVSS, see Figure 4b, page 137 in WO 2007070538 A2) and the amino acid sequences of the variable light chain of the antibody 3ee9 (VLk1:DIQMTQSPSSLSASVGDRVTITCRASQDINNYLSWYQQKPGKAPKLLIYGASNLQS GVPSRFSGSGSGTDFLTISLQPEDFAVYYCQQYYGRPTTFGQGTKVEIKRT, see Figure 4b, page 137 in WO 2007070538 A2).

In a preferred embodiment, the anti-carboanhydrase IX antibody 3ee9 is a IgG antibody.

In an especially preferred embodiment, the anti-carboanhydrase IX antibody 3ee9 is an IgG1 antibody (3ee9-IgG1),

wherein the amino acid sequence of the heavy chain comprises the following sequence:

QVELVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVSGISSLGSTTY
5 ADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGSPGTFMHGDHWGQGT
LTVVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
10 LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

and the amino acid sequence of the light chain comprises the following sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDINNYLSWYQQKPGKAPKLLIYGASNLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFAVYYCQQYYGRPTTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
15 LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKA
DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

anti-carboanhydrase IX antibody 3ee9-IgG1:

Another aspect of the present invention is supplying the anti-carboanhydrase IX antibody 3ee9-IgG1.

20 C4.4a antibody:

Especially preferred binders according to the invention are anti-C4.4a antibodies, in particular human or humanized anti-C4.4a antibodies. These antibodies have an affinity of preferably at least 10^{-7} M (as K_d value, i.e., preferably those with K_d values of less than 10^{-7} M), especially at least 10^{-8} M, most especially preferably in the range of 10^{-9} M to 10^{-11} M. The K_d values can be
25 determined by surface plasmon resonance spectroscopy, for example.

The antibody-drug conjugates according to the invention also have affinities in these ranges. Through conjugation of the active ingredients, the affinity is preferably not influenced significantly (the affinity is usually reduced by less than one order of magnitude, e.g., max. from 10^{-8} M to 10^{-7} M).

The antibodies used according to the invention are also preferably characterized by a high selectivity. A high selectivity occurs when the antibody according to the invention has a better affinity for the target protein than for another independent antigen, e.g., human serum albumin by a factor of at least 2, preferably by a factor of 5 or in particular preferably a factor of 10 (the
5 affinity can be determined, for example, by surface plasmon resonance spectroscopy).

Furthermore, the antibodies to be used according to the invention are preferably cross-reactive. To facilitate preclinical trials, e.g., toxicological studies or efficacy studies (e.g., in xenograft mice) and to be able to interpret them better, it is advantageous if the antibody to be used according to the invention not only binds the human target protein but also binds the species
10 target protein in the species used for the studies. In one embodiment, the antibody used according to the invention is additionally cross-reactive with the target protein of at least one other species in addition to the human target protein. For toxicological studies and efficacy studies, species of the rodent, dog and non-human primate families are preferably used. Preferred rodent species include the mouse and the rat. Preferred non-human primates include Rhesus monkeys,
15 chimpanzees and long-tailed macaques.

In one embodiment, the antibody used according to the invention is also cross-reactive with the target protein of at least one other species in addition to being cross-reactive with the human target protein, said additional species being selected from the group of species consisting of the mouse, rat and long-tailed macaque (*Macaca fascicularis*). Especially preferred antibodies for
20 use according to the invention include those that are cross-reactive with at least the mouse target protein in addition to being cross-reactive with the human target protein. The preferred cross-reactive antibodies are those whose affinity for the target protein of the additional non-human species does not differ from the affinity for the human target protein by a factor of more than 50, in particular more than 10.

25 Anti-C4.4a antibodies are described in WO 01/23553 or WO 2011070088, for example. These antibodies may be used according to the present invention.

Examples of C4.4a antibodies and antigen-binding fragments are described below. The sequences of the antibodies are given in Table 1, where each row shows the respective CDR amino acid sequences of the variable light chain and/or of the variable heavy chain of the
30 antibody listed in column 1. This table also shows the amino acid sequences of the variable light chain and of the variable heavy chain and also the amino acid sequence of the respective antibody listed lists in column 1.

In one embodiment, the anti-C4.4a antibodies or the antigen-binding antibody fragments bind to the S1 domain S1 (amino acid positions 1-85 of SEQ ID NO: 1) of C4.4a.

In one embodiment, the anti-C4.4a antibodies or the antigen-binding antibody fragments have cross-reactivity with human C4.4a (SEQ ID NO: 1) and with murine C4.4a (SEQ ID NO: 2).

- 5 In one exemplary embodiment, the anti-C4.4a antibodies or the antigen-binding antibody fragments thereof are internalized by the cell after binding to a C4.4a-expressing cell.

In another embodiment, the anti-C4.4a antibodies or the antigen-binding antibody fragments compete with the antibody M31-B01 and/or with the antibody M20-D02-S-A for binding to C4.4a. Antibodies M31-B01 and M20-D02-S-A compete for binding to C4.4a. Antibodies B01-1
10 to B01-12 were synthesized by affinity maturation from M31-B01 and compete with M31-B01 for binding to C4.4a. The antibodies D02-1 through D02-13 were synthesized by affinity maturation from M20-D02-S-A and compete with M20-D02-S-A for binding to C4.4a.

In another embodiment, the anti-4.4a antibodies or the antigen-binding antibody fragments comprise at least one, two or three of the CDR amino acid sequences listed in Table 1 or Table 2.

- 15 In another embodiment, the anti-4.4a antibodies or the antigen-binding antibody fragments comprise at least one, two or three CDR amino acid sequences of an antibody listed in Table 1 or Table 2.

In another embodiment, the anti-4.4a antibodies or the antigen-binding antibody fragments comprise at least one, two or three CDR amino acid sequences of the variable light chain and at
20 least one, two or three CDR amino acid sequences of the variable heavy chain of an antibody listed in Table 1 or Table 2.

In another embodiment, the anti-4.4a antibodies or the antigen-binding antibody fragments, which are at least 50%, 60%, 70%, 80%, 90% or 95% identical to the CDR amino acid sequences of the variable light chain and are identical with the CDR amino acid sequences of the variable
25 heavy chain comprise an antibody as listed in Table 1 or Table 2.

In another embodiment, the CDR sequences of the anti-C4.4a antibodies or of the antigen-binding fragments comprise:

CDR sequences of the heavy chain, which conform to CDR sequences SEQ ID NO: 297 (CDR H1), SEQ ID NO: 298 (CDR H2) and SEQ ID NO: 299 (CDR H3), and CDR sequences of the
30 light chain, which conform to CDR sequences SEQ ID NO: 300 (CDR L1), SEQ ID NO: 22 (CDR L2) and SEQ ID NO: 301 (CDR L3), or

CDR sequences of the heavy chain, which conform to CDR sequences SEQ ID NO: 302 (CDR H1), SEQ ID NO: 303 (CDR H2) and SEQ ID NO: 304 (CDR H3) and CDR sequences of the light chain, which conform to CDR sequences SEQ ID NO: 305 (CDR L1), SEQ ID NO: 306 (CDR L2) and SEQ ID NO: 307 (CDR L3).

- 5 In another embodiment, the anti-C4.4a antibodies or antigen-binding antibody fragments which are at least 50%, 60%, 70%, 80%, 90% or 95% identical to the variable light chain and to the variable heavy chain comprise an antibody as listed in Table 1 or Table 2.

In another embodiment, the anti-C4.4a antibodies or antigen-binding antibody fragments comprise the three CDR amino acid sequences of the variable light chain and the three CDR
10 amino acid sequences of the variable heavy chain as listed in Table 1 or Table 2.

In another embodiment, the anti-C4.4a antibodies or antigen-binding antibody fragments comprise a variable light chain and/or a variable heavy chain of an antibody as listed in Table 1 or Table 2.

In another embodiment, the anti-C4.4a antibodies or antigen-binding antibody fragments
15 comprise the variable light chain and the variable heavy chain of an antibody as listed in Table 1 or Table 2.

In a preferred embodiment, the C4.4a antibodies and the antigen-binding antibody fragments are selected from the group consisting of

antibody comprising the CDR sequences of the variable heavy chain represented by SEQ ID NO:
20 75-77 and which reflects the CDR sequences of the variable light chain, as represented by sequence SEQ ID NOS: 78-80 (B01-10),

antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 5, 9 and 13 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 17, 21 and 25 (M31-B01),

25 antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 6, 10 and 14 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 18, 22 and 26 (M20-D02-S-A),

antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 7, 11 and 15 and comprising the CDR sequences of the variable light
30 chain represented by the sequences SEQ ID NOS: 19, 23 and 27 (M60-G03),

antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 8, 12 and 16 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 20, 24 and 28(36-H02),

antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 45-47 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 48-50 (B01-3),

antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 55-57 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 58-60 (B01-5),

10 antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 65-67 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 68-70 (B01-7),

antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 85-87 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 88-90 (B01-12),

15 antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 95-97 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 98-100 (D02-4),

antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 105-107 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 108-110 (D02-6),

antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 115-117 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 118-120 (D02-7),

25 antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 125-127 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 128-130 (D02-11) and

antibody comprising the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NOS: 135-137 and comprising the CDR sequences of the variable light chain represented by the sequences SEQ ID NOS: 138-140 (D02-13).

30

In a preferred embodiment, the C4.4a antibodies and the antigen-binding antibody fragments are selected from the group consisting of antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 81 and comprising the amino acid sequence of the variable light chain represented by the sequence SEQ ID NO: 82 (B01-7),
5 antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NOS: 33 and comprising the amino acid sequence of the variable light chain represented by the sequence SEQ ID NO: 29 (M31-B01), antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 34 and comprising the amino acid sequence of the variable light chain represented by the sequence SEQ
10 ID NO: 30 (M20-D02 S-A), antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 35 and comprising the amino acid sequence of the variable light chain represented by the sequence SEQ ID NO: 31 (M60-G03), antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 36 and comprising the amino acid sequence of the variable light chain
15 represented by the sequence SEQ ID NO: 32 (M36-H02), antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 51 and comprising the amino acid sequence of the variable light chain represented by the sequence SEQ ID NO: 52 (B01-3), antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 61 and comprising the amino acid sequence of the
20 variable light chain represented by the sequence SEQ ID NO: 62 (B01-5), antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 71 and comprising the amino acid sequence of the variable light chain represented by the sequence SEQ ID NO: 72 (B01-7), antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 91 and comprising the amino acid sequence of
25 the variable light chain represented by the sequence SEQ ID NO: 92 (B01-12), antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 101 and comprising the amino acid sequence of the variable light chain represented by the sequence SEQ ID NO: 102 (D02-4), antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 111 and comprising the amino
30 acid sequence of the variable light chain represented by the sequence SEQ ID NO: 112 (D02-6), antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 121 and comprising the amino acid sequence of the variable light chain represented by the sequence SEQ ID NO: 122 (D02-7), antibodies comprising the amino acid sequence of the variable heavy chain represented by the sequence SEQ ID NO: 131 and
35 comprising the amino acid sequence of the variable light chain represented by the sequence SEQ ID NO: 132 (D02-11) and antibodies comprising the amino acid sequence of the variable heavy

chain represented by the sequence SEQ ID NO: 141 and comprising the amino acid sequence of the variable light chain represented by the sequence SEQ ID NO: 142 (D02-13).

In another embodiment, the anti-C4.4a antibodies comprise the light chain and the heavy chain of an antibody as listed in Table 2.

- 5 In a preferred embodiment, the anti-C4.4a antibodies comprise the light chain and the heavy chain of an antibody as listed in Table 2.

In an especially preferred embodiment, the C4.4a antibody is selected from the group consisting of:

- an antibody comprising the amino acid sequence of the light chain represented by SEQ ID NO: 346 and comprising the amino acid sequence of the heavy chain represented by SEQ ID NO: 347 (M31-B01),

an antibody comprising the amino acid sequence of the light chain represented by SEQ ID NO: 352 and comprising the amino acid sequence of the heavy chain represented by SEQ ID NO: 353 (B01-3),

- 15 an antibody comprising the amino acid sequence of the light chain represented by SEQ ID NO: 364 and comprising the amino acid sequence of the heavy chain represented by SEQ ID NO: 365 (B01-10) and

- an antibody comprising the amino acid sequence of the light chain represented by SEQ ID NO: 382 and comprising the amino acid sequence of the heavy chain represented by SEQ ID NO: 383 (D02-6).

Table 1: Sequences of the C4.4a antibodies

Antibody	SEQ ID NO: HCDR1	SEQ ID NO: HCDR2	SEQ ID NO: HCDR3	SEQ ID NO: LCDR1	SEQ ID NO: LCDR2	SEQ ID NO: LCDR3	SEQ ID NO: VH protein	SEQ ID NO: VL protein	SEQ ID NO: VH nucleotide	SEQ ID NO: VL nucleotide
M31-B01	5	9	13	17	21	25	33	29	41	37
M20-D02 S-A	6	10	14	18	22	26	34	30	42	38
M60-G03	7	11	15	19	23	27	35	31	43	39
M36-H02	8	12	16	20	24	28	36	32	44	40

Antibody	SEQ ID NO: HCDR1	SEQ ID NO: HCDR2	SEQ ID NO: HCDR3	SEQ ID NO: LCDR1	SEQ ID NO: LCDR2	SEQ ID NO: LCDR3	SEQ ID NO: VH protein	SEQ ID NO: VL protein	SEQ ID NO: VH nucleotide	SEQ ID NO: VL nucleotide
B01-3	45	46	47	48	49	50	51	52	53	54
B01-5	55	56	57	58	59	60	61	62	63	64
B01-7	65	66	67	68	69	70	71	72	73	74
B01-10	75	76	77	78	79	80	81	82	83	84
B01-12	85	86	87	88	89	90	91	92	93	94
D02-4	95	96	97	98	99	100	101	102	103	104
D02-6	105	106	107	108	109	110	111	112	113	114
D02-7	115	116	117	118	119	120	121	122	123	124
D02-11	125	126	127	128	129	130	131	132	133	134
D02-13	135	136	137	138	139	140	141	142	143	144
B01-nn1	145	146	147	148	149	150	151	152	308	309
B01-nn2	153	154	155	156	157	158	159	160	310	311
B01-nn3	161	162	163	164	165	166	167	168	312	313
B01-nn4	169	170	171	172	173	174	175	176	314	315
B01-nn5	177	178	179	180	181	182	183	184	316	317
B01-2	185	186	187	188	189	190	191	192	318	319
B01-4	193	194	195	196	197	198	199	200	320	321
B01-6	201	202	203	204	205	206	207	208	322	323
B01-8	209	210	211	212	213	214	215	216	324	325
B01-9	217	218	219	220	221	222	223	224	326	327
B01-11	225	226	227	228	229	230	231	232	328	329
B01-12	233	234	235	236	237	238	239	240	330	331
D02-ogl	241	242	243	244	245	246	247	248	332	333
D02-5	249	250	251	252	253	254	255	256	334	335
D02-8	257	258	259	260	261	262	263	264	336	337
D02-9	265	266	267	268	269	270	271	272	338	339
D02-10	273	274	275	276	277	278	279	280	340	341

Antibody	SEQ ID NO: HCDR1	SEQ ID NO: HCDR2	SEQ ID NO: HCDR3	SEQ ID NO: LCDR1	SEQ ID NO: LCDR2	SEQ ID NO: LCDR3	SEQ ID NO: VH protein	SEQ ID NO: VL protein	SEQ ID NO: VH nucleotide	SEQ ID NO: VL nucleotide
D02-11	281	282	283	284	285	286	287	288	342	343
D02-12	289	290	291	292	293	294	295	296	344	345

Table 2: Sequences of the light and heavy chains of the C4.4a antibodies

Antibody	Light chain SEQ ID NO:	Heavy chain SEQ ID NO:
M31-B01	346	347
B01-1	348	349
B01-2	350	351
B01-3	352	353
B01-4	354	355
B01-5	356	357
B01-6	358	359
B01-7	360	361
B01-8	362	363
B01-10	364	365
B01-11	366	367
B01-12	368	369
M20-D02 S-A	370	371
D02-1	372	373
D02-2	374	375
D02-3	376	377
D02-4	378	379
D02-5	380	381
D02-6	382	383
D02-7	384	385

Antibody	Light chain SEQ ID NO:	Heavy chain SEQ ID NO:
D02-8	386	387
D02-9	388	389
D02-10	390	391
D02-11	392	393
D02-12	394	395
D02-13	396	397

Anti-C4.4a antibody IgG:

Another aspect of the present invention is providing an anti-C4.4a IgG1 antibody comprising the amino acid sequence of the light chain and the heavy chain of an antibody as listed in Table 2.

- 5 One example of an antibody that binds the cancer target molecule HER2 is trastuzumab (Genentech). Trastuzumab is a humanized antibody used for treatment of breast cancer, among other things. An example of an antibody that binds the cancer target molecule CD20 is rituximab (Genentech). Rituximab (CAS No. 174722-31-7) is a chimeric antibody used for treating non-Hodgkin's lymphoma. One example of an antibody that binds the cancer target molecule CD52 is
- 10 alemtuzumab (Genzyme). Alemtuzumab (CAS No. 216503-57-0) is a humanized antibody that is used for treatment of chronic lymphatic leukemia.

Mesothelin antibody

- According to the invention an especially preferred binders are anti-mesothelin antibodies, in particular human or humanized anti-mesothelin antibodies. The antibodies preferably have an
- 15 affinity of at least 10^{-7} M (as Kd value, i.e., preferably those with Kd values less than 10^{-7} M), preferably of at least 10^{-8} M, especially preferably in the range from 10^{-9} M to 10^{-11} M. The Kd values can be determined by surface plasmon resonance spectroscopy.

- The antibody-drug conjugates according to the invention also have affinities in these ranges. Through conjugation of the active ingredients, the affinity is preferably not influenced
- 20 significantly (the affinity is usually reduced by less than one order of magnitude, e.g., max. from 10^{-8} M to 10^{-7} M).

The antibodies used according to the invention are also preferably characterized by a high selectivity. A high selectivity occurs when the antibody according to the invention has a better

affinity for the target protein than for another independent antigen, e.g., human serum albumin by a factor of at least 2, preferably by a factor of 5 or in particular preferably a factor of 10 (the affinity can be determined, for example, by surface plasmon resonance spectroscopy).

Furthermore, the antibodies used according to the invention are preferably cross-reactive. To facilitate preclinical trials, e.g., toxicological studies or efficacy studies (e.g., in xenograft mice) and to be able to interpret them better, it is advantageous if the antibody to be used according to the invention not only binds the human target protein but also binds the species target protein in the species used for these studies. In one embodiment, the antibody used according to the invention which is cross-reactive with the antibody used according to the invention but is also cross-reactive with the human target protein of at least one additional species. For toxicological studies and efficacy studies, species of the rodent, dog and non-human primate families are especially preferred. Preferred rodent species include the mouse and the rat. Preferred non-human primates include Rhesus monkeys, chimpanzees and long-tailed macaques.

In one embodiment, the antibody used according to the invention is also cross-reactive with the target protein of at least one additional species in addition to being cross-reactive with the human target protein, said additional species being selected from the group of species consisting of the mouse, the rat and the long-tailed macaque (*Macaca fascicularis*). Preferred antibodies in particular are those that are used according to the invention and are cross-reactive with at least the mouse target protein in addition to being cross-reactive with the human target protein. The preferred cross-reactive antibodies are those whose affinity for the target protein of the additional non-human species does not differ from the affinity for the human target protein by more than a factor of 50, in particular not more than a factor of 10.

The antibodies used according to the invention are additionally preferably characterized by invariant binding to mesothelin. Invariant binding is characterized, for example, in that the antibody used according to the invention binds to an epitope of mesothelin, which cannot be masked by another extracellular protein. Such an additional extracellular protein is, for example, the ovarian cancer antigen 125 protein (CA125). The antibodies used are preferably characterized in that their binding to mesothelin is not blocked by CA125.

Anti-mesothelin antibodies are described, for example, in WO 2009/068204. These antibodies may be used according to the invention.

Another aspect of the present invention is providing a novel anti-mesothelin antibody (MF-Ta) whose amino acid sequence comprises the CDR sequences of the variable heavy chain represented by the sequences SEQ ID NO:398 (HCDR1), SEQ ID NO:399 (HCDR2) and SEQ

ID NO:400 (HCDR3) and the CDR sequences of the variable light chain represented by the sequences SEQ ID NO:401 (LCDR1), SEQ ID NO:402 (LCDR2) and SEQ ID NO:403 (LCDR3).

5 In a preferred embodiment, the amino acid sequence of the anti-mesothelin antibody MF-Ta or the antigen-binding antibody fragment comprises a sequence of the variable heavy chain represented by the sequences SEQ ID NO: 404 and the sequence of the variable light chain represented by the sequence SEQ ID NO: 405. In a preferred embodiment, the amino acid sequence of the anti-mesothelin antibody MF-Ta or the antigen-binding antibody fragment comprises the sequence of the variable heavy chain, which is coded by the nucleic acid sequence
10 SEQ ID NO: 406 and the sequence of the variable light chain, which is coded by the nucleic acid sequence SEQ ID NO: 407.

In an especially preferred embodiment, the amino acid sequence of the anti-mesothelin antibody MF-Ta comprises the sequence of the heavy chain represented by the sequences SEQ ID NO: 408 and the sequence of the light chain represented by the sequence SEQ ID NO: 409.

15 In an especially preferred embodiment, the amino acid sequence of the anti-mesothelin antibody MF-Ta comprises the sequence of the heavy chain, which is coded by the nucleic acid sequence SEQ ID NO: 410 and the sequence of the light chain, which is coded by the nucleic acid sequence SEQ ID NO: 411.

20 Additional examples of antibodies that bind the cancer target molecule mesothelin are familiar to those skilled in the art and are described in WO 2009/068204, for example, and can be used for the binder-drug conjugates according to the invention.

In one embodiment of the binder-drug conjugates, the binder is an anti-mesothelin antibody or an antigen-binding antibody fragment wherein the antibody binds to mesothelin and has invariant binding.

25 In one embodiment, the binder-drug conjugate comprises an anti-mesothelin antibody or an antigen-binding antibody fragment comprises the amino acid sequences of the three CDR regions of the light chain and the amino acid sequences of the three CDR regions of the heavy chain of an antibody as described in WO 2009/068204 A1 (Table 7, pages 61-63).

30 In a preferred embodiment, the mesothelin antibodies or the antigen-binding antibody fragments are selected from the group consisting of:

anti-mesothelin antibodies or antigen-binding antibody fragments which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody MF-Ta,

5 anti-mesothelin antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody MF-J (WO 2009068204 A1; Table 7, page 61),

anti-mesothelin antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody MOR06640 (WO 2009/068204 A1; Table 7, page 61),

10 anti-mesothelin antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody MF-226 (WO 2009/068204 A1; Table 7, page 61), and

15 anti-mesothelin antibodies or antigen-binding antigen fragments thereof, which comprise the sequences of the three CDR regions of the light chain and the sequences of the three CDR regions of the heavy chain of the antibody MOR06626 (WO 2009/068204-A1; Table 7, page 61).

In an especially preferred embodiment the mesothelin antibodies or antigen-binding antibody fragments are selected from the group

20 anti-mesothelin antibodies or antigen-binding antibody fragments thereof comprising the sequence of the variable light chain and the sequence of the variable heavy chain of the antibody MF-Ta,

anti-mesothelin antibodies or antigen-binding antibody fragments thereof comprising the sequence of the variable light chain and the sequence of the variable heavy chain of the antibody MF-J (WO 2009/068204 A1; Table 7, page 61),

25 anti-mesothelin antibodies or antigen-binding antibody fragments thereof comprising the sequence of the variable light chain and the sequence of the variable heavy chain of the antibody MOR06640 (WO 2009/068204 A1; Table 7, page 61),

30 anti-mesothelin antibodies or antigen-binding antibody fragments thereof comprising the sequence of the variable light chain and the sequence of the variable heavy chain of the antibody MF-226 (WO 2009/068204 A1; Table 7, page 61), and

anti-mesothelin antibodies or antigen-binding antibody fragments thereof comprising the sequence of the variable light chain and the sequence of the variable heavy chain of the antibody MOR06626 (WO 2009/068204 A1; Table 7, page 61).

Additional antibodies:

- 5 Trastuzumab (Genentech) is an example of antibody that binds the cancer target molecule HER2. Trastuzumab is a humanized antibody used for treatment of breast cancer among other things. Rituximab (Genentech) is an example of an antibody that binds the cancer target molecule CD20. Rituximab (CAS No. 174722-31-7) is a chimeric antibody used for treatment of non-Hodgkin's lymphoma. Alemtuzumab (Genzyme) is an example of an antibody that binds the cancer target
10 molecule CD52. Alemtuzumab (CAS No. 216503-57-0) is a humanized antibody that is used for treatment of chronic lymphatic leukemia.

- Additional examples of antibodies that bind to HER2 in addition to trastuzumab (INN No. 7637, CAS No. 180288-69-1) and pertuzumab (CAS No.: 380610-27-5) also include antibodies such as those proposed in WO 2009123894 A2, WO2008140603 A2 or WO 2011044368 A2. One
15 example of anti-HER2 conjugate is trastuzumab emtansine (INN No. 9295).

Examples of antibodies that bind the cancer target molecule CD30 and can be used for treatment of cancer, e.g., Hodgkin's lymphoma include brentuximab, iratumumab and antibodies as disclosed in WO 2008092117, WO 2008036688 or WO 2006089232. An example of an anti-CD30 conjugate is brentuximab vedotin (INN No. 9144).

- 20 Examples of antibodies that bind the cancer target molecule CD22 and can be used for treatment of cancer, e.g., lymphoma include inotuzumab or epratuzumab. Examples of anti-CD22 conjugates include inotuzumab ozagamycin (INN No. 8574) or anti-CD22-MMAE and anti-CD22-MC-MMAE (CAS No.: 139504-50-0 and/or 474645-27-7).

- Examples of antibodies that bind the cancer target molecule CD33 and can be used for treatment
25 of cancer, e.g., leukemia include gemtuzumab or lintuzumab (INN No. 7580). One example of an anti-CD33 conjugate is gemtuzumab ozagamycin.

One example of an antibody that binds the cancer target molecule NMB and can be used for treatment of cancer, e.g., melanoma or breast cancer is glembatumumab (INN No. 9199). One example of an anti-NMB conjugate is glembatumumab vedotin (CAS No.: 474645-27-7).

- 30 One example of an antibody that binds the cancer target molecule CD56 and can be used for treatment of cancer, e.g., multiple myeloma, small-cell lung carcinoma, MCC or ovarian

carcinoma is lorvotuzumab. One example of an anti-CD56 conjugate is lorvotuzumab mertansine (CAS No.:139504-50-0).

5 Examples of antibodies that bind the cancer target molecule CD70 and can be used for treatment of cancer, e.g., non-Hodgkin's lymphoma or renal cell cancer are disclosed in WO 2007038637 A2 or WO 2008070593 A2. One example of an anti-CD70 conjugate is SGN-75 (CD70 MMAF)

One example of an antibody that binds the cancer target molecule CD74 and can be used for treatment of cancer, e.g., multiple myeloma, is milatuzumab. One example of an anti-CD74 conjugate is milatuzumab doxorubicin (CAS No.: 23214-92-8).

10 One example of an antibody that binds the cancer target molecule CD19 and can be used for treatment of cancer, e.g., non-Hodgkin's lymphoma is disclosed in WO 2008031056 A2. Additional antibodies and examples of an anti-CD19 conjugate (SAR3419) are disclosed in WO 2008047242 A2.

15 Examples of antibodies that bind the cancer target molecule Mucin-1 and can be used for treatment of cancer, e.g., non-Hodgkin's lymphoma include clivatuzumab or the antibodies disclosed in WO 2003106495 A2, WO 2008028686 A2. Examples of anti-mucin conjugates are disclosed in WO 2005009369 A2.

20 Examples of antibodies that bind the cancer target molecule CD138 and conjugate thereof that can be used for treatment of cancer, e.g., multiple myeloma are disclosed in WO 2009080829 A1, WO 2009080830 A1.

25 Examples of antibodies that bind the cancer target molecule integrin alphaV and can be used for treatment of cancer, e.g., melanoma, sarcoma or carcinoma include intetumumab (CAS No.: 725735-28-4), abciximab (CAS No.: 143653-53-6), etaracizumab (CAS No.: 892553-42-3) or the antibodies disclosed in US 7,465,449 B2, EP 719859 A1, WO 2002012501 A1 or WO 2006062779 A2. Examples of anti-integrin alphaV conjugates include intetumumab DM4 and additional ADCs disclosed in WO 2007024536 A2.

30 Examples of antibodies that bind the cancer target molecule TGF1 and can be used for treatment of cancer include the antibodies disclosed in WO 2002077033 A1, US 7,318,924, WO 2003083041 A2 and WO 2002088170 A2. Examples of anti-TGF1 conjugates are disclosed in WO2002088170-A2.

Examples of antibodies that bind the cancer target molecule and can be used for treatment of cancer, e.g., prostatic carcinoma are the antibodies disclosed in WO 199735615 A1, WO 199947554 A1 and WO 2001009192 A1. Examples of anti-PSMA conjugates are disclosed in WO 2009026274 A1.

- 5 Examples of antibodies that bind the cancer target molecule EPHA2 and can be used for producing a conjugate and for treatment of cancer are disclosed in WO 2004091375 A2.

Examples of antibodies that bind the cancer target molecule SLC44A4 and can be used for producing a conjugate and for treatment of cancer, e.g., pancreatic or prostatic carcinoma, are disclosed in WO 2009033094 A2 and US 20090175796 A1.

- 10 One example of an antibody that binds the cancer target molecule HLA-DOB is the antibody Lym-1 (CAS No.: 301344-99-0), which can be used for treatment of cancer, e.g., non-Hodgkin's lymphoma. Examples of anti-HLA-DOB conjugates are disclosed, for example, in WO 2005081711 A2.

- 15 Examples of antibodies that bind the cancer target molecule VTCN1 and can be used for producing a conjugate and for treatment of cancer, e.g., ovarian cancer, pancreatic, lung cancer or breast cancer are disclosed in WO 2006074418 A2.

- An example of an antibody that can be used to bind the cancer target molecule PDL1 is the antibody 3G10 from patent WO 2007005874 A2. The antibody 3G10 may be used, for example, in the human IgG1 format and as the anti-PDL1 used in the exemplary embodiments where the
20 antibody comprises the following sequences;

Light chain:

- EIVLTQSPATLSLSPGERATLSCRASQSVSSYLVWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISLEPEDFAVYYCQQRSNWPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQL
KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD
25 YEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

- QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYGFSWVRQAPGQGLEWMGWITAYNGNT
NYAQLKQGRVTMTTDTSTSTVYMEIRSLRSDDTAVYYCARDYFYGMVWGQGTITVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
30 GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPG

- 5 An example of an antibody that binds the cancer target molecule ICOSLG is the antibody 16H (SEQ ID NOS: 70 and 45) from WO 2007011941 A2. Anti-ICOSLG antibody 16H may be used in the human IgG1 format and as the anti-ICOSLG used in the exemplary embodiments comprising the following sequences:

Light chain:

10 DIQMTQSPSSLSASVGDRVTITCRASQGISNWLAWYQQKPEKAPKSLIYAASSLQSGVPSRF
SGSGSGTDFTLTISLQPEDFATYYCQQYDSYPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQL
KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

15 EVQLVESGGGLVQPGGSLRLSCAGSGFTFSSYWMSWVRQAPGKGLEWVAYIKQDGNK
YYVDSVKGRFTISRDNAKKSLYLQMNSLRAEDTAVYYCAREGILWFGDLPTFWGQGILVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
20 ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG

- 25 One example of an antibody that binds the target molecule FGFR3 is the antibody 15D8 (SEQ ID NO: 74 for the heavy chain and SEQ ID NO: 76 for the light chain) from WO 2010002862 A2. Anti-FGFR3 antibody 15D8 may be used in the human IgG1 format, for example, and as the anti-FGFR3 used in the exemplary embodiment, this antibody comprises the following sequences:

Light chain:

30 DIQLTQSPSSLSASVGDRVTITCSASSSVSYMYWFQQKPGKAPKPLIYLTSLASGV
PSRFSGSGSGTDFTLTISLQPEDFATYYCQQWSSYPLTFGGGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYNMYWVRQMPGKGLEWMGYIDPYNGGTS
YNQKFKGKATLTVDKSISTAYLQWSSLKASDTAMYYCAREGGNYEAWFAYWGQGTTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
5 SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPG

- 10 One example of an antibody that binds the target cancer molecule 5,6-dihydroxyindole-2-carboxylic acid oxidase (TYRP1) is the antibody 20D7S (SEQ ID NOS: 30 and 32) from patent WO 2009114585 A1. In the exemplary embodiments, anti-TYRP1 antibody 20D7S may be used in human IgG1 format, for example, and when used as the anti-TYRP1, the antibody comprises the following sequences:

15 Light chain

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISLEPEDFAVYYCQQRSNWLMYTFGQGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDDSTYSLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

20 Heavy chain:

QVQLVQSGSELKKPGASVKISCKASGYTFTSYAMNWVRQAPGQGLESMDGWINTNTGNPT
YAQGFTGRFVFSMDTSVSTAYLQISSLKAEDTAIYYCAPRYSSSWYLDYWGQGTTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
25 VFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPG

- 30 One example of an antibody that binds the cancer target molecule glypican-3 is the antibody that is known from the patent US 07776329 B2 and comprises the amino acid sequences SEQ ID NOS: 84 and 92 (human mouse chimera). The anti-glypican-3 antibody described above may be

used in human IgG1 format, for example, and as the anti-glypican-3 used in the exemplary embodiments, this antibody has the following sequences:

Light chain:

DVVMTQSPLSLPVTGPGEPAISCRSSQSLVHSNGNTYLHWYLQKPGQSPQLLIYKVSNRFS
5 GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQNTHPPTFGQGTKLEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

QVQLVESGAIEVKKPGASVKVSCKASGYTFDYMHWVRQAPGQGLEWMGALDPKTDG
10 TAYSQKFVKGRVTITADESTSTAYMELSSLRSEDTAVYYCARFYSYTYWGQGLTVTVSSAS
TKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVELTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
15 QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPG

The compounds according to the invention have valuable pharmacological properties and can be used to prevent and treat diseases in humans and animals.

The binder-drug conjugates (ADCs) of formula (Ia) according to the invention have a high and
20 specific cytotoxic activity with respect to tumor cells which can be demonstrated on the basis of
assays performed in the present experimental part (C-1 to C-7e). This high and specific cytotoxic
activity of the binder-drug conjugates (ADCs) of formula (Ia) according to the invention is
achieved by the suitable combination of the novel N,N-dialkylauristatin derivatives and binders
with linkers which have both an enzymatic, hydrolytic or reductively cleavable intended
25 breaking point for release of the toxophore as well as those not having any such intended
breaking point. The effect on the tumor cell is delineated very specifically by using stable linkers
in particular, which do not have any intended breaking points that can be cleaved enzymatically,
hydrolytically or reductively to release the toxophore and which still remain entirely or partially
intact after receiving the ADC into the tumor cell and after complete intracellular enzymatic
30 degradation of the antibody. The compatibility of ADCs with stable linkers presupposes, among
things, that the metabolites formed intracellularly will be formed with enough efficiency to reach
their target and be able to manifest their antiproliferative effect on the target there in adequate

potency without first being removed from the tumor cell by transporter proteins. The metabolites formed intracellularly after incorporation of the compounds of formula (Ia) according to the invention have a reduced potential as a substrate with respect to transporter proteins, so that a redistribution to the systemic circulation and thus the triggering of potential adverse effects are suppressed by the toxophore itself. In addition the basic character at the amino terminus of the monomethylauristatin peptide is preserved by the novel N-alkyl binding. In particular with the binder-drug conjugates (ADCs) of formula (Ia) according to the invention, the total charge of the antibody remains constant regardless of the number of toxophore-linker charges.

The compatibility of the ADCs with a stable linker chemistry and the respective target in conjunction with metabolites that represent a substrate for transporter proteins to a slight extent offers an enlarged therapeutic window.

Because of this profile of properties, the compounds according to the invention are therefore suitable to a particular extent for treatment of hyperproliferative diseases in humans and infants in general. These compounds may on the one hand block, inhibit, reduce or decrease cell proliferation and cell division on the one hand while on the other hand potentiating the apoptosis.

The hyperproliferative diseases for treatment of which the compounds according to the invention may be used include in particular the group of cancer and tumor diseases. These are understood to include in particular the following diseases within the scope of the present invention without being limited to these: breast cancer and breast tumors (ductile and lobular forms, also in situ), respiratory tract tumors (small cell and non-small-cell carcinomas, bronchial carcinoma), brain tumors (e.g., of the brain stem and the hypothalamus, astrocytoma, medulloblastoma, ependymoma and neuroectodermal and pineal tumors), tumors of the digestive tract (esophagus, stomach, gallbladder, small intestine, large intestine, rectum), liver tumors (including hepatocellular carcinoma, cholangiocarcinoma and mixed hepatocellular cholangiocarcinoma), tumors of the head and neck area (larynx, hypopharynx, nasopharynx, oropharynx, lips and oral cavity), skin tumors (squamous epithelial carcinoma, Kaposi's sarcoma, malignant melanoma, Merkel cell skin cancer and non-melanoma type skin cancer), tumors of the soft tissues (including soft tissue sarcomas, malignant fibrous histiocytoma, lymphosarcoma and rhabdomyosarcoma), tumors of the eyes (including intraocular melanoma and retinoblastoma), tumors of the endocrine and exocrine glands (e.g., thyroid and parathyroid glands, pancreatic gland and esophageal gland), tumors of the urinary tract (bladder, penis, kidney, renal pelvis and urethral tumors) as well as tumors of the reproductive organs (endometrium, cervical, ovarian, vaginal, vulval and uterine carcinomas in the woman and prostatic and testicular carcinomas in males). These also include proliferative blood diseases in solid form and as circulating blood

cells such as lymphomas, leukemias and myeloproliferative diseases, e.g., acute myeloid leukemia, acute lymphoblastic, chronic lymphocytic leukemia, chronic myelogenous leukemia and hairy cell leukemia as well as AIDS-related lymphomas, Hodgkin's lymphomas, non-Hodgkin's lymphomas, cutaneous T-cell lymphomas, Burkitt's lymphomas and lymphomas of the
5 central nervous system.

Preferred hyperproliferative diseases for anti-CA9 binder-drug conjugates

Hyperproliferative diseases for the treatment of which the compounds according to the invention may preferably be used include CA9 overexpressing tumors, breast cancers and breast tumors (ductile and lobular forms, also in situ); respiratory tract tumors (small cell and non-small-cell
10 carcinomas, bronchial carcinomas), of which preferably non-small-cell lung carcinoma; brain tumors (e.g., of the brain stem and of the hypothalamus, astrocytoma, medulloblastoma, ependymoma and/or neuroectodermal and pineal tumors); tumors of the digestive organs (esophagus, stomach, gallbladder, small intestine, large intestine, rectum), of which those that are especially preferred are stomach and intestinal tumors; liver tumors (including hepatocellular
15 carcinoma, cholangiocarcinoma and mixed hepatocellular cholangiocarcinoma); tumors of the head and neck area (larynx, hypopharynx, nasopharynx, oropharynx, lips, oral cavity, tongue and esophagus); tumors of the urinary tract (bladder, penis, kidney, renal pelvis and urethral tumors), of which the tumors of the kidneys and bladder are especially preferred; and/or tumors of the reproductive organs (endometrial, cervical, ovarian, vaginal, vulval and uterine carcinomas of the
20 woman and/or prostatic and testicular carcinomas in the man), of which cervical and uterine carcinomas are especially preferred.

Preferred hyperproliferative diseases for anti-EGFR binder-drug conjugates

Hyperproliferative diseases for the treatment of which the compounds according to the invention may preferably be used include EGFR overexpressing tumors, respiratory tract tumors (e.g.,
25 small cell and non-small-cell carcinoma, bronchial carcinoma), of which non-small-cell lung carcinoma is especially preferred; tumors of the digestive tract (e.g., esophagus, stomach, gallbladder, small intestine, large intestine, rectum), of which the intestinal tumors are especially preferred; tumors of the endocrine and exocrine glands (e.g., thyroid and parathyroid glands, pancreatic gland and salivary gland), of which the pancreas is preferred; tumors of the head and
30 neck area (e.g., larynx, hypopharynx, nasopharynx, oropharynx, lips, oral cavity, tongue and esophagus); and/or gliomas.

Preferred hyperproliferative diseases for anti-mesothelin binder-drug conjugates

Hyperproliferative diseases for treatment of which the compounds according to the invention are preferably used include mesothelin overexpressing tumors, tumors of the reproductive organs (endometrial, cervical, ovarian, vaginal, vulva and uterine carcinomas in the woman and/or
5 prostatic and testicular carcinomas in the man) of which ovarian carcinomas are preferred; tumors of the endocrine and exocrine glands (e.g., thyroid and parathyroid glands, pancreatic gland and salivary gland), of which the pancreas is preferred; respiratory tract tumors (e.g., small cell and non-small-cell carcinomas, bronchial carcinoma), of which non-small lung cancer is preferred; and/or mesotheliomas.

10 Preferred hyperproliferative diseases for anti-C4.4a binder-drug conjugates

Hyperproliferative diseases for treatment of which the compounds according to the invention are preferably used include C4.4a hyperexpressing tumors, squamous epithelial cell carcinomas (e.g., of the cervix, vulva, vagina, the anal canal, endometrium, fallopian tube, penis, scrotum, esophagus, breast, bladder, bile duct, endometrium, uterus and ovaries); breast cancer and breast
15 tumors (e.g., ductal and lobular forms, also *in situ*); respiratory tract tumors (e.g., small-cell and non-small-cell carcinomas, bronchial carcinoma), of which the non-small-cell lung cancer is preferred along with squamous epithelial cell and adenocarcinomas of the lungs; tumors of the head and neck area (e.g., larynx, hypopharynx, nasopharynx, oropharynx, lips, oral cavity, tongue and esophagus, squamous epithelial cell carcinomas of the head and neck area); tumors of
20 the urinary tract (bladder, penis, kidney, renal pelvis and urethral tumors, squamous epithelial cell carcinomas of the bladder), of which tumors of the kidney and bladder are especially preferred; skin tumors (squamous epithelial cell carcinoma, Kaposi's sarcoma, malignant melanoma, Merkel cell skin cancer and non-melanoma-type skin cancer), of which melanomas are especially preferred; tumors of the endocrine and exocrine glands (e.g., thyroid and
25 parathyroid glands, pancreatic gland and salivary gland), of which the pancreas is preferred; tumors of the digestive organs (e.g., esophagus, stomach, gallbladder, small intestine, large intestine, rectum), of which colorectal carcinomas are especially preferred; and/or tumors of the reproductive organs (endometrial, cervical, ovarian, vaginal, vulval and uterine carcinomas in the woman and/or prostatic and testicular carcinomas in the man), of which uterine carcinomas are
30 especially preferred.

These human diseases that have been described extensively can also occur with a comparable etiology in other mammals and can be treated there using the compounds according to the present invention.

The terms "treatment" or "to treat" is used in the conventional sense within the scope of this invention and refers to the care, treatment and consultation of a patient with the goal of combatting, reducing, diminishing or ameliorating a disease or health deviation and improving the quality of life which is impaired by this disease such as, for example, in a cancer.

- 5 An additional subject matter of the present invention is thus the use of the compounds according to the invention for treatment and/or prevention of diseases, in particular the diseases cited above.

An additional subject matter of the present invention is the use of the compounds according to the invention for producing a pharmaceutical drug for treatment and/or prevention of diseases, in
10 particular the diseases cited above.

An additional subject matter of the present invention is the use of the compounds according to the invention in a method for treatment and/or prevention of diseases, in particular the diseases cited above.

- 15 An additional subject matter of the present invention is a method for treatment and/or prevention of diseases, in particular the diseases cited above, using an effective amount of at least one of the compounds according to the invention.

The inventor-drug conjugate is preferably used for treating cancer in a patient, where the cancer cells of the patient to be treated express the target (preferably EGFR, CA9, mesothelin or C4.4a), preferably having a greater expression of this target than in non-tumorous tissue.

- 20 A method for identifying patients who will response advantageously to an anti-target binder-drug conjugate for treatment of cancer includes determining the target expression in the patient's cancer cells. In one embodiment, the target expression is determined by target gene expression analysis. Those skilled in the art are familiar with methods for gene expression analysis such as RNA detection, quantitative or qualitative polymerase chain reaction or fluorescence in situ
25 hybridization (FISH). In another preferred embodiment, the target expression is determined by means of immunohistochemistry using an anti-target antibody. Immunohistochemistry is preferably performed on tissue fixed in formaldehyde. The antibody used for the immunohistochemistry is the same antibody also used in the conjugate. The antibody used for the immunohistochemistry is a second antibody that recognizes the target protein/target, preferably
30 specifically.

The compounds according to the invention may be used alone or, if necessary, in combination with one or more other pharmacologically active substances as long as this combination does not

lead to adverse and unacceptable effects. Another subject matter of the present invention therefore relates to pharmaceutical drugs containing at least one of the compounds according to the invention and one or more additional active ingredients, in particular for treating and/or preventing the diseases listed above.

- 5 For example, the compounds according to the invention may be combined with known anti-hyperproliferative, cytostatic or cytotoxic for treatment of cancer. Suitable combination active ingredients and drugs that can be listed as examples include:

aldesleukin, alendronic acid, alfaferone, alitretinoin, allopurinol, aloprim, aloxi, altretamine, amino glutethimide, amifostine, amrubicin, amsacrine, anastrozol, anzmet, aranesp, arglabin,
 10 arsentrioxide, aromasine, 5-azacytidine, azathioprine, BCG or tice-BCG, bestatin, betamethasone acetate, betamethasone sodium phosphate, bexarotene, bleomycin sulfate, broxuridine, bortezomib, busulfane, calcitonine, campath, capecitabine, carboplatin, casodex, cefesone, celmoleukin, cerubidine, chlorambucil, cisplatin, cladribine, clodronic acid, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunoxome, decadrone, decadrone phosphate,
 15 delestrogen, denileukin diftitox, depo medrol, desloreline, dexrazoxane, diethylstilbestrol, diflucan, docetaxel, doxifluridine, doxorubicin, dronabinol, DW-166HC, eligard, elitek, ellence, emend, epirubicin, epoetin alfa, epogen, eptaplatin, ergamisole, estrace, estradiol, estramustine sodium phosphate, ethinyl estradiol, ethylol, etidronic acid, etopophos, etoposide, fadrozole, farstone, filgrastim, finasteride, fligastim, floxuridine, fluconazole, fludarabin,
 20 5-fluorodeoxyuridine monophosphate, 5-fluorouracil (5-FU), fluoxymesterone, flutamide, formestane, fosteabine, fotemustine, fulvestrant, gammagard, gemcitabine, gemtuzumab, gleevec, gliadel, gosereline, granisetron hydrochloride, histreline, hycamtine, hydrocortone, erythrohydroxynonyladenine, hydroxyurea, ibritumomab tiuxetan, idarubicin, ifosfamide, interferon-alpha, interferon-alpha-2, interferon-alpha-2 α , interferon-alpha-2 β , interferon-alpha-
 25 n1, interferon-alpha-n3, interferon-beta, interferon-gamma-1 α , interleukin-2, intron A, iressa, irinotecan, kytril, lentinane sulfate, letrozole, leucovorine, leuprolide, leuprolide acetate, levamisole, levofolic acid calcium salt, levothroid, levoxyl, lomustine, lonidamine, marinol, mechlorethamine, mecobalamine, medroxyprogesterone acetate, megestrole acetate, melphalan, menest, 6-mercaptopurine, mesna, methotrexate, metvix, miltefosine, minocycline, mitomycin C,
 30 mitotane, mitoxantrone, modrenal, myocet, nedaplatin, neulasta, neumega, neupogen, nilutamide, nolvadex, NSC-631570, OCT-43, octreotide, ondansetron hydrochloride, orapred, oxaliplatin, paclitaxel, pediapred, pegaspargase, pegasys, pentostatin, picibanil, pilocarpine hydrochloride, pirarubicin, plicamycin, porfimer sodium, prednimustine, prednisolone, prednisone, premarin, procarbazine, procrit, raltitrexed, rebif, rhenium 186 etidronate, rituximab, roferone A,
 35 romurtide, salagen, sandostatin, sargramostim, semustine, sizofiran, sobuzoxane, solu-medrol,

streptozocine, strontium-89 cehlorid, synthroid, tamoxifen, tamsulosine, tasonermine, tastolactone, taxoter, teceleukin, temozolomide, teniposide, testosterone propionate, testred, thioguanine, thiotepa, thyrotropin, tiludronic acid, topotecan, toremifen, tositumomab, tastuzumab, teosulfane, tretinoin, trexall, trimethylmelamine, trimetrexate, triptoreline acetate, 5 triptoreline pamoate, uft, uridine, valrubicin, vesnarinone, vinblastine, vincristine, vindesine, vinorelbine, virulizine, zinecard, zinostatin stimalamer, zofran; ABI-007, acolbifene, actimmune, affinitak, aminopterin, arzoxifene, asoprisnil, atamestane, atrasentane, avastin, BAY 43-9006 (sorafenib), CCI-779, CDC-501, celebrex, cetuximab, crisnatol, cyproterone acetate, decitabin, DN-101, doxorubicin MTC, dSLIM, dutasteride, edotecarin, eflornithine, exatecane, fenretinide, 10 histamine dihydrochloride, histreline hydrogel implant, holmium-166-DOTMP, ibandronic acid, interferon-gamma, intron PEG, ixabepilone, keyhole limpet hemocyanine, L-651582, lanreotide, lasofoxifen, libra, lonafarnib, miproxifen, minodronate, MS-209, liposomales MTP-PE, MX-6, nafareline, nemorubicin, neovastat, nolatrexed, oblimersen, onko-TCS, osidem, paclitaxel polyglutamate, pamidronate disodium, PN-401, QS-21, quazepam, Rr-1549, raloxifene, 15 ranpirnase, 13-*cis*-retinic acid, satraplatin, seocalcitol, T-138067, tarceva, taxoprexine, thymosine-alpha-1, tiazofurine, tipifarnib, tirapazamine, TLK-286, toremifene, transmid 107R, valspodar, vapreotide, vatalanib, verteporfin, vinflunine, Z-100, zoledronic acid as well as combinations thereof.

In a preferred embodiment, the compounds according to the present invention may be combined 20 with antihyperproliferative agents, which may include the following, for example, although this list is not conclusive:

aminogluthethimide, L-asparaginase, azathioprine, 5-azacytidine, bleomycin, busulfan, carboplatin, carmustin, chlorambucil, cisplatin, colaspase, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, diethylstilbestrol, 2',2'-difluorodeoxycytidine, 25 docetaxel, doxorubicin (adriamycin), epirubicin, epothilone and seine derivate, erythrohydroxynonyladenine, ethinyl estradiol, etoposide, fludarabine phosphate, 5-fluorodeoxyuridine, 5-fluorodeoxyuridine monophosphate, 5-fluoruracil, fluoxymesterone, flutamide, hexamethyl melamine, hydroxyurea, hydroxyprogesterone caproate, idarubicin, ifosfamide, interferon, irinotecan, leucovorin, lomustine, mechlorethamine, 30 medroxyprogesterone acetate, megestrol acetate, melphalan, 6-mercaptapurine, mesna, methotrexate, mitomycin C, mitotane, mitoxantrone, paclitaxel, pentostatin, n-phosphonoacetyl-L-aspartate (PALA), plicamycin, prednisolone, prednisone, procarbazine, raloxifene, semustine, streptozocine, tamoxifen, teniposide, testosterone propionate, thioguanine, thiotepa, topotecan, trimethylmelamine, uridine, vinblastine, vincristine, vindesine and vinorelbine.

In one very promising aspect, the compounds according to the invention can also be combined with biological therapeutic agents such as antibodies (e.g., Avastin, Rituxan, Erbitux, Herceptin). The compounds according to the invention may also achieve positive effects in combination with treatments directed against angiogenesis, for example, with Avastin, axitinib, recentin,
5 regorafenib, sorafenib or sunitinib. Combinations with inhibitors of the proteasome and of mTOR as well as combinations with antihormones and steroidal metabolic enzyme inhibitors are also especially suitable because of their favorable profile of side effects.

In general, the following goals can be pursued with the combination of compounds of the present invention with other active cytostatic or cytotoxic agents:

- 10 • an improved efficacy in retarding the growth of a tumor, in reducing its size or even completely eliminating it in comparison with a treatment with a single active ingredient;
- the possibility of using the chemotherapeutic agents that are used in a lower dosage than in monotherapy;
- the possibility of a tolerable therapy with few adverse effects in comparison with an
15 individual administration;
- the possibility of treatment of a broader spectrum of tumors;
- achieving a higher response rate to the treatment;
- a longer survival time for the patients in comparison with today's standard therapy.

In addition, the compounds according to the invention may also be used in combination with
20 radiation therapy and/or a surgical intervention.

An additional subject matter of the present invention is pharmaceutical drugs that contain at least one compound according to the invention, usually together with one or more inert nontoxic pharmaceutically suitable excipients as well as their use for the aforementioned purposes.

The compounds according to the invention may act systemically and/or locally. For this purpose
25 they are applied in a suitable way such as, for example, orally or parenterally. The compounds according to the invention may act systemically and/or locally. For this purpose they are applied in a suitable way such as, for example, parenterally, possibly by inhalation or as an implant and/or stent.

For these application methods, the compounds according to the invention may be administered in suitable dosage forms.

Parenteral administration may be performed in order to bypass a resorption step (e.g., intravenously, intra-arterially, intracardially, intraspinally or intralumbally) or with the inclusion
5 of resorption (e.g., intramuscularly, subcutaneously, intracutaneously, percutaneously or intraperitoneally). Suitable forms of administration for parenteral administration include infusion and injection preparations in the form of solutions, suspensions, emulsions or lyophilisates.

Parenteral administration in particular intravenous administration is preferred.

i.v. solution:

10 The compounds according to the invention may be converted to the dosage forms indicated. This may be done in the known way by "mixing with" and/or "dissolving in" inert nontoxic pharmaceutically suitable excipients (e.g., buffer substances, stabilizers, solubilizer, preservatives). These may include, for example: amino acids (glycine, histidine, methionine, arginine, lysine, leucine, isoleucine, threonine, glutamic acid, phenylalanine and others), sugars
15 and related substances (glucose, saccharose, mannitol, trehalose, sucrose, mannose, lactose, sorbitol), glycerol, sodium, potassium, ammonium and calcium salts (e.g., NaCl, KCl or Na₂HPO₄ and many more), acetate/acetic acid buffer systems, phosphate buffer systems, citric acid and citrate buffer systems, trometamol (TRIS and TRIS salts), polysorbates (e.g., polysorbate 80 and polysorbate 20), poloxamers (e.g., poloxamer 188 and poloxamer 171),
20 macrogols (PEG derivatives, e.g., 3350), Triton X-100, EDTA salts, glutathione, albumins (e.g., human), urea, benzyl alcohol, phenol, chlorocresol, metacresol, benzalkonium chloride and many others.

It has proven advantageous in general to administer doses of approx. 0.001 to 1 mg/kg, preferably approx. 0.01 to 0.5 mg/kg body weight to achieve effective results in parenteral
25 administration.

Nevertheless, it may be necessary under some circumstances to deviate from the stated amounts, namely depending on the body weight, the method of administration, the individual response to the active ingredient, the type of preparation and the point in time or interval at which the application occurs. Thus, in many cases, it may be sufficient to use less than the aforementioned
30 minimum amount, whereas in other cases the aforementioned upper limit must be exceeded. In the case of administration of larger amounts, it may be advisable to distribute the larger amount among several individual doses throughout the day.

The following examples are presented to illustrate the invention, although the invention is not limited to these examples.

The percentage amounts specified in the following tests and examples are percent by weight (wt%), unless otherwise indicated; parts refer to parts by weight. Solvent ratios, dilution ratios

5 and concentration specifications for liquid-liquid solutions are each based on volume.

A. Examples

Abbreviations and acronyms:

A431NS	human tumour cell line
A549	human tumour cell line
ABCB1	ATP-binding cassette sub-family B member 1 (synonym for P-gp and MDR1)
abs.	absolute
ADC	antibody-drug-conjugate
Ac	acetyl
aq.	aqueous, aqueous solution
ATP	adenosine triphosphate
BCRP	breast cancer resistance protein, an efflux transporter
Boc	<i>tert</i> -butoxycarbonyl
br.	broad (in NMR)
Ex.	example
<i>ca.</i>	<i>circa</i> , approximately
CAIX	carboanhydrase IX
CI	chemical ionization (in MS)
d	doublet (in NMR)
d	day(s)
TLC	thin-layer chromatography
DCI	direct chemical ionization (in MS)
dd	doublet of a doublet (in NMR)
DMAP	4- <i>N,N</i> -dimethylaminopyridine
DME	1,2-dimethoxyethane
DMEM	Dulbecco's modified eagle medium (standardized nutrient medium for the cell culture)
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulphoxide

DPBS, D-PBS, PBS Dulbecco's phosphate-buffered saline solution
 PBS = DPBS = D-PBS, pH7.4, from Sigma, No. D8537

Composition:

0.2 g KCl
 0.2 g KH₂PO₄ (anhydrous)
 8.0 g NaCl
 1.15 g Na₂HPO₄ (anhydrous)
 make up to 1 l with H₂O

dt	doublet of a triplet (in NMR)
DTT	DL-dithiothreitol
EDC	<i>N</i> '-(3-dimethylaminopropyl)- <i>N</i> -ethylcarbodiimide hydrochloride
EGFR	epidermal growth factor receptor
EI	electron impact ionization (in MS)
ELISA	enzyme-linked immunosorbent assay
eq.	equivalent(s)
ESI	electrospray ionization (in MS)
ESI-MicroTofq	<u>ESI</u> - MicroTofq (name of the mass spectrometer, with ToF = time of flight and q = quadrupole)
FCS	foetal calf serum
Fmoc	(9 <i>H</i> -fluoren-9-ylmethoxy)carbonyl
sat.	Saturated
GTP	guanosine 5'-triphosphate
h	hour(s)
HATU	<i>O</i> -(7-azabenzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluroniumhexafluorophosphate
HCT-116	human tumour cell line
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulphonic acid
HOAc	acetic acid
HOBt	1-hydroxy-1 <i>H</i> -benzotriazole hydrate

HOSu	<i>N</i> -hydroxysuccinimide
HPLC	high-pressure, high-performance liquid chromatography
HT29	human tumour cell line
IC ₅₀	half-maximum inhibitory concentration
i.m.	intramuscular, administration into the muscle
i.v.	intravenous, administration into the vein
conc.	Concentrated
LC-MS	liquid chromatography-coupled mass spectrometry
LLC-PK1 cells	Lewis lung carcinoma pork kidney cell line
L-MDR	human MDR1 transfected LLC-PK1 cells
m	multiplet (in NMR)
MDR1	multidrug resistance protein 1
min	minute(s)
MS	mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2 <i>H</i> -tetrazolium bromide
NCI-H292	human tumour cell line
NCI-H520	human tumour cell line
NMM	<i>N</i> -methylmorpholine
NMP	<i>N</i> -methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance spectrometry
NMRI	mouse strain, originating from Naval Medical Research Institute (NMRI)
Nude mice	experimental animals
NSCLC	non-small cell lung cancer (non-small cell bronchial carcinoma)
PBS	phosphate-buffered saline solution
Pd/C	palladium on activated carbon
P-gp	P-glycoprotein, a transporter protein
PNGaseF	enzyme for sugar elimination
quant.	quantitative (for yield)
quart	quartet (in NMR)
quint	quintet (in NMR)
R _f	retention index (for TLC)
RT	room temperature
R _t	retention time (for HPLC)
s	singlet (in NMR)
s.c.	subcutaneous, administration beneath the skin

SCC-4	human tumour cell line
SCC-9	human tumour cell line
SCID mice	experimental mice with a severe combined immunodeficiency
t	triplet (in NMR)
<i>tert</i>	tertiary
TFA	trifluoroacetic acid
THF	tetrahydrofuran
UV	ultraviolet spectrometry
v/v	volumeto volume ratio (of a solution)
Z	benzyloxycarbonyl

HPLCand LC-MS methods:

Method 1 (LC-MS):

Instrument: Waters Acquity SQD UPLC System; column: Waters Acquity UPLC HSS T3 1.8μ
 5 50 mm x 1 mm; eluent A: 1 l water + 0.25 ml 99% strengthformic acid, eluent B: 1 l acetonitrile
 + 0.25 ml 99% strengthformic acid; gradient: 0.0 min 90% A → 1.2 min 5% A → 2.0 min 5% A;
 flow rate: 0.40 ml/min; oven: 50°C; UV detection: 210-400 nm.

Method 2 (LC-MS):

Instrument: Micromass QuattroPremier with Waters UPLC Acquity; column: Thermo Hypersil
 10 GOLD 1.9μ 50 mm x 1 mm; eluent A: 1 l water + 0.5 ml 50% strengthformic acid, eluent B: 1 l
 acetonitrile + 0.5 ml 50% strengthformic acid; gradient: 0.0 min 90% A → 0.1 min 90% A → 1.5
 min 10% A → 2.2 min 10% A; flow rate: 0.33 ml/min; oven: 50°C; UV detection: 210 nm.

Method 3 (LC-MS):

Instrument: Micromass Quattro Micro MS with HPLC Agilent Series 1100; column: Thermo
 15 Hypersil GOLD 3μ 20 mm x 4 mm; eluent A: 1 l water + 0.5 ml 50% strengthformic acid, eluent
 B: 1 l acetonitrile + 0.5 ml 50% strengthformic acid; gradient: 0.0 min 100% A → 3.0 min 10%
 A → 4.0 min 10% A → 4.01 min 100% A (flow rate 2.5 ml/min) → 5.00 min 100% A; oven:
 50°C; flow rate: 2 ml/min; UV detection: 210 nm.

Method 4 (LC-MS):

MS instrument: Micromass ZQ; HPLC instrument: HP 1100 Series; UV DAD; column: Phenomenex Gemini 3 μ 30 mm x 3.00 mm; eluent A: 1 l water + 0.5 ml 50% strengthformic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strengthformic acid; gradient: 0.0 min 90% A $\rightarrow$ 2.5 min 30% A $\rightarrow$ 3.0 min 5% A $\rightarrow$ 4.5 min 5% A; flow rate: 0.0 min 1 ml/min $\rightarrow$ 2.5 min/3.0 min/4.5 min 2 ml/min; oven: 50°C; UV detection: 210 nm.

Method 5 (HPLC):

Instrument: HP 1090 Series II; column: Merck Chromolith SpeedROD RP-18e, 50 mm x 4.6 mm; preliminary column: Merck Chromolith Guard Cartridge Kit RP-18e, 5 mm x 4.6 mm; injection volume: 5 μ l; eluent A: 70% HClO₄ in water (4 ml/litre), eluent B: acetonitrile; gradient: 0.00 min 20% B $\rightarrow$ 0.50 min 20% B $\rightarrow$ 3.00 min 90% B $\rightarrow$ 3.50 min 90% B $\rightarrow$ 3.51 min 20% B $\rightarrow$ 4.00 min 20% B; flow rate: 5 ml/min; column temperature: 40°C.

Method 6 (HPLC):

Instrument: Waters 2695 with DAD 996; column: Merck Chromolith SpeedROD RP-18e, 50 mm x 4.6 mm; Ord. No.: 1.51450.0001, preliminary column: Merck Chromolith Guard Cartridge Kit RP-18e, 5 mm x 4.6 mm; Ord. No.: 1.51470.0001, eluent A: 70% HClO₄ in water (4 ml/litre), eluent B: acetonitrile; gradient: 0.00 min 5% B $\rightarrow$ 0.50 min 5% B $\rightarrow$ 3.00 min 95% B $\rightarrow$ 4.00 min 95% B; flow rate: 5 ml/min.

Method 7 (LC-MS):

MS instrument: Waters ZQ; HPLC instrument: Agilent 1100 Series; UV DAD; column: Thermo Hypersil GOLD 3 μ 20 mm x 4 mm; eluent A: 1 l water + 0.5 ml 50% strengthformic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strengthformic acid; gradient: 0.0 min 100% A $\rightarrow$ 3.0 min 10% A $\rightarrow$ 4.0 min 10% A $\rightarrow$ 4.1 min 100% A (flow rate 2.5 ml/min); oven: 55°C; flow rate: 2 ml/min; UV detection: 210 nm.

Method 8 (LC-MS):

MS instrument: Waters ZQ; HPLC instrument: Agilent 1100 Series; UV DAD; column: Thermo Hypersil GOLD 3 μ 20 mm x 4 mm; eluent A: 1 l water + 0.5 ml 50% strengthformic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strengthformic acid; gradient: 0.0 min 100% A $\rightarrow$ 2.0 min 60% A $\rightarrow$ 2.3 min 40% A $\rightarrow$ 3.0 min 20% A $\rightarrow$ 4.0 min 10% A $\rightarrow$ 4.2 min 100% A (flow rate 2.5 ml/min); oven: 55°C; flow rate: 2 ml/min; UV detection: 210 nm.

Method 9 (LC-MS):

Instrument: Waters Acquity SQD UPLC System; column: Waters Acquity UPLC HSS T3 1.8µ
50 mm x 1 mm; eluent A: 1 l water + 0.25 ml 99% strengthformic acid, eluent B: 1 l acetonitrile
+ 0.25 ml 99% strengthformic acid; gradient: 0.0 min 95% A → 6.0 min 5% A → 7.5 min 5% A;
5 oven: 50°C; flow rate: 0.35 ml/min; UV detection: 210-400 nm.

Method 10 (HPLC):

Instrument: Agilent 1200 Series; column: Agilent Eclipse XDB-C18 5µ 4.6 mm x 150 mm;
preliminary column: Phenomenex KrudKatcher Disposable Pre-Column; injection volume: 5 µl;
eluent A: 1 l water + 0.01% trifluoroacetic acid; eluent B: 1 l acetonitrile + 0.01% trifluoroacetic
10 acid; gradient: 0.00 min 10% B → 1.00 min 10% B → 1.50 min 90% B → 5.5 min 10% B; flow
rate: 2 ml/min; column temperature: 30°C.

For all reactants or reagents whose preparation is not explicitly described below, they were
obtained commercially from generally available sources. For all other reactants or reagents
whose preparation is likewise not described below, and which were not available commercially
15 or were obtained from sources which are not generally available, a reference is given to the
published literature in which their preparation is described.

Method 11 (LC-MS):

Instrument: Waters ACQUITY SQD UPLC System; column: Waters Acquity UPLC HSS T3 1.8
µ 30 x 2 mm; eluent A: 1 l water + 0.25 ml 99% strength formic acid , eluent B: 1 l acetonitrile +
20 0.25 ml 99% strengthformic acid; gradient: 0.0 min 90% A → 1.2 min 5% A → 2.0 min 5% A
oven: 50°C; flow rate: 0.60 ml/min; UV detection: 208 – 400 nm.

Method 12 (HPLC):

Instrument: Agilent 1200 Series with column oven and DAD; column: Merck Chromolith
25 SpeedROD RP-18e, 50 mm x 4.6 mm; Ord. No.: 1.51450.0001; preliminary column: Merck
Chromolith Guard Cartridge Kit RP-18e, 5 mm x 4.6 mm; Ord. No.: 1.51470.0001; eluent A:
70% HClO₄ in water (4 ml/litre), eluent B: acetonitrile; gradient: 0.00 min 5% B → 0.50 min 5%
B → 3.00 min 95% B → 4.00 min 95% B; flow rate: 5 ml/min; column temperature: 30°C.

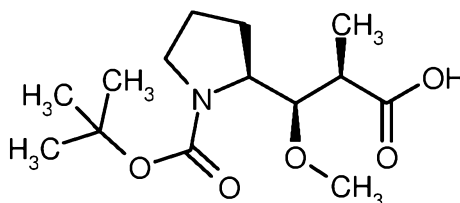
Method 13 (LC-MS):

MS instrument: Waters (Micromass) Quattro Micro; Instrument HPLC: Agilent 1100 Series;
column: YMC-Triart C18 3 μ 50 x 3 mm; eluent A: 1 l water + 0.01 mol ammoniumcarbonate,
eluent B: 1 l acetonitrile; gradient: 0.0 min 100% A $\rightarrow$ 2.75 min 5% A $\rightarrow$ 4.5 min 5% A; oven:
5 40°C; flow rate: 1.25 ml/min; UV detection: 210 nm.

Starting Compounds and Intermediates:

Starting Compound 1

(2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid
(Boc-dolaproine)

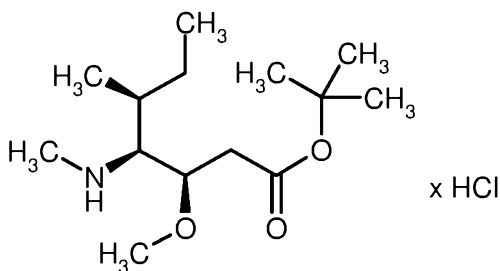


5

The title compound can be prepared in various ways according to literature methods; see, for example, Pettit *et al.*, *Synthesis* 1996, 719; Shioiri *et al.*, *Tetrahedron Lett.* 1991, 32, 931; Shioiri *et al.*, *Tetrahedron* 1993, 49, 1913; Koga *et al.*, *Tetrahedron Lett.* 1991, 32, 2395; Vidal *et al.*, *Tetrahedron* 2004, 60, 9715; Poncet *et al.*, *Tetrahedron* 1994, 50, 5345. It was prepared either as
10 the free acid or as a 1:1 salt with dicyclohexylamine.

Starting Compound 2a

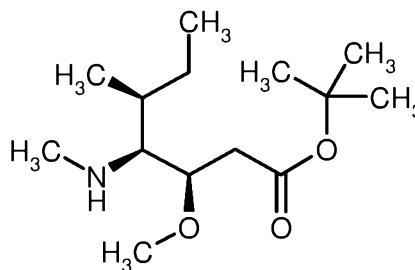
tert-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-(methylamino)heptanoate hydrochloride
(dolaisoleucine-OtBu x HCl)



15 The title compound can be prepared in various ways according to literature methods; see, for example, Pettit *et al.*, *J. Org. Chem.* 1994, 59, 1796; Koga *et al.*, *Tetrahedron Lett.* 1991, 32, 2395; Shioiri *et al.*, *Tetrahedron Lett.* 1991, 32, 931; Shioiri *et al.*, *Tetrahedron* 1993, 49, 1913.

Starting Compound 2b

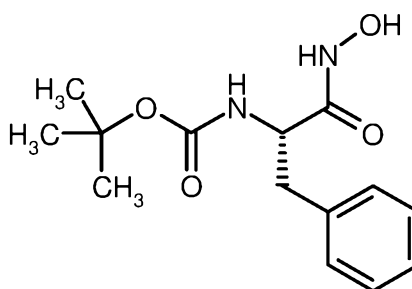
tert-butyl-(3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-(methylamino)heptanoate
20 (dolaisoleucine-O^tBu)



The compound was prepared in analogy to starting compound 2a, except that the hydrogenation was performed without addition of 1N hydrochloric acid.

Starting Compound 3

- 5 N^{α} -(*tert*-butoxycarbonyl)-*N*-hydroxy-L-phenylalaninamide



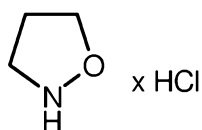
The title compound was prepared by the literature method (A. Ritter *et al.*, *J. Org. Chem.* 1994, 59, 4602).

Yield: 750 mg (75% of theory)

- 10 LC-MS (Method 3): $R_t = 1.67$ min; MS (ESIpos): $m/z = 281$ ($M+H$)⁺.

Starting Compound 4

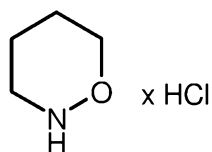
1,2-oxazolidine hydrochloride



- The title compound can be prepared by literature methods (see, for example, H. King, *J. Chem. Soc.* 1942, 432); it is also commercially available.

Starting Compound 5

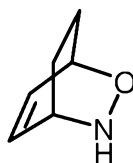
1,2-oxazinane hydrochloride



The title compound can be prepared by literature methods (see, for example, H. King, *J. Chem. Soc.* 1942, 432).

Starting Compound 6

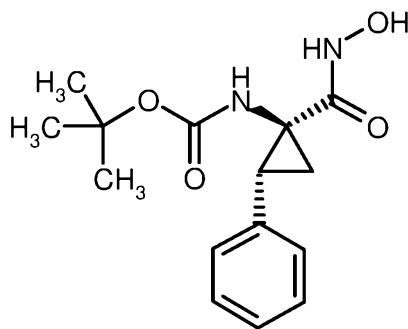
2-oxa-3-azabicyclo[2.2.2]oct-5-ene



The title compound can be prepared in Boc-protected form by the literature method (see, for example, C. Johnson *et al.*, *Tetrahedron Lett.* 1998, 39, 2059); the deprotection was effected in a customary manner by treatment with trifluoroacetic acid and subsequent neutralization.

Yield: 149 mg (89% of theory)

Starting Compound 7

tert-butyl (1*S*,2*R*)-1-(hydroxycarbamoyl)-2-phenylcyclopropyl carbamate

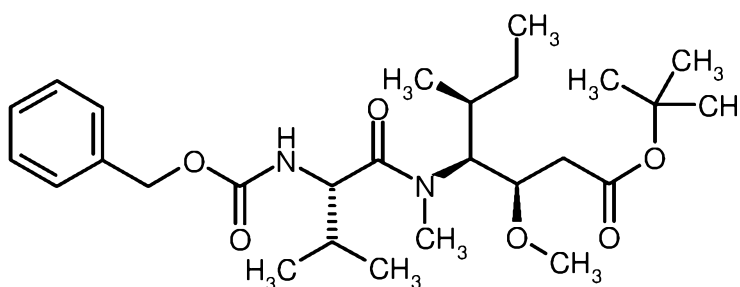
The title compound was prepared by a literature method (A. Ritter *et al.*, *J. Org. Chem.* 1994, 59, 4602) proceeding from commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid (C. Cativiela *et al.*, *Chirality* 1999, 11, 583).

Yield: 339 mg (59% of theory)

- 5 LC-MS (Method 1): $R_t = 0.82$ min; MS (ESIpos): $m/z = 293$ ($M+H$)⁺.

Intermediate 1

tert-butyl-(3*R*,4*S*,5*S*)-4-[{*N*-[(benzyloxy)carbonyl]-L-valyl}(methylamino)-3-methoxy-5-methylheptanoate



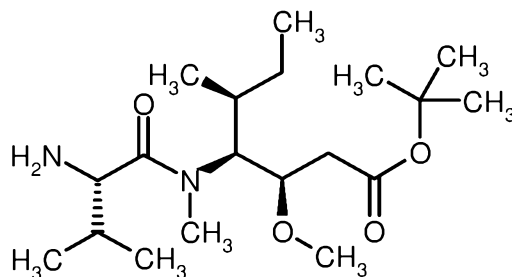
- 10 10.65 g (41.058 mmol) of *tert*-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-(methylamino)-heptanoate (starting compound 2b) were taken up in 250 ml of dichloromethane, and the solution was cooled to -10°C. Then, while stirring, 10.317 g (41.058 mmol) of *N*-[(benzyloxy)carbonyl]-L-valine, 16.866 g (61.586 mmol) of 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP) and 28.6 ml of *N,N*-diisopropylethylamine were added, and the mixture was subsequently stirred at
- 15 RT for 20 h. The reaction mixture was then diluted with dichloromethane and shaken twice with saturated sodium chloride solution, dried over sodium sulphate, filtered and concentrated. The residue was purified by flash chromatography on silica gel with 4:1 petroleum ether/ethyl acetate as the eluent. The corresponding fractions were concentrated, and the residue was dried under high vacuum overnight. 10.22 g (51% of theory) of the title compound were obtained as a
- 20 yellowish oil.

HPLC (Method 5): $R_t = 2.3$ min;

LC-MS (Method 2): $R_t = 1.59$ min; MS (ESIpos): $m/z = 493$ ($M+H$)⁺.

Intermediate 2

tert-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-[methyl(L-valyl)amino]heptanoate



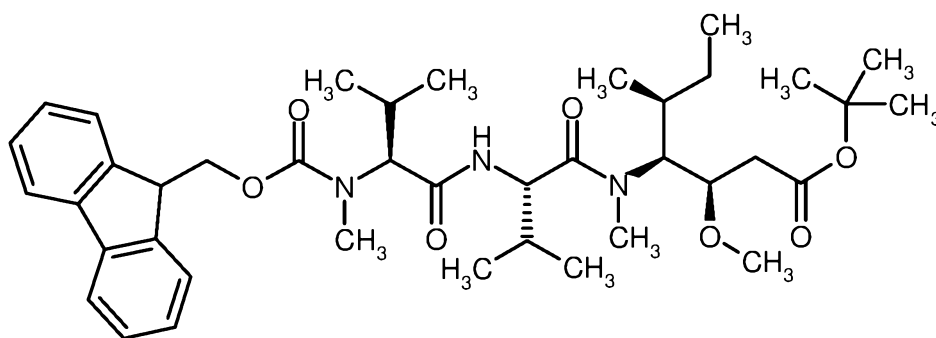
500 mg (1 mmol) of *tert*-butyl (3*R*,4*S*,5*S*)-4-[[*N*-[(benzyloxy)carbonyl]-*L*-valyl](methyl)amino]-3-methoxy-5-methylheptanoate (intermediate 1) were dissolved in 50 ml of methanol and, after addition of 100 mg of 10% palladium on activated carbon, hydrogenated under standard hydrogen pressure at RT for 1 h. The catalyst was then filtered off, and the solvent was removed under reduced pressure. This gave 370 mg (quant.) of the title compound as a virtually colourless oil.

HPLC (Method 5): $R_t = 1.59$ min;

LC-MS (Method 1): $R_t = 0.74$ min; MS (ESIpos): $m/z = 359$ ($M+H$)⁺.

10 Intermediate 3

N-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-1-*tert*-butoxy-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide



4.64 g (13.13 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-*L*-valine were dissolved in 20 ml of DMF and admixed successively with 4.28 g (11.94 mmol) of *tert*-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-[methyl(*L*-valyl)amino]heptanoate (Intermediate 2), 2.75 g (14.33 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 2.2 g (14.33 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate. The mixture was stirred at RT overnight. The reaction mixture was then poured into a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over

magnesium sulphate, filtered and concentrated. The residue was used directly in the next stage, without further purification.

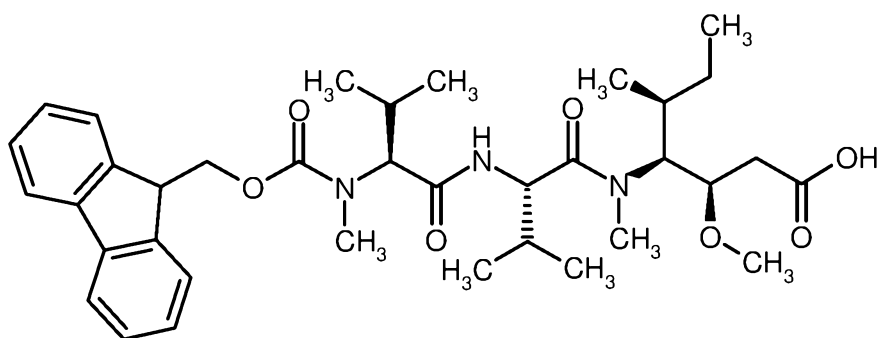
Yield: 9.1 g (quant., 60% purity)

HPLC (Method 5): $R_t = 2.7$ min;

- 5 LC-MS (Method 2): $R_t = 1.99$ min; MS (ESIpos): $m/z = 694$ (M+H)⁺.

Intermediate 4

N-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide



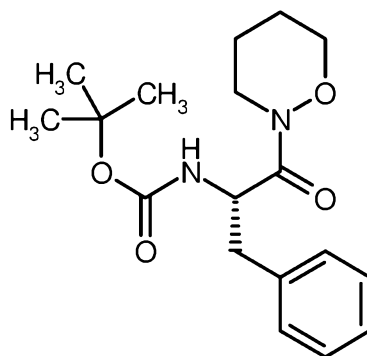
- 10 9.1 g of the crude product *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-*tert*-butoxy-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 3) were taken up in 56.6 ml of dichloromethane, 56.6 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 2 h. Subsequently, the reaction mixture was concentrated *in vacuo* and the remaining residue was purified by flash chromatography, using
- 15 dichloromethane, 3:1 dichloromethane/ethyl acetate and 15:5:0.5 dichloromethane/ethyl acetate/methanol as eluent. After purification of the corresponding fractions and concentration, 5.8 g (86% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): $R_t = 2.2$ min;

LC-MS (Method 1): $R_t = 1.3$ min; MS (ESIpos): $m/z = 638$ (M+H)⁺.

- 20 Intermediate 5

tert-butyl (2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl carbamate



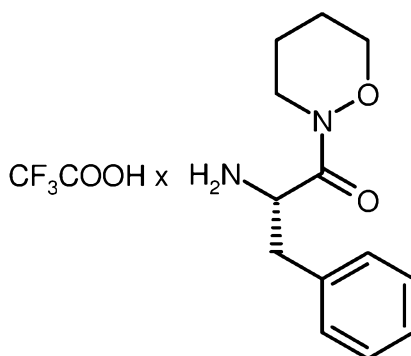
500 mg (1.9 mmol) of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine were dissolved in 10 ml of DMF and admixed successively with 466 mg (3.8 mmol) of 1,2-oxazinan-2-yl hydrochloride (Starting Compound 5), 433 mg (2.3 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 382 mg (2.8 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate and 731 mg (5.7 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT overnight. The reaction mixture was then poured into a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over magnesium sulphate, filtered and concentrated. 620 mg (98% of theory) of the title compound were obtained.

HPLC (Method 5): $R_t = 1.8$ min;

LC-MS (Method 2): $R_t = 1.62$ min; MS (ESIpos): $m/z = 235$ ($M - C_4H_8 - CO_2 + H$)⁺.

Intermediate 6

(2*S*)-2-amino-1-(1,2-oxazinan-2-yl)-3-phenylpropan-1-one trifluoroacetate



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620 mg (1.85 mmol) of *tert*-butyl (2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl carbamate (Intermediate 5) were taken up in 5 ml of dichloromethane, 10 ml of trifluoroacetic acid were added and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture

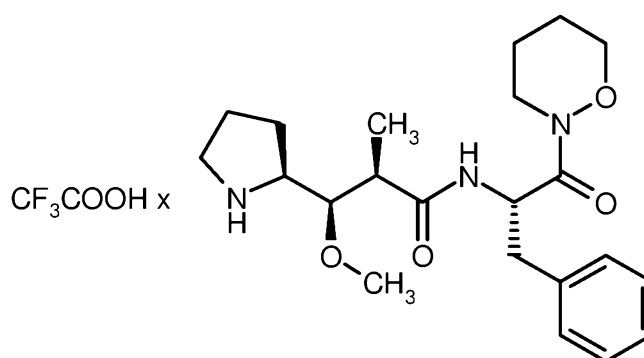
was concentrated *in vacuo*, and the remaining residue was lyophilized from water/acetonitrile. In this way, 779 mg (91% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): $R_t = 0.45$ min;

LC-MS (Method 3): $R_t = 1.09$ min; MS (ESIpos): $m/z = 235$ (M+H)⁺.

5 Intermediate 7

(2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate



- 360 mg (1.25 mmol) of (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1) were taken up in 10 ml of DMF and admixed successively with 579.2 mg (1.25 mmol) of (2*S*)-2-amino-1-(1,2-oxazinan-2-yl)-3-phenylpropan-1-one trifluoroacetate (Intermediate 6), 714.5 mg (1.88 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 655 μ l of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 16 h. The reaction mixture was then concentrated, and the residue was taken up in ethyl acetate and extracted by shaking first with 5% aqueous citric acid solution, then with 5% aqueous sodium hydrogencarbonate solution and subsequently with saturated sodium chloride solution. The organic phase was concentrated, and the residue was purified by flash chromatography on silica gel with 16:4 dichloromethane/methanol as the eluent. The corresponding fractions were combined and the solvent was removed under *in vacuo*. After the residue had been dried under high vacuum, 503.5 mg (74% of theory) of the Boc-protected intermediate *tert*-butyl (2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidine-1-carboxylate were obtained.

HPLC (Method 12): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 1.12$ min; MS (ESIpos): $m/z = 504$ (M+H)⁺.

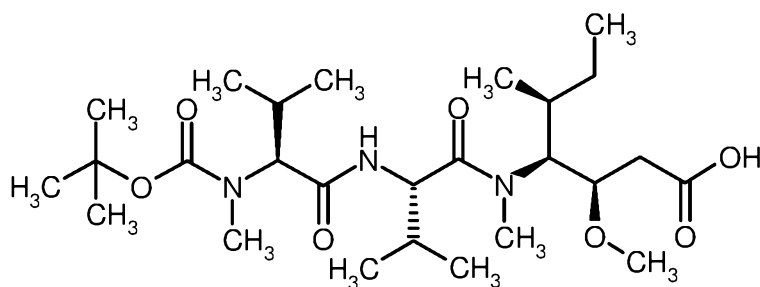
503 mg (1 mmol) of this intermediate were taken up in 20 ml of dichloromethane, 10 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated *in vacuo* and redistilled with dichloromethane. The remaining residue was precipitated from ethyl acetate with n-pentane, and the solvent was decanted off. The residue thus obtained was dissolved in water and extracted by shaking with ethyl acetate, and the aqueous phase was subsequently lyophilized. In this way, 462 mg (89% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 12): $R_t = 1.53$ min;

10 LC-MS (Method 11): $R_t = 0.57$ min; MS (ESIpos): $m/z = 404$ (M+H)⁺.

Intermediate 8

N-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide



15 51 mg (0.08 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were dissolved in 10 ml of DMF, and 0.5 ml of piperidine was added. After stirring at RT for 10 min, the reaction mixture was concentrated *in vacuo*, and the residue was stirred with diethyl ether. The insoluble constituents were filtered off and washed repeatedly with diethyl ether. Then the filter residue was taken up in 5 ml of dioxane/water, and the solution was adjusted to pH 11 with 1 N sodium hydroxide solution. Under ultrasound treatment, a total of 349 mg (1.6 mmol) of di-*tert*-butyl dicarbonate were added in several portions, in the course of which the pH of the solution was kept at 11. After the reaction had ended, the dioxane was evaporated off and the aqueous solution was adjusted to a pH of 2-3 with citric acid. The mixture was extracted twice, with 50 ml each time of ethyl acetate. The organic phases were combined, dried over magnesium sulphate and concentrated under reduced pressure. The residue was taken up in diethyl ether and the of the title compound was precipitated with pentane. The solvent was removed by

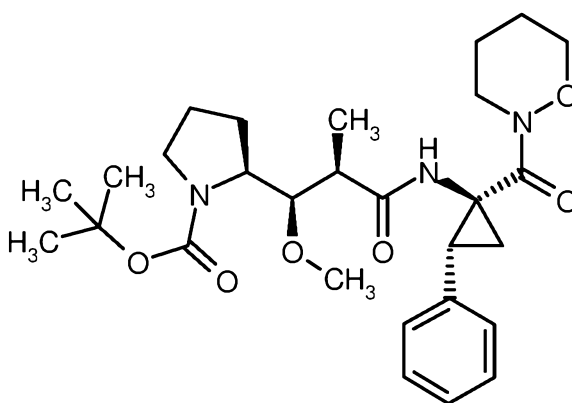
decantation. The residue was digested several times more with pentane and finally dried under high vacuum. 40 mg (97% of theory) of the title compound were thus obtained.

HPLC (Method 6): $R_t = 2.2$ min;

LC-MS (Method 2): $R_t = 1.32$ min; MS (ESIpos): $m/z = 516$ (M+H)⁺.

5 Intermediate 9

tert-butyl-(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidine-1-carboxylate



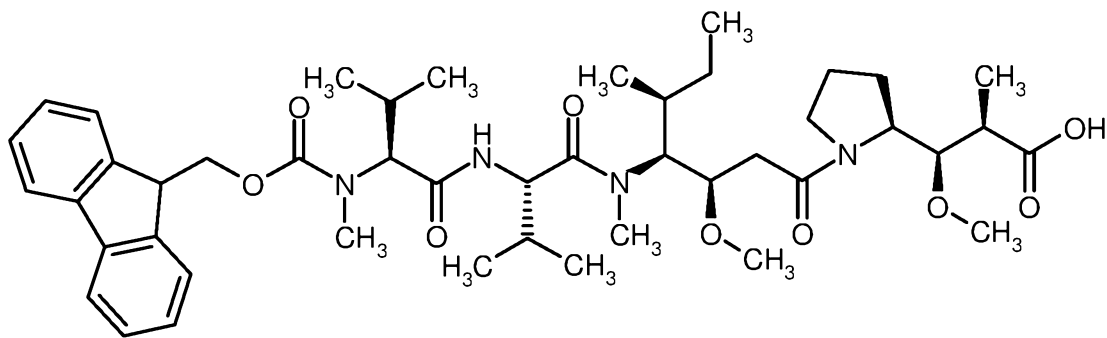
- The title compound was prepared in analogy to the synthesis of Intermediates 5, 6 and 7 over three stages by coupling of commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with 1,2-oxazinan hydrochloride (Starting Compound 5), subsequent deprotection with trifluoroacetic acid and coupling with Starting Compound 1. The end product was purified by preparative HPLC.

HPLC (Method 5): $R_t = 2.12$ min;

- 15 LC-MS (Method 2): $R_t = 1.25$ min; MS (ESIpos): $m/z = 516$ (M+H)⁺.

Intermediate 10

N-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



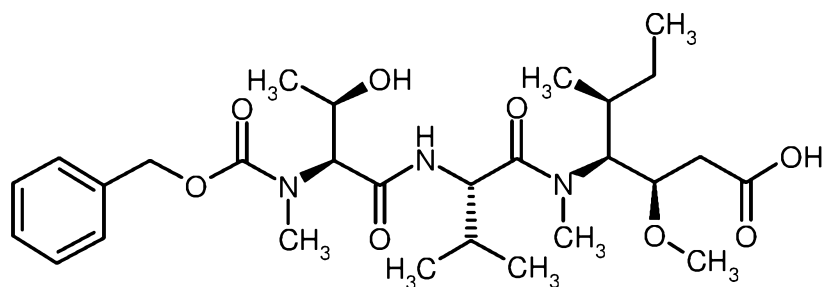
315 mg (0.494 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were dissolved in 12 ml of DMF and admixed with 104 mg (0.543 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 83 mg (0.543 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate, and the mixture was stirred at RT for 90 min. Subsequently, 112 μ l of *N,N*-diisopropylethylamine and 149 mg (0.494 mmol) of (2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoic acid trifluoroacetate, which had been prepared beforehand from Starting Compound 1 by elimination of the Boc protecting group by means of trifluoroacetic acid, were added. The mixture was stirred at RT for 2 h and then concentrated under high vacuum. The remaining residue was purified twice by preparative HPLC. 140 mg (35% of theory) of the title compound were obtained in the form of a colourless foam.

HPLC (Method 5): R_t = 2.40 min;

LC-MS (Method 1): R_t = 1.38 min; MS (ESIpos): m/z = 807 ($M+H$)⁺.

15 Intermediate 11

N-[(benzyloxy)carbonyl]-*N*-methyl-L-threonyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide



First, *N*-[(benzyloxy)carbonyl]-*N*-methyl-L-threonine was released from 237 mg (0.887 mmol) of its dicyclohexylamine salt thereof by taking it up in ethyl acetate and extractive shaking with 5% aqueous sulphuric acid. The organic phase was dried over magnesium sulphate, filtered and

concentrated. The residue was taken up in 16 ml of DMF and admixed successively with 365 mg (1 mmol) of *tert*-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-[methyl(L-valyl)amino]heptanoate (Intermediate 2), 185 mg (0.967 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 148 mg (0.967 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate. The mixture was stirred at RT for 2 h. The reaction mixture was then poured into a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over magnesium sulphate, filtered and concentrated. The residue was purified by preparative HPLC. 283 mg (53% of theory) of the *tert*-butyl ester intermediate *N*-[(benzyloxy)carbonyl]-*N*-methyl-L-threonyl-*N*-[(3*R*,4*S*,5*S*)-1-*tert*-butoxy-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were thus obtained.

HPLC (Method 5): $R_t = 2.17$ min.

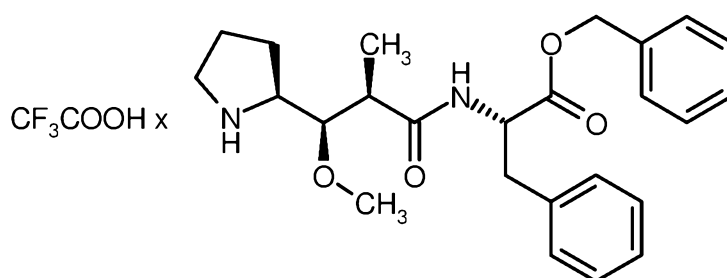
283 mg (0.466 mmol) of this intermediate were taken up in 5 ml of dichloromethane, 5 ml of anhydrous trifluoroacetic acid were added, and the mixture was stirred at RT for 2 h. Subsequently, the reaction mixture was concentrated under high vacuum and the remaining residue was purified by means of preparative HPLC. This gave 156 mg (61% of theory) of the title compound as a colourless foam.

HPLC (Method 5): $R_t = 1.50$ min;

LC-MS (Method 2): $R_t = 1.09$ min; MS (ESIpos): $m/z = 552$ ($M+H$)⁺.

20 Intermediate 12

Benzyl-*N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-phenylalaninate trifluoroacetate



In the first step, Starting Compound 1 was released from 600 mg (1.28 mmol) of the corresponding dicyclohexylammonium salt by dissolving the salt in 100 ml of ethyl acetate and extractive shaking, first with 50 ml of 0.5% sulphuric acid and then with saturated sodium

chloride solution. Then the organic phase was dried over magnesium sulphate, filtered, concentrated and reacted immediately with benzyl L-phenylalaninate in analogy to the synthesis of Intermediate 7, and then deprotected.

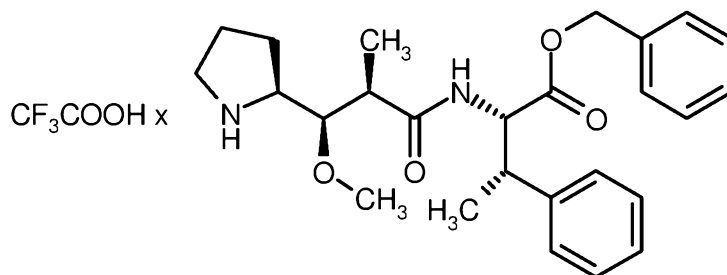
Yield: 650 mg (94% over 2 stages)

5 HPLC (Method 6): $R_t = 1.76$ min;

LC-MS (Method 2): $R_t = 1.68$ min; MS (ESIpos): $m/z = 425$ (M+H)⁺.

Intermediate 13

Benzyl-(βS)-N-[(2R,3R)-3-methoxy-2-methyl-3-[(2S)-pyrrolidin-2-yl]propanoyl]-β-methyl-L-phenylalaninate trifluoroacetate



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First, (2R,3R)-3-[(2S)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid was released from 351 mg (0.75 mmol) of the dicyclohexylamine salt (Starting Compound 1) by taking it up in ethyl acetate and extractive shaking with aqueous 5% potassium hydrogensulphate solution. The organic phase was dried over magnesium sulphate, filtered and concentrated. The residue was taken up in 10 ml of DMF and admixed successively with 373 mg (0.75 mmol) of benzyl-(βS)-β-methyl-L-phenylalaninate trifluoroacetate [prepared from commercially available (βS)-N-(*tert*-butoxycarbonyl)-β-methyl-L-phenylalanine by EDC/DMAP-mediated esterification with benzyl alcohol and subsequent cleaving of the Boc protecting group with trifluoroacetic acid], 428 mg (1.125 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 392 μl of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 20 h. The reaction mixture was then poured onto a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, and subsequently concentrated. The residue was purified by means of preparative HPLC. This gave 230 mg (57% of theory) of the Boc-protected

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intermediate benzyl-(β S)-N-{(2R,3R)-3-[(2S)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoyl}- β -methyl-L-phenylalaninate.

HPLC (Method 6): R_t = 2.3 min;

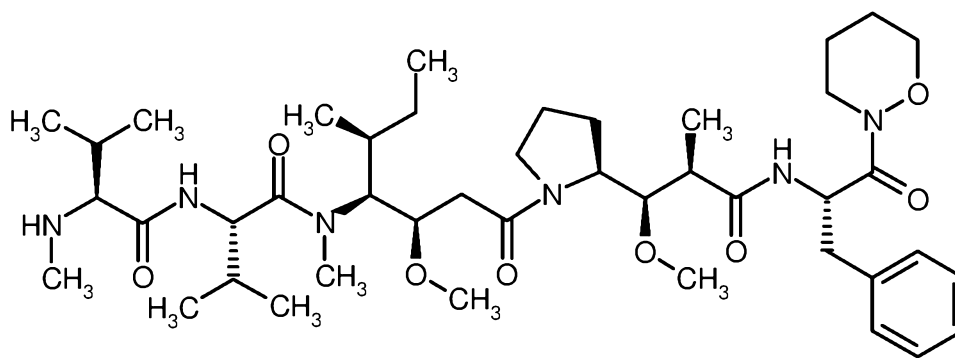
LC-MS (Method 1): R_t = 1.36 min; MS (ESIpos): m/z = 539 (M+H)⁺.

- 5 230 mg (0.42 mmol) of this intermediate were taken up in 5 ml of dichloromethane, 5 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated *in vacuo*. The remaining residue was the reaction mixture dried further *in vacuo* and then lyophilized from acetonitrile/water. In this way, 230 mg (quant.) of the title compound were obtained.

- 10 HPLC (Method 6): R_t = 1.6 min.

Intermediate 14

N-methyl-L-valyl-*N*-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-[(2S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



x CF₃COOH

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- 143 mg (0.223 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2R,3S,4S)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were taken up in 15 ml of DMF and admixed successively with 141 mg (0.22 mmol) of (2R,3R)-3-methoxy-2-methyl-*N*-[(2S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]-3-[(2S)-pyrrolidin-2-yl]propanamide trifluoroacetate (Intermediate 7), 102 mg (0.27 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 128 μ l (0.74 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 3 h. The reaction mixture was then poured into a mixture of semisaturated aqueous ammonium chloride solution
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and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over magnesium sulphate, filtered and concentrated. This gave 275 mg (quant.) of the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

HPLC (Method 5): $R_t = 2.73$ min;

LC-MS (Method 4): $R_t = 3.19$ min; MS (ESIpos): $m/z = 1023$ (M+H)⁺.

46 mg (0.045 mmol) of this intermediate were dissolved in 4 ml of DMF. After 1 ml of piperidine had been added, the reaction mixture was stirred at RT for 1 h. Subsequently, the reaction mixture was concentrated *in vacuo* and the residue was purified by means of preparative HPLC (eluent: acetonitrile + 0.01% TFA/water + 0.01% TFA). 22 mg (54% of theory) of the title compound were obtained as a colourless foam.

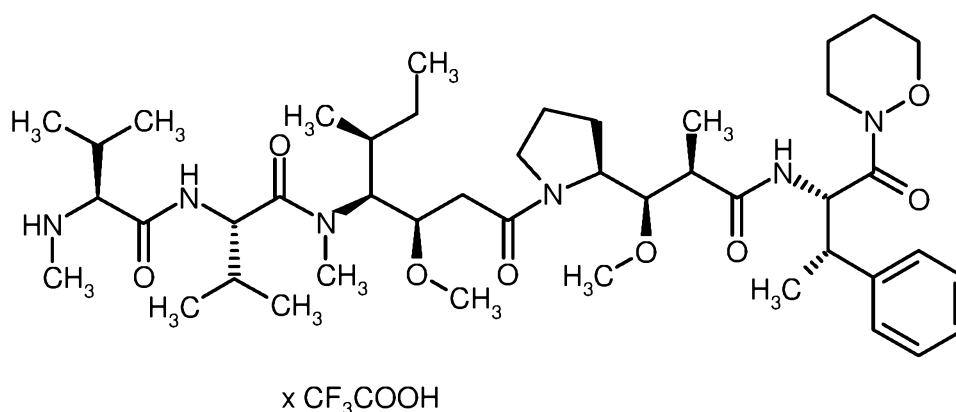
HPLC (Method 5): $R_t = 1.68$ min;

LC-MS (Method 2): $R_t = 1.03$ min; MS (ESIpos): $m/z = 801$ (M+H)⁺

¹H NMR (600 MHz, DMSO-*d*₆): $\delta = 8.8$ (m, 2H), 8.7 (m, 1H), 8.42 and 8.15 (2d, 1H), 7.3-7.1 (m, 5H), 5.12 and 4.95 (2m, 1H), 4.70 and 4.62 (2m, 1H), 4.62 and 4.50 (2t, 1H), 4.1-3.9 (m, 3H), 3.85 (m, 1H), 3.75-3.6 (m, 2H), 3.23, 3.18, 3.17, 3.14, 3.02 and 2.96 (6s, 9H), 3.1-2.9 and 2.75 (2m, 2H), 2.46 (m, 3H), 2.4-2.1 (m, 2H), 2.05 (br. m, 2H), 1.85-1.55 (br. m, 6H), 1.5-1.2 (br. m, 3H), 1.1-0.8 (m, 18H), 0.75 (t, 3H) [further signals hidden under H₂O peak].

Intermediate 15

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*,3*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



126 mg (0.198 mmol) of *N*-[*(9H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were taken up in 10 ml of DMF and admixed successively with 105 mg (0.198 mmol) of

5 (2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(2*S*,3*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate (Intermediate 17), 41.6 mg (0.217 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 33 mg (0.217 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate and 79 μ l (0.454 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT overnight. The reaction mixture was then poured into a mixture of

10 semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over magnesium sulphate, filtered and concentrated. This gave 220 mg (quant.) of the Fmoc-protected intermediate *N*-[*(9H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*,3*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

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HPLC (Method 5): $R_t = 2.77$ min;

LC-MS (Method 1): $R_t = 1.5$ min; MS (ESIpos): $m/z = 1037$ (M+H)⁺.

220 mg (0.212 mmol) of this intermediate were dissolved in 5 ml of DMF. After 1 ml of piperidine had been added, the reaction mixture was stirred at RT for 1 h. Subsequently, the reaction mixture was concentrated under reduced pressure and the residue was purified by means of preparative HPLC (eluent: acetonitrile + 0.01% TFA / water + 0.01% TFA). 91 mg (46% of theory) of the title compound were obtained as a colourless foam.

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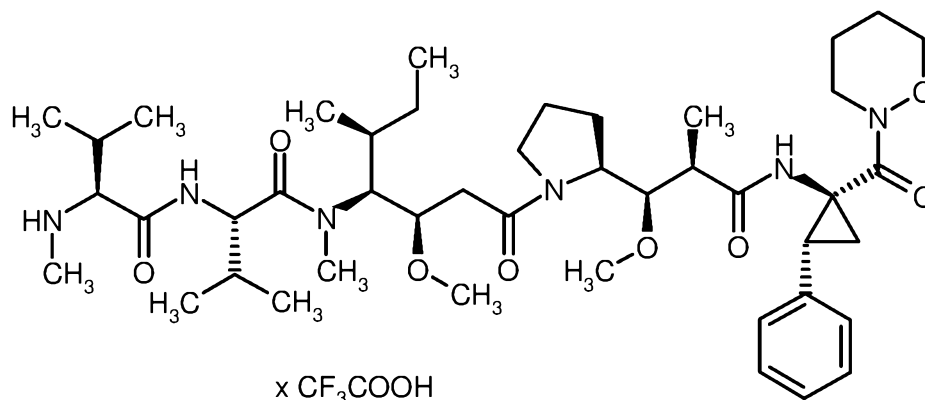
HPLC (Method 5): $R_t = 1.71$ min;

25 LC-MS (Method 1): $R_t = 0.9$ min; MS (ESIpos): $m/z = 815$ (M+H)⁺

¹H NMR (600 MHz, DMSO-d₆): δ = 8.87 and 8.80 (2d, 2H), 8.75 (m, 1H), 8.40 and 7.98 (2d, 1H), 7.3-7.1 (m, 5H), 5.45 and 5.2 (2t, 1H), 4.78 and 4.62 (2m, 1H), 4.73 and 4.58 (2t, 1H), 4.2-4.0 (m, 3H), 3.7-3.6 (m, 1H), 3.35, 3.20, 3.18, 3.14, 3.12 and 3.00 (6s, 9H), 3.1 and 2.95 (2m, 2H), 2.46 (m, 3H), 2.4-2.0 (m, 4H), 1.9-1.6 (m, 4H), 1.6-1.2 (m, 5H), 1.1-0.75 (m, 21H), 0.80 (t, 3H) [further signals hidden under H₂O peak].

Intermediate 16

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



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617 mg (1.2 mmol) of *tert*-butyl (2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidine-1-carboxylate (Intermediate 24) were taken up in 44 ml of dichloromethane, 4.4 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated *in vacuo* and the remaining residue was lyophilized from dioxane/water. 702 mg (quant.) of the deprotected compound (2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate were obtained as a crude product, which was used in the following stage without further purification.

470 mg (0.74 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were taken up in 57 ml of DMF and admixed successively with 390 mg (approx. 0.74 mmol) of the above-obtained (2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate, 336 mg (0.88 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 423 μl (2.4 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 2 h. The reaction

25

mixture was then poured into a mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed successively with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution, dried over sodium sulphate, filtered and concentrated. The residue was purified by preparative HPLC. This gave 453 mg (59% of theory) of the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

HPLC (Method 5): $R_t = 2.58$ min;

LC-MS (Method 1): $R_t = 3.10$ min; MS (ESIpos): $m/z = 1035$ ($M+H$)⁺.

453 mg (0.438 mmol) of this intermediate were dissolved in 24 ml of DMF. After 2.4 ml of piperidine had been added, the reaction mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC (eluent: acetonitrile/0.1% TFA in water). 260 mg (64% of theory) of the title compound were obtained as a colourless foam.

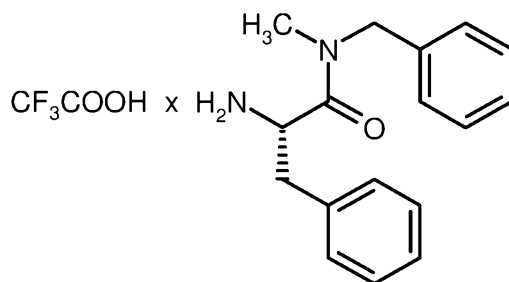
HPLC (Method 5): $R_t = 1.64$ min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 813$ ($M+H$)⁺

¹H NMR (400 MHz, DMSO-*d*₆): $\delta = 8.8$ (m, 2H), 8.65 (m, 2H), 7.3-7.1 (m, 5H), 4.8-4.05 (m, 2H), 4.0 and 3.82 (2m, 2H), 3.8-3.5 (m, 8H), 3.32, 3.29, 3.20, 3.19, 3.12 and 3.00 (6s, 9H), 2.65 (t, 1H), 2.5-2.45 (m, 3H), 2.4-1.3 (m, 15H), 1.15-0.85 (m, 18H), 0.8 and 0.75 (2d, 3H) [further signals hidden under H₂O peak].

Intermediate 17

N-benzyl-*N*-methyl-L-phenylalaninamide trifluoroacetate



1000 mg (3.77 mmol) of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine were dissolved in 10 ml of DMF and admixed with 457 mg (3.77 mmol) of *N*-methylbenzylamine, 2150 mg (5.65 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 657 μ l of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT for 30 min and then concentrated *in vacuo*. The residue was taken up in dichloromethane and extracted by shaking three times with water. The organic phase was dried over magnesium sulphate and concentrated. The residue was purified by flash chromatography on silica gel with 3:1 petroleum ether/ethyl acetate as the eluent. The product fractions were concentrated, and the residue was dried under high vacuum. This gave 1110 mg (75% of theory) of the Boc-protected intermediate *N*-benzyl-*N* ^{α} -(*tert*-butoxycarbonyl)-*N*-methyl-L-phenylalaninamide.

HPLC (Method 6): $R_t = 2.1$ min;

LC-MS (Method 1): $R_t = 1.14$ min; MS (ESIpos): $m/z = 369$ (M+H)⁺.

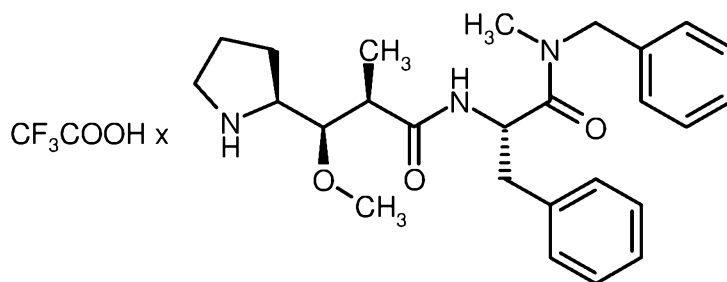
1108 mg (3,007 mmol) of this intermediate were taken up in 30 ml of dichloromethane, 10 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated *invacuo*, the remaining residue was stirred with dichloromethane, and the solvent was distilled off. The residue was stirred twice more with pentane, the solvent was decanted off again each time and the of the title compound was finally dried under high vacuum. 1075 mg (93% of theory) of the title compound were thus obtained as a resin.

HPLC (Method 6): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.6$ min; MS (ESIpos): $m/z = 269$ (M+H)⁺.

Intermediate 18

N-benzyl-*N* ^{α} -{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-*N*-methyl-L-phenylalaninamide trifluoroacetate



First, (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1) was released from 141 mg (0.491 mmol) of its dicyclohexylamine salt by taking it up in ethyl acetate and extractive shaking with 5% aqueous sulphuric acid. The organic phase was dried over magnesium sulphate, filtered and concentrated. The residue was taken up in 10 ml of DMF and 187.6 mg (0.49 mmol) of *N*-benzyl-*N*-methyl-L-phenylalaninamide trifluoroacetate (Intermediate 9), 190.3 mg (1.47 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 256 µl of *N,N*-diisopropylethylamine were added. The mixture was stirred at RT for 1 h. The reaction mixture was then concentrated, the residue was taken up in ethyl acetate, and the solution was subsequently extracted by shaking successively with saturated ammonium chloride solution, saturated sodium hydrogencarbonate solution and water. The organic phase was dried over magnesium sulphate and concentrated. The residue was purified by flash chromatography on silica gel with 30:1 acetonitrile/water as the eluent. The product fractions were concentrated and the residue was dried under high vacuum. This gave 168 mg (64% of theory) of the Boc-protected intermediate *tert*-butyl (2*S*)-2-[(1*R*,2*R*)-3-((2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl)amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidine-1-carboxylate.

HPLC (Method 6): $R_t = 2.2$ min;

LC-MS (Method 2): $R_t = 1.22$ min; MS (ESIpos): $m/z = 538$ (M+H)⁺.

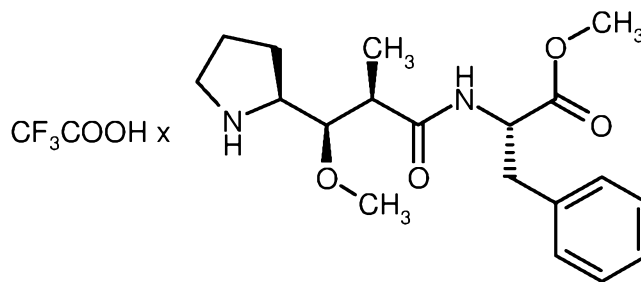
168 mg (0.312 mmol) of this intermediate were taken up in 15 ml of dichloromethane, 3 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated *in vacuo*. The remaining residue was stirred first with dichloromethane, then with diethyl ether, and the solvent was distilled off again each time. After drying under high vacuum, 170 mg (99% of theory) of the title compound were obtained as a resin.

HPLC (Method 6): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.73$ min; MS (ESIpos): $m/z = 438$ (M+H)⁺.

Intermediate 19

Methyl-*N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-phenylalaninate trifluoroacetate



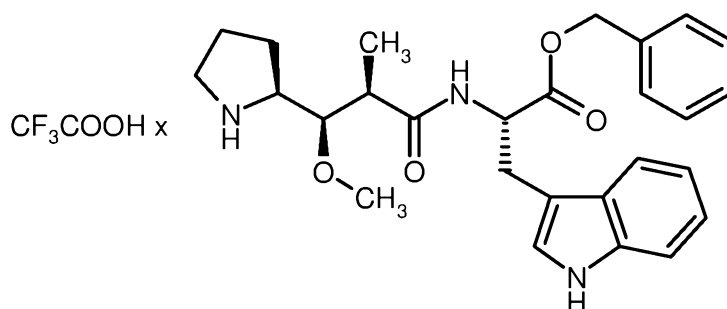
The title compound was prepared in analogy to the synthesis of Intermediate 18, proceeding from (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1), which was released from the dicyclohexylamine salt, and methyl L-phenylalaninate hydrochloride.

HPLC (Method 5): $R_t = 0.6$ min;

LC-MS (Method 3): $R_t = 1.17$ min; MS (ESIpos): $m/z = 349$ (M+H)⁺.

Intermediate 20

Benzyl-*N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-tryptophanate trifluoroacetate



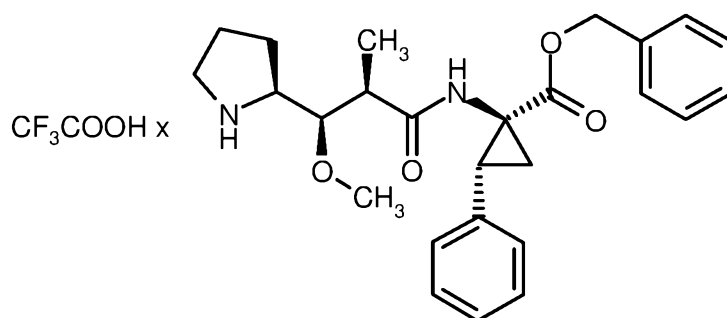
The title compound was prepared in analogy to the synthesis of Intermediate 18, proceeding from (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1), which was released from the dicyclohexylamine salt, and benzyl L-tryptophanate.

HPLC (Method 6): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 0.8$ min; MS (ESIpos): $m/z = 464$ (M+H)⁺.

Intermediate 21

Benzyl-(1*S*,2*R*)-1-((2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl)amino)-2-phenylcyclopropanecarboxylate trifluoroacetate



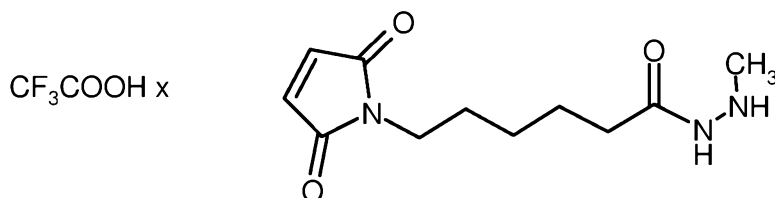
- 5 The title compound was prepared in analogy to the synthesis of Intermediate 18, proceeding from (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1), which was released from the dicyclohexylamine salt, and benzyl (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate. Benzyl-(1*S*,2*R*)-1-amino-2-phenylcyclopropane-carboxylate had been prepared beforehand by standard methods, by esterifying commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with benzyl alcohol and subsequent Boc cleaving with trifluoroacetic acid.

HPLC (Method 5): $R_t = 1.5$ min;

LC-MS (Method 2): $R_t = 0.93$ min; MS (ESIpos): $m/z = 437$ ($M+H$)⁺.

15 Intermediate 22

6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*'-methylhexanehydrazide trifluoroacetate



- 100 mg (473 μ mol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoic acid were dissolved in 71 μ l of DMF and then admixed with 139 mg (947 μ mol) of *tert*-butyl-1-methylhydrazinecarboxylate, 182 mg (947 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 145 mg (947 μ mol) of 1-hydroxy-1*H*-benzotriazole

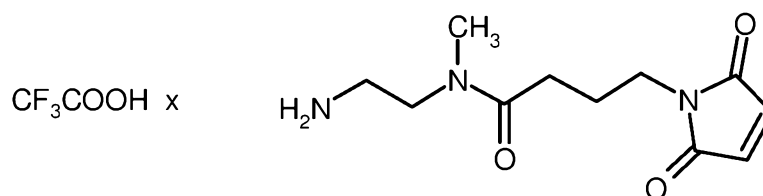
hydrate. The mixture was stirred at RT overnight and then concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane/water, 129 mg (80% of theory) of the protected intermediate were obtained as a colourless foam.

Subsequently, the 129 mg (380 μ mol) were deblocked with 2 ml of trifluoroacetic acid in 8 ml of dichloromethane. After stirring at RT for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was lyophilized from acetonitrile/water, which left 125 mg (83% of theory) of the title compound as a colourless foam.

LC-MS (Method 1): $R_t = 0.38$ min; MS (ESIpos): $m/z = 240$ (M+H)⁺

Intermediate 23

10 N-(2-aminoethyl)-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-methylbutanamide trifluoroacetate



First, 35 mg (164 μ mol) of tert-butyl-[2-(methylamino)ethyl]carbamate-hydrochloride-trifluoroacetate, 30 mg (164 μ mol) of 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoic acid, 75 mg (197 μ mol) of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium-hexafluorophosphate and 57 μ l of N,N-diisopropylethylamine were combined in 5 ml of DMF and stirred at RT overnight. Subsequently, the solvent was removed *in vacuo*, and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were concentrated and, by lyophilization from dioxane/water, 35 mg (63% of theory) of the protected intermediate were obtained.

HPLC (Method 12): $R_t = 1.6$ min;

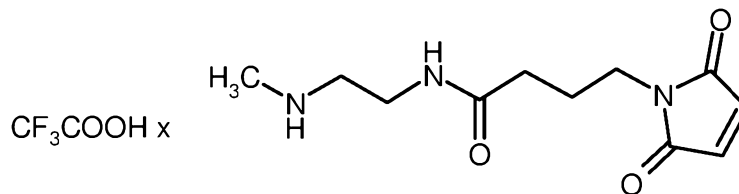
LC-MS (Method 1): $R_t = 0.71$ min; MS (ESIpos): $m/z = 340$ (M+H)⁺.

Subsequently, the entire amount of the protected intermediate was deblocked with 1 ml of trifluoroacetic acid in 5 ml of dichloromethane to obtain 28 mg (77% of theory) of the title compound.

LC-MS (Method 3): $R_t = 0.75$ min; MS (ESIpos): $m/z = 240$ (M+H)⁺.

Intermediate 24

4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-[2-(methylamino)ethyl]butanamide trifluoroacetate



First, 35 mg (164 μ mol) of tert-butyl-(2-aminoethyl)methylcarbamate hydrochloride
 5 trifluoroacetate, 30 mg (164 μ mol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid, 75
 mg (197 μ mol) of O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium
 hexafluorophosphate and 57 μ l of *N,N*-diisopropylethylamine were combined in 5 ml of DMF
 and stirred at RT for 30 min. Subsequently, the solvent was removed *in vacuo*, and the remaining
 residue was purified by means of preparative HPLC. The corresponding fractions were
 10 concentrated and, by lyophilization from dioxane/water, 51 mg (91% of theory) of the protected
 intermediate were obtained.

HPLC (Method 12): R_t = 1.6 min;

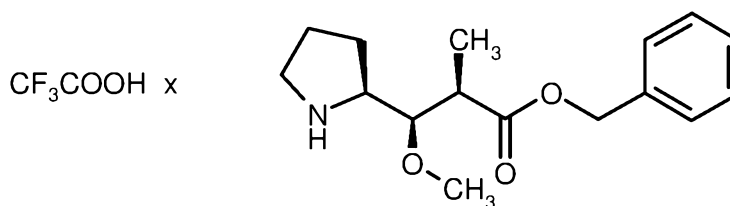
LC-MS (Method 1): R_t = 0.77 min; MS (ESIpos): m/z = 340 ($M+H$)⁺.

Subsequently, the entire amount was deprotected with 1 ml of trifluoroacetic acid in 5 ml of
 15 dichloromethane to obtain 45 mg (69% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.19 min; MS (ESIpos): m/z = 240 ($M+H$)⁺.

Intermediate 25

Benzyl-(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoate trifluoroacetate



20 First, (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic
 acid was released from 1.82 g (3.88 mmol) of its dicyclohexylamine salt by taking it up in 150
 ml of ethyl acetate and extractive shaking with 100 ml of 0.5% sulphuric acid. The organic phase

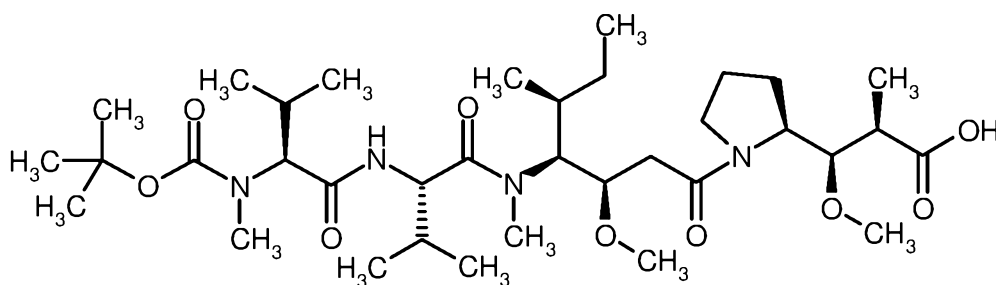
- was dried over magnesium sulphate, filtered and concentrated. The residue was taken up in 10 ml of dioxane and 10 ml of water, 1517 mg (4.66 mmol) of caesium carbonate were added, and the mixture was treated in an ultrasound bath for 5 min, then concentrated under *in vacuo* and redistilled once with DMF. The residue was then taken up in 15 ml of dichloromethane, and 990 mg (11.64 mmol) of benzyl bromide were added to this. The mixture was treated in an ultrasound bath for 15 min and then concentrated *in vacuo*. The residue was partitioned between ethyl acetate and water, the organic phase was removed and extracted by shaking with saturated sodium chloride solution and then concentrated. The residue was then purified by preparative HPLC. This gave 1170 mg (80% of theory) of the Boc-protected intermediate.
- Subsequently, these 1170 mg were deprotected immediately with 5 ml of trifluoroacetic acid in 15 ml of dichloromethane. After stirring at RT for 15 min, the reaction mixture was concentrated *in vacuo*. The residue was lyophilized from dioxane. After drying under high vacuum, there remained 1333 mg (84% of theory) of the title compound as a yellow oil.

HPLC (Method 6): $R_t = 1.5$ min;

- LC-MS (Method 1): $R_t = 0.59$ min; MS (ESIpos): $m/z = 278$ (M+H)⁺.

Intermediate 26

N-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



- 1200 mg (2.33 mmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 5) were combined with 910.8 mg (2.33 mmol) of benzyl (2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoate trifluoroacetate (Intermediate 14), 1327 mg (3.49 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 2027 μ l of *N,N*-diisopropylethylamine in 50 ml of DMF, and the mixture was stirred at RT for 5 min. Thereafter, the solvent was removed *in vacuo*. The remaining residue was taken up in ethyl acetate and extracted by shaking it successively with 5% aqueous citric acid solution and saturated sodium

hydrogencarbonate solution. The organic phase was removed and concentrated. The residue was purified by means of preparative HPLC. The product fractions were combined and concentrated, and the residue was dried under high vacuum. This gave 1000 mg (55% of theory) of the benzyl ester intermediate *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-(benzyloxy)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide as a resin.

LC-MS (Method 1): $R_t = 1.56$ min; MS (ESIpos): $m/z = 775$ ($M+H$)⁺.

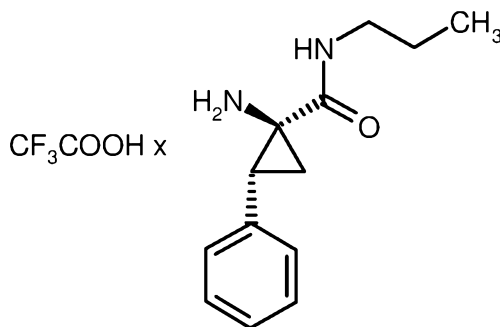
The entire amount of this intermediate obtained was taken up in 25 ml of a mixture of methanol and dichloromethane (20:1), and the benzyl ester group was removed by hydrogenation under standard hydrogen pressure with 10% palladium on activated carbon as a catalyst. After stirring at RT for 30 min, the catalyst was filtered off and the filtrate was concentrated *in vacuo*. This gave 803 mg (91% of theory) of the title compound as a white solid.

HPLC (Method 6): $R_t = 2.1$ min;

LC-MS (Method 1): $R_t = 1.24$ min; MS (ESIpos): $m/z = 685$ ($M+H$)⁺.

15 Intermediate 27

(1*S*,2*R*)-1-amino-2-phenyl-*N*-propylcyclopropanecarboxamide trifluoroacetate

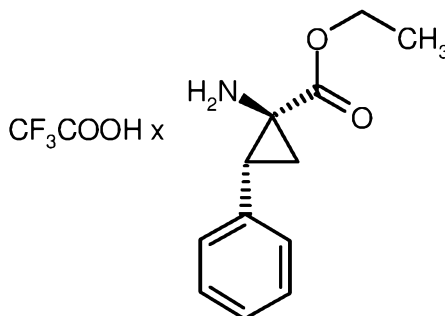


The title compound was prepared by coupling commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with *n*-propylamine in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and subsequent Boc cleaving with trifluoroacetic acid (yield: 85% of theory over both stages).

HPLC (Method 6): $R_t = 1.2$ min;

LC-MS (Method 1): $R_t = 0.52$ min; MS (ESIpos): $m/z = 219$ ($M+H$)⁺.

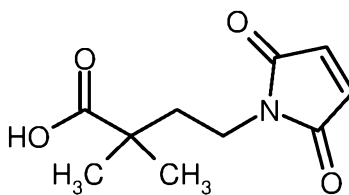
Intermediate 28

Ethyl-(1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate trifluoroacetate

The title compound was prepared according to standard methods by esterifying commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with ethanol and subsequent Boc cleaving with trifluoroacetic acid.

LC-MS (Method 1): R_t = 0.50 min; MS (ESIpos): m/z = 206 ($M+H$)⁺.

Intermediate 29

4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoic acid

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To a solution of 1.39 g (8.95 mmol) of N-methoxycarbonylmaleimide in 44 ml of saturated sodium hydrogencarbonate solution were added, at 0°C, 1.5 g (8.95 mmol) of 4-amino-2,2-dimethylbutyric acid, and the mixture was stirred for 40 min. Subsequently, the cooling bath was removed, and the reaction mixture was stirred for 1 h more. While cooling with ice, the reaction mixture was then adjusted to pH 3 by adding sulphuric acid, then extracted with ethyl acetate. The combined organic phases were dried over magnesium sulphate and concentrated. 1.17 g (79% purity, 49% of theory) of the title compound were obtained.

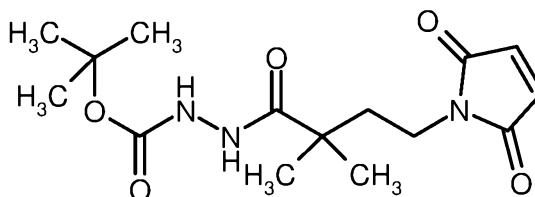
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LC-MS (Method 1): R_t = 0.64 min; m/z = 212 ($M+H$)⁺.

Intermediate 30

tert-butyl2-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-

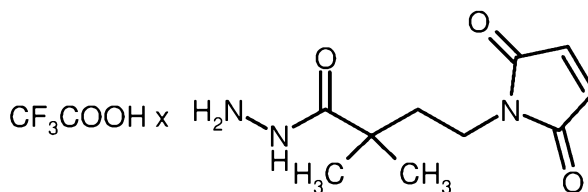
dimethylbutanoyl]hydrazinecarboxylate



- 5 To a solution of 50 mg (237 μmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoic acid in 2 ml of THF were added, at 0°C, first 26 μl (237 μmol) of 4-methylmorpholine and then 31 μl (237 μmol) of isobutyl chloroformate. After removing the cooling bath and stirring at RT for a further 15 min, 31.3 mg (237 μmol) of *tert*-butyloxycarbonyl hydrazide were added. The reaction mixture was stirred overnight and then concentrated. The
- 10 residue was purified by preparative HPLC. 50.8 mg (66% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.71 min; m/z = 324 ($M-H$)⁻.

Intermediate 31

4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanehydrazide trifluoroacetate

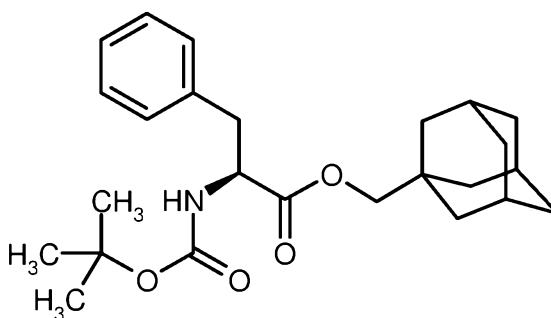
15

50 mg (154 μmol) of *tert*-butyl 2-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoyl]hydrazinecarboxylate were dissolved in 2 ml of dichloromethane, and 0.4 ml of trifluoroacetic acid was added. The reaction mixture was stirred at RT for 30 min and then concentrated. 55.2 mg (93% purity, 99% of theory) of the title compound were obtained.

- 20 LC-MS (Method 1): R_t = 0.36 min; m/z = 226 ($M+H$)⁺.

Intermediate 32

Adamantan-1-ylmethyl *N*-(*tert*-butoxycarbonyl)-*L*-phenylalaninate

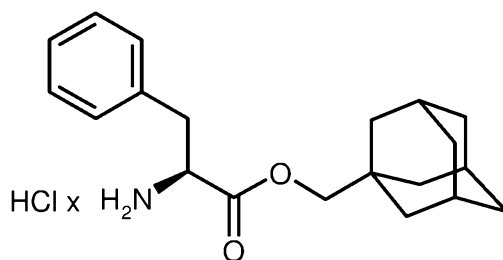


To a solution of 500 mg (1.89 mmol) of *N*-Boc-*L*-phenylalanine in 25 ml of dichloromethane were added, at RT, 1192 mg (6.2 mmol) of EDC, 578 μ l (4.1 mmol) of triethylamine, 345 mg (2.8 mmol) of DMAP and 345 mg (2.1 mmol) of 1-adamantylmethanol. The reaction mixture
 5 was stirred overnight, then diluted with 50 ml of dichloromethane, and was successively washed with 10% aqueous citric acid solution, water and saturated sodium chloride solution. The organic phase was dried over magnesium sulphate, then concentrated, and the residue was purified by preparative HPLC. 769 mg (90% of theory) of the title compound were obtained.

LC-MS (Method 2): R_t = 1.84 min; m/z = 414 ($M+H$)⁺.

10 Intermediate 33

Adamantan-1-ylmethyl *L*-phenylalaninate hydrochloride

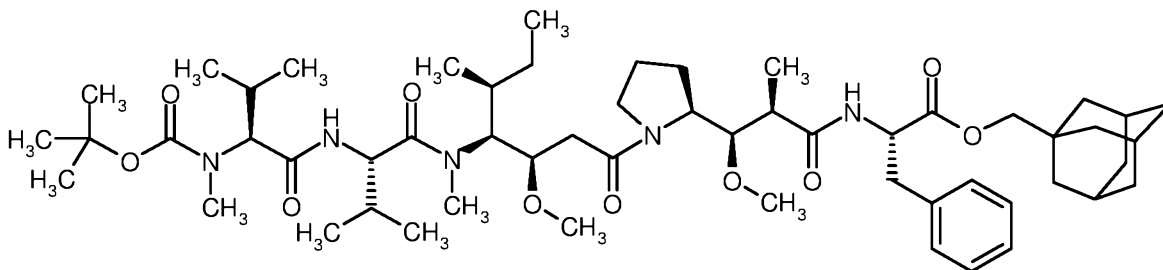


769 mg (1.86 mmol) of adamantan-1-ylmethyl *N*-(*tert*-butoxycarbonyl)-*L*-phenylalaninate (Intermediate 13) were dissolved in 25 ml of a 4 N solution of hydrogen chloride in dioxane and
 15 stirred at RT for 1 h. Subsequently, the reaction mixture was concentrated, and the residue was dried *in vacuo*. 619 mg (95% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.82 min; m/z = 314 ($M+H$)⁺.

Intermediate 34

N-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(adamantan-1-ylmethoxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide



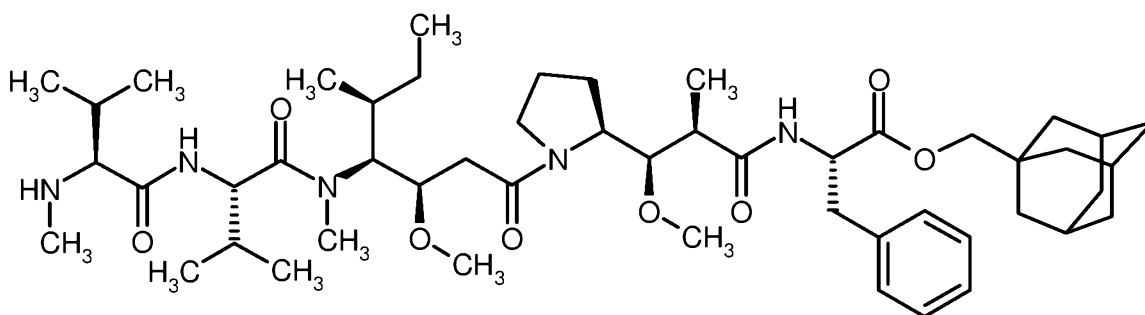
5

To a solution of 20 mg (29 μ mol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide in 1 ml of DMF were added, at RT, 15.3 μ l (88 μ mol) of *N,N*-diisopropylethylamine, 6.7 mg (44 μ mol) of HOBt and 6.7 mg (35 μ mol) of EDC, and the mixture was stirred for 30 min. Subsequently, 10.1 mg (32 μ mol) of adamantan-1-yl *L*-phenylalaninate hydrochloride were added. After stirring overnight, the reaction mixture was separated directly into its components via preparative HPLC. 27.5 mg (93% of theory) of the title compound were obtained.

15 LC-MS (Method 1): R_t = 1.70 min; m/z = 980 ($M+H$)⁺.

Intermediate 35

N-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(adamantan-1-ylmethoxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide trifluoroacetate



20

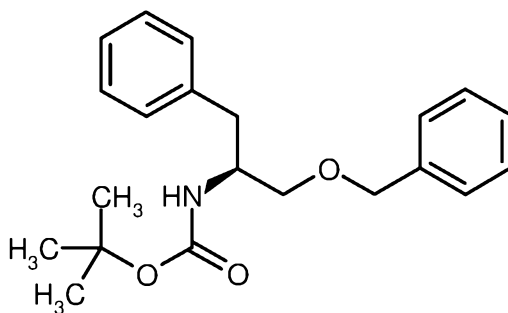
x CF₃COOH

27.5 mg (28 μ mol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(adamantan-1-ylmethoxy)-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 1.8 ml of dichloromethane, and 361 μ l of TFA were added. The reaction mixture was stirred for 30 min and then concentrated. The residue was taken up in water and lyophilized. 22.7 mg (81% of theory) of the title compound were obtained.

LC-MS (Method 1): $R_t = 1.14$ min; $m/z = 880$ ($M+H$)⁺.

Intermediate 36

tert-butyl (2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl carbamate



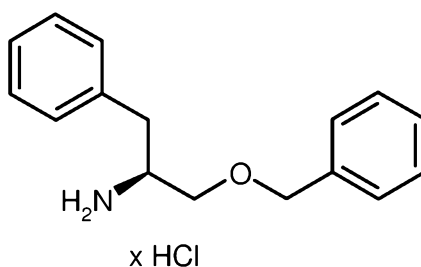
10

Under an argon atmosphere, 500 mg (1.99 mmol) of *N*-Boc-*L*-phenylalaninol were dissolved in 5 ml of DMF and cooled to 0°C. Subsequently, 159 mg (3.98 mmol) of a 60% suspension of sodium hydride in paraffin oil were added. The reaction mixture was stirred until the evolution of gas had ended, and then 260 μ l (2.19 mmol) of benzyl bromide were added. The cooling bath was removed, and the reaction mixture was stirred at RT for 2 h. Thereafter, the reaction mixture was concentrated, the residue was taken up in ice water, and the mixture was extracted with dichloromethane. The organic phase was washed with saturated sodium chloride solution, dried over magnesium sulphate and concentrated. The residue was purified by means of preparative HPLC. 226 mg (33% of theory) of the title compound were obtained.

20 LC-MS (Method 1): $R_t = 1.28$ min; $m/z = 342$ ($M+H$)⁺.

Intermediate 37

(2*S*)-1-(benzyloxy)-3-phenylpropan-2-amine hydrochloride

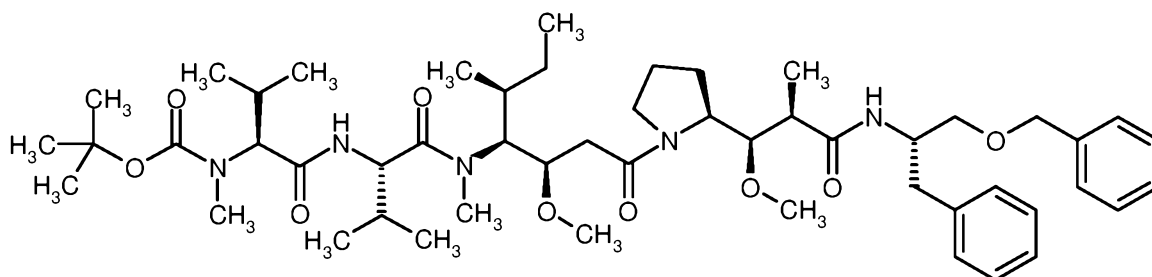


220 mg (644 μ mol) of *tert*-butyl (2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl carbamate were dissolved in 11 ml of a 4 N solution of hydrogen chloride in dioxane and stirred at RT for 1 h. Then the reaction mixture was concentrated, and the residue was dried *in vacuo*. 138 mg (77% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.65 min; m/z = 242 ($M+H$)⁺.

Intermediate 38

N-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

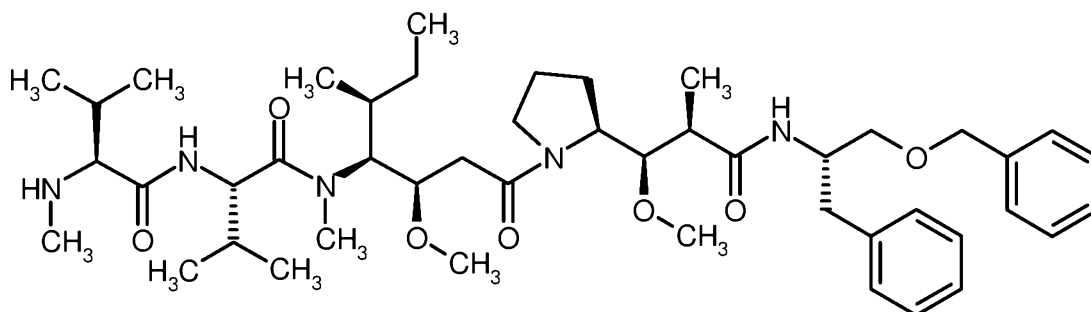


To a solution of 20 mg (29 μ mol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide in 1 ml of DMF were added, at RT, 15.3 μ l (88 μ mol) of *N,N*-diisopropylethylamine, 6.7 mg (44 μ mol) of HOBt and 6.7 mg (35 μ mol) of EDC, and the mixture was stirred for 30 min. Subsequently, 7.8 mg (32 μ mol) of (2*S*)-1-(benzyloxy)-3-phenylpropan-2-amine hydrochloride were added. After stirring overnight, the reaction mixture was separated directly into its components via preparative HPLC. 26 mg (98% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 1.51 min; m/z = 909 ($M+H$)⁺.

Intermediate 39

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



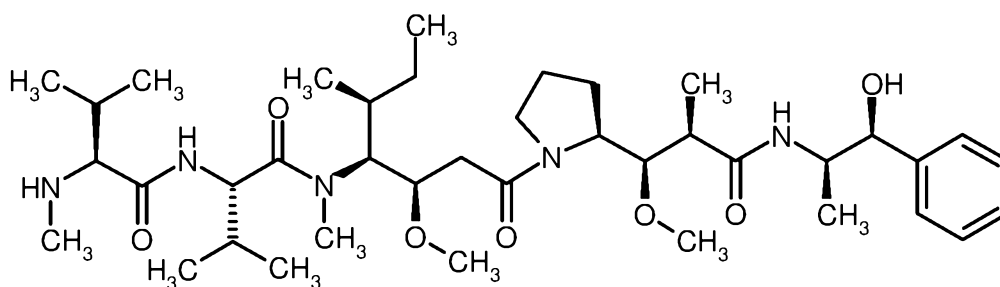
5 x CF₃COOH

26 mg (29 μmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 1.8 ml of dichloromethane, and 370 μl of TFA were added. The reaction mixture was stirred at RT for 30 min and then concentrated. The residue was taken up in water and lyophilized. 26.4 mg (quant.) of the title compound were obtained.

LC-MS (Method 1): *R*_t = 0.97 min; *m/z* = 809 (M+H)⁺.

Intermediate 40

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



50 mg (70 μmol) of Intermediate 26 and 11 mg (70 μmol) of (1*S*, 2*R*)-2-amino-1-phenylpropan-1-ol in 10 ml of DMF were admixed with 42 mg (0.11 μmol) of O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 25 μl of *N,N*-diisopropylethylamine,

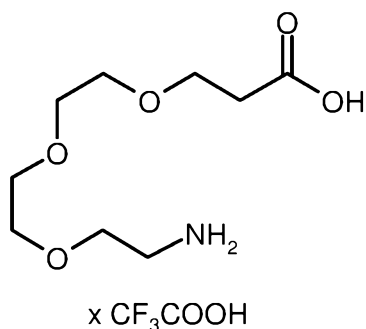
- and the reaction mixture was stirred at RT for 5 min. This was followed by concentration and purification of the residue by means of preparative HPLC. After combining the corresponding fractions, concentrating and drying under high vacuum, 49 mg (81%) of the protected intermediate were obtained. Subsequently, the Boc group was cleaved using known conditions with trifluoroacetic acid in dichloromethane. Concentration was followed by the purification of the title compound via preparative HPLC, and 26 mg (52%) of the title compound were obtained.

HPLC (Method 12): $R_t = 1.65$ min;

LC-MS (Method 1): $R_t = 0.77$ min; MS (ESIpos): $m/z = 718$ (M+H)⁺.

Intermediate 41

- 10 3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoic acid trifluoroacetate

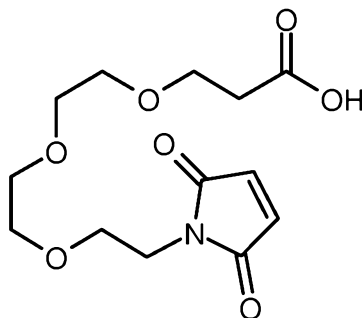


- 150 mg (541 μmol) of *tert*-butyl 3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoate were dissolved in 3 ml of dichloromethane, 1.5 ml of trifluoroacetic acid were added, and the reaction mixture was stirred at RT for 1 h, then concentrated.
- 15 181 mg (100% of theory) of the title compound were obtained.

MS (EI): m/z 222 (M+H)⁺

Intermediate 42

3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)propanoic acid



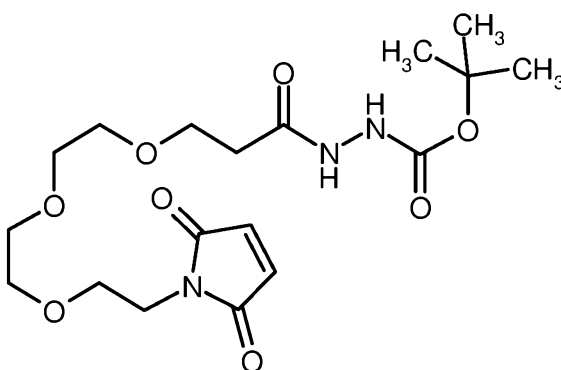
186 mg (555 μ mol) of 3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoic acid trifluoroacetate were dissolved in 2.6 ml of saturated sodium hydrogencarbonate solution and admixed at 0°C with 86 mg (555 μ mol) of N-methoxycarbonylmaleimide. The reaction mixture was stirred at 0°C for 40 min and at RT for 1 h, then cooled again to 0°C, adjusted to pH 3 with sulphuric acid and extracted 3x with 25 ml of ethyl acetate. The combined organic phases were dried over magnesium sulphate and concentrated.

126 mg (75% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.53 min; m/z = 302 ($M+H$)⁺.

10 Intermediate 43

tert-butyl-15-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-4-oxo-7,10,13-trioxa-2,3-diazapentadecan-1-oate



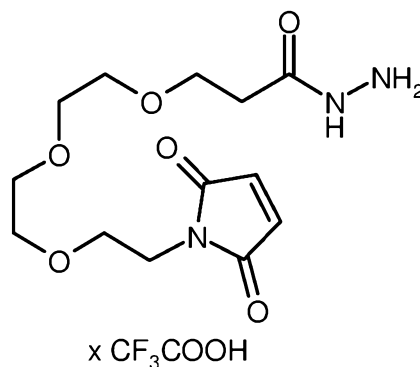
125 mg (417 μ mol) of 3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)propanoic acid were dissolved at 0°C in 2.1 ml of THF and admixed with 46 μ l (417 mmol) of 4-methylmorpholine and 54.5 μ l (417 μ mol) of isobutyl chloroformate. The ice bath was removed, and the reaction mixture was stirred at RT for 30 min. Subsequently, at 0°C, 55 mg (417 μ mol) of *tert*-butyloxycarbonyl hydrazide were added. The reaction mixture was warmed to RT overnight, concentrated and purified via preparative HPLC.

60 mg (33% of theory) of the title compound were obtained.

LC-MS (Method 1): $R_t = 0.66$ min; $m/z = 416$ ($M+H$)⁺.

Intermediate 44

3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)propanehydrazide
5 trifluoroacetate



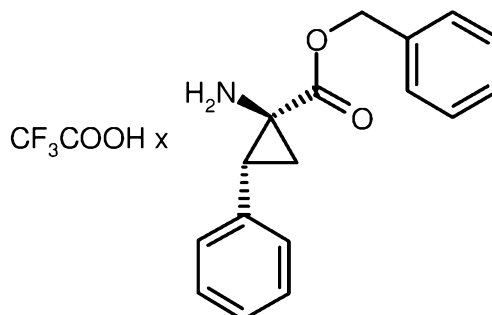
60 mg (145 μmol) of *tert*-butyl-15-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-4-oxo-7,10,13-trioxa-
2,3-diazapentadecan-1-oate were dissolved in 1 ml of dichloromethane, and 0.2 ml of
10 trifluoroacetic acid was added. The reaction mixture was stirred at RT for 30 min and then
concentrated.

62 mg (100% of theory) of the title compound were obtained.

LC-MS (Method 1): $R_t = 0.35$ min; $m/z = 316$ ($M+H$)⁺.

Intermediate 45

Benzyl-(1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate trifluoroacetate

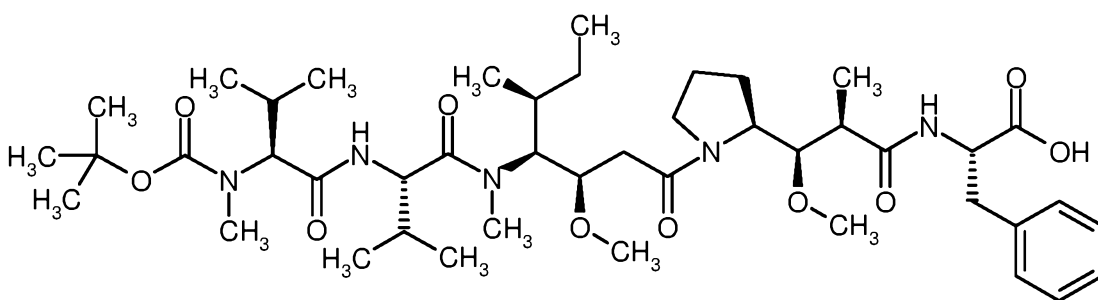


The title compound was prepared according to standard methods by esterifying commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with benzyl alcohol and subsequent Boc cleaving with trifluoroacetic acid.

LC-MS (Method 1): $R_t = 0.72$ min; MS (ESIpos): $m/z = 268$ (M+H)⁺.

5 Intermediate 46

N-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*)-1-carboxy-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



- 10 383 mg (0.743 mmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 8) were combined with 485 mg (0.743 mmol) of benzyl-*N*-[(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl]-L-phenylalaninate trifluoroacetate (Intermediate 12), 424 mg (1.114 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 388 μ l of *N,N*-diisopropylethylamine in 15 ml of DMF, and the mixture was stirred at RT for 10 min.
- 15 Subsequently, the solvent was removed *in vacuo*. The remaining residue was taken up in ethyl acetate and extracted by shaking successively with 5% aqueous citric acid solution and saturated sodium hydrogencarbonate solution. The organic phase was removed and concentrated, and the residue was purified by means of preparative HPLC. The product fractions were combined and
- 20 concentrated, and the residue was dried under high vacuum. 335 mg (48% of theory) of the benzyl ester intermediate were obtained as a foam.

LC-MS (Method 1): $R_t = 1.49$ min; MS (ESIpos): $m/z = 922$ (M+H)⁺.

- 100 mg (0.11 mmol) of this intermediate were taken up in 15 ml of methanol and the benzyl ester group was removed by hydrogenation under standard hydrogen pressure with 10% palladium on
- 25 activated carbon as a catalyst. After stirring at RT for 1 h, the catalyst was filtered off and the

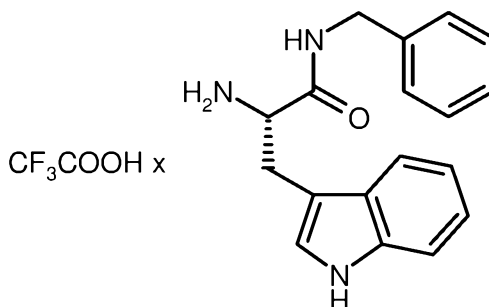
filtrate was concentrated *in vacuo*. After lyophilization from dioxane, 85 mg (94% of theory) of the title compound were obtained as a solid.

HPLC (Method 12): $R_t = 2.4$ min;

LC-MS (Method 1): $R_t = 1.24$ min; MS (ESIpos): $m/z = 832$ (M+H)⁺.

5 Intermediate 47

N-benzyl-L-tryptophanamide trifluoroacetate



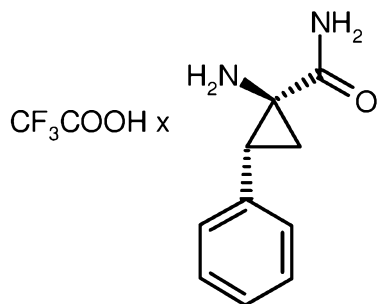
202 mg (0.5 mmol) of 2,5-dioxopyrrolidin-1-yl *N*-(tert-butoxycarbonyl)-L-tryptophanate and 45 mg (0.42 mmol) of benzylamine were dissolved in 10 ml of DMF, and 110 μ l (630 μ mol) of *N,N*-diisopropylethylamine were added. The reaction mixture was stirred at RT for 3 h. Subsequently, the reaction mixture was concentrated *in vacuo* and the residue was purified by flash chromatography on silica gel (eluent: 20:0.5:0.05 dichloromethane/methanol/17% aq. ammonia). The corresponding fractions were combined and concentrated. The resulting residue was digested with diethyl ether and then dried under high vacuum. Subsequently, this residue was taken up in 10 ml of dichloromethane, and 3 ml of anhydrous trifluoroacetic acid were added. After stirring at RT for 45 minutes, the mixture was concentrated, and the residue was purified via preparative HPLC. After drying *in vacuo*, 117 mg (57% of theory over both stages) of the title compound were obtained.

HPLC (Method 12): $R_t = 1.6$ min;

20 LC-MS (Method 1): $R_t = 0.66$ min; MS (ESIpos): $m/z = 294$ (M+H)⁺.

Intermediate 48

(1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxamide trifluoroacetate



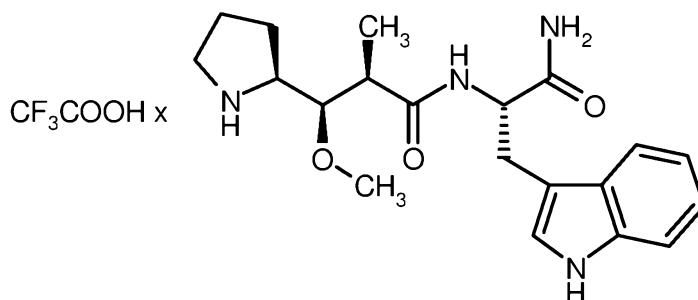
50 mg (180 μ mol) of commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid were dissolved in 5 ml of DMF, 94 μ l (541 μ mol) of *N,N*-diisopropylethylamine, 31 mg (270 μ mol) of *N*-hydroxysuccinimide and 41.5 mg (216 μ mol) of EDC were added, and then the mixture was stirred at RT overnight. The reaction mixture was then concentrated, the residue was taken up in dioxane, 71 mg (901 μ mol) of ammonium hydrogencarbonate were added, and the reaction mixture was then left to stand at RT for 3 days. The reaction mixture was then diluted with a 1:1 mixture of ethyl acetate and water. The organic phase was removed, dried over magnesium sulphate and concentrated. The resulting residue was subsequently taken up in 3 ml of dichloromethane, and 3 ml of anhydrous trifluoroacetic acid were added. Stirring at RT for 1 h was followed by concentration. The residue was stirred with pentane, suctioned off and lyophilized from dioxane. In this way, 32 mg (62% of theory over both stages) of the title compound were obtained.

HPLC (Method 6): R_t = 0.38 min;

15 LC-MS (Method 1): R_t = 0.20 min; MS (ESIpos): m/z = 177 ($M+H$)⁺.

Intermediate 49

N ^{α} -{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-tryptophanamide trifluoroacetate



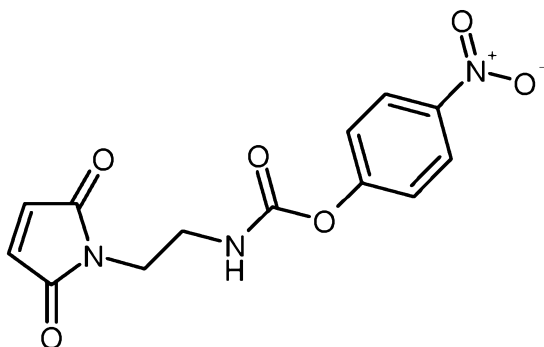
20 The title compound was prepared in analogy to the synthesis of Intermediate 13 from Starting Compound 1 and L-tryptophanamide hydrochloride.

HPLC (Method 5): $R_t = 1.4$ min;

LC-MS (Method 1): $R_t = 0.92$ min; MS (ESIpos): $m/z = 473$ (M+H)⁺.

Intermediate 50

4-nitrophenyl 2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl carbamate



5

813 mg (3.1 mmol) of triphenylphosphine were dissolved in 25 ml of THF and cooled to -70°C under argon. After the dropwise addition of 627 mg (3.1 mmol) of diisopropyl azodicarboxylate, the mixture was stirred for 5 min. Subsequently, 500 mg (3.1 mmol) of tert-butyl-(2-aminoethyl) carbamate dissolved in 5 ml of THF were added dropwise, and the reaction mixture was stirred at -70°C for another 5 min. Then 136.6 mg (1.55 mmol) of 2,2-dimethyl-1-propanol dissolved in 1 ml of THF and 301 mg (3.1 mmol) of maleimide were added, the reaction mixture was stirred at -70°C for another 10 min, and then the mixture was warmed to RT. After stirring at RT for another 16 h, the solvent was removed *in vacuo*, and the residue was purified by means of preparative HPLC. This gave 463 mg (62%) of the protected intermediate.

15 After removing the Boc protecting group under standard conditions, 652 mg of 1-(2-aminoethyl)-1*H*-pyrrole-2,5-dione were obtained as trifluoroacetate.

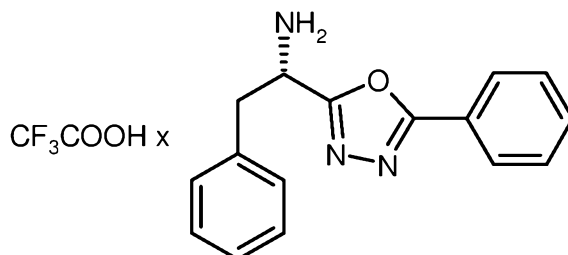
112.9 mg (543 μmol) of nitrophenyl chloroformate were dissolved in 30 ml of THF and, after the addition of 100 mg (271.6 μmol) of 1-(2-aminoethyl)-1*H*-pyrrole-2,5-dione trifluoroacetate, the mixture was stirred at RT for 30 min. The mixture was filtered, and the filtrate was concentrated to dryness and then slurried with diethyl ether. After suctioning off and drying, 60 mg (95% of theory) of the title compound were obtained.

20

HPLC (Method 5): $R_t = 0.65$ min;

LC-MS (Method 1): $R_t = 0.74$ min; MS (ESIpos): $m/z = 306$ (M+H)⁺.

Intermediate 51

(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethanamine trifluoroacetate

200 mg (0.75 mmol) of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine were initially provided at 0°C
 5 in 5.5 ml of dichloromethane, and 128 mg (0.79 mmol) of 1,1'-carbonyldiimidazole were added
 to this. After 30 min, 103 mg (0.75 mmol) of benzoyl hydrazide were added. After a further 45
 min at 0°C, 500 mg (1.5 mmol) of carbon tetrabromide and 395 mg (1.5 mmol) of
 triphenylphosphine were finally added. The reaction mixture was stirred first at 0°C for 2 h and
 then at RT overnight. The mixture was subsequently concentrated on a rotary evaporator, and the
 10 residue was dried under high vacuum. The crude product thus obtained was purified by means of
 preparative HPLC. 217 mg (78% of theory) of the Boc-protected intermediate *tert*-butyl-[(1*S*)-2-
 phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl carbamate were obtained.

LC-MS (Method 12): $R_t = 1.15$ min; MS (ESIpos): $m/z = 366$ (M+H)⁺

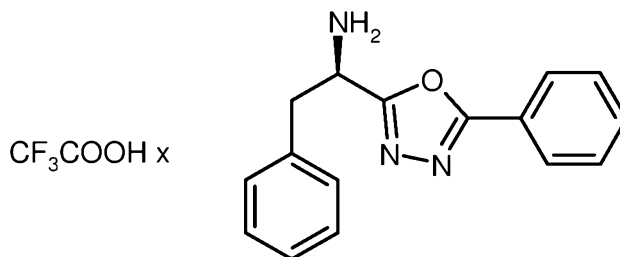
217 mg (0.59 mmol) of this intermediate were taken up in 3 ml of dichloromethane, 0.6 ml of
 15 trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the
 reaction mixture was concentrated *in vacuo*. The remaining residue was the reaction mixture and
 was dried further *in vacuo*, then lyophilized from dioxane. In this way, 214 mg (90% of theory)
 of the title compound were obtained.

LC-MS (Method 11): $R_t = 0.62$ min; MS (ESIpos): $m/z = 266$ (M+H)⁺

20 Intermediate 52

(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethanamine trifluoroacetate

- 370 -



200 mg (0.75 mmol) of *N*-(*tert*-butoxycarbonyl)-D-phenylalanine were initially provided at 0°C in 5.5 ml of dichloromethane, and 128.3 mg (0.79 mmol) of 1,1'-carbonyldiimidazole were added to this. After 30 min, 103 mg (0.75 mmol) of benzoyl hydrazide were added. After another 45
 5 min at 0°C, 500 mg (1.5 mmol) of carbon tetrabromide and 395 mg (1.5 mmol) of triphenylphosphine were finally added. The reaction mixture was stirred first at 0°C for 2 h and then at RT overnight. The mixture was subsequently concentrated on a rotary evaporator, and the residue was dried under high vacuum. The crude product thus obtained was purified by means of preparative HPLC. 219 mg (80% of theory) of the Boc-protected intermediate *tert*-butyl (1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl carbamate were obtained.
 10

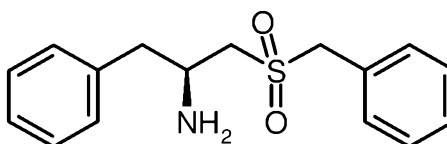
LC-MS (Method 2): $R_t = 1.36$ min; MS (ESIpos): $m/z = 366$ ($M+H$)⁺

219 mg (0.6 mmol) of this intermediate were taken up in 3 ml of dichloromethane, 0.6 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated *in vacuo*. The remaining residue was the reaction mixture and
 15 was dried further *in vacuo*, then lyophilized from dioxane. In this way, 196 mg (86% of theory) of the title compound were obtained as a solid.

HPLC (Method 10): $R_t = 2.41$ min

Intermediate 53

(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-amine



20

200 mg (1.13 mmol) of (4*S*)-4-benzyl-1,3-oxazolidin-2-one were initially provided in 3 ml of *tert*-butanol, and 280 mg (2.26 mmol) of benzyl mercaptan were added to this. The mixture was subsequently heated under reflux for 2 days. Thereafter, the reaction mixture was concentrated

on a rotary evaporator, and the resulting intermediate (2*S*)-1-(benzylsulphanyl)-3-phenylpropan-2-amine was directly converted further, without workup.

HPLC (Method 10): $R_t = 2.63$ min

LC-MS (Method 1): $R_t = 0.67$ min; MS (ESIpos): $m/z = 258$ (M+H)⁺

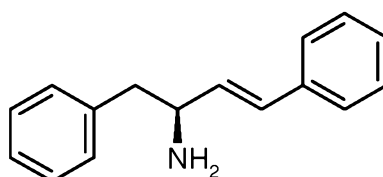
- 5 The crude intermediate obtained above was dissolved in a solution of 2 ml of 30% hydrogen peroxide and 5 ml of formic acid, and the mixture was stirred at RT for 12 h. Then the reaction mixture was added to saturated sodium sulphate solution and extracted three times with ethyl acetate. The organic phase was dried over magnesium sulphate and concentrated *in vacuo*. The obtained crude product was purified by means of preparative HPLC. 343 mg (61% of theory) of the title compound were thus obtained.
- 10

HPLC (Method 10): $R_t = 2.40$ min;

LC-MS (Method 12): $R_t = 0.65$ min; MS (ESIpos): $m/z = 290$ (M+H)⁺

Intermediate 54

(2*S*,3*E*)-1,4-diphenylbut-3-en-2-amine



15

- 552.7 mg (9.85 mmol) of potassium hydroxide were dissolved in methanol, adsorbed onto 1.1 g of neutral aluminium oxide and then dried under high vacuum. To a solution of 240 mg (0.82 mmol) of (2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-amine and 1.56 g of the potassium hydroxide on aluminium oxide thus prepared in 6.2 ml of *n*-butanol were added dropwise, at 5-10°C, 307 µl (3.3 mmol) of dibromodifluoromethane. The reaction mixture was stirred at RT for 2 h, then filtered through Celite, and the residue was washed thoroughly with dichloromethane afterwards. The filtrate was concentrated, and the resulting residue was dried *in vacuo*. The crude product thus obtained was purified by means of preparative HPLC. 98 mg (35% of theory) of the title compound were obtained with an *E/Z* diastereomer ratio of 4:1.
- 20
- 25

HPLC (Method 10): $R_t = 2.46$ min;

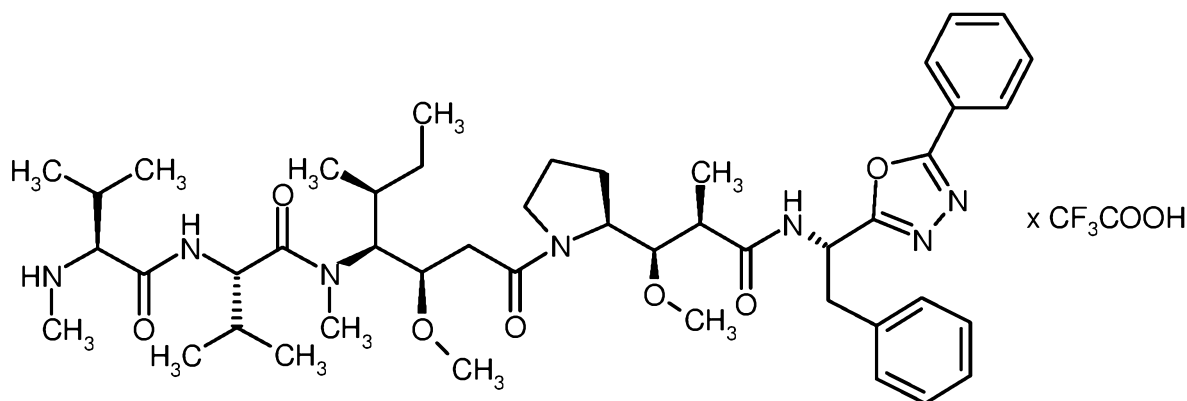
LC-MS (Method 12): $R_t = 0.75$ min; MS (ESIpos): $m/z = 224$ ($M+H$)⁺

The *E/Z* diastereomer mixture obtained above was dissolved in 2 ml of ethanol and 0.2 ml of *N,N*-diisopropylethylamine and separated by means of HPLC on chiral phase [column: Daicel Chiralpak AD-H, 5 μ m, 250 mm x 20 mm, eluent: hexane/(ethanol + 0.2% diethylamine) 50:50 v/v; UV detection: 220 nm; temperature: 30°C]. The appropriate fractions were concentrated on a rotary evaporator, and the residue was dried *in vacuo*. 45 mg of the title compound were obtained.

¹H NMR (400 MHz, DMSO-*d*₆) δ [ppm] = 2.62 - 2.83 (m, 2 H) 3.52 - 3.71 (m, 1 H) 6.18 - 6.30 (m, 1 H) 6.34 - 6.46 (m, 1 H) 6.98 - 7.57 (m, 10 H) [further signals hidden under solvent peaks].

10 Intermediate 55

N-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide trifluoroacetate



15

20 mg (29 μ mol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide were dissolved in 1 ml of DMF, 13.3 mg (35 μ mol) of HATU and 15.3 μ l (88 μ mol) of *N,N*-diisopropylethylamine were added, and the mixture was stirred at RT for 30 min. Subsequently, 12.2 mg (32 μ mol) of (1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethanamine trifluoroacetate were added. The reaction mixture was stirred at RT overnight and then separated by means of preparative HPLC. This gave 22 mg (81% of theory) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide.

25

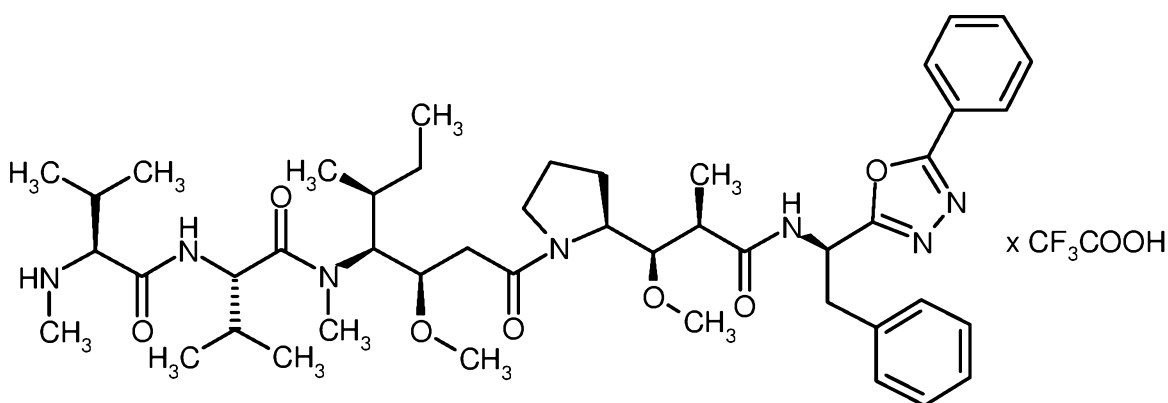
LC-MS (Method 12): $R_t = 1.45$ min; MS (ESIpos): $m/z = 933$ ($M+H$)⁺

By subsequently cleaving the BOC protecting group with trifluoroacetic acid, 22.4 mg (98% of theory) of the title compound were then obtained.

LC-MS (Method 11): $R_t = 0.85$ min; MS (ESIpos): $m/z = 833$ ($M+H$)⁺

5 Intermediate 56

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino]propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



10

N-(*tert*-Butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino]propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared in analogy to the synthesis of Intermediate 55, by reacting 20 mg (29 μ mol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide with 12.2 mg (32 μ mol) of (1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethanamine trifluoroacetate.

15

Yield: 17 mg (64% of theory)

20 HPLC (Method 10): $R_t = 3.74$ min;

LC-MS (Method 1): $R_t = 1.45$ min; MS (ESIpos): $m/z = 933$ ($M+H$)⁺

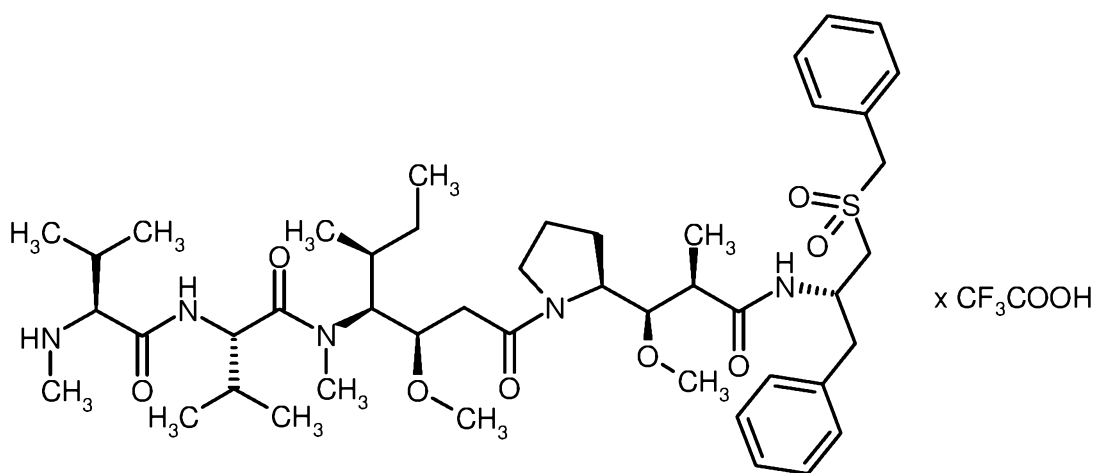
By subsequently cleaving the BOC protecting group with trifluoroacetic acid, 17.1 mg (99% of theory) of the title compound were thus obtained.

HPLC (Method 10): $R_t = 2.55$ min;

LC-MS (Method 11): $R_t = 0.85$ min; MS (ESIpos): $m/z = 833$ (M+H)⁺

Intermediate 57

5 *N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide trifluoroacetate



10 *N*-(*tert*-Butoxycarbonyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide was prepared in analogy to the synthesis of Intermediate 55, by reacting 20 mg (29 μ mol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide with 9.3 mg (20 μ mol) of (2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-amine.

15 Yield: 19.2 mg (68 % of theory)

HPLC (Method 10): $R_t = 3.5$ min;

LC-MS (Method 12): $R_t = 1.41$ min; MS (ESIpos): $m/z = 957$ (M+H)⁺

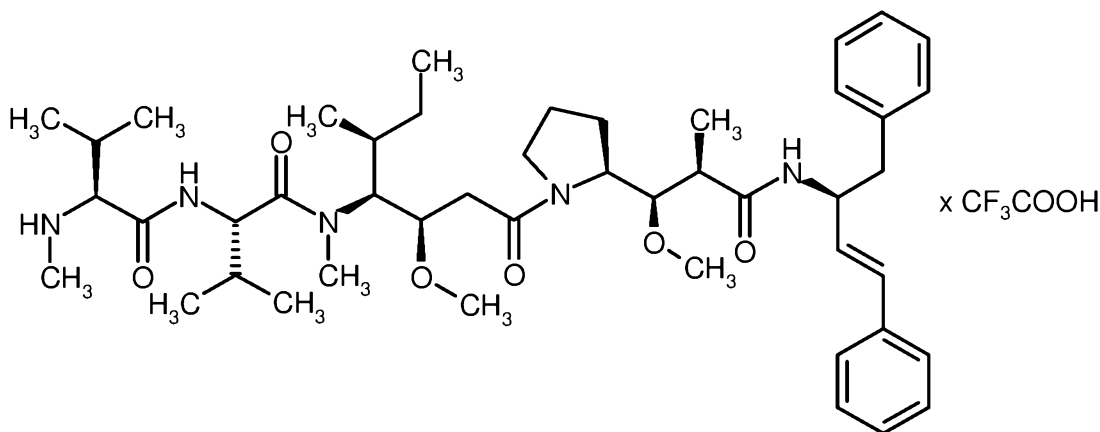
By subsequently cleaving the BOC protecting group with trifluoroacetic acid, 19.3 mg (99% of theory) of the title compound were thus obtained.

20 HPLC (Method 10): $R_t = 2.52$ min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 857$ (M+H)⁺

Intermediate 58

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



N-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared in analogy to the synthesis of Intermediate 55, by reacting 20 mg (29 μ mol) *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide with 7.1 mg (32 μ mol) of (2*S*,3*E*)-1,4-diphenylbut-3-en-2-amine.

Yield: 15.1 mg (58 % of theory)

HPLC (Method 10): $R_t = 4.2$ min;

LC-MS (Method 12): $R_t = 1.51$ min; MS (ESIpos): $m/z = 891$ (M+H)⁺

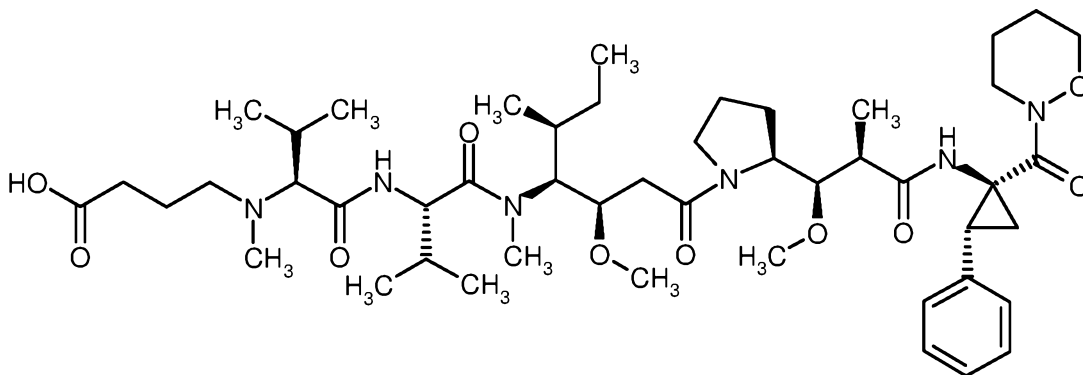
By subsequently cleaving the BOC protecting group with trifluoroacetic acid, 15.7 mg (99% of theory) of the title compound were then obtained.

HPLC (Method 10): $R_t = 2.62$ min;

LC-MS (Method 12): $R_t = 0.97$ min; MS (ESIpos): $m/z = 791$ (M+H)⁺

Intermediate 61

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



5

50 mg (0.054 mmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-((2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[[*(1S,2R)*-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 16) were dissolved in 8 ml of dioxane/water, and 70 ml (0.108 mmol) of a 15% solution of 4-oxobutanoic acid in water were added. The reaction mixture was subsequently stirred at 100°C for 1 h. After cooling to RT, 3.7 mg (0.059 mmol) of sodium cyanoborohydride were added, and the mixture was adjusted to a pH of 3 by adding about 300 µl of 0.1 N hydrochloric acid. The reaction mixture was then stirred at 100°C for another 2 h. After cooling, another 70 ml (0.108 mmol) of the 15% 4-oxobutanoic acid solution were added, and the reaction mixture was stirred again at 100°C for 1 h. Then a further 3.7 mg (0.059 mmol) of sodium cyanoborohydride were added, and about 300 µl of 0.1 N hydrochloric acid were subsequently used to readjust the pH to 3. The reaction mixture was then stirred at 100°C for another 2 h. For a conversion that was still incomplete, this procedure was repeated for a third time. The reaction mixture was finally concentrated, and the residue was purified by means of preparative HPLC. In this way, 32 mg (65% of theory) of the title compound were obtained in the form of a colourless foam.

HPLC (Method 5): $R_t = 1.64$ min;

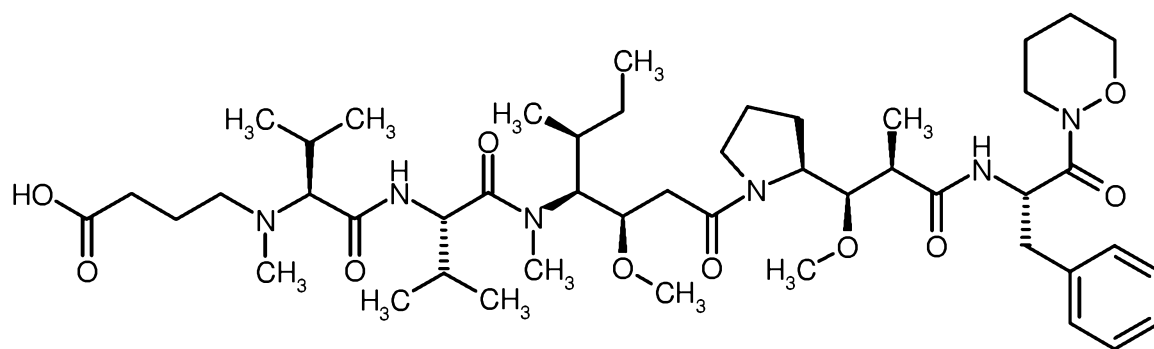
LC-MS (Method 9): $R_t = 4.76$ min; MS (ESIpos): $m/z = 899$ ($M+H$)⁺

¹H NMR (500 MHz, DMSO-d₆): δ = 8.95 and 8.8 (2m, 1H), 8.88 and 8.65 (2s, 1H), 7.4-7.1 (m, 5H), 5.0, 4.78, 4.65 and 4.55 (4m, 2H), 4.1-3.7 (m, 5H), 3.32, 3.29, 3.20, 3.12, 3.1 and 3.0 (6s,

9H), 2.75 (m, 2H), 2.63 (t, 1H), 2.4-2.2 (m, 4H), 2.1-1.2 (m, 12H), 1.2-0.8 (m, 16H), 0.75 (m, 3H) [further signals hidden under H₂O and DMSO peaks].

Intermediate 62

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{{(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl}amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



The title compound was prepared in analogy to the synthesis of Intermediate 61, by reacting 50 mg of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{{(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl}amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 14) with 4-oxobutanoic acid.

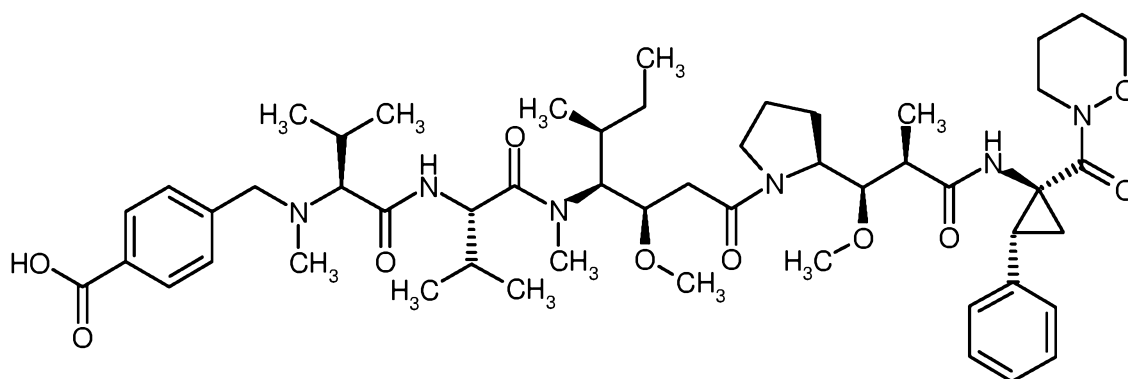
Yield: 34 mg (70% of theory)

HPLC (Method 5): *R*_t = 1.64 min;

LC-MS (Method 9): *R*_t = 4.77 min; MS (ESIpos): *m/z* = 887 (*M*+*H*)⁺.

Intermediate 63

N-(4-carboxybenzyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{{(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl}amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



The title compound was prepared in analogy to the synthesis of Intermediate 61 by reacting 15 mg of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 16) with 4-formylbenzoic acid.

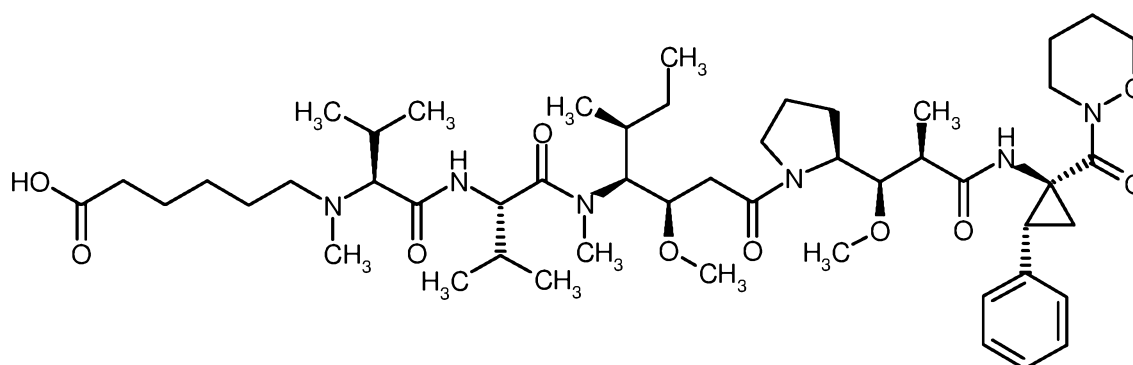
Yield: 7.5 mg (48% of theory)

HPLC (Method 5): $R_t = 1.75$ min;

LC-MS (Method 1): $R_t = 0.97$ min; MS (ESIpos): $m/z = 947$ ($M+H$)⁺.

10 Intermediate 64

N-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



10 mg (0.011 mmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate

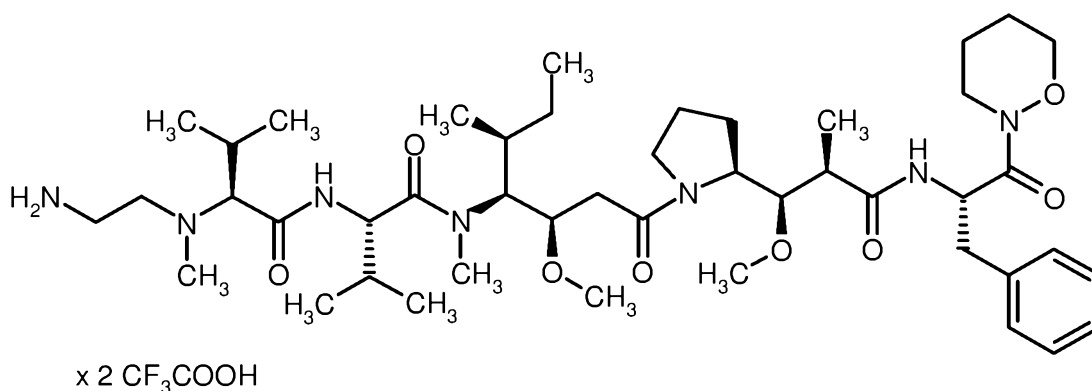
(Intermediate 16) were dissolved in 2 ml of dioxane/water, and 2.8 mg (0.022 mmol) of 6-oxohexanoic acid were added. The reaction mixture was subsequently stirred at 100°C for 1 h. After cooling to RT, 0.75 mg (0.012 mmol) of sodium cyanoborohydride were added, and the mixture was adjusted to a pH of 3 by adding 0.1 N hydrochloric acid. The reaction mixture was then stirred at 100°C for another hour. After cooling, another 2.8 mg (0.022 mmol) of 6-oxohexanoic acid were added, and the reaction mixture was again stirred at 100°C for 1 h. A further 0.75 mg (0.012 mmol) of sodium cyanoborohydride were added, and 0.1 N hydrochloric acid was subsequently used to readjust the pH to 3. The reaction mixture was then stirred at 100°C for another 1 h. This procedure was then repeated for a third time. The reaction mixture was finally concentrated, and the crude product was purified by means of preparative HPLC. This gave 6.4 mg (64% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 5): $R_t = 1.68$ min;

LC-MS (Method 9): $R_t = 4.86$ min; MS (ESIpos): $m/z = 927$ (M+H)⁺.

Intermediate 65

N-(2-aminoethyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide bistrifluoroacetate



The title compound was prepared by reacting 68 mg of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 14) with *tert*-butyl-(2-oxoethyl)carbamate and subsequent cleaving of the Boc protecting group with trifluoroacetic acid.

Yield: 49 mg (62% of theory over two stages)

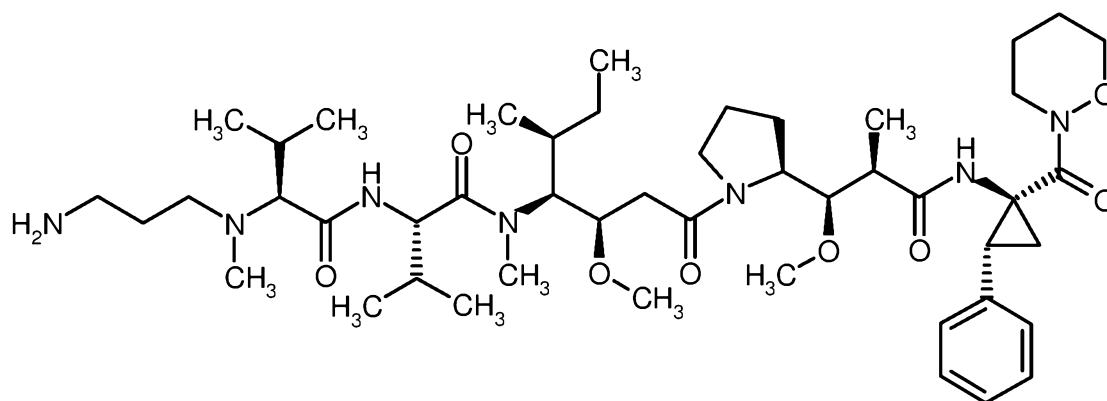
HPLC (Method 5): $R_t = 1.58$ min;

LC-MS (Method 2): $R_t = 1.05$ min; MS (ESIpos): $m/z = 844$ ($M+H$)⁺

¹H NMR (600 MHz, DMSO-d₆): $\delta = 8.25$ (m, 1H), 8.45 and 8.15 (2d, 1H), 7.65-7.55 (m, 3H), 7.23-7.1 (m, 5H), 5.12 and 4.95 (2m, 1H), 4.72 and 4.62 (2m, 1H), 4.6 and 4.52 (2t, 1H), 4.2-3.8 (m, 4H), 3.7 (d, 1H), 3.23, 3.20, 3.19, 3.18, 3.03 and 2.98 (6s, 9H), 3.0-2.7 (m, 6H), 2.4-1.2 (m, 15H), 1.05, 1.0, 0.88 and 0.82 (4d, 6H), 0.92 (m, 6H), 0.73 (m, 6H) [further signals hidden under H₂O peak].

Intermediate 66

N-(3-aminopropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



The title compound was prepared in analogy to the synthesis of Intermediate 65 by reacting 25 mg (0.027 mmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 16) with benzyl-(3-oxopropyl)carbamate and subsequent hydrogenolytic cleaving of the Z protecting group (with 10% palladium on charcoal as a catalyst, in ethanol as a solvent).

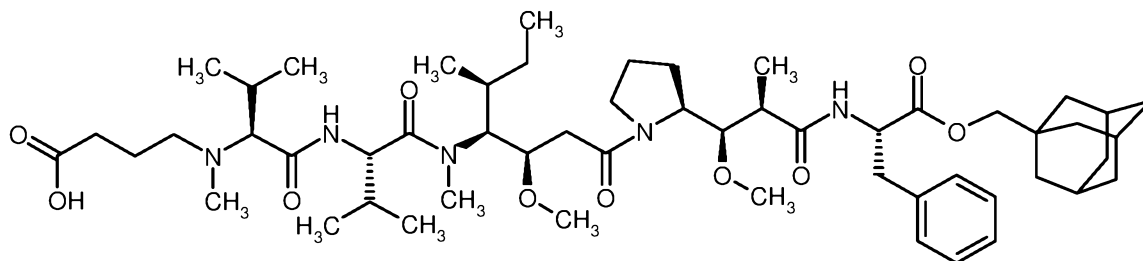
Yield: 11 mg (41% of theory over two stages)

HPLC (Method 5): $R_t = 1.53$ min;

LC-MS (Method 1): $R_t = 0.72$ min; MS (ESIpos): $m/z = 870$ ($M+H$)⁺.

Intermediate 67

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(adamantan-1-ylmethoxy)-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



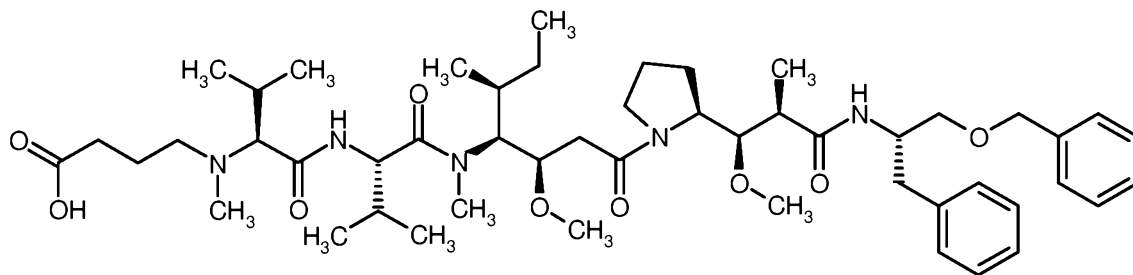
5

26 mg (26 μ mol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(adamantan-1-ylmethoxy)-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate and
 10 33.9 μ l of a 15% aqueous succinaldehydic acid solution (53 μ mol) were dissolved in 957 μ l of a 1:1-dioxane/water mixture and heated to 100°C for 1 h. After brief cooling, 1.81 mg (29 μ mol) of sodium cyanoborohydride were added. The reaction mixture was adjusted to pH 3 by adding 0.1 N hydrochloric acid, and the mixture was heated to 100°C for another 2 h. After again adding the same amounts of succinaldehydic acid solution, sodium cyanoborohydride and hydrochloric acid,
 15 acid, the mixture was heated once again to 100°C for 2 h. The reaction mixture was then directly separated into its components by means of preparative HPLC. 18.5 mg (73% of theory) of the title compound were thus obtained.

LC-MS (Method 1): R_t = 1.17 min; m/z = 967 ($M+H$)⁺.

Intermediate 68

20 *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

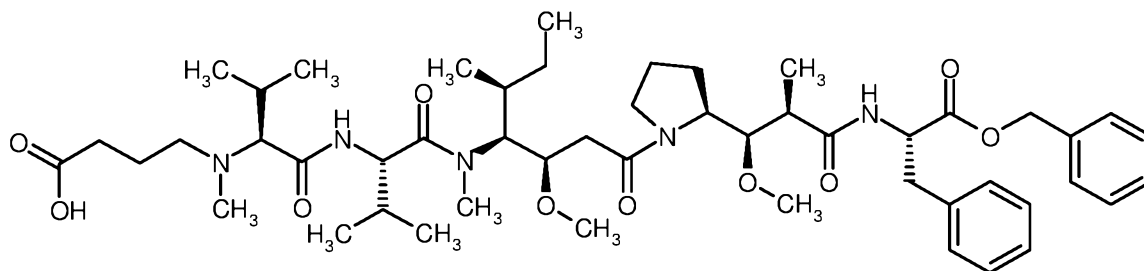


24 mg (26 μ mol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate and 33.7 μ l of a 15% aqueous succinaldehydic acid solution (52 μ mol) were dissolved in 953 μ l of a 1:1-dioxane/water mixture and heated to 100°C for 1 h. After brief cooling, 1.80 mg (29 μ mol) of sodium cyanoborohydride were added. The reaction mixture was adjusted to pH 3 by adding 0.1 N hydrochloric acid and the mixture was heated to 100°C for another 2 h. After adding the same amounts of succinaldehydic acid solution, sodium cyanoborohydride and hydrochloric acid again, the mixture was heated once again to 100°C for 2 h. The reaction mixture was then directly separated into its components by means of preparative HPLC. 15.2 mg (65% of theory) of the title compound were thus obtained.

LC-MS (Method 1): R_t = 1.01 min; m/z = 895 ($M+H$)⁺

15 Intermediate 69

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



20

53 mg (84 μ mol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) and 45 mg (84 μ mol) of benzyl *N*-[(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl]-L-

phenylalaninate trifluoroacetate (Intermediate 12) were taken up in 2 ml of DMF, 19 µl of *N,N*-diisopropylethylamine, 14 mg (92 µmol) of HOBt and 17.6 mg (92 µmol) of EDC were added, and then the mixture was stirred at RT overnight. Subsequently, the reaction mixture was concentrated and the residue was purified by means of preparative HPLC. This gave 59 mg (68% of theory) of the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

LC-MS (Method 1): $R_t = 1.55$ min; $m/z = 1044$ (M+H)⁺.

57 mg (0.055 mmol) of this intermediate were treated with 1.2 ml of piperidine in 5 ml of DMF to cleave the Fmoc protecting group. After concentration and purification by means of preparative HPLC, 39 mg (76% of theory) of the free amine intermediate *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as the trifluoroacetate.

HPLC (Method 5): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 1.01$ min; $m/z = 822$ (M+H)⁺.

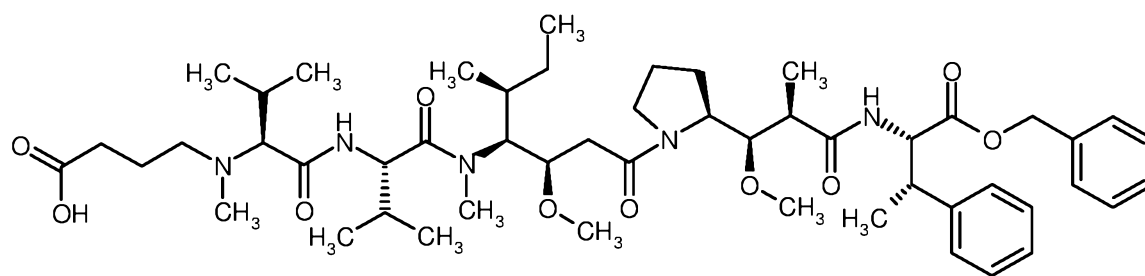
37 mg (0.045 mmol) of this intermediate were dissolved in 5 ml of dioxane/water and, in analogy to the preparation of the compound in Intermediate 66, reacted with a 15% aqueous solution of 4-oxobutanoic acid in the presence of sodium cyanoborohydride. 16 mg (39% of theory) of the title compound were obtained in the form of a colourless foam.

HPLC (Method 6): $R_t = 2.1$ min;

LC-MS (Method 1): $R_t = 1.01$ min; MS (ESIpos): $m/z = 908$ (M+H)⁺.

Intermediate 70

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 14, proceeding from Intermediates 4 and 26, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared.

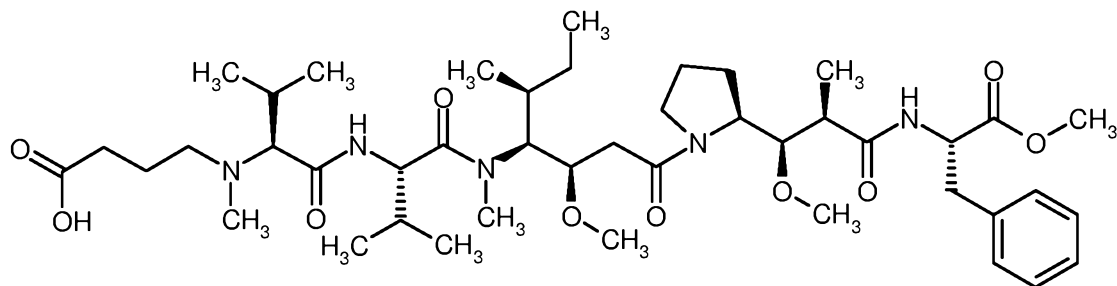
30 mg (0.032 mmol) of this compound were dissolved in 6 ml of dioxane/water, and 41 μ l (0.063 mmol) of a 15% aqueous solution of 4-oxobutanoic acid were added. The reaction mixture was subsequently stirred at 100°C for 1 h. After cooling to RT, 2.2 mg (0.035 mmol) of sodium cyanoborohydride were added, and the mixture was adjusted to a pH of 3 by adding about 300 μ l of 0.1 N hydrochloric acid. The reaction mixture was then stirred at 100°C for another 2 h. After cooling, another 41 μ l (0.063 mmol) of the 15% 4-oxobutanoic acid solution were added, and the reaction mixture was again stirred at 100°C for 1 h. Then a further 2.2 mg (0.035 mmol) of sodium cyanoborohydride were added, and about 300 μ l of 0.1 N hydrochloric acid were subsequently used to adjust the pH back to 3. The reaction mixture was then stirred at 100°C for another 2 h. In the event of the conversion still being incomplete, this procedure was repeated for a third time. The reaction mixture was finally concentrated and the crude product was purified by means of preparative HPLC. This gave 24 mg (82% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 5): $R_t = 1.9$ min;

LC-MS (Method 9): $R_t = 5.15$ min; MS (ESIpos): $m/z = 922$ ($M+H$)⁺.

Intermediate 71

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-3-[(2*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino}-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 14, proceeding from Intermediates 4 and 39, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-3-{[(2*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino}-2-methyl-3-

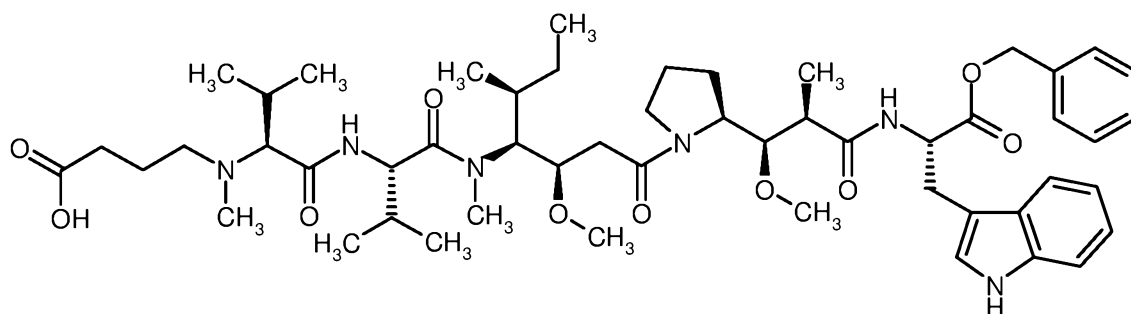
5 oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared. 7
mg (0.009 mmol) of this compound were then used, in analogy to the preparation of Intermediate
61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain
2 mg (22% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 6): $R_t = 1.9$ min;

10 LC-MS (Method 2): $R_t = 1.06$ min; MS (ESIpos): $m/z = 832$ ($M+H$)⁺.

Intermediate 72

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



15

212 mg (411 μ mol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 8) and 237 mg (411 μ mol) of benzyl-*N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-tryptophanate trifluoroacetate (Intermediate 20) were taken up in 30 ml of DMF, and 188 mg (493 μ mol) of *O*-20 (7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 215 μ l *N,N*-diisopropylethylamine were added. The reaction mixture was stirred at RT for 20 h, then

concentrated *in vacuo*, and the residue was purified by means of preparative HPLC. The product fractions were combined and concentrated, and the residue was dried under high vacuum. This gave 315 mg (80% of theory) of the Boc-protected intermediate *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{(2*S*)-1-(benzyloxy)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide as a colourless foam.

LC-MS (Method 1): $R_t = 1.45$ min; $m/z = 961$ ($M+H$)⁺.

50 mg (52 μ mol) of this intermediate were treated with 1 ml of trifluoroacetic acid in 9 ml of dichloromethane to cleave the Boc protecting group. After concentration and purification by means of preparative HPLC, 29 mg (57% of theory) of the free amine intermediate *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{(2*S*)-1-(benzyloxy)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as trifluoroacetate.

LC-MS (Method 1): $R_t = 0.99$ min; $m/z = 861$ ($M+H$)⁺.

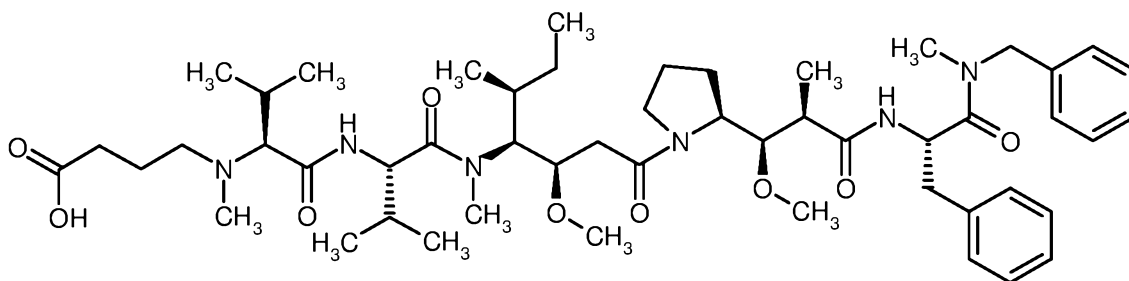
29 mg (0.03 mmol) of this intermediate were dissolved in 6 ml of dioxane/water, and 39 μ l (0.059 mmol) of a 15% aqueous solution of 4-oxobutanoic acid were added. The reaction mixture was subsequently stirred at 100°C for 1 h. After cooling to RT, 2 mg (0.033 mmol) of sodium cyanoborohydride were added, and the mixture was adjusted to a pH of 3 by adding about 300 μ l of 0.1 N hydrochloric acid. The reaction mixture was then stirred at 100°C for further 2 h. After cooling, another 39 μ l (0.059 mmol) of the 15% 4-oxobutanoic acid solution were added, and the reaction mixture was again stirred at 100°C for 1 h. Then a further 2 mg (0.033 mmol) of sodium cyanoborohydride were added, and about 300 μ l of 0.1 N hydrochloric acid were subsequently used to adjust the pH back to 3. The mixture was then stirred at 100°C for another 2 h. Thereafter, the reaction mixture was poured onto a 1:1 mixture of semisaturated aqueous ammonium chloride solution and ethyl acetate. The organic phase was removed, washed with saturated sodium chloride solution, dried over sodium sulphate and concentrated. The residue was freeze-dried from water/ acetonitrile. This gave 27 mg (94% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 5): $R_t = 2.2$ min;

LC-MS (Method 9): $R_t = 5.04$ min; MS (ESIpos): $m/z = 947$ ($M+H$)⁺.

Intermediate 73

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



5

First, in analogy to the synthesis described in Intermediate 14, proceeding from Intermediates 4 and 38, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared. 25 mg (0.026 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 13 mg (54% of theory) of the title compound in the form of a colourless foam.

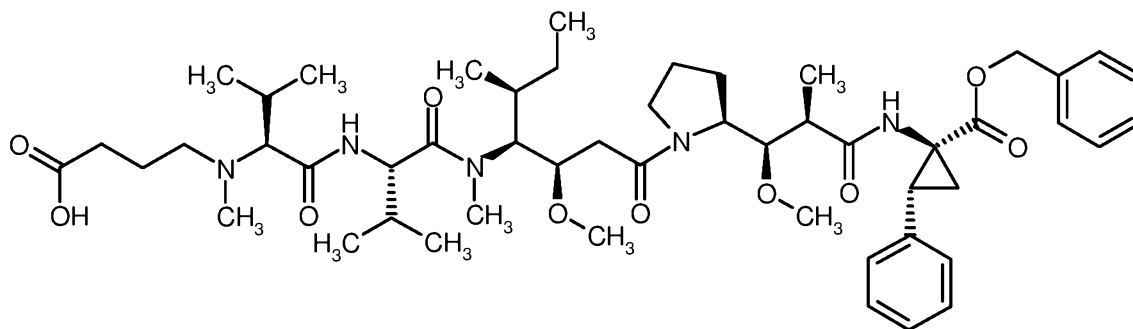
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HPLC (Method 12): $R_t = 2.2$ min;

15 LC-MS (Method 9): $R_t = 5.01$ min; MS (ESIpos): $m/z = 921$ ($M+H$)⁺.

Intermediate 74

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-[(benzyloxy)carbonyl]-2-phenylcyclopropyl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



50 mg (73 μ mol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and 28 mg (73 μ mol) of benzyl (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate trifluoroacetate (Intermediate 45) were taken up in 5 ml of DMF, and 42 mg (110 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 38 μ l of *N,N*-diisopropylethylamine were added. The reaction mixture was stirred at RT for 5 h, then concentrated *in vacuo*, and the residue was purified by means of preparative HPLC. The product fractions were combined and concentrated. After lyophilization from dioxane/water, 35 mg (51% of theory) of the Boc-protected intermediate *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-[(benzyloxy)carbonyl]-2-phenylcyclopropyl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as a colourless foam.

LC-MS (Method 1): R_t = 1.52 min; m/z = 934 ($M+H$)⁺.

35 mg of this intermediate were treated with 1 ml of trifluoroacetic acid in 5 ml of dichloromethane to cleave the Boc protecting group. After concentration and lyophilization from dioxane/water, 34 mg (97% of theory) of the free amine intermediate *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-[(benzyloxy)carbonyl]-2-phenylcyclopropyl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as the trifluoroacetate.

LC-MS (Method 1): R_t = 0.91 min; m/z = 834 ($M+H$)⁺.

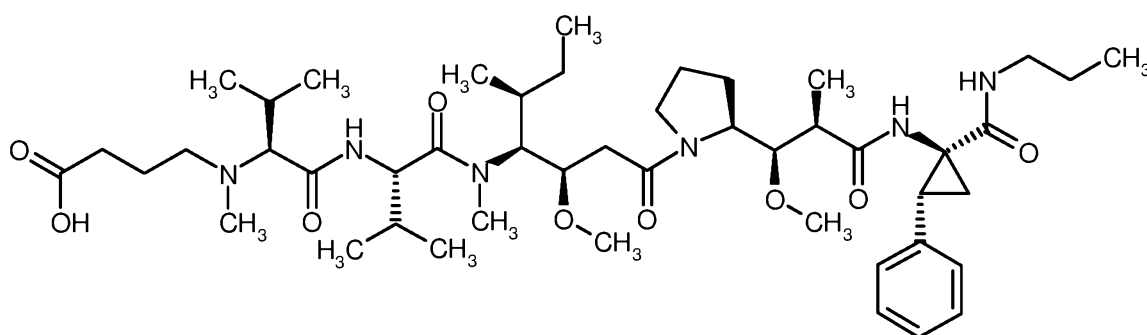
11 mg (0.011 mmol) of this intermediate were then used, in analogy to the preparation of Intermediate 66, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 2.5 mg (24% of theory) of the title compound in the form of a colourless foam.

HPLC (Method 12): $R_t = 2.2$ min;

LC-MS (Method 9): $R_t = 5.1$ min; MS (ESIpos): $m/z = 920$ (M+H)⁺.

Intermediate 75

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*S*,2*R*)-2-phenyl-1-(propylcarbamoyl)cyclopropyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



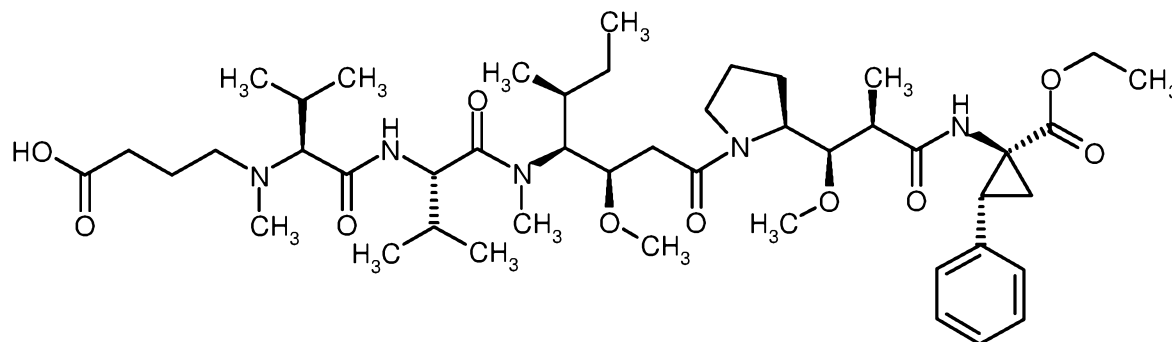
First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and (1*S*,2*R*)-1-amino-2-phenyl-*N*-propylcyclopropanecarboxamide trifluoroacetate (Intermediate 27) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*S*,2*R*)-2-phenyl-1-(propylcarbamoyl)cyclopropyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as trifluoroacetate. 14 mg (0.016 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 11.3 mg (83% of theory) of the title compound.

HPLC (Method 6): $R_t = 1.9$ min;

LC-MS (Method 2): $R_t = 1.27$ min; MS (ESIpos): $m/z = 871$ (M+H)⁺.

Intermediate 76

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-(ethoxycarbonyl)-2-phenylcyclopropyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



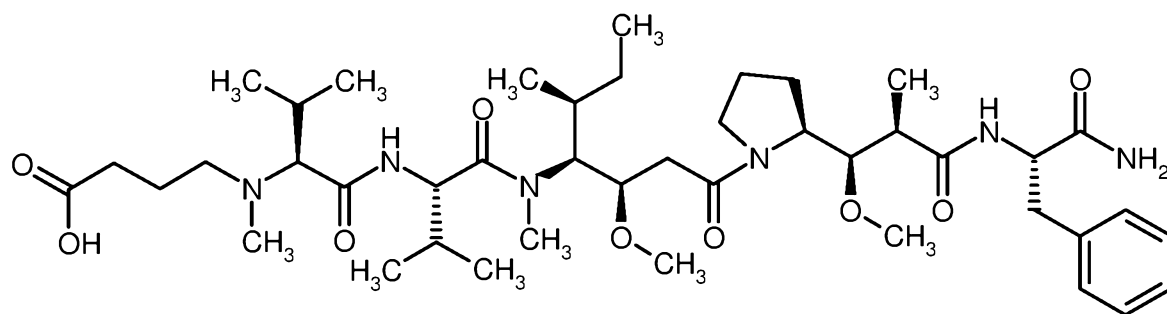
- 5 First, by coupling of Intermediate 46 (*N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide) with Intermediate 48 (ethyl (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate trifluoroacetate) in the presence of O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent Boc cleaving, the starting compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-(ethoxycarbonyl)-2-phenylcyclopropyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate was prepared. 70 mg (0.079 mmol) of this starting material were then used, by reaction with 4-oxobutanoic acid, in analogy to Intermediate 61, to obtain 46 mg (68% of theory) of the title compound.

HPLC (Method 6): $R_t = 1.9$ min;

LC-MS (Method 2): $R_t = 1.28$ min; MS (ESIpos): $m/z = 858$ ($M+H$)⁺

Intermediate 77

- 20 *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 75, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and L-phenylalaninamide hydrochloride in the presence of O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 47 mg (0.049 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 39 mg (96% of theory) of the title compound.

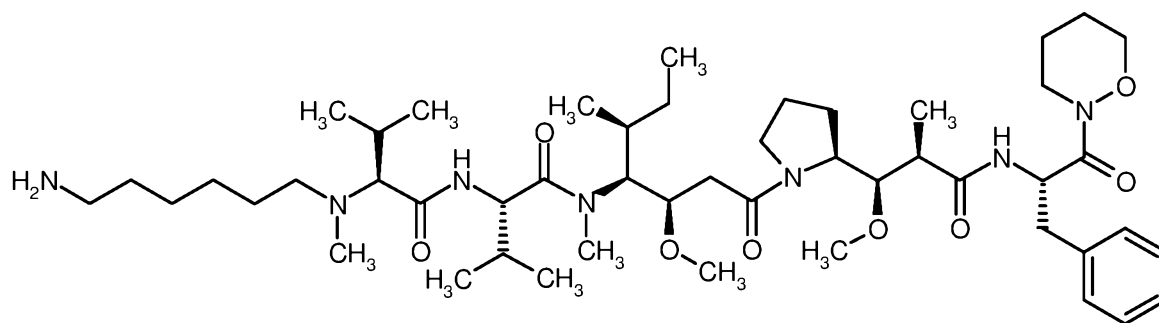
HPLC (Method 6): $R_t = 1.7$ min;

LC-MS (Method 9): $R_t = 4.44$ min; MS (ESIpos): $m/z = 817$ ($M+H$)⁺

¹H NMR (500 MHz, DMSO-*d*₆): $\delta = 8.95$ and 8.8 (2m, 1H), 8.25 and 8.0 (2d, 1H), 7.45 , 7.35 and 7.0 (3s, broad, 2H), 7.3 - 7.1 (m, 5H), 4.8 - 4.4 (2m, 3H), 3.95 (m, 1H), 3.82 (m, 1H), 3.72 (d, 1H), 3.22 , 3.18 , 3.15 , 3.05 and 3.00 (5s, 9H), 2.85 - 2.7 (m, 4H), 2.45 - 1.6 (m, 12H), 1.5 - 1.2 (m, 3H), 1.1 - 0.7 (m, 21H) [further signals hidden under solvent peaks].

Intermediate 78

N-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 66 over 2 stages, proceeding from 20 mg (16 μ mol) of the compound from Intermediate 14 and benzyl-(6-oxohexyl)carbamate, and the hydrogenation was performed in methanol as the solvent.

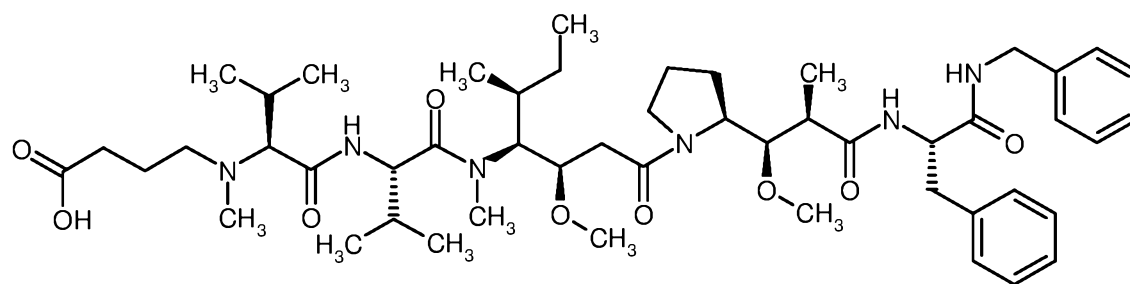
- 5 Yield: 7.6 mg (55% of theory over 2 stages)

HPLC (Method 6): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.7 min; MS (ESIpos): m/z = 901 ($M+H$)⁺.

Intermediate 79

- 10 *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



- 36 mg (43 μ mol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*)-1-carboxy-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 46) and 4.6 mg (43 μ mol) of benzylamine were taken up in 5 ml of DMF, 7.5 μ l (88 μ mol) of *N,N*-diisopropylethylamine, 10 mg (65 μ mol) of HOBt and 10 mg (52 μ mol) of EDC were added, and then the mixture was stirred at RT overnight. Subsequently, the reaction mixture was concentrated and the residue was purified by means of preparative HPLC. 29 mg (73% of theory) of the Boc-protected intermediate *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino]-1-
- 15
- 20

methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained.

LC-MS (Method 1): $R_t = 1.43$ min; $m/z = 921$ ($M+H$)⁺.

29 mg of this intermediate were treated with 1 ml of trifluoroacetic acid in 6 ml of dichloromethane to cleave the Boc protecting group. After concentration and lyophilization from dioxane/water, 30 mg (quant.) of the free amine intermediate *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-
5 {(*2S*)-2-[(1*R*,2*R*)-3-[(*2S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as the trifluoroacetate.

10 LC-MS (Method 1): $R_t = 0.95$ min; $m/z = 821$ ($M+H$)⁺.

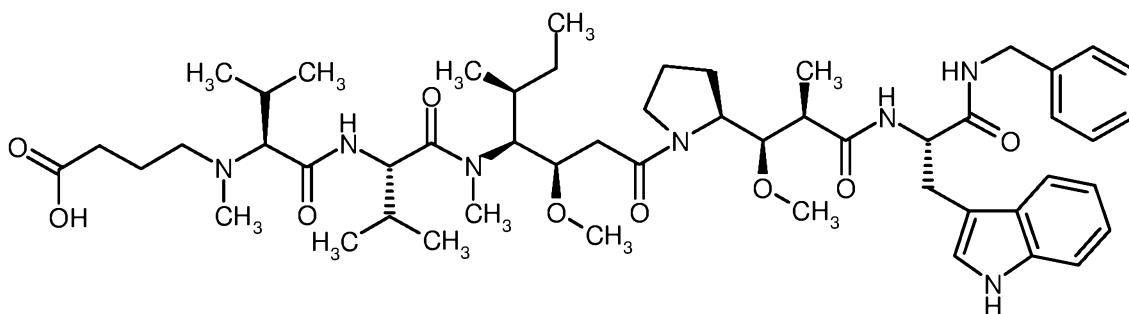
17 mg (0.018 mmol) of this intermediate were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 13 mg (80% of theory) of the title compound in the form of a colourless foam.

15 HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 9): $R_t = 4.97$ min; MS (ESIpos): $m/z = 907$ ($M+H$)⁺.

Intermediate 80

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(*2S*)-2-[(1*R*,2*R*)-3-[(*2S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-
20 oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(*2S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

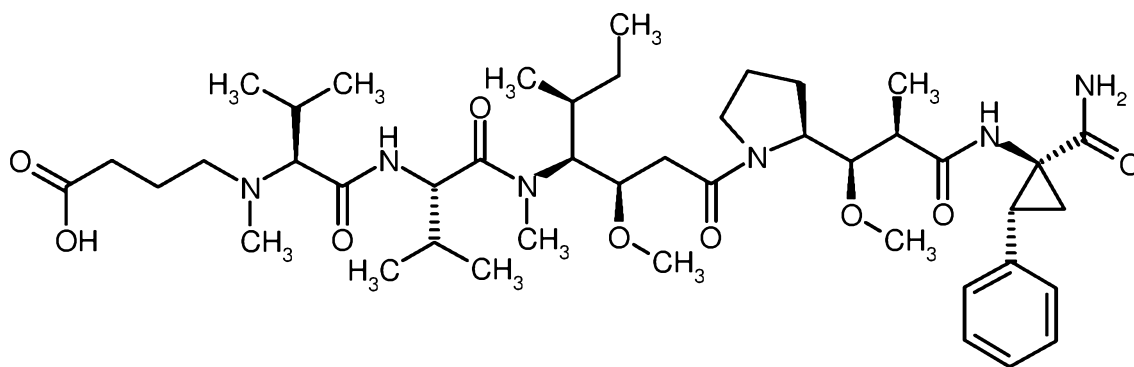
(Intermediate 26) and *N*-benzyl-L-tryptophanamide trifluoroacetate (Intermediate 47) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 10 mg (0.01 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 2.5 mg (26% of theory) of the title compound.

10 HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 2): $R_t = 1.13$ min; MS (ESIpos): $m/z = 946$ (M+H)⁺.

Intermediate 81

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-carbamoyl-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxamide trifluoroacetate (Intermediate 48) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-carbamoyl-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 14 mg (0.0163 mmol) of this compound were then used, in

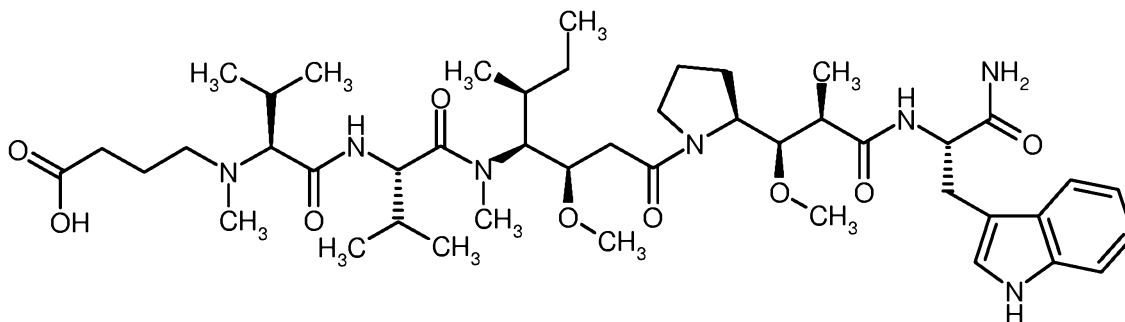
analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 8 mg (57% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 9): $R_t = 4.64$ min; MS (ESIpos): $m/z = 829$ (M+H)⁺.

5 Intermediate 82

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



- 10 First, in analogy to the synthesis described in Intermediate 69, by coupling of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) and *N*^α-[(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl]-L-tryptophanamide trifluoroacetate (Intermediate 49) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and
- 15 subsequent cleaving of the Fmoc protecting group by means of piperidine, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 78 mg (0.088 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61,
- 20 by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 68 mg (90% of theory) of the title compound.

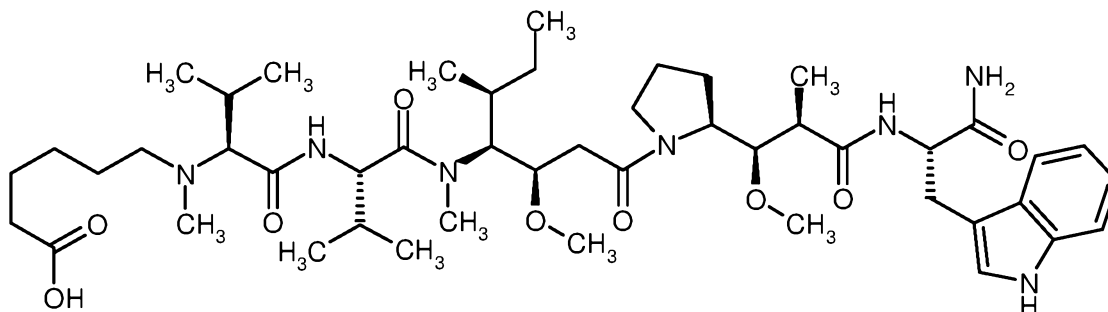
HPLC (Method 5): $R_t = 1.8$ min;

LC-MS (Method 9): $R_t = 4.49$ min; MS (ESIpos): $m/z = 856$ (M+H)⁺.

Intermediate 83

N-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

5



This compound was prepared in analogy to the compound in Intermediate 82, proceeding from 20 mg (26 μ mol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride.

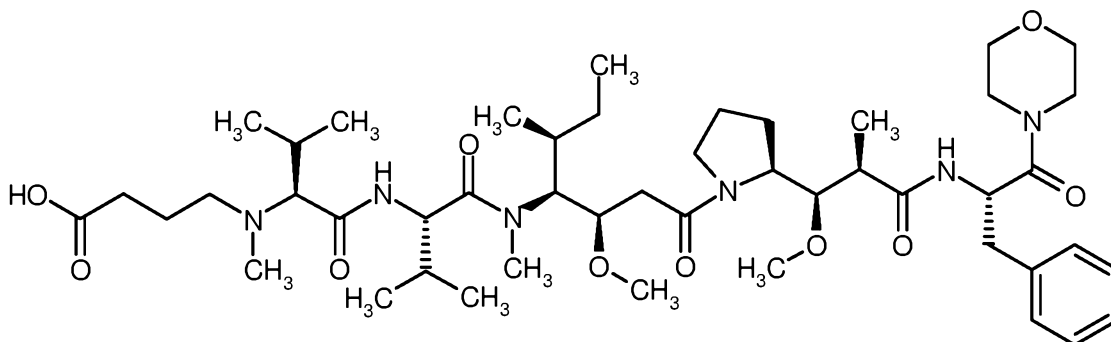
Yield: 5 mg (25% of theory)

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 11): R_t = 0.72 min; MS (ESIpos): m/z = 884 ($M+H$)⁺.

Intermediate 84

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(morpholin-4-yl)-1-oxo-3-phenylpropan-2-yl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



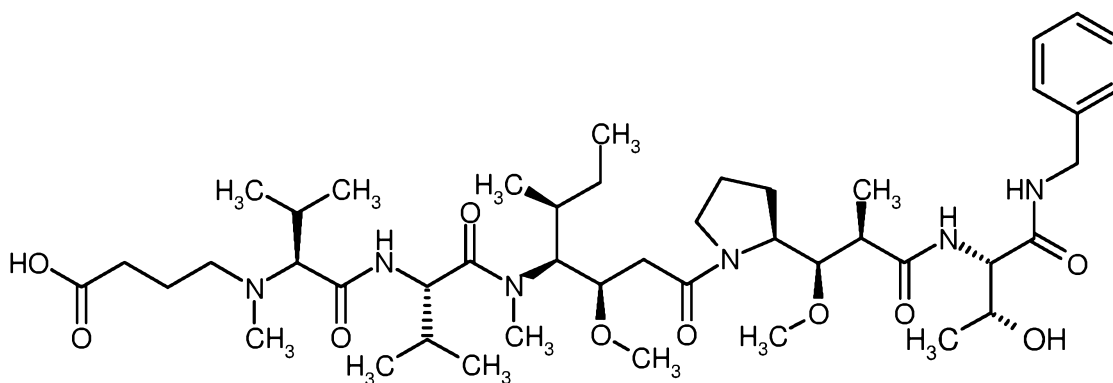
First, in analogy to the synthesis described in Intermediate 79, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*)-1-carboxy-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 46) and morpholine in the presence of EDC and HOBt and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(morpholin-4-yl)-1-oxo-3-phenylpropan-2-yl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 30 mg (0.033 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 22 mg (76% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 9): $R_t = 4.58$ min; MS (ESIpos): $m/z = 887$ (M+H)⁺.

Intermediate 85

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*R*)-1-(benzylamino)-3-hydroxy-1-oxobutan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 79, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*)-1-carboxy-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 46) and *N*-benzyl-L-threoninamide trifluoroacetate in the presence of HATU and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*R*)-1-(benzylamino)-3-hydroxy-1-oxobutan-2-yl]amino]-1-methoxy-2-methyl-

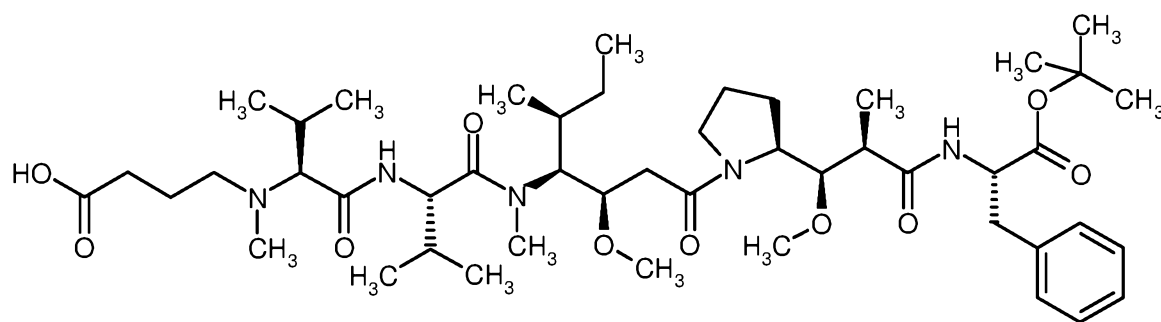
3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 21 mg (0.024 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 20 mg (97% of theory) of the title compound.

5 HPLC (Method 5): $R_t = 1.54$ min;

LC-MS (Method 9): $R_t = 4.49$ min; MS (ESIpos): $m/z = 861$ ($M+H$)⁺.

Intermediate 86

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-*tert*-butoxy-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and *tert*-butyl-L-phenylalaninate hydrochloride in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid to obtain the *tert*-butyl ester (stirring with trifluoroacetic acid in dichloromethane for 40 minutes), the amine compound

20 *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-*tert*-butoxy-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 22 mg (0.02 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain

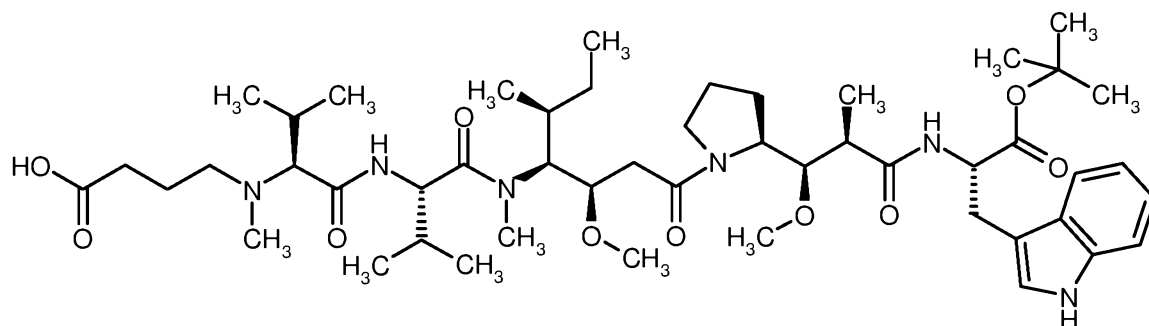
25 16 mg (94% of theory) of the title compound.

HPLC (Method 5): $R_t = 2.0$ min;

LC-MS (Method 9): $R_t = 5.05$ min; MS (ESIpos): $m/z = 874$ (M+H)⁺.

Intermediate 87

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-*tert*-butoxy-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 86, proceeding from 230 mg (336 μ mol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and *tert*-butyl-L-tryptophanate hydrochloride over 3 stages.

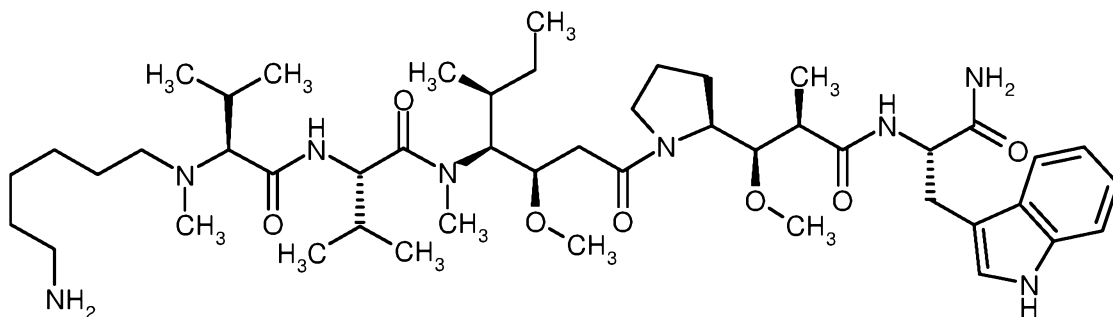
Yield: 95 mg (31% of theory over 3 stages)

HPLC (Method 5): $R_t = 2.0$ min;

LC-MS (Method 9): $R_t = 5.05$ min; MS (ESIpos): $m/z = 913$ (M+H)⁺.

Intermediate 88

N-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the syntheses described in Intermediate 69, by coupling of *N*-[(9H-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) and *N*^α-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-tryptophanamide trifluoroacetate (Intermediate 49) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Fmoc protecting group by means of piperidine, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 30 mg (0.03 mmol) of this compound were then used, in analogy to the preparation of the compound of Intermediate 61, by reaction with benzyl-(6-oxohexyl)carbamate, which had been obtained beforehand by oxidation of benzyl-(6-hydroxyhexyl)carbamate, in the presence of sodium cyanoborohydride, to obtain 17 mg (45% of theory) of the *Z*-protected compound. Subsequently, hydrogenolysis in methanol over 10% palladium/activated carbon yielded the title compound.

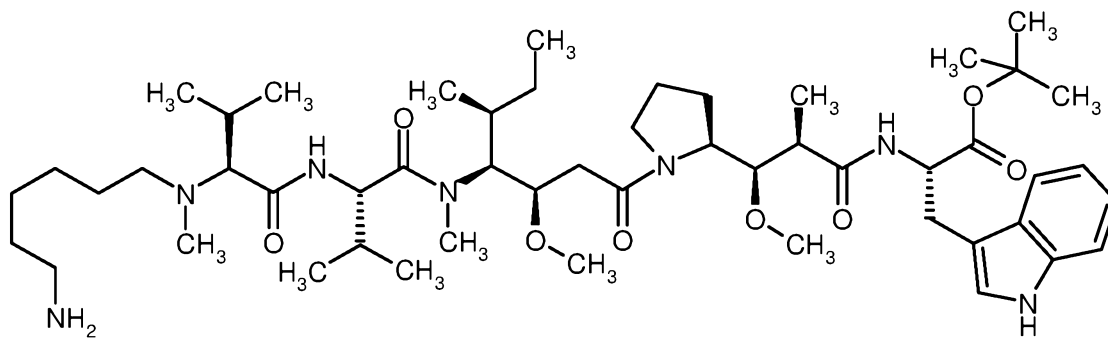
Yield: 14 mg (95% of theory)

HPLC (Method 5): *R*_t = 1.5 min;

LC-MS (Method 1): *R*_t = 0.73 min; MS (ESIpos): *m/z* = 869 (*M*+*H*)⁺.

Intermediate 89

N-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-*tert*-butoxy-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 86, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and *tert*-butyl-L-tryptophanate hydrochloride in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid to obtain the *tert*-butyl ester (stirring with 1:10 trifluoroacetic acid/dichloromethane for 30 min), the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-*tert*-butoxy-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 71 mg (0.075 mmol) of this compound were then used, in analogy to the preparation of the compound of Intermediate 61, by reaction with benzyl-(6-oxohexyl)carbamate, which had been obtained beforehand by oxidation of benzyl-(6-hydroxyhexyl)carbamate, in the presence of sodium cyanoborohydride, to obtain 35 mg (44% of theory) of the *Z*-protected compound. Subsequently, hydrogenolysis in methanol over 10% palladium/activated carbon yielded the title compound.

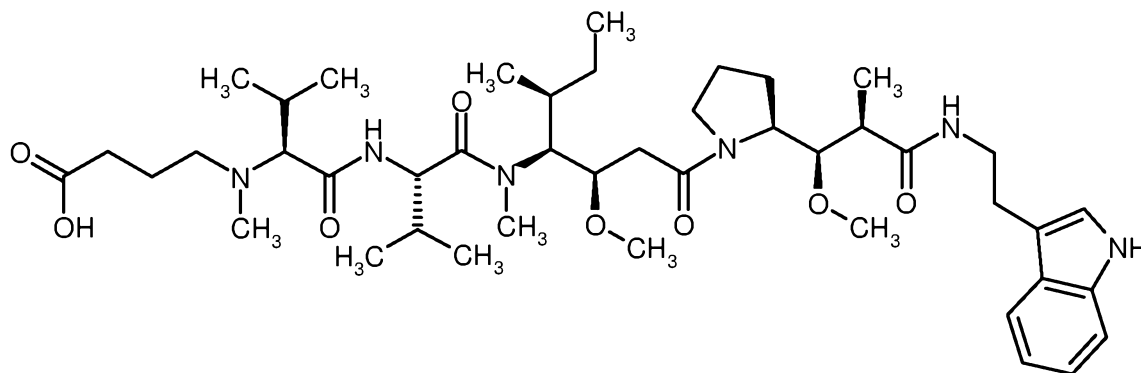
Yield: 30 mg (98% of theory)

HPLC (Method 5): *R*_t = 1.9 min;

LC-MS (Method 1): *R*_t = 0.77 min; MS (ESIpos): *m/z* = 926 (*M*+*H*)⁺.

20 Intermediate 90

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[[2-(1*H*-indol-3-yl)ethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



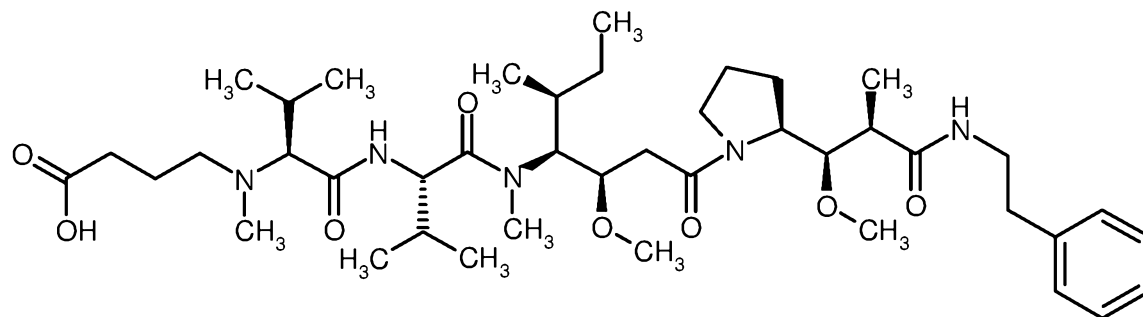
First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-((2*S*)-2-((1*R*,2*R*)-2-carboxy-1-methoxypropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and 2-(1*H*-indol-3-yl)ethanamine in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-((2*S*)-2-((1*R*,2*R*)-3-((2-(1*H*-indol-3-yl)ethyl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 100 mg (0.119 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 50 mg (49% of theory) of the title compound. The title compound was purified here by flash chromatography on silica gel with dichloromethane/methanol/17% ammonia as the eluent, in the course of which the mixing ratio was switched from initially 15/2/0.2 to 15/4/0.5.

HPLC (Method 6): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.87$ min; MS (ESIpos): $m/z = 813$ ($M+H$)⁺.

Intermediate 91

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-((2*S*)-2-((1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-((2-phenylethyl)amino)propyl)pyrrolidin-1-yl)-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



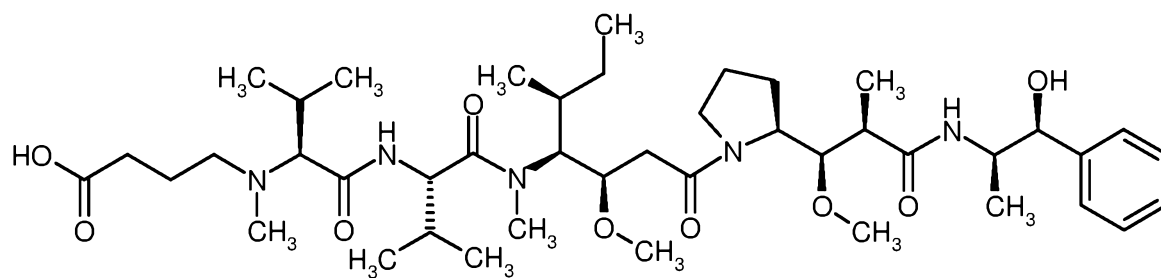
First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and phenylethylamine in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(2-phenylethyl)amino]propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 57 mg (0.071 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 44 mg (80% of theory) of the title compound. The title compound can also be purified here by flash chromatography on silica gel with dichloromethane/methanol/17% ammonia as the eluent (15/2/02 -> 15/4/0.5).

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 9): $R_t = 4.64$ min; MS (ESIpos): $m/z = 774$ ($M+H$)⁺.

Intermediate 92

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



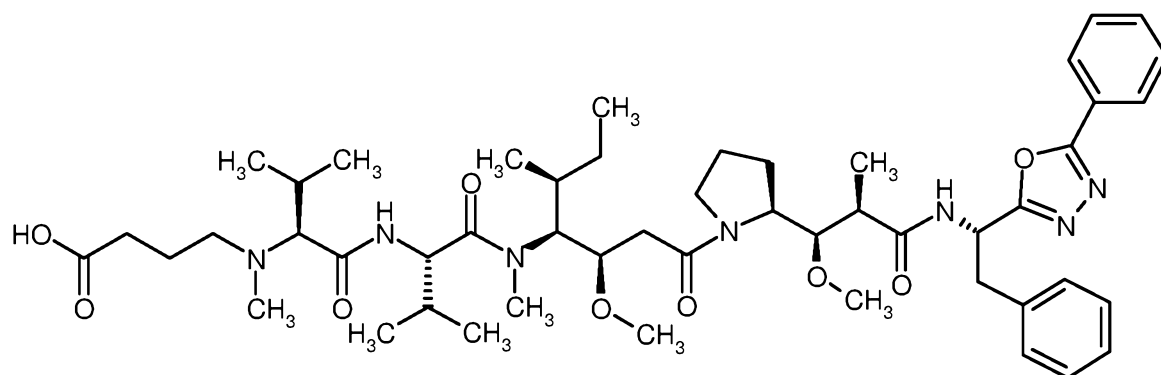
100 mg (0.139 mmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 40) were used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 94 mg (84% of theory) of the title compound. The title compound was purified by preparative HPLC.

HPLC (Method 5): $R_t = 1.5$ min;

10 LC-MS (Method 9): $R_t = 4.46$ min; MS (ESIpos): $m/z = 804$ ($M+H$)⁺.

Intermediate 93

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



15

22.4 mg (24 μ mol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate were dissolved in 1.4 ml of dioxane/water and, analogously to the preparation of Intermediate 61, reacted with 15% aqueous solution of 4-oxobutanoic acid in the presence of

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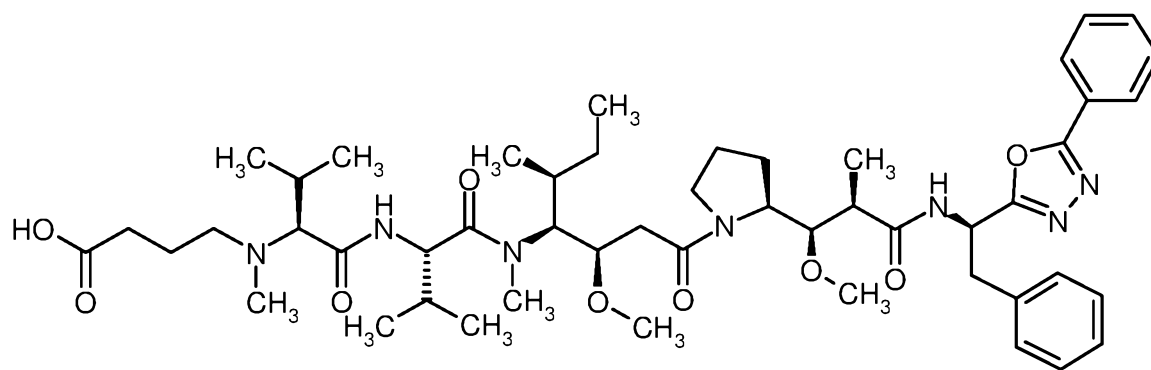
sodium cyanoborohydride. After lyophilization from dioxane, 8.2 mg (38% of theory) of the title compound were obtained in the form of a white solid.

HPLC (Method 10): $R_t = 2.54$ min

LC-MS (Method 12): $R_t = 0.94$ min; MS (ESIpos): $m/z = 919$ ($M+H$)⁺

5 Intermediate 94

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



10

17.1 mg (18 μ mol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate were dissolved in 1.1 ml of dioxane/water and, analogously to the preparation of

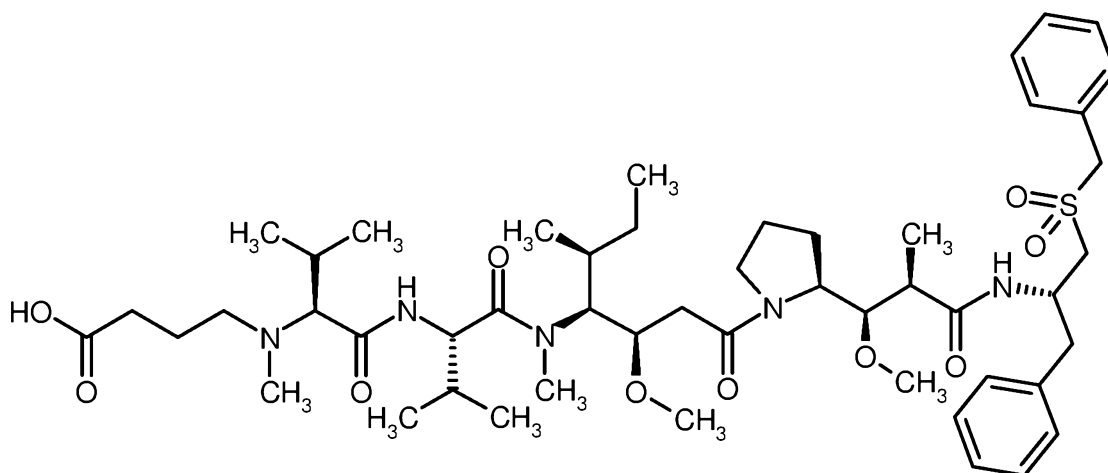
15 Intermediate 61, reacted with 15% aqueous solution of 4-oxobutanoic acid in the presence of sodium cyanoborohydride. After lyophilization from dioxane, 14.8 mg (89% of theory) of the title compound were obtained in the form of a white solid.

HPLC (Method 10): $R_t = 2.54$ min;

LC-MS (Method 12): $R_t = 0.92$ min; MS (ESIpos): $m/z = 919$ ($M+H$)⁺

20 Intermediate 95

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

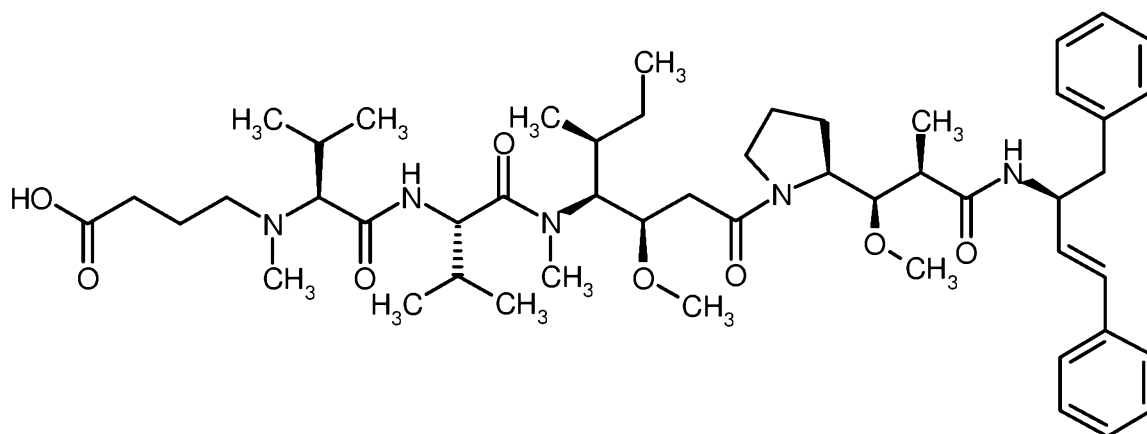


19.3 mg (20 μ mol) *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate were dissolved in 1.2 ml of dioxane/water and, analogously to the preparation of Intermediate 61, reacted with 15% aqueous solution of 4-oxobutanoic acid in the presence of sodium cyanoborohydride. After lyophilization from dioxane, 8.6 mg (45% of theory) of the title compound were obtained in the form of a solid.

LC-MS (Method 11): R_t = 0.85 min; MS (ESIpos): m/z = 943 ($M+H$)⁺

10 Intermediate 96

N-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



15.5 mg (10 μ mol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-

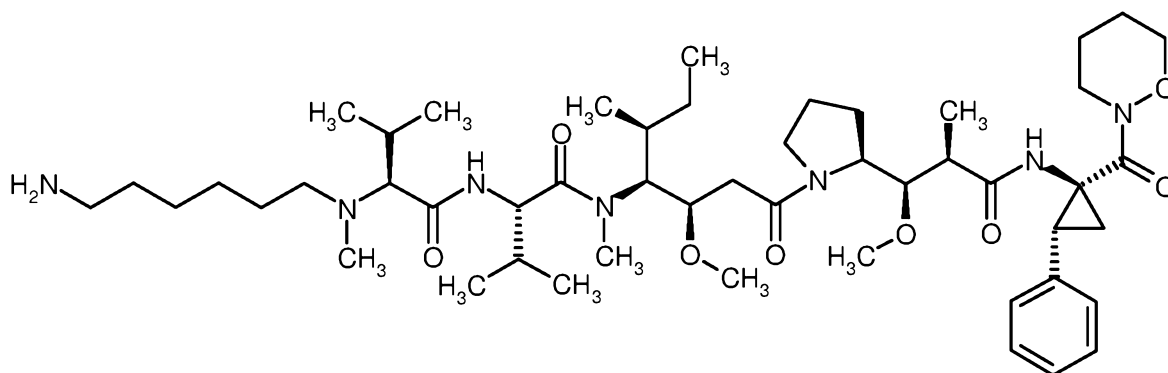
methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate were dissolved in 1.0 ml of dioxane/water and, analogously to the preparation of Intermediate 61, reacted with 15% aqueous solution of 4-oxobutanoic acid in the presence of sodium cyanoborohydride. After lyophilization from dioxane, 10.3 mg (68% of theory) of the title compound were obtained in the form of a white solid.

HPLC (Method 10): $R_t = 2.59$ min;

LC-MS (Method 11): $R_t = 0.94$ min; MS (ESIpos): $m/z = 877$ ($M+H$)⁺

Intermediate 97

N-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



The title compound was prepared in analogy to the synthesis of Intermediate 66, by reaction of 200 mg (0.108 mmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 16) with benzyl-(6-oxohexyl)carbamate and subsequent hydrogenolytic cleaving of the Z protecting group (with 5% palladium on charcoal as a catalyst, in methanol as a solvent).

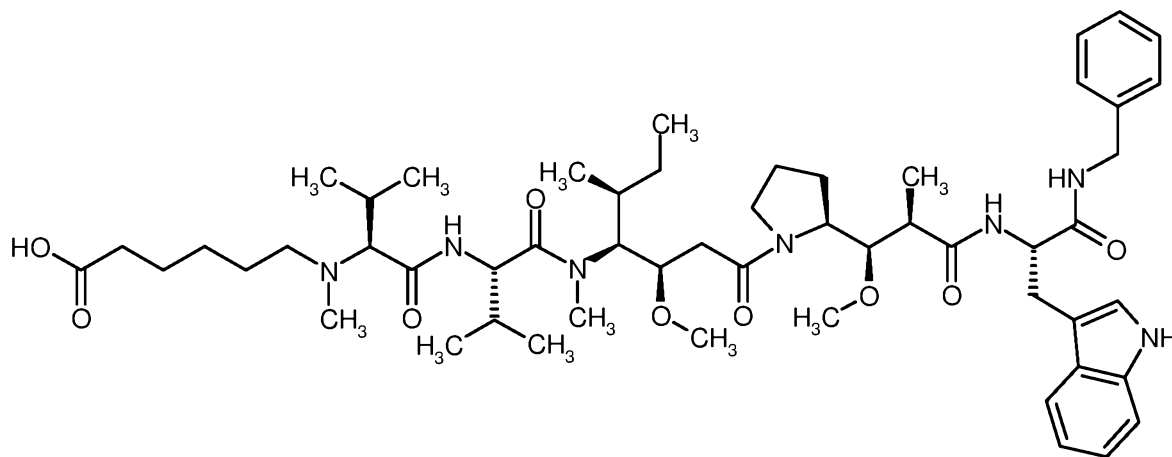
Yield: 69 mg (65% of theory over two stages)

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.76$ min; MS (ESIpos): $m/z = 912$ ($M+H$)⁺.

Intermediate 98

N-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



5

This compound was prepared in analogy to the synthesis described in Intermediate 80. The purification was effected by preparative HPLC.

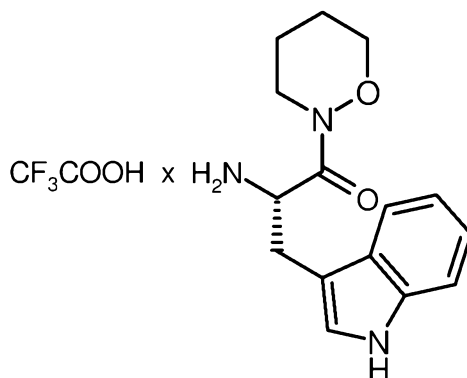
Yield: 40 mg (29% of theory over 3 stages)

HPLC (Method 5): $R_t = 1.9$ min;

10 LC-MS (Method 1): $R_t = 0.92$ min; MS (ESIpos): $m/z = 974$ ($M+H$)⁺.

Intermediate 99

(2*S*)-2-amino-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)propan-1-one trifluoroacetate



324 mg (0.81 mmol) of 2,5-dioxopyrrolidin-1-yl *N*-(*tert*-butoxycarbonyl)-L-tryptophanate were
 15 dissolved in 20 ml of DMF, and 200 mg (1.62 mmol) of 1,2-oxazinan-2-yl hydrochloride (Starting

Compound 5) and 850 μl of *N,N*-diisopropylethylamine were added. The reaction mixture was stirred at 50°C overnight and then concentrated under *in vacuo*. The residue was taken up in dichloromethane and extracted with water. The organic phase was dried over magnesium sulphate and concentrated. The residue was purified by flash chromatography on silica gel with 4:1 dichloromethane/ethyl acetate as the eluent. The product fractions were concentrated, and the residue was dried under high vacuum. This gave 147.5 mg (48% of theory) of the Boc-protected intermediate.

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 1.03$ min; MS (ESIpos): $m/z = 374$ (M+H)⁺.

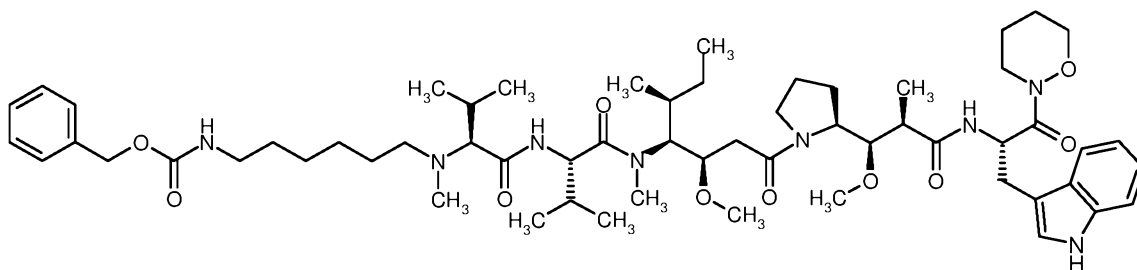
Using 166 mg (444.5 μmol) of this intermediate, under standard conditions with 3 ml of trifluoroacetic acid in 20 ml of dichloromethane, the Boc protecting group was cleaved and, after HPLC purification, 155 mg (86% of theory) of the title compound were obtained.

HPLC (Method 12): $R_t = 1.43$ min;

LC-MS (Method 11): $R_t = 0.56$ min; MS (ESIpos): $m/z = 274$ (M+H)⁺.

Intermediate 100

N-(6-([(benzyloxy)carbonyl]amino)hexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



177 mg (260 μmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and 100 mg (260 μmol) of (2*S*)-2-amino-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)propan-1-one trifluoroacetate (Intermediate 99) were taken up in 15 ml of DMF, and 118 mg (310 μmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 140 μl of *N,N*-diisopropylethylamine were added. The reaction mixture was stirred at RT for 30 min, then concentrated *in vacuo*, and the residue

was purified by means of preparative HPLC. The product fractions were combined and concentrated. After lyophilization from dioxane, 170 mg (68% of theory) of the Boc-protected intermediate were obtained.

LC-MS (Method 1): $R_t = 1.36$ min; $m/z = 940$ (M+H)⁺.

- 5 170 mg of this intermediate were treated with 3 ml of trifluoroacetic acid in 30 ml of dichloromethane for 30 min for cleaving the Boc protecting group. Then the reaction mixture was concentrated *in vacuo*, and the residue was purified by means of preparative HPLC to obtain 155 mg (86% of theory) of the deprotected N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide intermediate.
- 10

HPLC (Method 12): $R_t = 1.85$ min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 840$ (M+H)⁺.

- 50 mg (0.052 mmol) of this intermediate were then used, in analogy to the preparation of Intermediate 97, with benzyl-(6-oxohexyl)carbamate in the presence of sodium cyanoborohydride and subsequent hydrogenolytic cleaving of the Z protecting group (with 5% palladium on charcoal as a catalyst, in methanol as a solvent), to prepare the title compound.
- 15

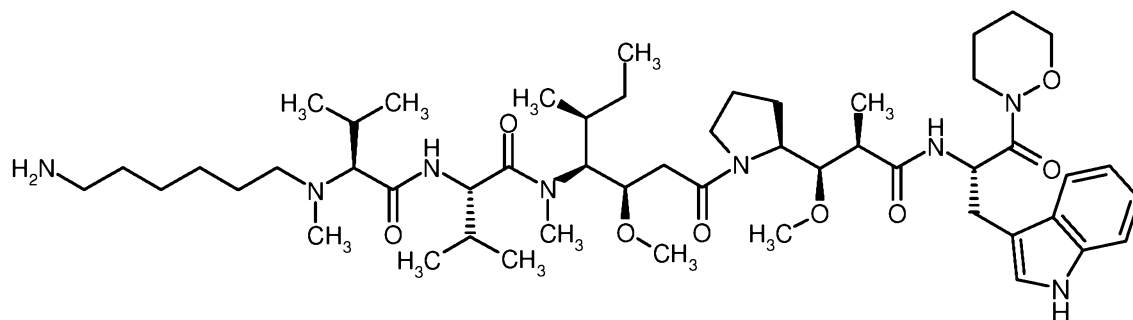
Yield: 21 mg (37% of theory)

HPLC (Method 12): $R_t = 2.1$ min;

- 20 LC-MS (Method 1): $R_t = 1.02$ min; MS (ESIpos): $m/z = 1073$ (M+H)⁺.

Intermediate 101

N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



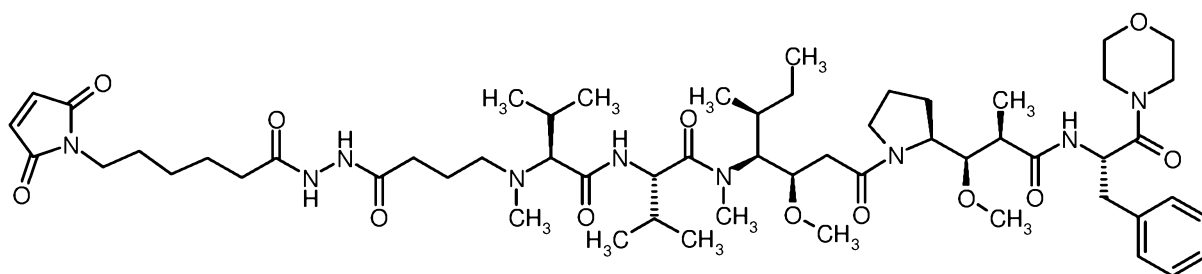
- 26.7 mg (24.87 μmol) of Intermediate 100 were dissolved in 10 ml of methanol and hydrogenated over palladium/activated carbon (5%) under standard hydrogen pressure for 30 min. The catalyst was filtered off and the solvent was evaporated off *in vacuo*. After the residue had been dried under high vacuum, 22.5 mg (96% of theory) of the title compound were obtained.

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.76$ min; MS (ESIpos): $m/z = 939$ ($M+H$) $^+$.

Intermediate 102

- N*-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(morpholin-4-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



- This compound was prepared in analogy to the synthesis described in Intermediate 157 from *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(morpholin-4-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

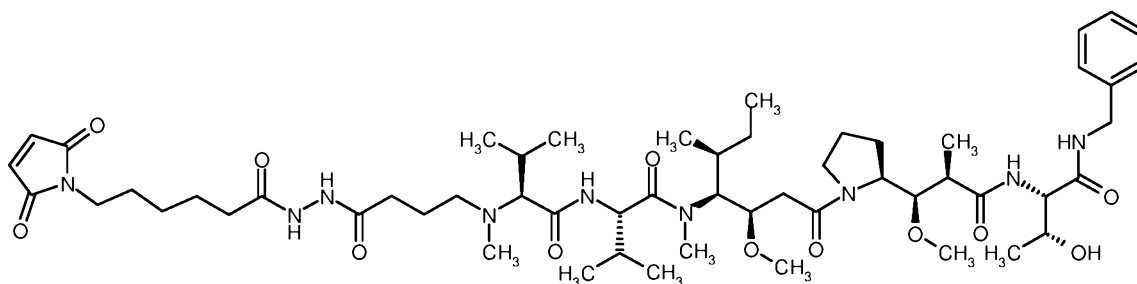
- Yield: 8 mg (71% of theory)

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.87$ min; MS (ESIpos): $m/z = 1094$ (M+H)⁺.

Intermediate 103

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*R*)-1-(benzylamino)-3-hydroxy-1-oxobutan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 157 from *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*R*)-1-(benzylamino)-3-hydroxy-1-oxobutan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

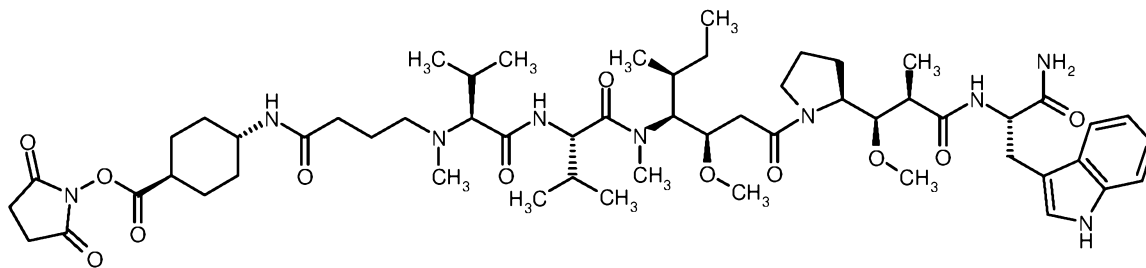
Yield: 3 mg (22% of theory)

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.78$ min; MS (ESIpos): $m/z = 1069$ (M+H)⁺.

Intermediate 104

N-{4-[(trans-4-[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl)cyclohexyl]amino}-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, benzyl *trans*-4-aminocyclohexanecarboxylate trifluoroacetate was prepared from *trans*-4-aminocyclohexanecarboxylic acid by introducing the Boc protecting group, then introducing the benzyl ester protecting group and subsequently cleaving the Boc protecting group by
 5 conventional peptide chemistry methods.

15 mg (18 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-
 {(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]
 pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were then
 10 dissolved in 5 ml of dimethylformamide and subsequently admixed with 13 mg (35 μ mol) of *O*-
 (7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 9 μ l of *N,N*-
 diisopropylethylamine and with 15 mg (44 μ mol) of benzyl *trans*-4-
 aminocyclohexanecarboxylate trifluoroacetate. The mixture was stirred at RT for 1 h and then
 concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. The
 corresponding fractions were combined and the solvent was evaporated off *in vacuo*. After the
 15 residue had been dried under high vacuum, 14.7 mg (78% of theory) of the protected
 intermediate were obtained as a colourless foam.

HPLC (Method 6): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.95 min; MS (ESIpos): m/z = 1072 ($M+H$)⁺.

From this protected intermediate, the benzyl ester was first removed by hydrogenolytic means,
 20 and the free carboxyl component was obtained in quantitative yield. 14 mg (14 μ mol; 1 equiv.) of
 the deprotected compound were taken up in 5 ml of DMF and admixed with 3.3 mg (29 μ mol;
 2.1 equiv.) of *N*-hydroxysuccinimide in the presence of 4.1 mg (21 μ mol; 1.5 equiv.) of 1-(3-
 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 7.5 μ l (44 μ mol; 3.1 equiv.) of *N,N*-
 diisopropylethylamine and 0.9 mg (7 μ mol; 0.5 equiv.) of 4-dimethylaminopyridine, and the
 25 mixture was stirred at RT overnight. Then another 10 equiv. of *N*-hydroxysuccinimide, 5 equiv.
 of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 5 equiv. of *N,N*-
 diisopropylethylamine and 0.5 equiv. of 4-dimethylaminopyridine were added, and the reaction
 mixture was treated in an ultrasound bath for 5 h. Subsequently, the solvent was evaporated off,
 the residue was purified by means of preparative HPLC and the corresponding fractions were

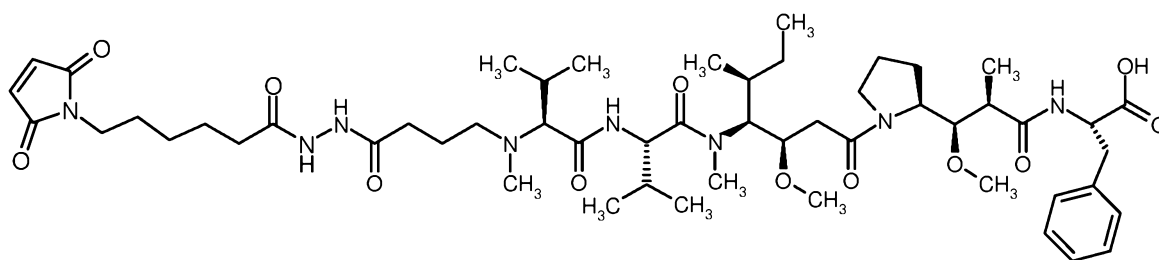
combined and concentrated. After lyophilization of the residue from dioxane, 9.7 mg (62% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 6): $R_t = 1.8$ min;

LC-MS (Method 11): $R_t = 0.77$ min; MS (ESIpos): $m/z = 1078$ (M+H)⁺.

5 Intermediate 105

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



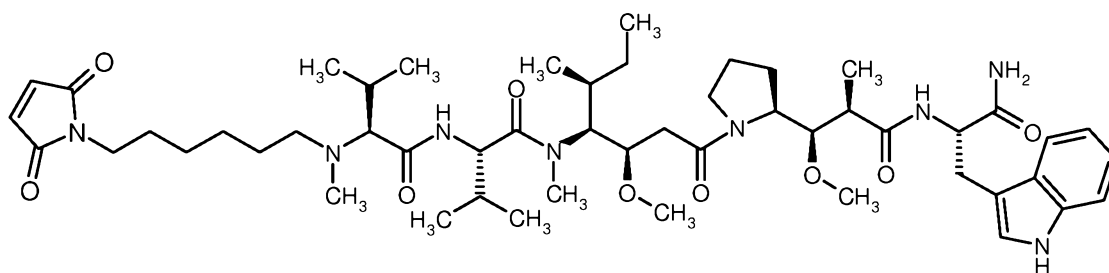
This compound was prepared in analogy to the synthesis described in Intermediate 157, proceeding from 4-[(2*S*)-1-[(2*S*)-1-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-*tert*-butoxy-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)amino}-3-methylbutan-2-yl]amino}-3-methyl-1-oxobutan-2-yl](methyl)amino}butanoic acid and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The ester intermediate was obtained in 42% yield. In a second step, 6 mg (6 μ mol) of this intermediate were cleaved with trifluoroacetic acid the *tert*-butyl ester. After HPLC purification, 3.4 mg (48% of theory) of the title compound were obtained.

HPLC (Method 5): $R_t = 1.66$ min;

LC-MS (Method 2): $R_t = 1.04$ min; MS (ESIpos): $m/z = 1025$ (M+H)⁺.

Intermediate 106

N-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



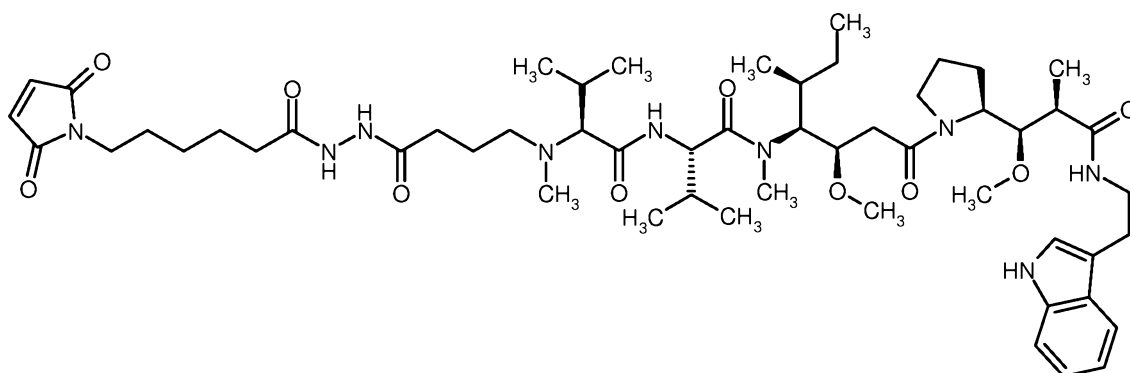
14 mg (16 μ mol) of *N*-(6-aminoheptyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-((2*S*)-2-[(1*R*,2*R*)-3-[[[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 88) were taken up in 750 μ l of dioxane and admixed with 1.5 ml of saturated sodium hydrogencarbonate solution and then with 3.2 mg (21 μ mol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate. The reaction mixture was stirred at RT for 1 h and then concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization, 5.5 mg (36% of theory) of the title compound were obtained.

10 HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 949 ($M+H$)⁺.

Intermediate 107

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-((2*S*)-2-[(1*R*,2*R*)-3-{[2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



38 mg (47 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-((2*S*)-2-[(1*R*,2*R*)-3-[[2-(1*H*-indol-3-yl)ethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-

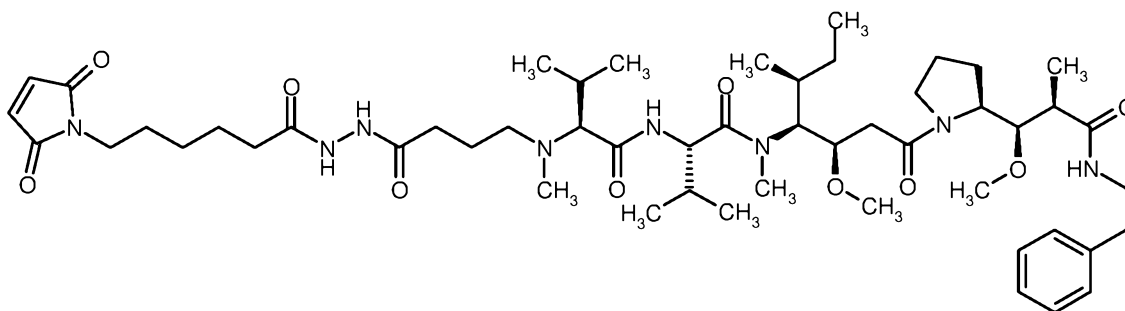
- methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 37 ml of DMF and then admixed with 71 mg (187 μmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 33 μl of *N,N*-diisopropylethylamine and with 37 mg (140 μmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT for 1 h. This was followed by concentration under high vacuum and purification of the remaining residue by means of preparative HPLC. Thus, 12.2 mg (26% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.85$ min; MS (ESIpos): $m/z = 1020$ ($M+H$)⁺.

10 Intermediate 108

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-{(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-{(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(2-phenylethyl)amino]propyl}pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl}-*N*-methyl-L-valinamide



- 15 The compound was prepared in analogy to Intermediate 107.

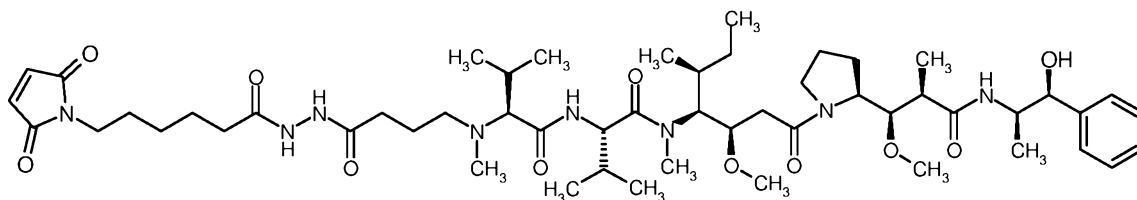
Yield: 2.5 mg (30% of theory)

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.9$ min; MS (ESIpos): $m/z = 981$ ($M+H$)⁺.

Intermediate 109

- 20 *N*-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



The compound was prepared in analogy to Intermediate 107 from the compound in Intermediate 92.

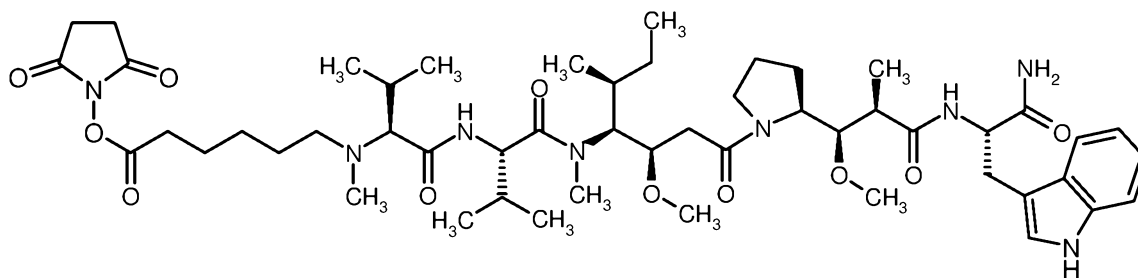
5 Yield: 35 mg (65% of theory)

HPLC (Method 5): $R_t = 1.9$ min;

LC-MS (Method 11): $R_t = 0.76$ min; MS (ESIpos): $m/z = 1011$ ($M+H$)⁺.

Intermediate 110

10 *N*-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



15 This compound was prepared in analogy to Intermediate 147 from the compound in Intermediate 83.

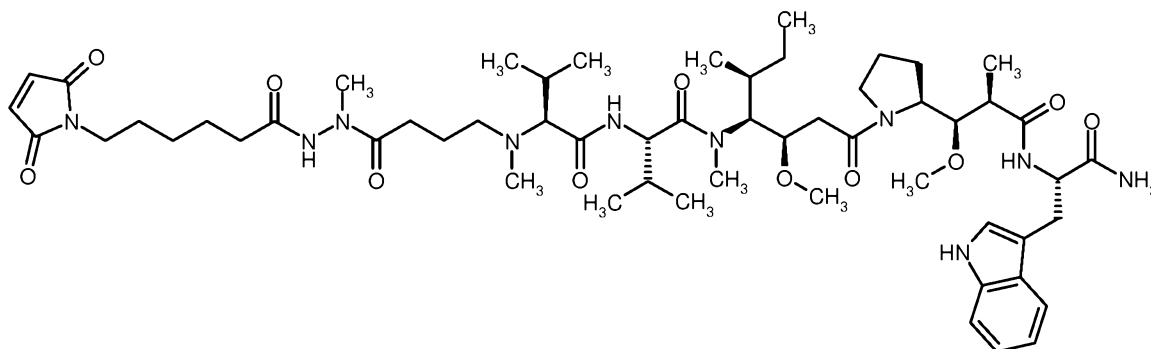
Yield: 2.4 mg (24% of theory)

HPLC (Method 6): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.87$ min; MS (ESIpos): $m/z = 981$ ($M+H$)⁺.

Intermediate 111

- N*-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]-1-methylhydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 140 from Intermediate 82 and Intermediate 22.

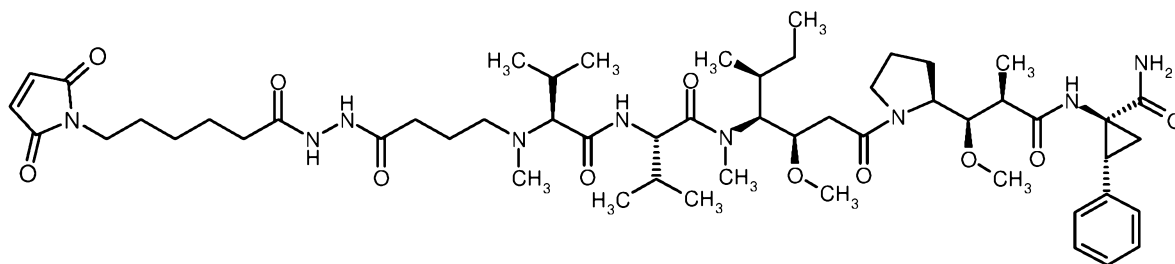
- Yield: 6.5 mg (51% of theory)

HPLC (Method 6): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 4.71$ min; MS (ESIpos): $m/z = 1077$ ($M+H$)⁺.

Intermediate 112

- N*-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*,2*R*)-1-carbamoyl-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 157 from the compound in Intermediate 81.

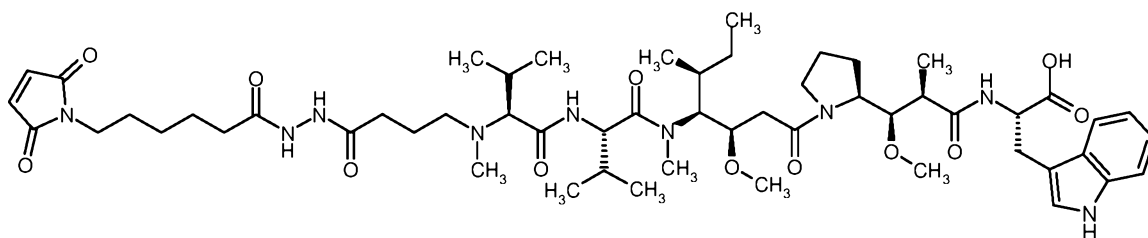
Yield: 5.7 mg (57 % of theory)

HPLC (Method 5): $R_t = 1.6$ min;

5 LC-MS (Method 1): $R_t = 0.87$ min; MS (ESIpos): $m/z = 1036$ ($M+H$)⁺.

Intermediate 113

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



95 mg (104 μ mol) of 4-[[[(2*S*)-1-[[[(2*S*)-1-[[[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[[[(2*S*)-1-*tert*-butoxy-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)amino}-3-methylbutan-2-yl]amino}-3-methyl-1-oxobutan-2-yl](methyl)amino}butanoic acid were dissolved in DMF and then admixed with 79.5 mg (209 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 73 μ l of *N,N*-diisopropylethylamine and with 68 mg (261 μ mol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT for 2 h. This was followed by concentration under high vacuum and purification of the remaining residue by means of preparative HPLC. Thus, 104 mg (89% of theory) of the *tert*-butyl ester of the title compound were obtained as a colourless foam.

HPLC (Method 5): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 0.93$ min; MS (ESIpos): $m/z = 1121$ ($M+H$)⁺.

The intermediate was taken up in 33.4 ml of dichloromethane, 17 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 1 h. Subsequently, the reaction mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC.

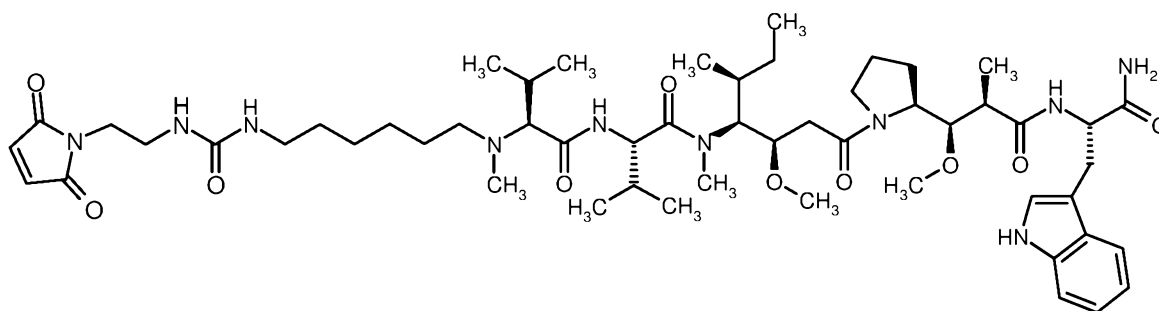
Thus, 61 mg (62 % of theory) of the title compound were obtained as a colourless foam.

5 HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 1064$ (M+H)⁺.

Intermediate 114

N-[6-([2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]carbamoyl)amino]hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



5 mg (5 μ mol) of *N*-(6-aminoheptyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were taken up in 885 μ l of DMF and admixed with 5.3 mg (8 μ mol) of 4-nitrophenyl-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]carbamate and 2.8 μ l of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT for 2 h and then concentrated to dryness. The residue was purified by means of preparative HPLC.

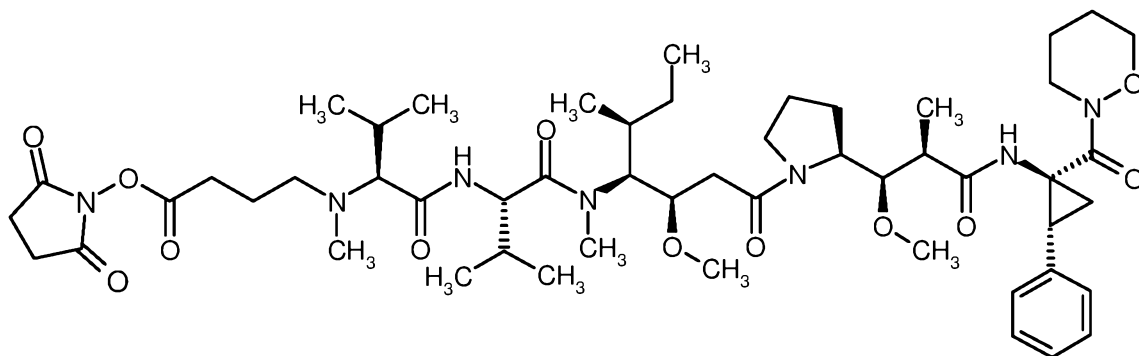
Yield: 0.58 mg (11% of theory) of a colourless foam

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.83$ min; MS (ESIpos): $m/z = 1035$ (M+H)⁺.

Intermediate 115

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the compound in Intermediate 147, starting from 8 mg (9 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide. After concentration, the activated ester was purified by means of preparative HPLC and, after removal of the solvent *in vacuo*, reacted immediately with the antibody.

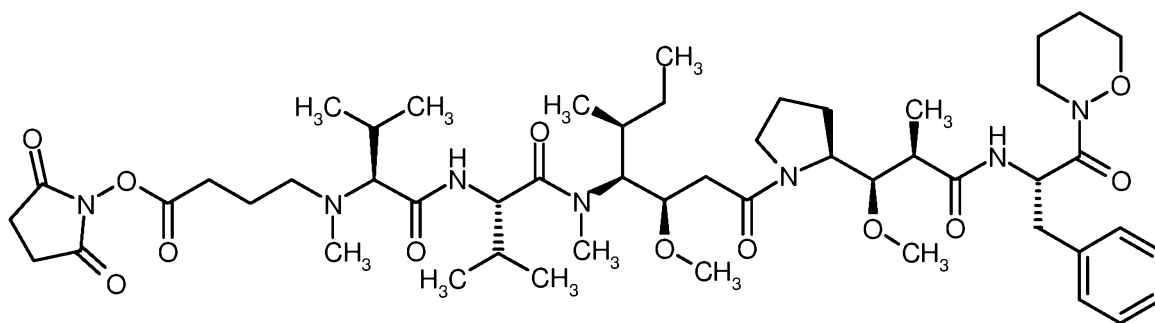
Yield: 3 mg (27% of theory) (hydrolysis-sensitive)

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 996 ($M+H$)⁺.

Intermediate 116

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the compound in Intermediate 147, starting from 5 mg (6 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide. After concentration, the activated ester was purified by means of preparative HPLC and, after removal of the solvent *in vacuo*, reacted immediately with the antibody.

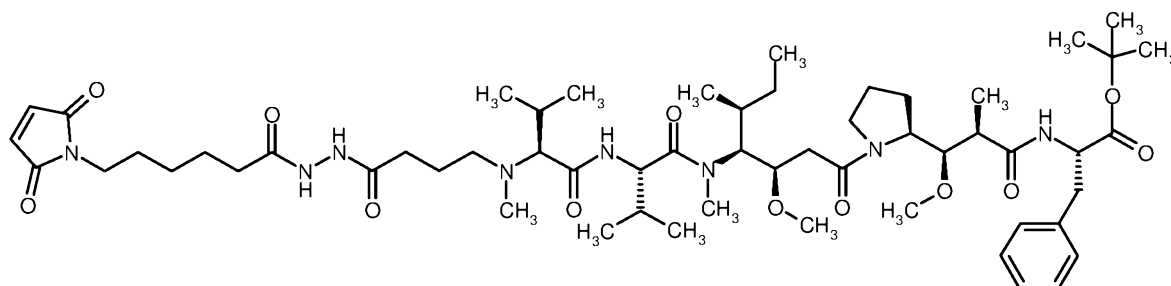
Yield: 3.2 mg (43% of theory) (hydrolysis-sensitive)

10 HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 984 ($M+H$)⁺.

Intermediate 117

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-tert-butoxy-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 157 from the compound in Intermediate 86.

20

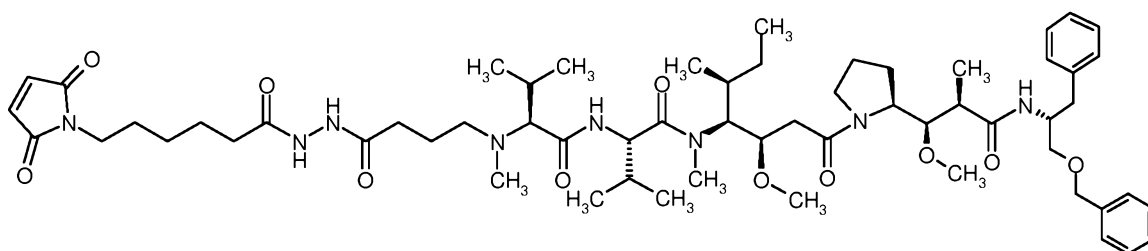
Yield: 7 mg (42% of theory)

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.94$ min; MS (ESIpos): $m/z = 1081$ (M+H)⁺.

Intermediate 118

- 5 *N*-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-[(2*R*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



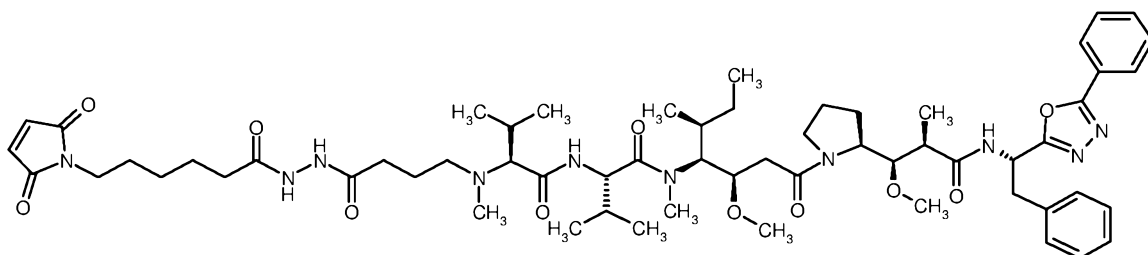
10

The target compound was prepared in analogy to Intermediate 157 from 7 mg (7.8 μ mol) of the compound in Intermediate 68. Yield: 6.3 mg (53% of theory)

LC-MS (Method 1): $R_t = 1.00$ min; MS (ESIpos): $m/z = 1102$ (M+H)⁺.

Intermediate 119

- 15 *N*-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



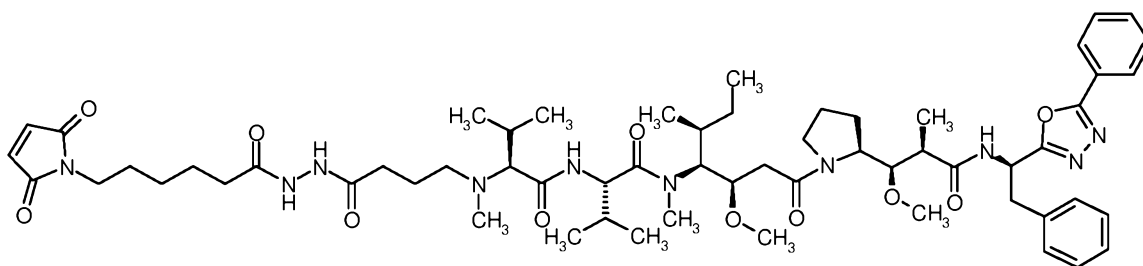
20

7.4 mg (8.1 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 6.3 mg (24.2 mmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide hydrochloride were coupled and worked up in analogy to Intermediate 157. 1.6 mg (13% of theory) of the title compound were obtained as a solid.

LC-MS (Method 11): $R_t = 0.89$ min; MS (ESIpos): $m/z = 1126$ (M+H)⁺

Intermediate 120

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

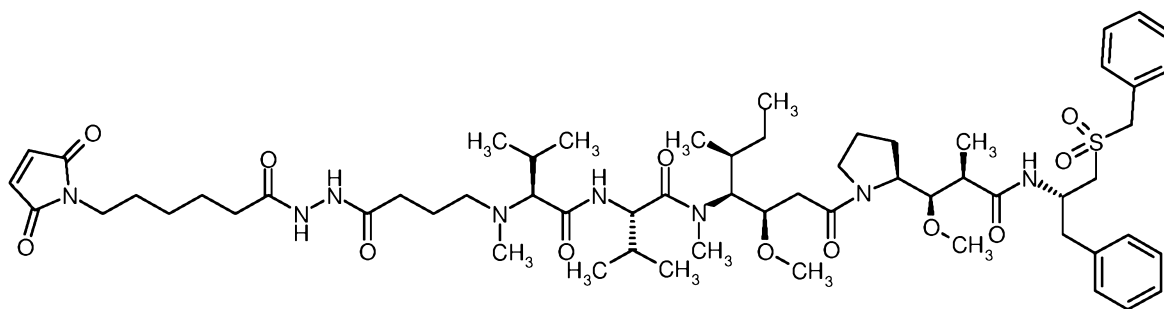


12.8 mg (13.9 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 10.9 mg (41.8 mmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide hydrochloride were coupled and worked up in analogy to Intermediate 157. 10.8 mg (59% of theory) of the title compound were obtained as a solid.

LC-MS (Method 11): $R_t = 0.90$ min; MS (ESIpos): $m/z = 1126$ (M+H)⁺

Intermediate 121

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

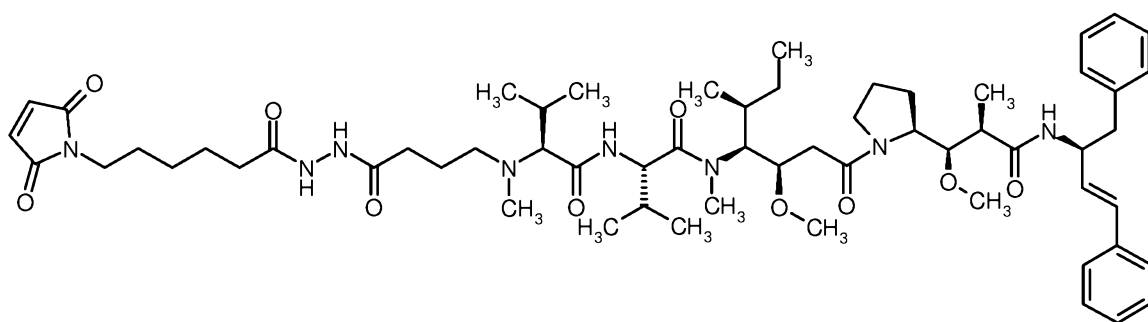


7.4 mg (7.9 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylsulfonyl)-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 6.2 mg (23.5 mmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide hydrochloride were coupled and worked up in analogy to Intermediate 157. 6.9 mg (74% of theory) of the title compound were obtained as a solid.

LC-MS (Method 11): $R_t = 0.87$ min; MS (ESIpos): $m/z = 1150$ ($M+H$)⁺

10 Intermediate 122

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



15

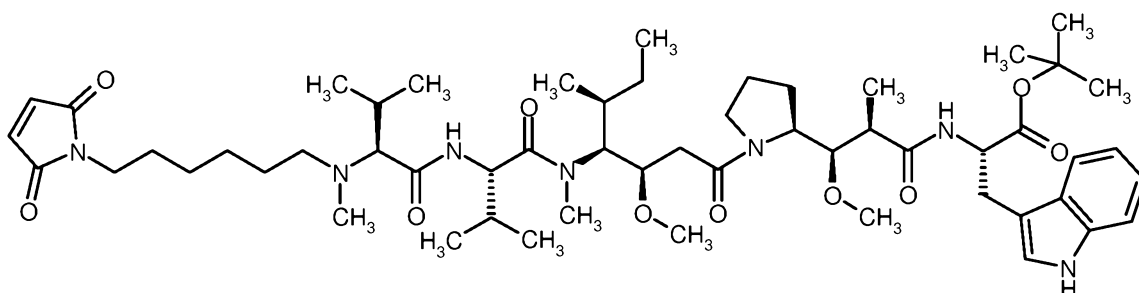
8 mg (9.1 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 7.2 mg (27.4 mmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide hydrochloride were coupled and worked up in analogy to Intermediate 157. 8.2 mg (82% of theory) of the title compound were obtained as a white solid.

20

LC-MS (Method 11): $R_t = 0.95$ min; MS (ESIpos): $m/z = 1083$ ($M+H$)⁺

Intermediate 123

N-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-*tert*-butoxy-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



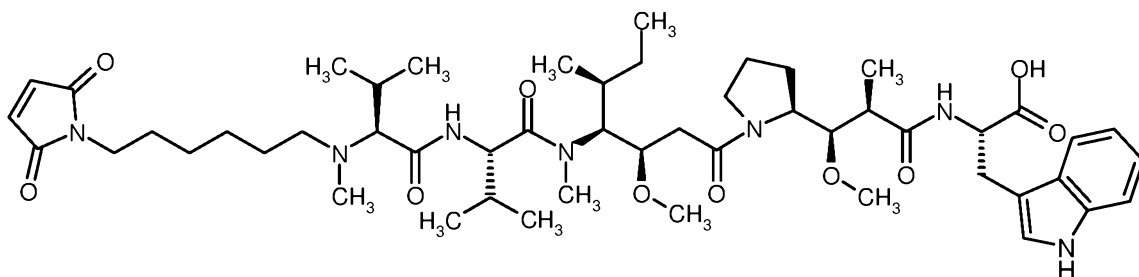
30 mg (30 μ mol) of Intermediate 89 were taken up in 2 ml of 1,4-dioxane and admixed with 4 ml of saturated sodium hydrogencarbonate solution and then with 7.5 mg (50 μ mol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate. The reaction mixture was stirred at RT for 1 h and then concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization, 24 mg (74% of theory) of the title compound were obtained.

HPLC (Method 5): $R_t = 2.2$ min;

15 LC-MS (Method 1): $R_t = 1.01$ min; MS (ESIpos): $m/z = 1006$ ($M+H$)⁺.

Intermediate 124

N-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



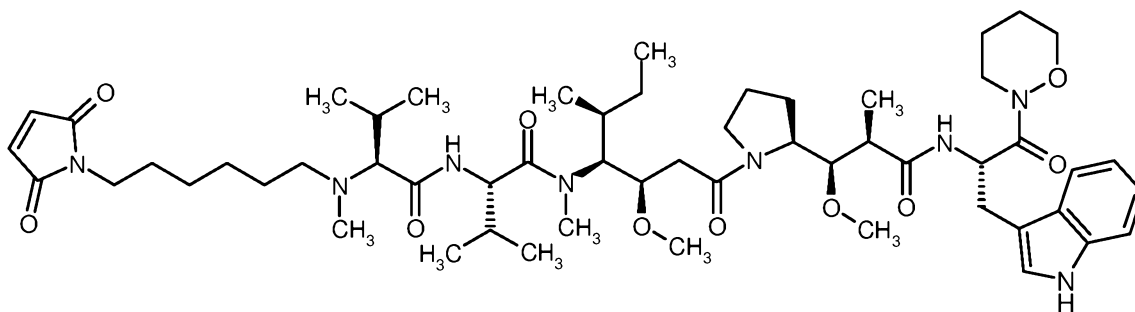
22 mg (20 μ mol) of Intermediate 123 were reacted with 4 ml of trifluoroacetic acid in 8 ml of dichloromethane at RT for 1 h. Thereafter, the reaction mixture was concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization, 11 mg (54% of theory) of the title compound were obtained.

5 HPLC (Method 5): $R_t = 1.8$ min;

LC-MS (Method 11): $R_t = 0.85$ min; MS (ESIpos): $m/z = 950$ ($M+H$)⁺.

Intermediate 125

N-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[[*(2S)*-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



22.5 mg (20 μ mol) of Intermediate 101 were taken up in 2 ml of 1:1 dioxane/water and then
 15 admixed with 5.6 mg (40 μ mol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate and
 with 0.25 ml of saturated sodium hydrogencarbonate solution. The reaction mixture was stirred
 at RT for 30 min. Then another 0.25 ml of the saturated sodium hydrogencarbonate solution were
 added, and the reaction mixture was stirred at RT for another 15 min and then concentrated *in*
vacuo. The remaining residue was purified by means of preparative HPLC. After lyophilization,
 20 12.8 mg (50% of theory) of the title compound were obtained as a colourless foam.

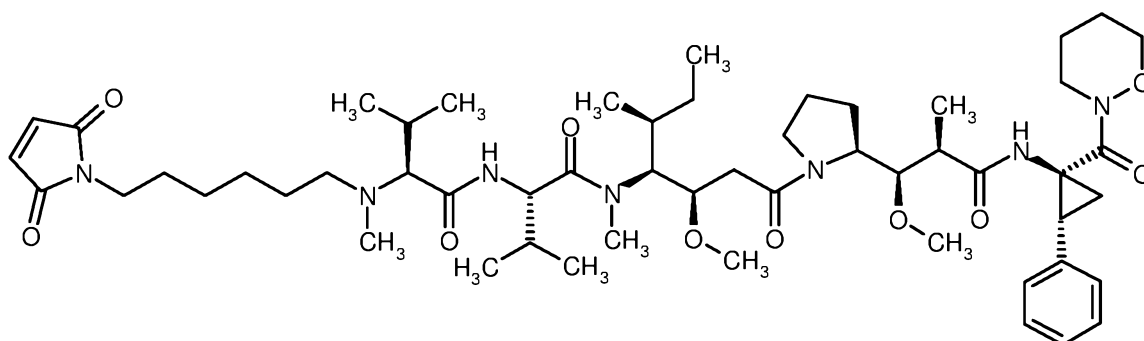
HPLC (Method 5): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.95$ min; MS (ESIpos): $m/z = 1019$ ($M+H$)⁺.

Intermediate 126

N-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-
 25 1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-

phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



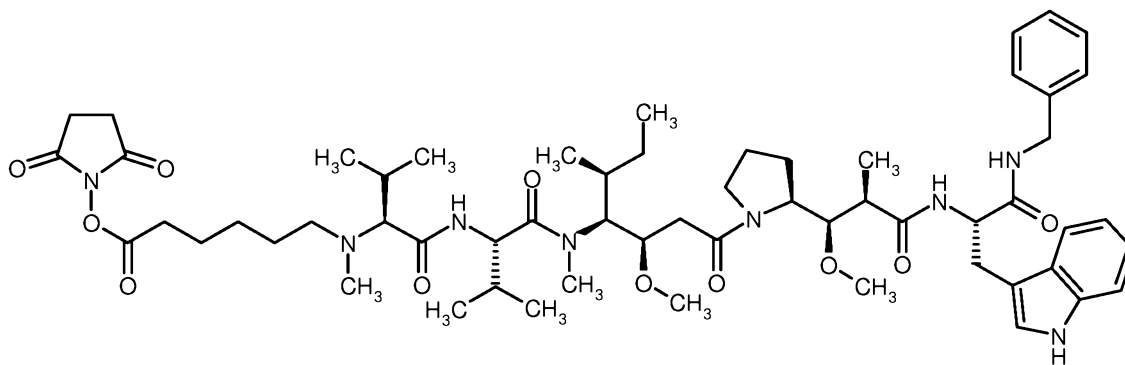
5 64 mg (70 μ mol) of *N*-(6-aminoheptyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 97) were taken up in 3 ml of 1:1 dioxane/water, then adjusted to pH 9 with 4 ml of saturated sodium hydrogencarbonate solution and subsequently admixed with 16.3 mg (110
10 μ mol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate. The reaction mixture was stirred at RT for 1 h and then concentrated *in vacuo*. Then another 8 mg (55 μ mol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate were added, and the reaction mixture was adjusted again to pH 9 and stirred at RT for another hour. This was followed by concentration and purification of the remaining residue by means of preparative HPLC. At first, 31 mg of an as yet
15 uncyclized intermediate were obtained. 27 mg of this intermediate were taken up again in 2 ml of 1:1 dioxane/water and then admixed with 250 μ l of saturated sodium hydrogencarbonate solution. After stirring at RT for 2 hours, the reaction mixture was concentrated, and the residue was purified by means of preparative HPLC. After lyophilization, 20 mg (29% of theory) of the title compound were obtained.

20 HPLC (Method 5): R_t = 1.96 min;

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 992 ($M+H$)⁺.

Intermediate 127

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-
25 methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



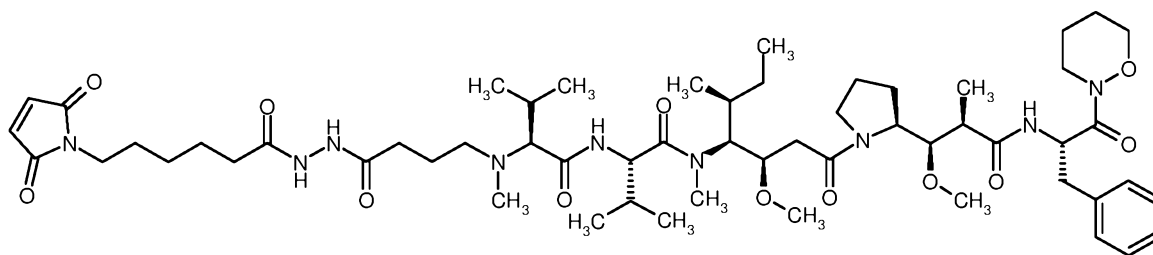
17 mg (18 μ mol) of *N*-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-
 5 {[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-
 oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide
 (Intermediate 98) were dissolved in 2.8 ml of dichloromethane and admixed with 20 mg (174
 mmol) of 1-hydroxypyrrolidine-2,5-dione and then admixed with 10 mg (52 μ mol) of 1-(3-
 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.21 mg (0.17 μ mol) of DMAP.
 After stirring at RT for 4 h, the reaction mixture was concentrated *in vacuo*. The remaining
 residue was purified by means of preparative HPLC. After lyophilization, 8.2 mg (43% of
 10 theory) of the title compound were obtained.

HPLC (Method 5): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.98 min; MS (ESIpos): m/z = 1071 ($M+H$)⁺.

Intermediate 128

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-
 15 L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(1,2-
 oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-
 oxoheptan-4-yl]-*N*-methyl-L-valinamide



20 5 mg (5.6 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-
 [(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-

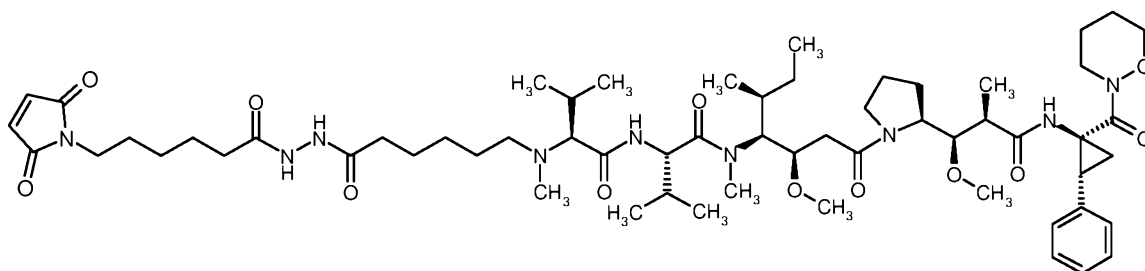
yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide were dissolved in 845 μ l of DMF and then admixed with 3.2 mg (17 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 2.6 mg (17 μ mol) of 1-hydroxy-1*H*-benzotriazole hydrate, 1.96 μ l of *N,N*-diisopropylethylamine and with 5.9 mg (22.5 μ mol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 2.2 mg (36% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 1094 ($M+H$)⁺.

Intermediate 129

N-(6-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-6-oxohexyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide



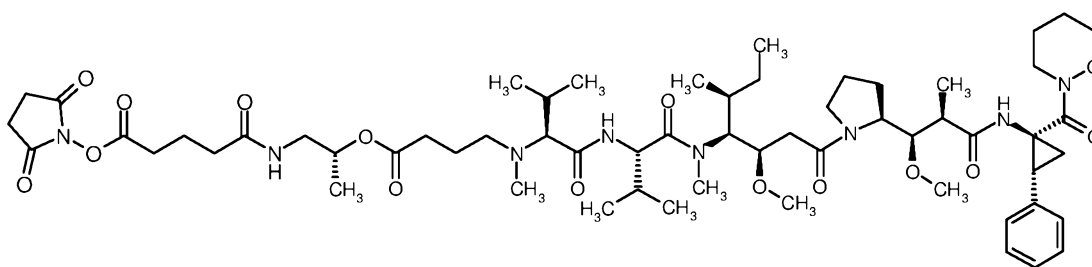
4 mg (4.3 μ mol) of *N*-(5-carboxypentyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide were dissolved in 646 μ l of DMF and then admixed with 2.5 mg (13 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 2.0 mg (13 μ mol) of 1-hydroxy-1*H*-benzotriazole hydrate, 2.25 μ l of *N,N*-diisopropylethylamine and with 4.5 mg (17 μ mol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT for 3 h and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 1.9 mg (39% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 9): $R_t = 4.9$ min; MS (ESIpos): $m/z = 1134$ (M+H)⁺.

Intermediate 130

N-(4-{[(2*R*)-1-({5-[(2,5-dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}amino)propan-2-yl]oxy}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



10.5 mg (11.7 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl] amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 3.7 ml of dichloromethane and then admixed with 6.7 mg (35 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 0.7 mg (5.8 μ mol) of 4-dimethylaminopyridine and with 8.2 mg (47 μ mol) of commercially available *tert*-butyl-[2*R*)-2-hydroxypropyl]carbamate. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 7.5 mg (61% of theory) of the Boc-protected intermediate were obtained as a colourless foam.

HPLC (Method 5): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 1.03$ min; MS (ESIpos): $m/z = 1056$ (M+H)⁺.

Subsequently, the Boc protecting group was cleaved with trifluoroacetic acid. 4.9 mg (0.005 mmol) of the deprotected crude product were then, without further purification, taken up in 1.8 ml of dichloromethane and admixed with 3.7 mg (0.011 mmol) of 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, 2.4 μ l (0.014 mmol) of *N,N*-diisopropylethylamine and 0.6 mg (5 μ mol) of 4-dimethylaminopyridine. The mixture was stirred at RT for 2 h and then

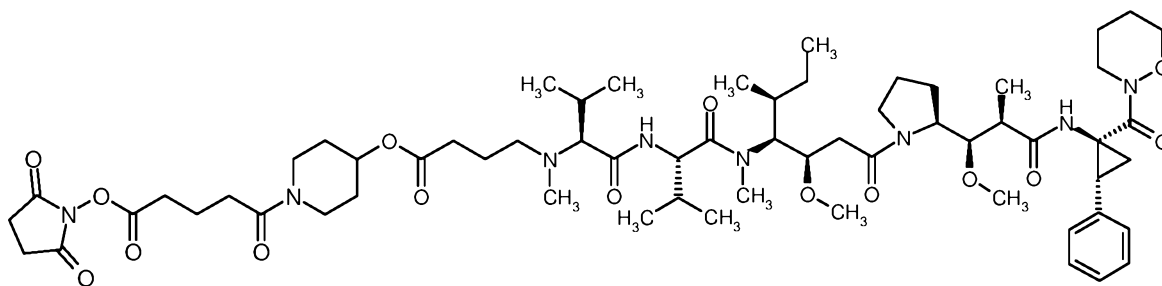
concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 0.77 mg (15% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.93$ min; MS (ESIpos): $m/z = 1167$ ($M+H$)⁺.

5 Intermediate 131

N-{4-[(1-{5-[(2,5-dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}piperidin-4-yl)oxy]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



10

10 mg (11 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 2 ml of dichloromethane and then admixed with 4.3 mg (22 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 0.88 mg (6 μ mol) of 4-dimethylaminopyridine and with 5.2 mg (22 μ mol) of commercially available benzyl 4-hydroxypiperidine-1-carboxylate. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 5 mg (40% of theory) of the *Z*-protected intermediate were obtained as a colourless foam.

20

HPLC (Method 5): $R_t = 2.1$ min;

LC-MS (Method 1): $R_t = 1.04$ min; MS (ESIpos): $m/z = 1116$ ($M+H$)⁺.

Subsequently, the *Z* protecting group was cleaved by hydrogenolytic means in ethanol over palladium/activated carbon. 4.6 mg (0.005 mmol) of the deprotected crude product were then, without further purification, taken up in 1.8 ml of dichloromethane and admixed with 3.8 mg (0.012 mmol) of 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, 0.8 μ l (0.005

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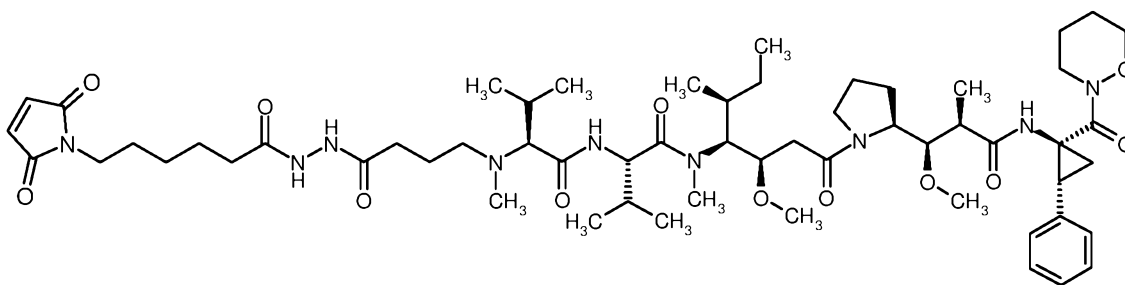
mmol) of *N,N*-diisopropylethylamine and 0.6 mg (5 µmol) of 4-dimethylaminopyridine. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 0.96 mg (16% of theory) of the title compound were obtained as a colourless foam.

5 HPLC (Method 5): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.94$ min; MS (ESIpos): $m/z = 1193$ (M+H)⁺.

Intermediate 132

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazinyl}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



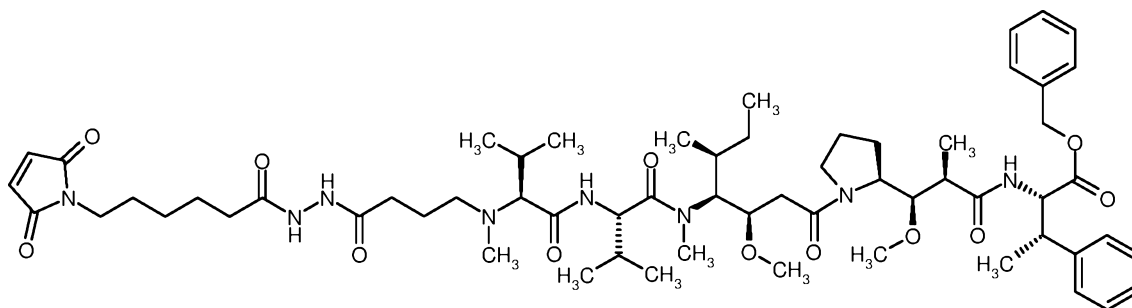
15 mg (16.7 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl] amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 2500 µl of DMF and then admixed with 9.6 mg (50 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 7.6 mg (50 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 5.8 µl of *N,N*-diisopropylethylamine and with 17.4 mg (67 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 11.2 mg (52% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 2): $R_t = 1.09$ min; MS (ESIpos): $m/z = 1106$ (M+H)⁺.

Intermediate 133

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazinyl}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



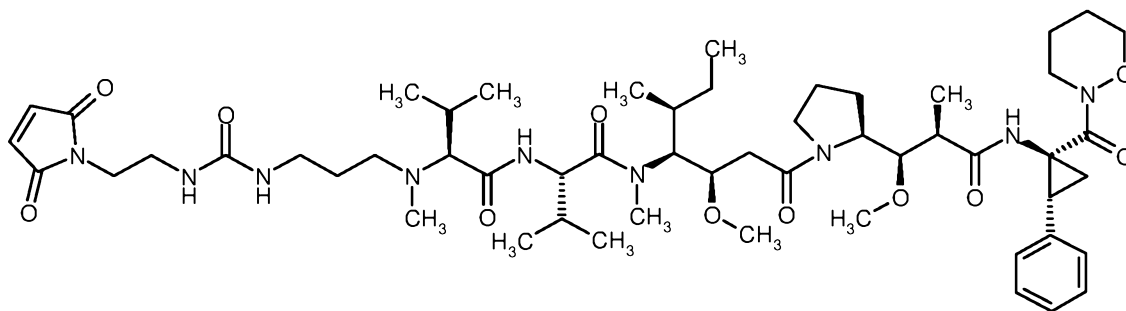
5.8 mg (6.3 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 943 μ l of DMF and then admixed with 3.6 mg (19 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 2.9 mg (19 μ mol) of 1-hydroxy-1*H*-benzotriazole hydrate, 2.2 μ l of *N,N*-diisopropylethylamine and with 6.6 mg (25 μ mol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 4.5 mg (64% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 2.0 min;

LC-MS (Method 1): R_t = 1.03 min; MS (ESIpos): m/z = 1129 ($M+H$)⁺.

20 Intermediate 134

N-[3-({[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]carbamoyl}amino)propyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



First, 4-nitrophenyl 2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl carbamate was prepared under standard conditions, starting from commercially available 1-(2-aminoethyl)-1*H*-pyrrole-2,5-dione trifluoroacetate and 4-nitrophenyl chlorocarbonate.

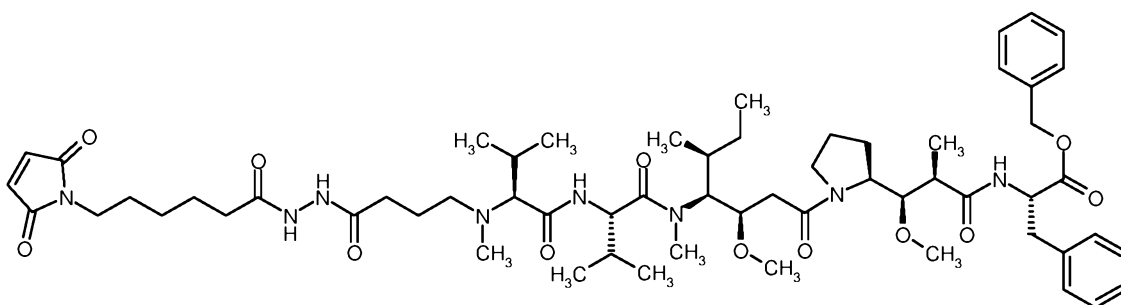
- 5 5 mg (6 μ mol) of *N*-(3-aminopropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 1000 μ l of DMF and then admixed with 2 μ l of *N,N*-diisopropylethylamine and with 2.2 mg (9 μ mol) of 4-nitrophenyl-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]carbamate.
- 10 The mixture was stirred at RT for 1 h and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 1.6 mg (23% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 2): R_t = 1.09 min; MS (ESIpos): m/z = 1036 ($M+H$)⁺.

15 Intermediate 135

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



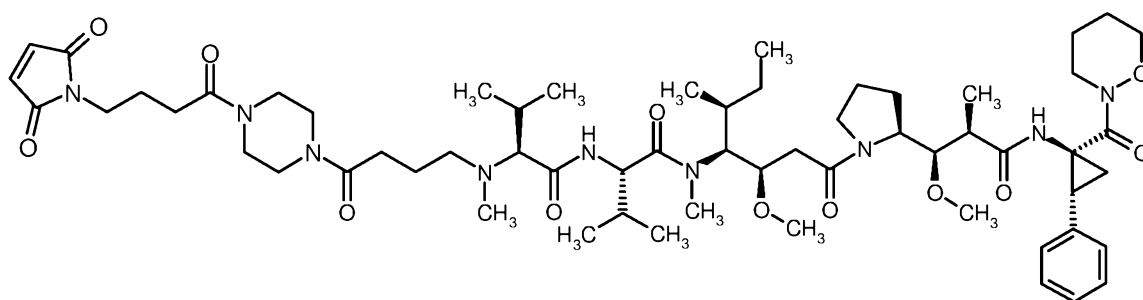
10 mg (11 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-
 {(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-
 oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide
 were dissolved in 4000 μ l of DMF and then admixed with 6.3 mg (33 μ mol) of 1-(3-
 5 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 4.5 mg (33 μ mol) of 1-hydroxy-1*H*-
 benzotriazole hydrate, 5.7 μ l of *N,N*-diisopropylethylamine and with 11.5 mg (44 μ mol) of
 commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture
 was stirred at RT overnight and then concentrated under high vacuum. The remaining residue
 was purified by means of preparative HPLC. Thus, 2.6 mg (14% of theory) of the title compound
 10 were obtained as a colourless foam.

HPLC (Method 6): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.01 min; MS (ESIpos): m/z = 1115 ($M+H$)⁺.

Intermediate 136

N-(4-{4-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoyl]piperazin-1-yl}-4-oxobutyl)-*N*-
 15 methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-
 1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-
 methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



20 First, 1-[4-oxo-4-(piperazin-1-yl)butyl]-1*H*-pyrrole-2,5-dione trifluoroacetate was prepared
 under standard conditions, starting from tert-butyl piperazine-1-carboxylate and 4-(2,5-dioxo-
 2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid over 2 stages.

5 mg (5.6 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-
 [(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]
 25 amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were
 dissolved in 1000 μ l of DMF and then admixed with 2.1 mg (11 μ mol) of 1-(3-
 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1.7 mg (11 μ mol) of 1-hydroxy-1*H*-

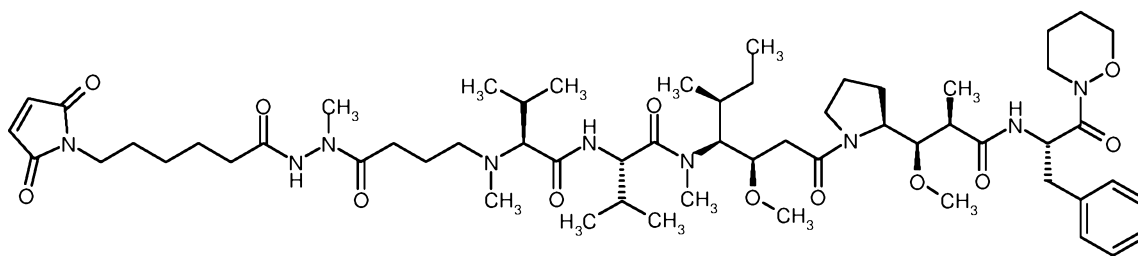
benzotriazole hydrate, 2 µl of *N,N*-diisopropylethylamine and with 3.5 mg (5.6 µmol) of 1-[4-oxo-4-(piperazin-1-yl)butyl]-1*H*-pyrrole-2,5-dione trifluoroacetate. The mixture was stirred at RT overnight. Then 2.1 mg (5.6 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate were added, and the reaction mixture was stirred at RT for another 3 h. Subsequently, the solvent was removed *in vacuo*, and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were concentrated and, by lyophilization from water, 0.6 mg (10% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 6): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.9$ min; MS (ESIpos): $m/z = 1132$ ($M+H$)⁺.

Intermediate 137

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]-1-methylhydrazino}-4-oxobutyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide



First, 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N'*-methylhexanehydrazide trifluoroacetate was prepared under standard conditions, starting from commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoic acid and *tert*-butyl 1-methylhydrazinecarboxylate over 2 stages.

6.9 mg (8 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide were dissolved in 2540 µl of DMF and then admixed with 3.6 mg (9 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 3 µl of *N,N*-diisopropylethylamine and with 4.1 mg (12 µmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N'*-methylhexanehydrazide trifluoroacetate. The mixture was stirred at RT overnight.

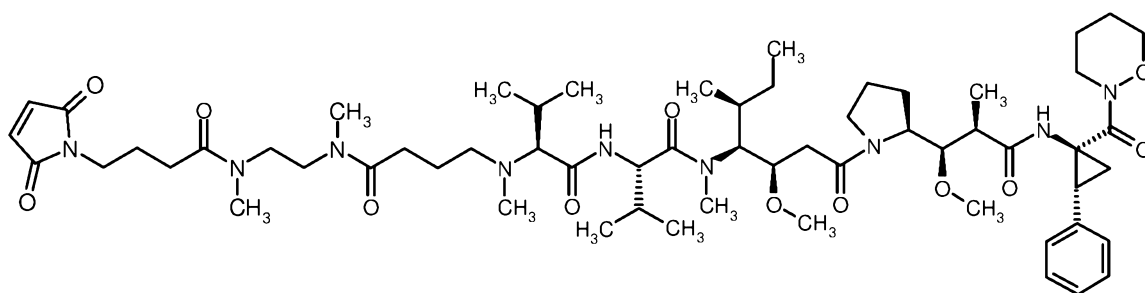
Subsequently, the solvent was removed *in vacuo*, and the remaining residue was purified by means of preparative HPLC. Thus, 3.9 mg (45% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): $R_t = 1.8$ min;

5 LC-MS (Method 1): $R_t = 0.93$ min; MS (ESIpos): $m/z = 1108$ (M+H)⁺.

Intermediate 138

N-{4-[(2-{[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoyl](methyl)amino}ethyl)(methyl)amino]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



Starting from *tert*-butylmethyl[2-(methylamino)ethyl]carbamate and 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid, over 2 stages, 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-methyl-*N*-[2-(methylamino)ethyl]butanamide trifluoroacetate was prepared first.

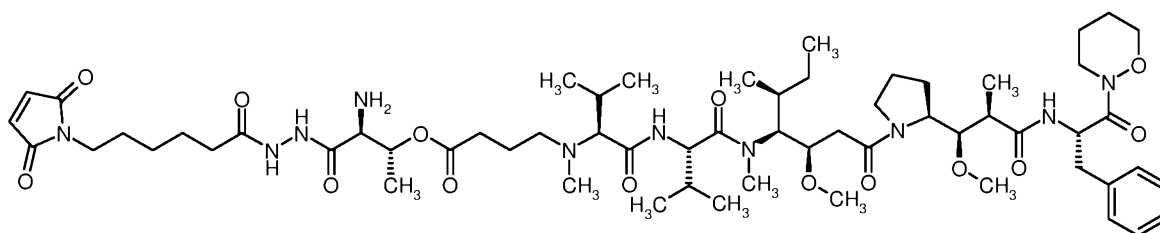
6.6 mg (7.3 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 2000 μ l of DMF and then admixed with 5.6 mg (14.7 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 2.6 μ l of *N,N*-diisopropylethylamine and with 4.1 mg (9 μ mol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-methyl-*N*-[2-(methylamino)ethyl]butanamide trifluoroacetate. After stirring at RT for 3 h, the same amounts of HATU and *N,N*-diisopropylethylamine were added once more, and the reaction mixture was then stirred at RT overnight. Subsequently, the solvent was removed *in vacuo*, and the remaining residue was purified by means of preparative HPLC. Thus, 4 mg (44% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 6): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 0.91$ min; MS (ESIpos): $m/z = 1134$ ($M+H$)⁺.

Intermediate 139

(2*R*,3*S*)-3-amino-4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutan-2-yl (3*R*,4*S*,7*S*,10*S*)-4-[(2*S*)-butan-2-yl]-7,10-diisopropyl-3-(2-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



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13 mg (14.7 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 10 ml of dichloromethane and then admixed with 8.4 mg (44 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 5.4 mg (44 μ mol) of 4-dimethylaminopyridine and with 9 mg (29.3 μ mol) of commercially available benzyl *N*-(*tert*-butoxycarbonyl)-L-threoninate. The mixture was stirred at RT for 5 h. Subsequently, the reaction mixture was extracted twice by shaking with water, and the organic phase was dried over sodium sulphate and concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane/water, 14 mg (81% of theory) of the protected intermediate were obtained as a colourless foam.

HPLC (Method 12): $R_t = 2.3$ min;

LC-MS (Method 1): $R_t = 1.13$ min; MS (ESIpos): $m/z = 1178$ ($M+H$)⁺.

Subsequently, the Z protecting group was cleaved by hydrogenolytic means in methanol over 10% palladium/activated carbon. 9.5 mg (0.0087 mmol) of the deprotected crude product were then, without further purification, taken up in 5 ml of DMF and admixed with 5 mg (26.2 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 4 mg (26.2 μ mol) of 1-

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hydroxy-1*H*-benzotriazole hydrate, 54.6 μ l of *N,N*-diisopropylethylamine and with 9.1 mg (34.9 μ mol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT for 1 h and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane, 9.5 mg (84% of theory) of the Boc-protected intermediate were obtained.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 1295 ($M+H$)⁺.

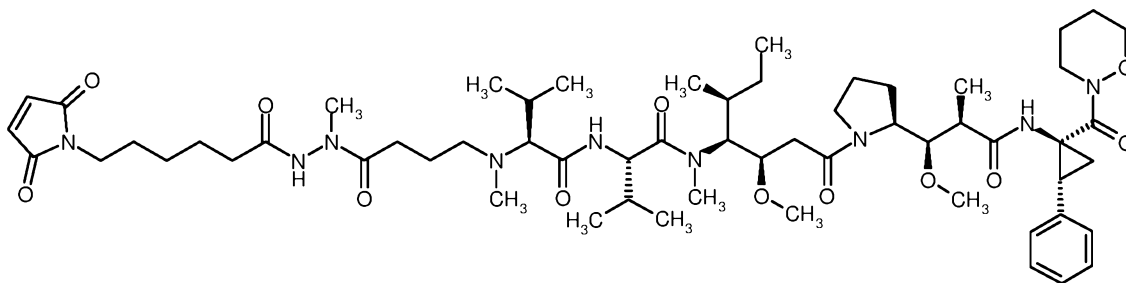
Subsequently, 9.5 mg (7.3 μ mol) were deprotected with 0.5 ml of trifluoroacetic acid in 2 ml of dichloromethane of the Boc-protected intermediate and, after lyophilization from dioxane, 9 mg (82% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 1195 ($M+H$)⁺.

Intermediate 140

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]-1-methylhydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[[[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



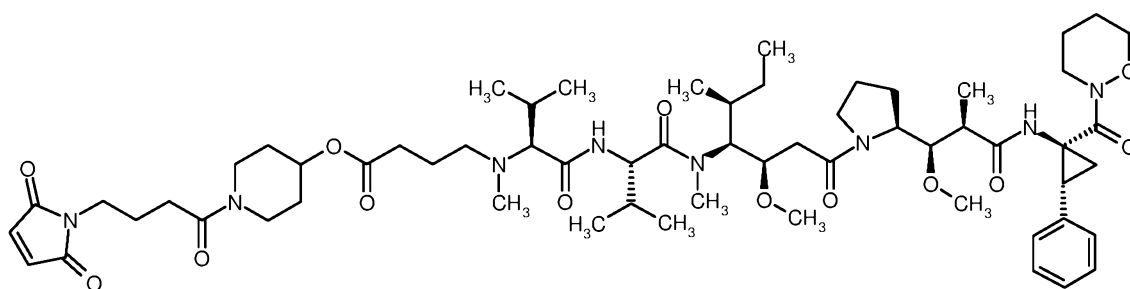
4.1 mg (12 μ mol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N'*-methylhexanehydrazide trifluoroacetate (Intermediate 22) were dissolved together with 6.9 mg (8 μ mol) of the compound from Intermediate 61 in 2.5 ml of DMF and then admixed with 3.5 mg (9 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 3 μ l of *N,N*-diisopropylethylamine. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane, 2.6 mg (30% of theory) of the title compound were obtained.

HPLC (Method 5): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.90$ and 0.91 min; MS (ESIpos): $m/z = 1120$ ($M+H$)⁺.

Intermediate 141

N-[4-({1-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoyl]piperidin-4-yl}oxy)-4-oxobutyl]-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide



44 mg (49 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide were dissolved in 2 ml of dichloromethane and then admixed with 18.8 mg (98 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 3.8 mg (24 μ mol) of 4-dimethylaminopyridine and with 23 mg (98 μ mol) of commercially available benzyl 4-hydroxypiperidine-1-carboxylate. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 22 mg (40% of theory) of the *Z*-protected intermediate were obtained as a colourless foam.

HPLC (Method 5): $R_t = 2.1$ min;

LC-MS (Method 1): $R_t = 1.04$ min; MS (ESIpos): $m/z = 1116$ ($M+H$)⁺.

Subsequently, the *Z* protecting group was cleaved by hydrogenolytic means in ethanol over palladium/activated carbon.

19 mg (19 μ mol) of the deprotected crude product were then, without further purification, taken up in 4 ml of DMF and admixed with 7 mg (39 μ mol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid, 11 mg (29 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 5 μ l of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 1

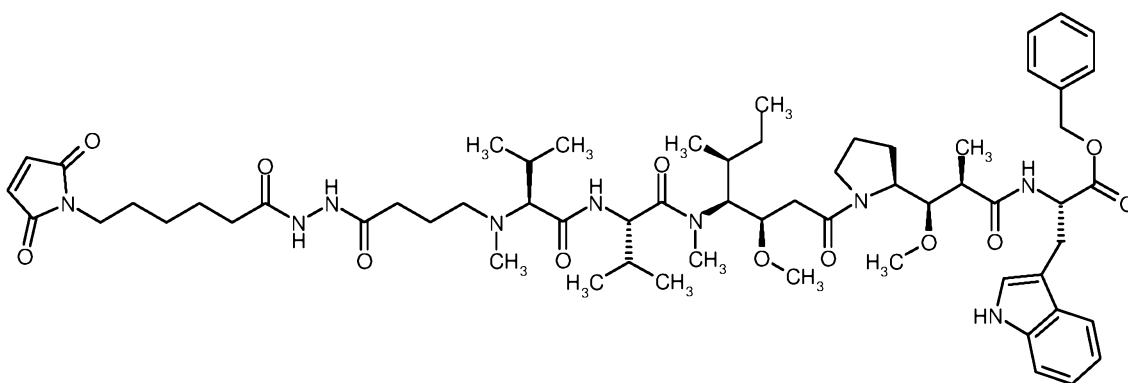
h and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane, 7.5 mg (34% of theory) of the title compound were obtained.

HPLC (Method 5): $R_t = 1.8$ min;

5 LC-MS (Method 1): $R_t = 0.94$ min; MS (ESIpos): $m/z = 1147$ (M+H)⁺.

Intermediate 142

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



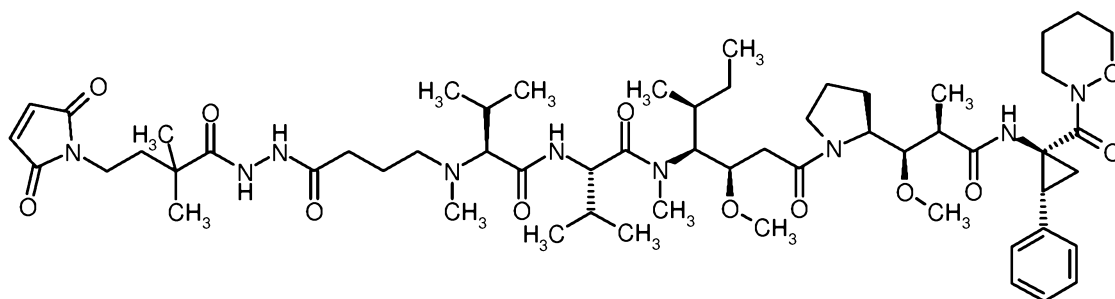
9 mg (9.5 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 72) were dissolved in 1000 μ l of DMF and then admixed with 10 mg (38 μ mol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide, 7.2 mg (19 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 8 μ l of *N,N*-diisopropylethylamine, and the reaction mixture was stirred at RT for 1 h. Subsequently, the solvent was removed *in vacuo* and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were concentrated and, by lyophilization, 6.4 mg (58% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.99$ min; MS (ESIpos): $m/z = 1154$ (M+H)⁺.

Intermediate 143

N-(4-{2-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[[[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino]-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



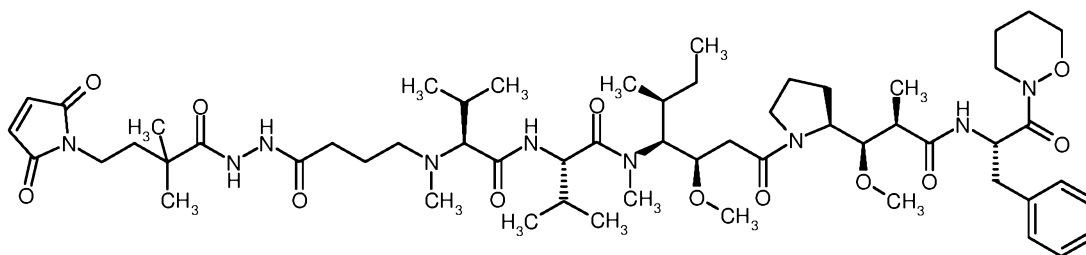
6 mg (6.7 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[[[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino]-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 61) were reacted with 3 mg (8.7 μ mol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanehydrazide trifluoroacetate in analogy to Intermediate 142 to yield 2 mg (27% of theory) of the title compound.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 3): R_t = 1.92 min; MS (ESIpos): m/z = 1106 ($M+H$)⁺.

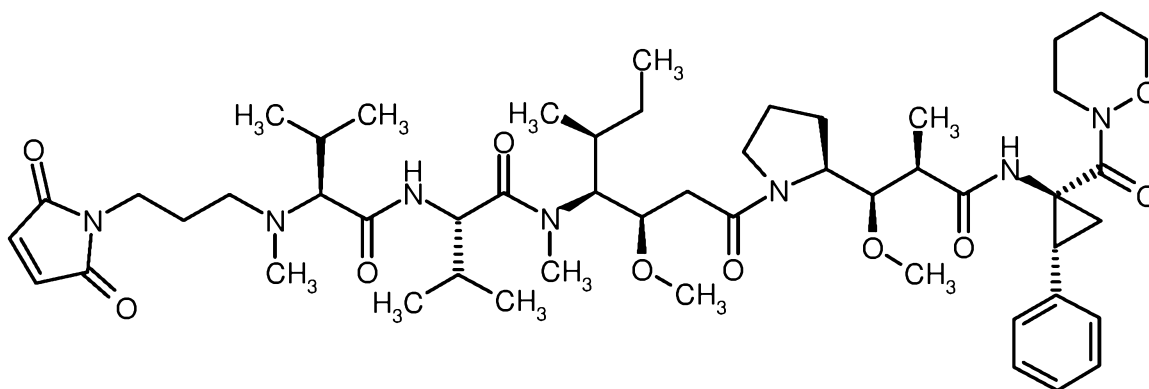
Intermediate 144

N-(4-{2-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[[[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino]-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



LC-MS (Method 3): $R_t = 1.86$ min; $m/z = 1094$ ($M+H$)⁺.

N-[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenyl cyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



12 mg (14 μ mol) of *N*-(3-aminopropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-
20 [(1*R*,2*R*)-1-methoxy-2-methyl-3-{(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]
amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide
(Intermediate 66) were taken up in 750 μ l of dioxane and admixed with 1.5 ml of saturated
sodium hydrogencarbonate solution and then with 3.2 mg (21 μ mol) of methyl 2,5-dioxo-2,5-
dihydro-1*H*-pyrrole-1-carboxylate. The reaction mixture was stirred at RT for 1 h and then
25 concentrated under reduced pressure. The remaining residue was purified by means of

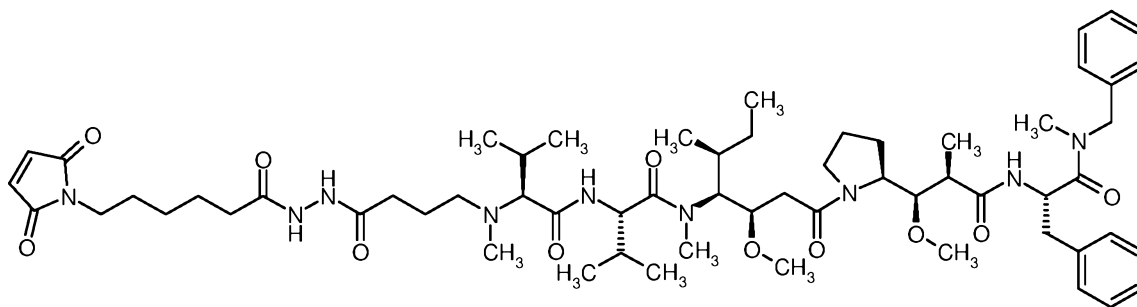
preparative HPLC. After lyophilization, 4.2 mg (32% of theory) of the title compound were obtained.

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.94$ min; MS (ESIpos): $m/z = 950$ ($M+H$)⁺.

5 Intermediate 146

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



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9 mg (9.8 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 73) were reacted in analogy to Intermediate 133 with 10 mg (39 μ mol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide to yield 1.8 mg (15% of theory) of the title compound.

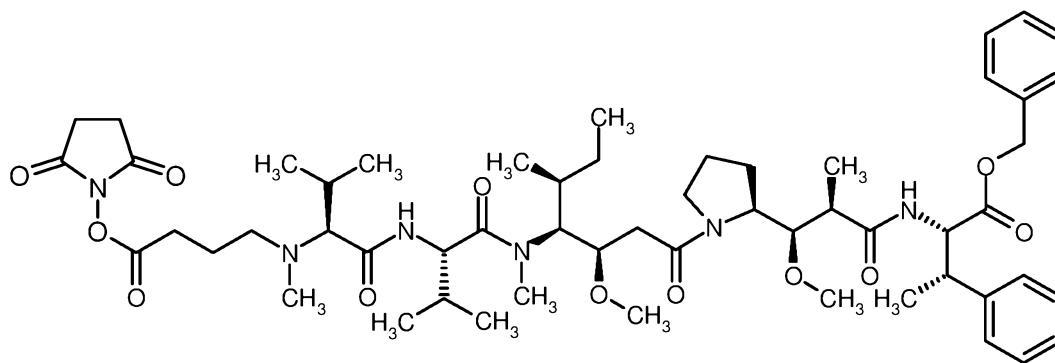
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HPLC (Method 12): $R_t = 2.2$ min;

LC-MS (Method 9): $R_t = 5.11$ min; MS (ESIpos): $m/z = 1128$ ($M+H$)⁺.

20 Intermediate 147

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



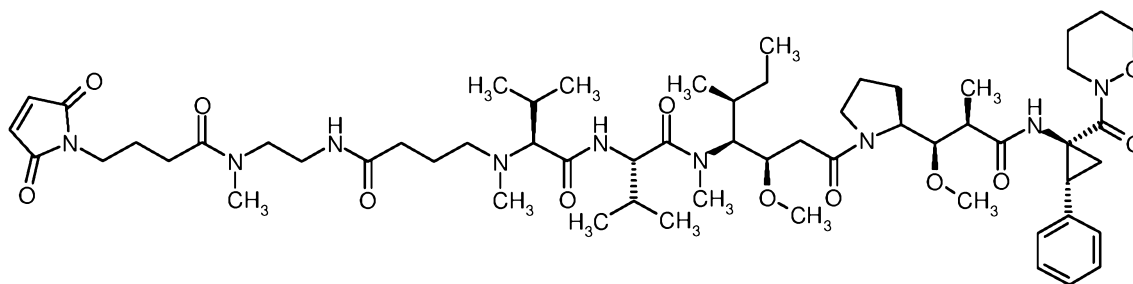
16 mg (17 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

- 5 (Intermediate 70) were dissolved in 2 ml of dichloromethane and admixed with 2.6 mg (23 mmol) of 1-hydroxypyrrolidine-2,5-dione and then with 4 mg (21 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. After stirring at RT for 2 h, the same amounts of 1-hydroxypyrrolidine-2,5-dione and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were added once again. Then stirring at RT overnight, the reaction mixture was
- 10 concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization, 10 mg (56% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 2.0 min;

Intermediate 148

- N*-{4-[(2-{[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoyl](methyl)amino}ethyl)amino]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide
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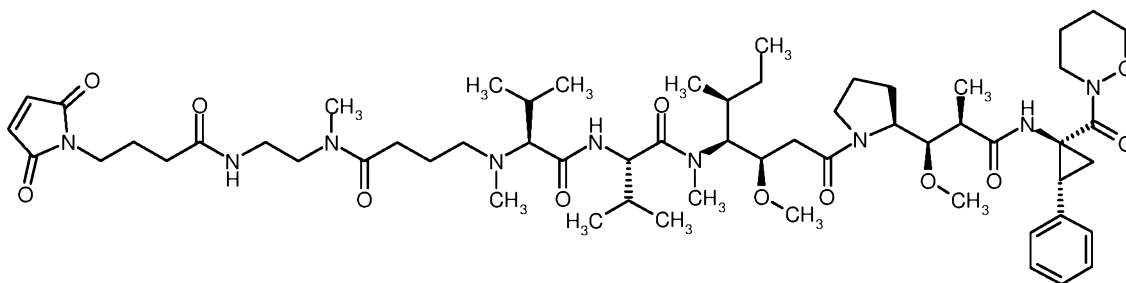
6 mg (7 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 61) were combined with 2.8 mg (8 μ mol) of *N*-(2-aminoethyl)-4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-methylbutanamide trifluoroacetate, 10.1 mg (27 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 5 μ l of *N,N*-diisopropylethylamine in 2 ml of DMF and stirred at RT overnight. Then another 5 mg (23.5 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 3 μ l of *N,N*-diisopropylethylamine were added. After stirring at RT for a further 5 h, the solvent was removed *in vacuo*, and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were concentrated and, by lyophilization from dioxane, 1.3 mg (15% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 2): R_t = 1.21 min; MS (ESIpos): m/z = 1120 ($M+H$)⁺.

Intermediate 149

N-{4-[(2-{[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoyl]amino}ethyl)(methyl)amino]-4-oxobutyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



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6 mg (7 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 61) were combined with 3.1 mg (9 μ mol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-[2-(methylamino)ethyl]butanamide trifluoroacetate, 10.1 mg (27 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 5 μ l of *N,N*-diisopropylethylamine in 2 ml of DMF, and the mixture was stirred at RT for 4 h. Then the

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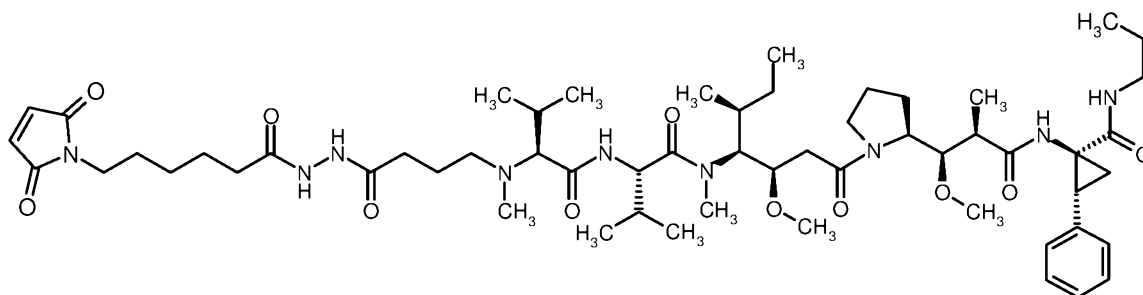
solvent was removed *in vacuo*, and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were concentrated and, by lyophilization from dioxane, 1 mg (13.4% of theory) of the title compound were obtained.

HPLC (Method 12): $R_t = 2.1$ min;

5 LC-MS (Method 1): $R_t = 0.89$ min; MS (ESIpos): $m/z = 1121$ (M+H)⁺.

Intermediate 150

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*,2*R*)-2-phenyl-1-(propylcarbamoyl)cyclopropyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



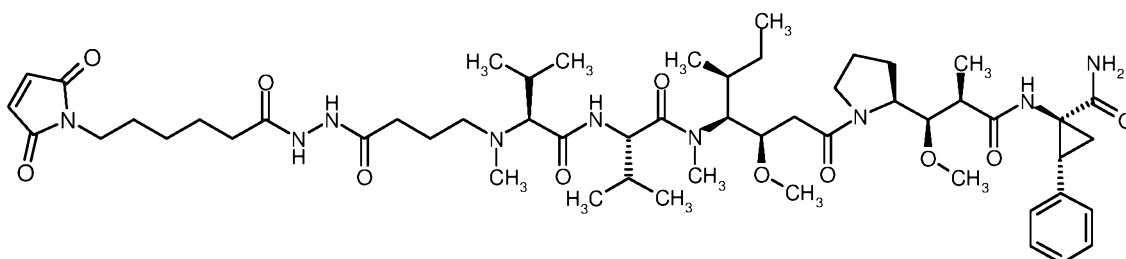
7.9 mg (9 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*,2*R*)-2-phenyl-1-(propylcarbamoyl)cyclopropyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 3 ml of DMF and then admixed with 10.4 mg (54 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 8.3 mg (54 μ mol) of 1-hydroxy-1*H*-benzotriazole hydrate, 9 μ l of *N,N*-diisopropylethylamine and with 9.5 mg (36 μ mol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 4.3 mg (22% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 6): $R_t = 1.9$ min;

LC-MS (Method 9): $R_t = 4.93$ min; MS (ESIpos): $m/z = 1078$ (M+H)⁺.

Intermediate 151

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-carbamoyl-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



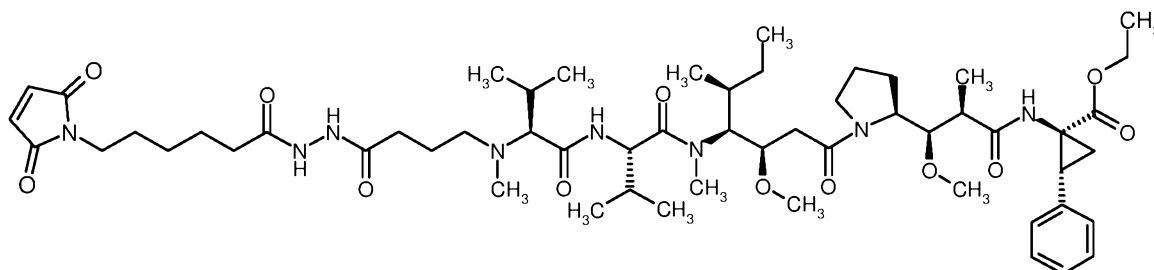
The compound was prepared in analogy to Intermediate 150 starting from the compound in Intermediate 81.

HPLC (Method 5): $R_t = 1.7$ min;

10 LC-MS (Method 1): $R_t = 0.87$ min; MS (ESIpos): $m/z = 1036$ ($M+H$)⁺.

Intermediate 152

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-(ethoxycarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



10 mg (12 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-(ethoxycarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 3 ml of DMF and then admixed with 8.9 mg (23 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 10 μ l of *N,N*-diisopropylethylamine and

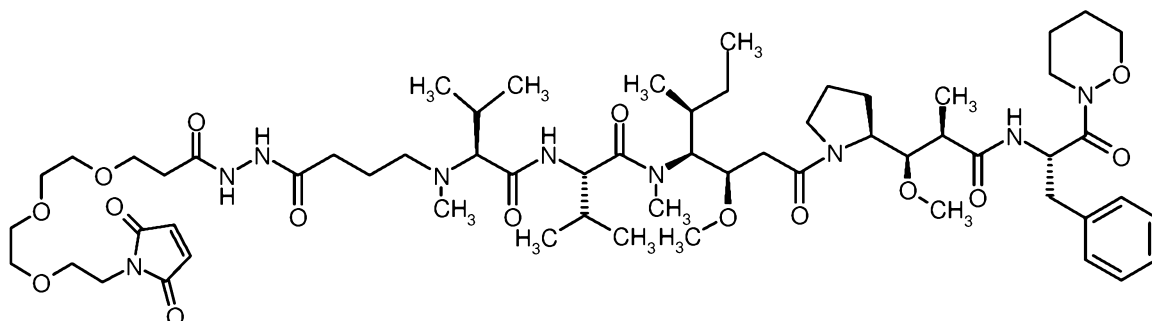
with 12 mg (47 μ mol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The mixture was stirred at RT for 1 h. This was followed by concentration under high vacuum and purification of the remaining residue by means of preparative HPLC. Thus, 5.8 mg (37 % of theory) of the title compound were obtained as a colourless foam.

5 HPLC (Method 6): R_t = 2.0 min;

LC-MS (Method 9): R_t = 4.99 min; MS (ESIpos): m/z = 1066 ($M+H$)⁺.

Intermediate 153

N-[1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-12,15-dioxo-3,6,9-trioxa-13,14-diazaoctadecan-18-yl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-
10 {[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



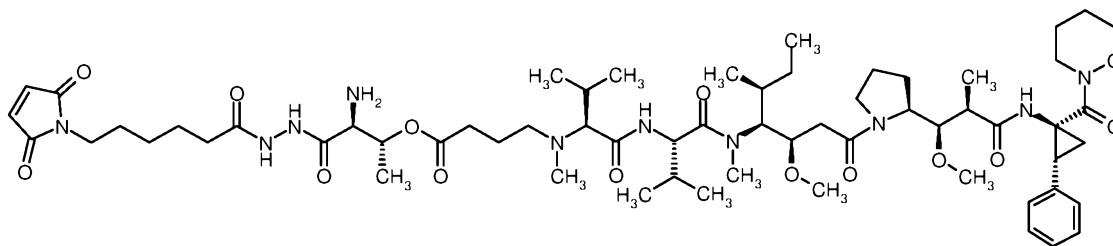
To a solution of 5 mg (5.6 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-
15 phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide in 1 ml of DMF were added 9.7 mg (22.5 μ mol) of 3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)propanehydrazide trifluoroacetate, 3.2 mg (16.9 μ mol) of EDC, 1.96 μ l (11.3 μ mol) of *N,N*-diisopropylethylamine and 2.6 mg (16.9 μ mol) of HOBT. The reaction mixture was stirred at RT for 3 h. Subsequently, another 1.2 mg (2.8 μ mol)
20 of 3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)propanehydrazide trifluoroacetate were added. The reaction mixture was stirred at RT overnight and then purified by means of preparative HPLC.

3.6 mg (51% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.90 min; m/z = 1185 ($M+H$)⁺.

Intermediate 154

(2*R*,3*S*)-3-amino-4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutan-2-yl-(3*R*,4*S*,7*S*,10*S*)-4-[(2*S*)-butan-2-yl]-7,10-diisopropyl-3-(2-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



15 mg (17 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 10 ml of dichloromethane and then admixed with 12.8 mg (67 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 10 mg (83 μ mol) of 4-dimethylaminopyridine and with 10.3 mg (33 μ mol) of commercially available benzyl *N*-(*tert*-butoxycarbonyl)-L-threoninate. The mixture was heated to reflux for 4 h. Then the same amounts of coupling reagent and 4-dimethylaminopyridine were added again, and the reaction mixture was heated overnight with reflux. Subsequently, the reaction mixture was diluted with dichloromethane and extracted by shaking once with water, the organic phase was removed and concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. Thus, 7.7 mg (37% of theory) of the protected intermediate were obtained as a colourless foam.

HPLC (Method 12): R_t = 2.5 min;

LC-MS (Method 1): R_t = 1.13 min; MS (ESIpos): m/z = 1190 ($M+H$)⁺.

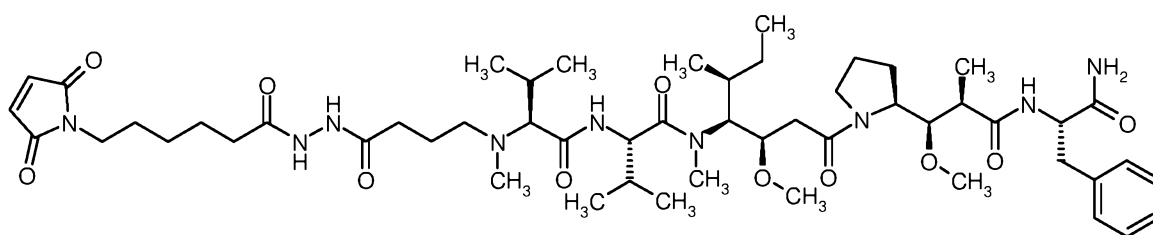
Subsequently, the benzyl ester protecting group was removed by hydrogenation under standard hydrogen pressure in methanol over 10% palladium/activated carbon, and the acid thus obtained, as described in Intermediate 151, was coupled to 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. In a last step, the Boc protecting group was detached with trifluoroacetic acid. The remaining residue was purified by means of preparative HPLC. Thus, 0.22 mg (2.5% of theory over 3 stages) of the title compound was obtained as a colourless foam.

HPLC (Method 12): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 0.81$ min; MS (ESIpos): $m/z = 1207$ (M+H)⁺.

Intermediate 155

- 5 *N*-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



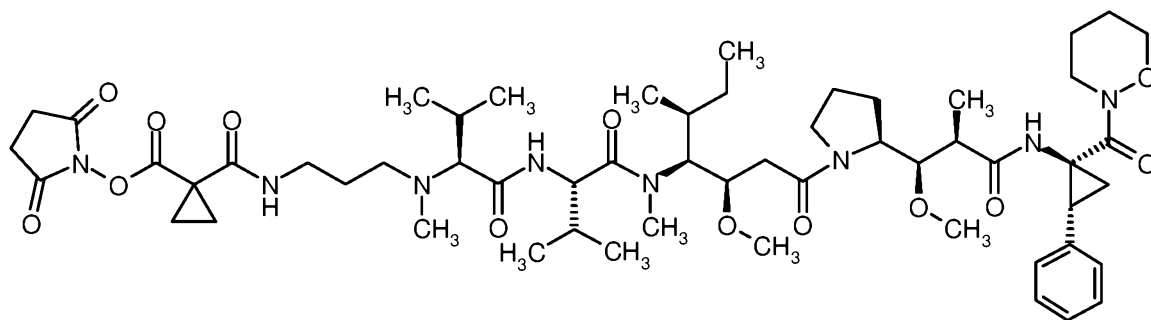
- 10 This compound was prepared in analogy to the synthesis described in Intermediate 152 from *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

- 15 HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.82$ min; MS (ESIpos): $m/z = 1024$ (M+H)⁺.

Intermediate 156

- 20 *N*-(3-{[(1-{[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}cyclopropyl)carbonyl]amino}propyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



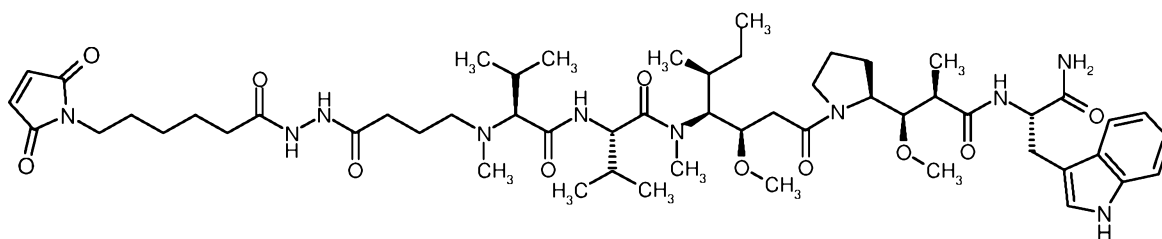
This compound was prepared in analogy to the synthesis described in the last stage of Intermediate 131 from *N*-(3-aminopropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 1,1'-[cyclopropane-1,1-diylbis(carbonyloxy)]dipyrrolidine-2,5-dione, which had been obtained from the corresponding dicarboxylic acid beforehand.

HPLC (Method 12): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 0.92$ min; MS (ESIpos): $m/z = 1080$ ($M+H$)⁺.

Intermediate 157

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



15 mg (18 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 3.8 ml of DMF and then admixed with 27 mg (70 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 12 μ l of *N,N*-diisopropylethylamine and with 14 mg (53 μ mol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. The reaction mixture was stirred at RT for 1 h. This

was followed by concentration under high vacuum and purification of the remaining residue by means of preparative HPLC. Thus, 6.2 mg (33% of theory) of the title compound were obtained as a colourless foam.

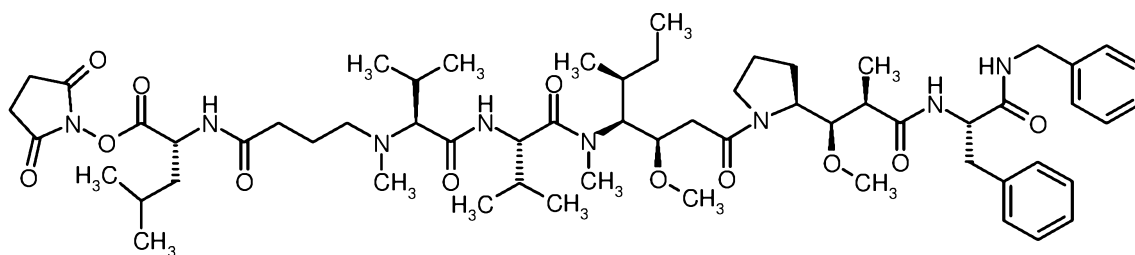
HPLC (Method 5): $R_t = 1.6$ min;

5 LC-MS (Method 1): $R_t = 0.85$ min; MS (ESIpos): $m/z = 1063$ (M+H)⁺.

¹H-NMR (500 MHz, DMSO-d₆, characteristic signals): $\delta = 10.8$ (d, 1H), 9.8-9.7 (m, 2H), 9.6 and 9.4 (2m, 1H), 8.9, 8.88, 8.78 and 8.75 (4d, 1H), 8.08 and 7.85 (2d, 1H), 7.6-6.9 (m, 9H), 4.7-4.4 (m, 3H), 3.4 (t, 2H), 3.23, 3.2, 3.18, 3.0, and 2.99 (5s, 9H), 2.8 (m, 3H), 2.1 (t, 2H), 1.06 and 1.01 (2d, 3H), 0.95-0.8 (m, 15H), 0.8-0.75 (dd, 3H).

10 Intermediate 158

N-[4-((2*R*)-1-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-methyl-1-oxopentan-2-yl)amino]-4-oxobutyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



15

13 mg (14.7 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

20 were dissolved in 4 ml of dimethylformamide and then admixed with 9.4 mg (25 μ mol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 6 μ l of *N,N*-diisopropylethylamine and with 7 mg (31 μ mol) of commercially available *tert*-butyl-D-leucinate hydrochloride. The mixture was stirred at RT for 5 h and then concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization from
25 dioxane/water, 6.5 mg (49% of theory) of the protected intermediate were obtained as a colourless foam.

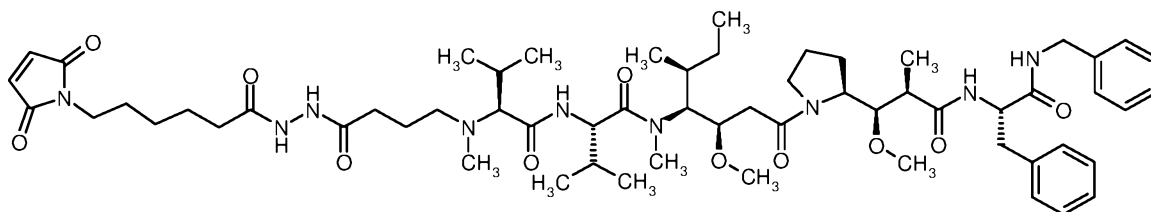
HPLC (Method 5): $R_t = 2.2$ min;

LC-MS (Method 1): $R_t = 1.21$ min; MS (ESIpos): $m/z = 1076$ (M+H)⁺.

Trifluoroacetic acid in dichloromethane was first used to cleave the Boc protecting group from this protected intermediate, yielding 6.2 mg (99% of theory) of the deprotected compound. 5.2 mg (5 μ mol) of this intermediate were taken up in 1.5 ml of dichloromethane and reacted with 0.8 mg (7 μ mol) of *N*-hydroxysuccinimide, in the presence of 1.2 mg (6 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.16 mg (1 μ mol) of 4-dimethylaminopyridine. After stirring at RT for 2 h, the reaction mixture was concentrated and purified by means of preparative HPLC. 1.3 mg of the title compound were obtained, some of which was hydrolysed into an educt.

Intermediate 159

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 157 from *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

Yield: 6 mg (53% of theory)

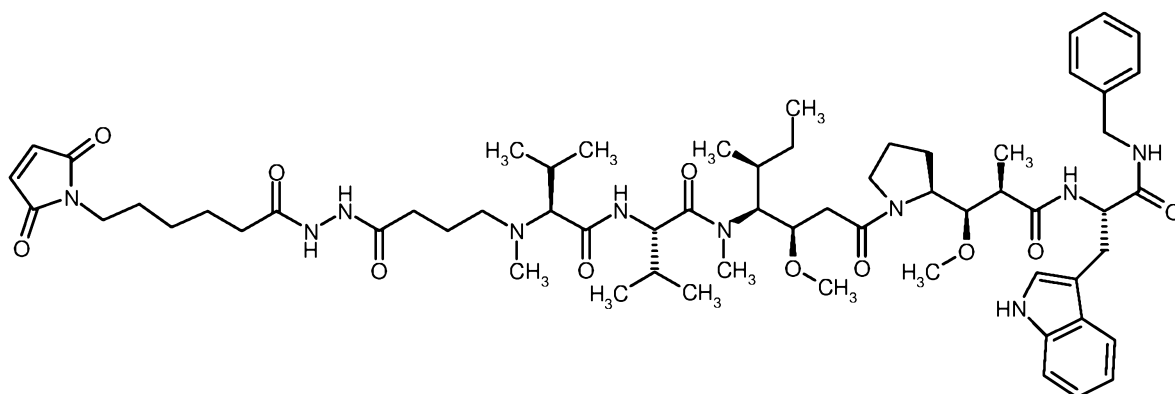
HPLC (Method 5): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.94$ min; MS (ESIpos): $m/z = 1114$ (M+H)⁺.

Intermediate 160

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-

oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 157 from 20
 5 mg (21 μ mol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-((2*S*)-2-[(1*R*,2*R*)-3-
 {[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-
 oxopropyl]pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and
 commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

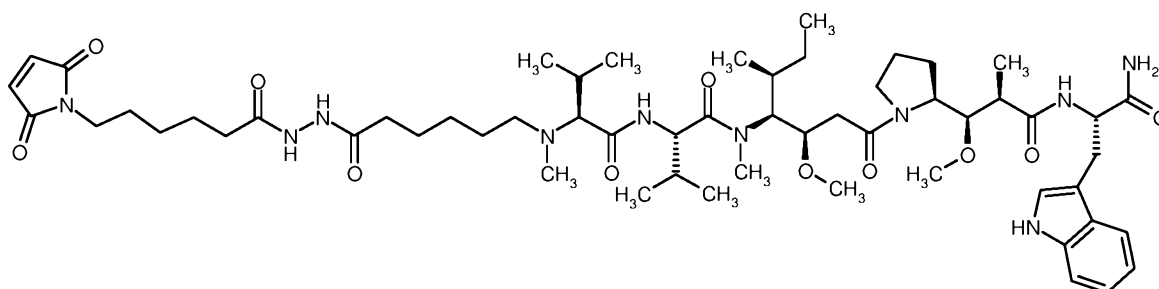
Yield: 13 mg (52% of theory)

10 HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 1153 ($M+H$)⁺.

Intermediate 161

N-(6-{2-[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]hydrazino}-6-oxohexyl)-*N*-methyl-
 L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-((2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-
 15 yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-
 4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 157 from *N*-
 (5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-((2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-amino-3-(1*H*-
 20 indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl)-3-

methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

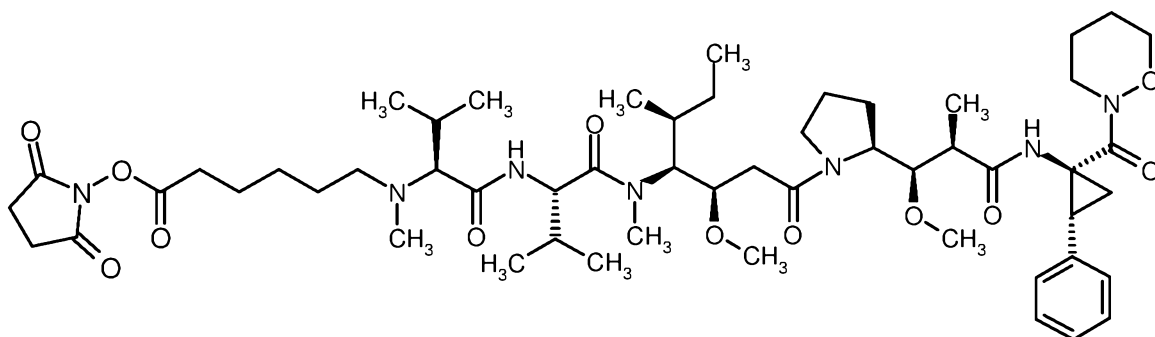
Yield: 0.8 mg (16% of theory)

HPLC (Method 5): $R_t = 1.6$ min;

5 LC-MS (Method 1): $R_t = 0.78$ min; MS (ESIpos): $m/z = 1092$ ($M+H$)⁺.

Intermediate 162

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



18 mg (20 μ mol) of *N*-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino]-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

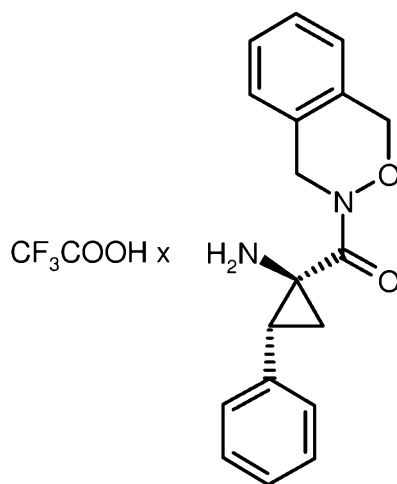
(Intermediate 64) were dissolved in 3.2 ml of dichloromethane and admixed with 22 mg (190 mmol) of 1-hydroxypyrrolidine-2,5-dione and then with 11 mg (60 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.24 mg (0.17 μ mol) of DMAP. After stirring at RT for 2 h, another 22 mg (190 mmol) of 1-hydroxypyrrolidine-2,5-dione, 11 mg (60 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.24 mg (0.17 μ mol) of DMAP were added, and the reaction mixture was stirred at RT for another hour. This was followed by concentration *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization, 8.2 mg (41% of theory) of the title compound were obtained.

25 HPLC (Method 5): $R_t = 2.0$ min;

LC-MS (Method 11): $R_t = 0.9$ min; MS (ESIpos): $m/z = 1024$ (M+H)⁺.

Intermediate 163

[(1*S*,2*R*)-1-amino-2-phenylcyclopropyl](1,4-dihydro-3*H*-2,3-benzoxazin-3-yl)methanone trifluoroacetate



5

First, starting with 265 mg (0.82 mmol) of *tert*-butyl (1*S*,2*R*)-1-(hydroxycarbonyl)-2-phenylcyclopropyl carbamate (Starting Compound 7), and by reaction with 1,2-bis(bromomethyl)benzene analogously to a literature method (see H. King, *J. Chem. Soc.* 1942, 432), the Boc-protected intermediate *tert*-butyl-[(1*S*,2*R*)-1-(1,4-dihydro-3*H*-2,3-benzoxazin-3-ylcarbonyl)-2-phenylcyclopropyl]carbamate was prepared.

10

Yield: 108 mg (34% of theory)

LC-MS (Method 2): $R_t = 1.3$ min; MS (ESIpos): $m/z = 395$ (M+H)⁺.

108 mg (0.27 mmol) of this intermediate were taken up in 3.7 ml of dichloromethane, 1.8 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 15 min. This was followed by concentration *in vacuo* and lyophilization of the remaining residue from dioxane. 112 mg of the title compound were obtained in quantitative yield as a colourless foam.

15

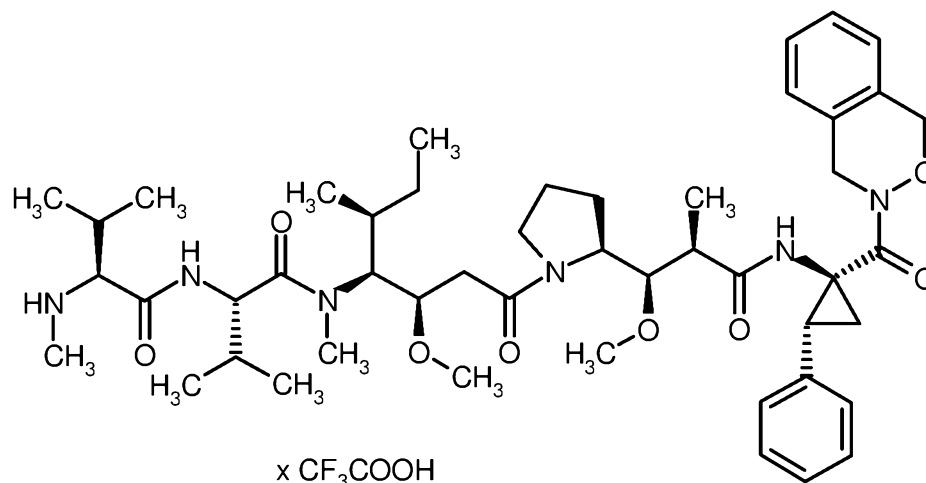
LC-MS (Method 1): $R_t = 0.7$ min; MS (ESIpos): $m/z = 295$ (M+H)⁺.

Intermediate 164

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*,2*R*)-1-(1,4-dihydro-3*H*-2,3-benzoxazin-3-ylcarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-

20

oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



166 mg (0.196 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-
 5 [(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-
 methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 10) were taken up in 40 ml of
 DMF and admixed successively with 80 mg (0.196 mmol) of [(1*S*,2*R*)-1-amino-2-
 phenylcyclopropyl](1,4-dihydro-3*H*-2,3-benzoxazin-3-yl)methanone trifluoroacetate
 (Intermediate 163), 112 mg (0.294 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-
 10 tetramethyluronium hexafluorophosphate (HATU) and 682 μ l (3.9 mmol) of *N,N*-
 diisopropylethylamine. The mixture was subsequently stirred at RT overnight. The reaction
 mixture was then concentrated *in vacuo*, the residue was taken up in ethyl acetate, and the
 solution was washed with saturated aqueous sodium chloride solution. The organic phase was
 dried over magnesium sulphate, filtered and concentrated. The residue was finally purified by
 15 means of preparative HPLC. In this way, 19 mg (9% of theory) of the Fmoc-protected
 intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-
 [(1*R*,2*R*)-3-[(1*S*,2*R*)-1-(1,4-dihydro-3*H*-2,3-benzoxazin-3-ylcarbonyl)-2-
 phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-
 methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained.

20 HPLC (Method 5): R_t = 1.68 min;

LC-MS (Method 1): R_t = 1.51 min; MS (ESIpos): m/z = 1083 ($M+H$)⁺.

19 mg (0.015 mmol) of this intermediate were dissolved in 4 ml of DMF. After adding 817 μ l of
 piperidine, the reaction mixture was stirred at RT for 5 min. This was followed by concentration
in vacuo, and the residue was first digested with diethyl ether and then purified by means of

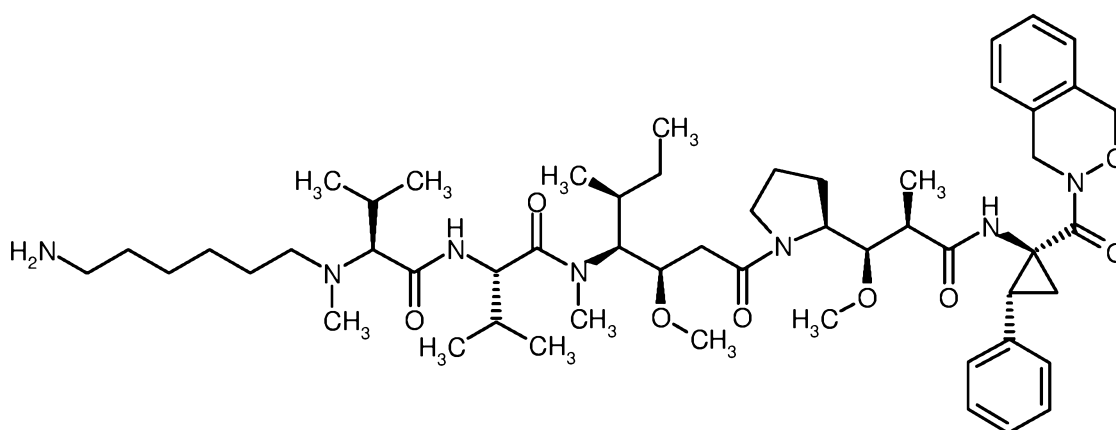
preparative HPLC (eluent: acetonitrile + 0.1% TFA / 0.1% aq. TFA). The corresponding fractions were combined, the solvent was removed *in vacuo*, and then the residue was lyophilized from dioxane/water. 12 mg (92% of theory) of the title compound were obtained as a colourless foam.

5 HPLC (Method 6): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 0.94$ min; MS (ESIpos): $m/z = 861$ (M+H)⁺.

Intermediate 165

N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-[(1S,2R)-1-(1,4-dihydro-3H-2,3-benzoxazin-3-ylcarbonyl)-2-phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



20 mg (0.021 mmol) of Intermediate 164 were used, in analogy to the preparation of Intermediate 97, together with benzyl-(6-oxohexyl)carbamate in the presence of sodium cyanoborohydride and with subsequent hydrogenolytic cleaving of the Z protecting group (using 5% palladium on carbon as catalyst, in methanol as a solvent), to prepare the title compound.

Yield: 4.5 mg (23% of theory over 2 stages)

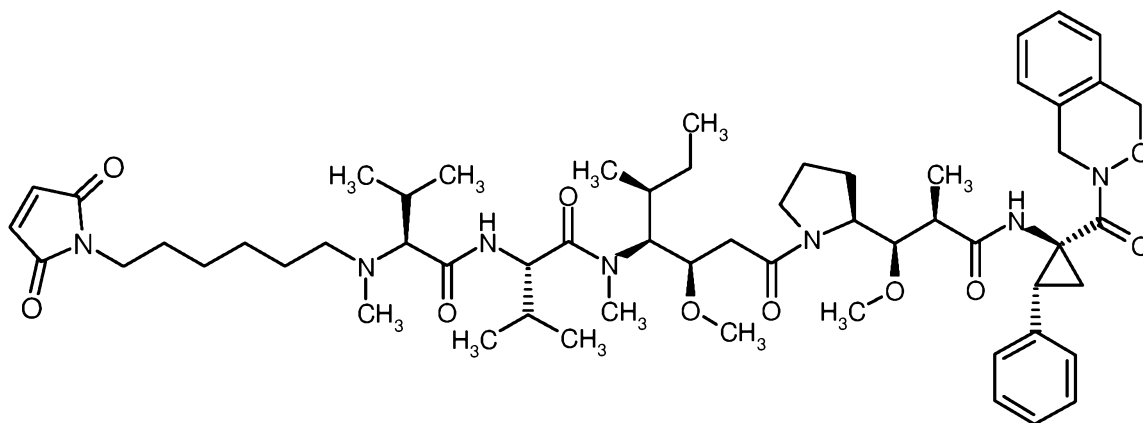
HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.9$ min; MS (ESIpos): $m/z = 960$ (M+H)⁺.

Intermediate 166

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-[(1S,2R)-1-(1,4-dihydro-3H-2,3-benzoxazin-3-ylcarbonyl)-2-

phenylcyclopropyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



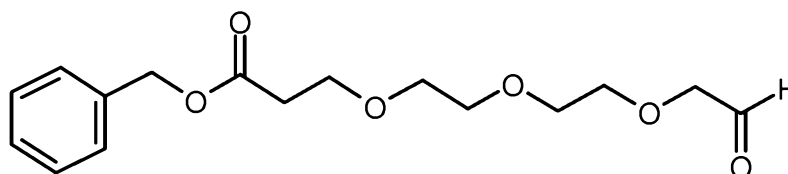
- 5 4.4 mg (4.5 μ mol) of Intermediate 165 were taken up in 1 ml of 1:1 dioxane/water and then admixed with 1 mg (6.8 μ mol) of methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate and with 50 μ l of saturated aqueous sodium hydrogencarbonate solution. The reaction mixture was stirred at RT for 30 min. Then another 50 μ l of the saturated aqueous sodium hydrogencarbonate solution were added, and the reaction mixture was stirred at RT for a further 15 min and then
- 10 concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization, 1 mg (21% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 12): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.08 min; MS (ESIpos): m/z = 1040 ($M+H$)⁺.

Intermediate 167

- 15 Benzyl 3-{2-[2-(2-oxoethoxy)ethoxy]ethoxy}propanoate



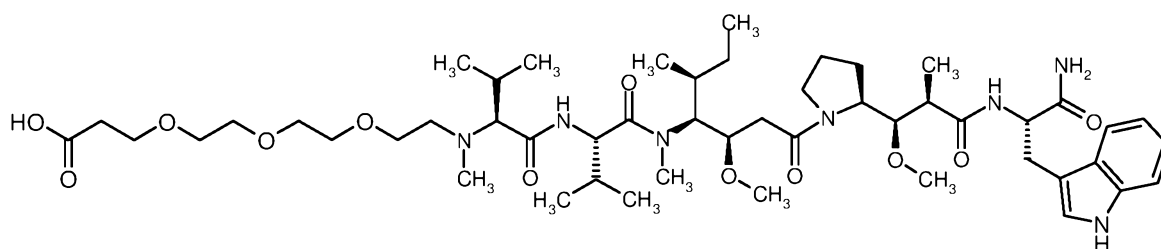
- The title compound was prepared from 6 g (21.55 mmol) of commercially available 3-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}propanoic acid under standard conditions, first by esterification with benzyl chloride and caesium carbonate and subsequent oxidation with sulphur trioxide-pyridine complex.
- 20

Yield: 611 mg (10% of theory over 2 stages)

LC-MS (Method 2): $R_t = 1.69$ min; MS (ESIpos): $m/z = 311$ ($M+H$)⁺.

Intermediate 168

N-(2-{2-[2-(2-carboxyethoxy)ethoxy]ethoxy}ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-
5 [(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 69, by coupling of *N*-(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) and *N*^α-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-tryptophanamide trifluoroacetate (Intermediate 49) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Fmoc protecting group by means of piperidine, the amine compound
15 *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate.

25 mg (0.028 mmol) of this compound and 17.5 mg (0.06 mmol) of Intermediate 167 were combined in 2 ml of methanol and admixed with 12.6 mg (0.14 mmol) of borane-pyridine complex and 2.5 ml of acetic acid. The reaction mixture was stirred at RT overnight. Then, the same amounts of borane-pyridine complex and acetic acid were added once more, and the reaction mixture was stirred at RT for another 24 h. This was followed by concentration *in vacuo*, and the residue was purified by means of preparative HPLC. After concentration of the corresponding fractions and lyophilization from 1:1 dioxane/water, 26.5 mg (88% of theory) of the *Z*-protected title compound were obtained.
25

HPLC (Method 12): $R_t = 2.04$ min;

LC-MS (Method 1): $R_t = 0.97$ min; MS (ESIpos): $m/z = 1064$ ($M+H$)⁺.

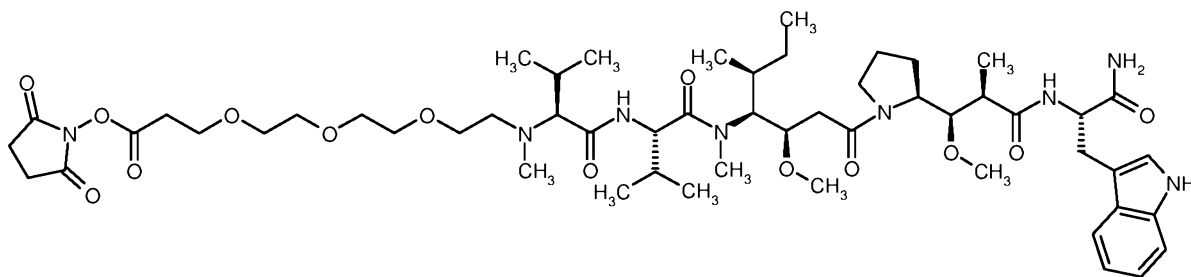
25 mg (0.024 mmol) of this intermediate were taken up in 10 ml of methanol and hydrogenated over 10% palladium on activated carbon under standard hydrogen pressure at RT for 45 min. The catalyst was then filtered off, and the solvent was removed *in vacuo*. After lyophilization from dioxane, 19.7 mg (85% of theory) of the title compound were obtained.

5 HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.83$ min; MS (ESIpos): $m/z = 974$ (M+H)⁺.

Intermediate 169

N-{2-[2-(2-{3-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-oxopropoxy}ethoxy)ethoxy]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



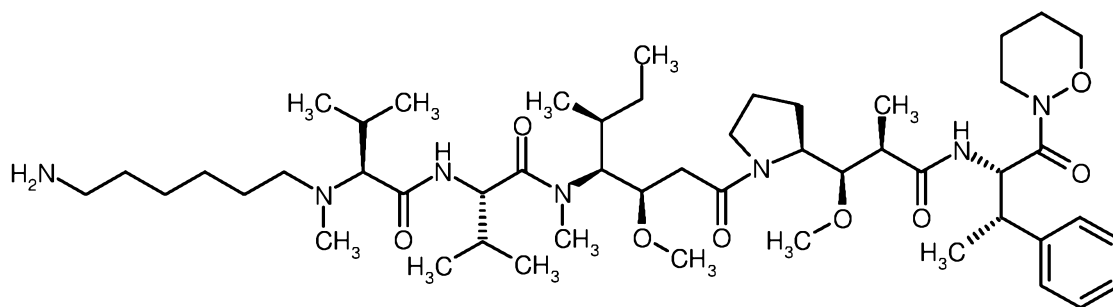
10 mg (10 μ mol) of Intermediate 168 were dissolved in 3 ml of DMF and admixed with 3.5 mg (30 mmol) of 1-hydroxypyrrolidine-2,5-dione and then with 2.4 mg (10 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 5 μ l of N,N-diisopropylethylamine. After stirring at RT for 20 h, 8 mg (0.02 mmol) of HATU were added, and the reaction mixture was stirred once again at RT overnight and then concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane, 8.6 mg (64% of theory) of the title compound were obtained.

20 HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 11): $R_t = 0.81$ min; MS (ESIpos): $m/z = 1071$ (M+H)⁺.

Intermediate 170

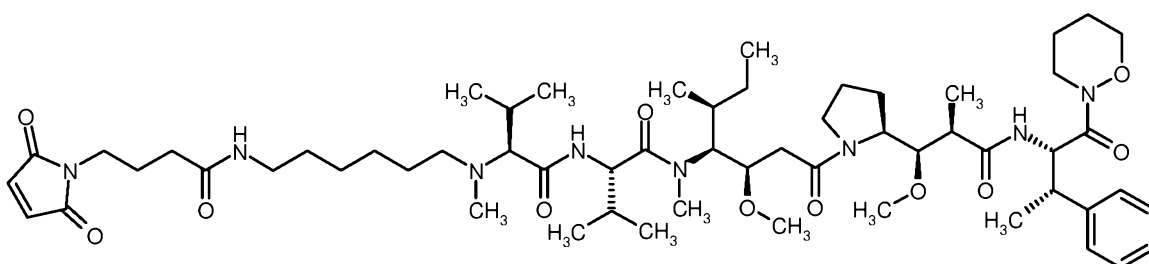
N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S,3S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



Yield: 16.7 mg (63% of theory over 2 stages)

LC-MS (Method 1): $R_t = 0.81$ min; MS (ESIpos): $m/z = 914$ ($M+H$)⁺.

N-(6-([4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoyl]amino)hexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-((2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-((2S,3S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino)-3-oxopropyl]pyrrolidin-1-yl)-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



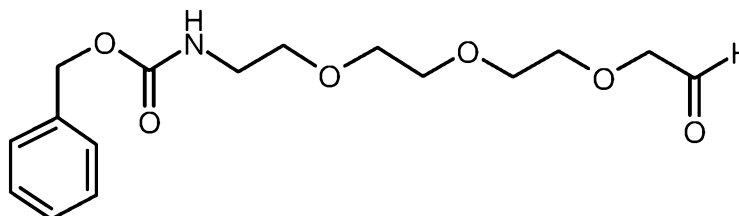
6.7 mg (7.3 μmol) of the compound from Intermediate 170 and 3 mg (14.7 μmol) of
15 commercially available 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoic acid were taken up in
2 ml of DMF and admixed with 5.6 mg (14.7 μmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-
tetramethyluronium hexafluorophosphate (HATU) and 2 μl of *N,N*-diisopropylethylamine. The
mixture was stirred at RT for 30 min. The reaction mixture was concentrated, and the residue
was purified by means of preparative HPLC. The corresponding fractions were combined, the
20 solvent was removed *in vacuo*, and then the residue was lyophilized from dioxane. Thus, 4.5 mg
(56% of theory) of the title compound were obtained.

HPLC (Method 12): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 1.12$ min; MS (ESIpos): $m/z = 1079$ ($M+H$)⁺.

Intermediate 172

Benzyl 2-{2-[2-(2-oxoethoxy)ethoxy]ethoxy}ethyl)carbamate



5

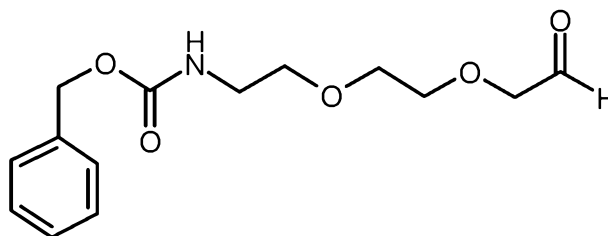
The title compound was prepared from commercially available 2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethanol under standard conditions by first introducing the Z protecting group and then oxidizing with sulphur trioxide-pyridine complex.

HPLC (Method 12): $R_t = 1.4$ min;

10 LC-MS (Method 11): R_t = 0.65 min; MS (ESIpos): m/z = 326 (M+H)⁺.

Intermediate 173

Benzyl {2-[2-(2-oxoethoxy)ethoxy]ethyl}carbamate



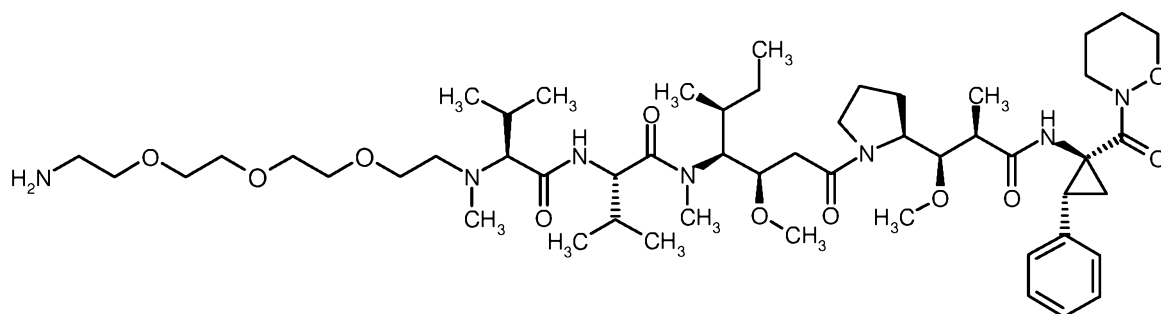
The title compound was prepared in analogy to Intermediate 172 from commercially available 2-[2-(2-aminoethoxy)ethoxy]ethanol under standard conditions by first introducing the Z protecting group and then oxidizing with sulphur trioxide-pyridine complex.

HPLC (Method 12): $R_t = 1.3$ min;

LC-MS (Method 11): $R_t = 0.68$ min; MS (ESIpos): $m/z = 282$ ($M+H$)⁺.

Intermediate 174

N-(2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



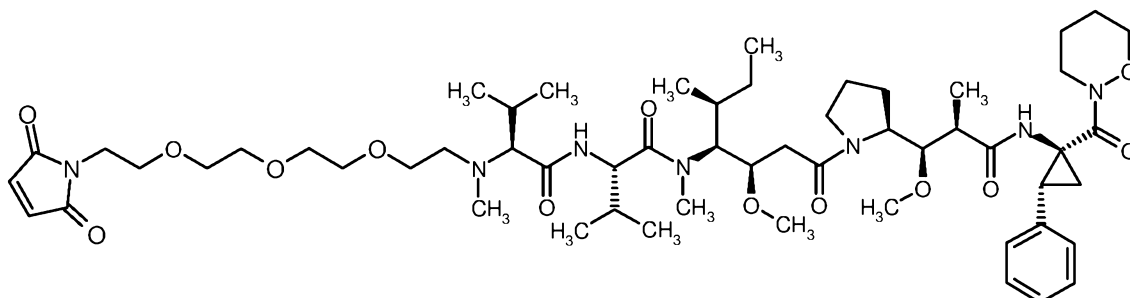
47 mg (0.05 mmol) of Intermediate 16 were reductively aminated in analogy to the preparation of Intermediate 167 with benzyl-(2-{2-[2-(2-oxoethoxy)ethoxy]ethoxy}ethyl)carbamate in the presence of borane-pyridine complex. Subsequently, the Z protecting group was removed by hydrogenolytic means with 5% palladium on carbon as a catalyst and in methanol as a solvent, and 38 mg (66% of theory over 2 stages) of the title compound were prepared.

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.8$ min; MS (ESIpos): $m/z = 988$ ($M+H$)⁺.

Intermediate 175

N-[2-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)ethyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



The preparation was done in analogy zu Intermediate 166 starting from 34 mg (0.03 mmol) of Intermediate 174.

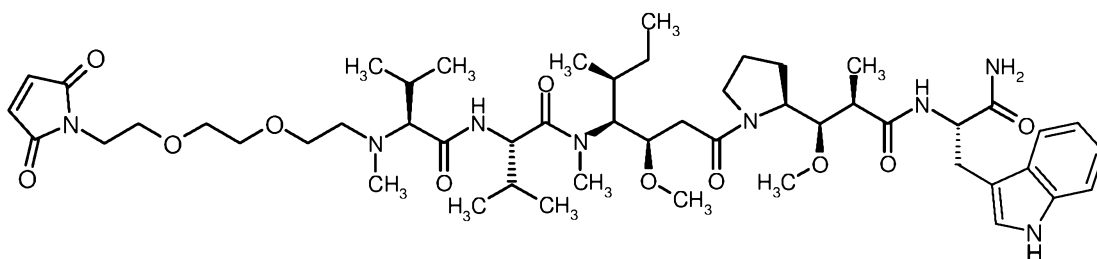
Yield: 8.3 mg (23% of theory)

HPLC (Method 5): $R_t = 1.9$ min;

5 LC-MS (Method 1): $R_t = 0.97$ min; MS (ESIpos): $m/z = 1068$ (M+H)⁺.

Intermediate 176

N-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}ethyl)-N-methyl-L-valyl-N-
 [(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-
 1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-
 10 methyl-L-valinamide



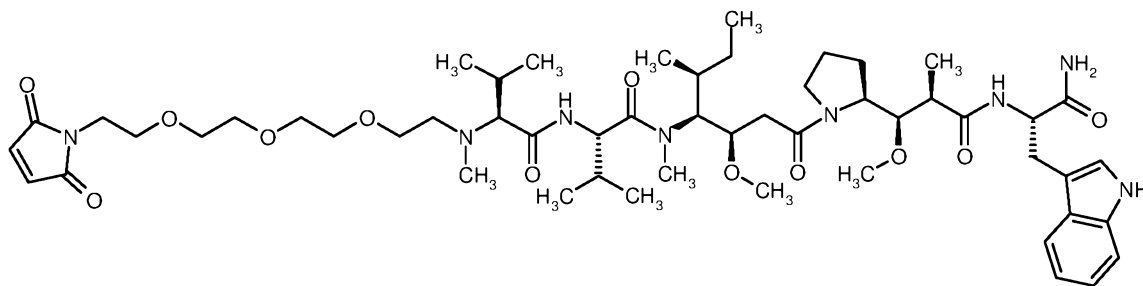
The preparation was done in analogy to Intermediates 174 and 175 starting with the reductive amination of Intermediate 16 with Intermediate 173, subsequent deprotection and formation of the maleimide.

15 HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 11): $R_t = 0.8$ min; MS (ESIpos): $m/z = 981$ (M+H)⁺.

Intermediate 177

N-[2-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)ethyl]-N-methyl-L-
 valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-
 20 yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-
 4-yl]-N-methyl-L-valinamide



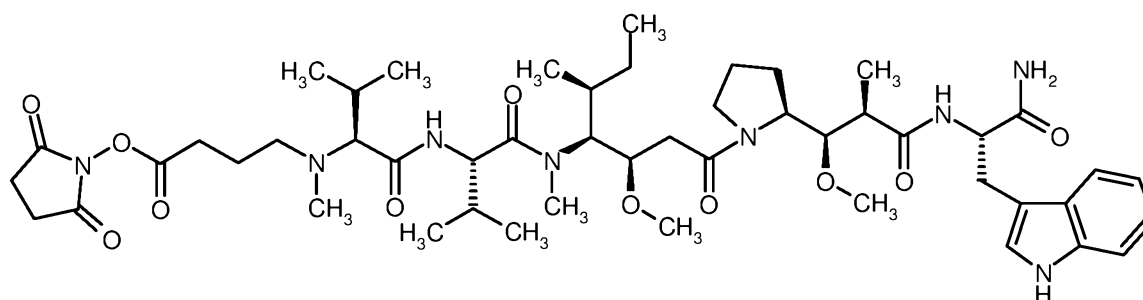
The preparation was done in analogy to Intermediates 174 and 175 starting with the reductive amination of Intermediate 16 with Intermediate 172, subsequent deprotection and formation of
5 the maleimide.

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 1025$ ($M+H$)⁺.

Intermediate 178

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-
10 [(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

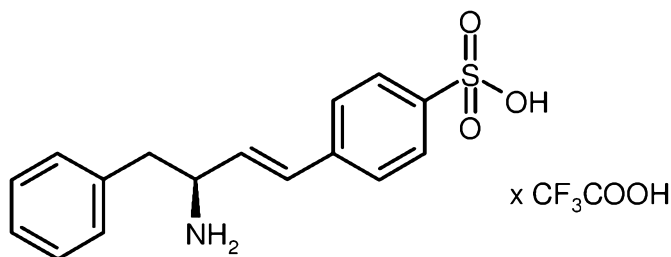


The preparation was done in analogy to Intermediate 162 starting from 6 mg of Intermediate 82.

15 LC-MS (Method 1): $R_t = 0.82$ min; MS (ESIpos): $m/z = 953$ ($M+H$)⁺.

Intermediate 179

4-[(1E,3S)-3-amino-4-phenylbut-1-en-1-yl]benzenesulphonic acid trifluoroacetate



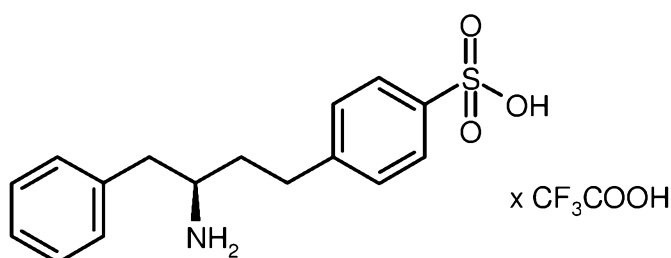
A mixture of 13.6 mg (0.06 mmol) of palladium(II) acetate, 469 mg (1.46 mmol) of potassium 4-iodobenzenesulphonate, 300 mg (1.21 mmol) of (S)-tert-butyl-1-phenylbut-3-en-2-yl-carbamate, 16.5 mg (0.12 mmol) of phenylurea and 167.6 mg (1.21 mmol) of potassium carbonate in 7.5 ml
 5 of DMF was heated in a microwave for 15 min to 160°C. The crude product was subsequently purified directly by means of preparative HPLC. This yielded 312 mg of a mixture of 31% of the BOC-protected compound and 69% of the free amine.

This mixture was subsequently taken up in 30 ml of dichloromethane, admixed with 1 ml of trifluoroacetic acid and stirred at RT for 20 h. After concentrating *in vacuo*, the residue was
 10 stirred in with diethyl ether, and the precipitate that formed was suctioned off and washed with diethyl ether. This yielded 200 mg (62% of theory) of the title compound.

LC-MS (Method 11): $R_t = 0.44$ min; MS (ESIpos): $m/z = 304$ ($M+H$)⁺.

Intermediate 180

4-[(3R)-3-amino-4-phenylbutyl]benzenesulphonic acid



15

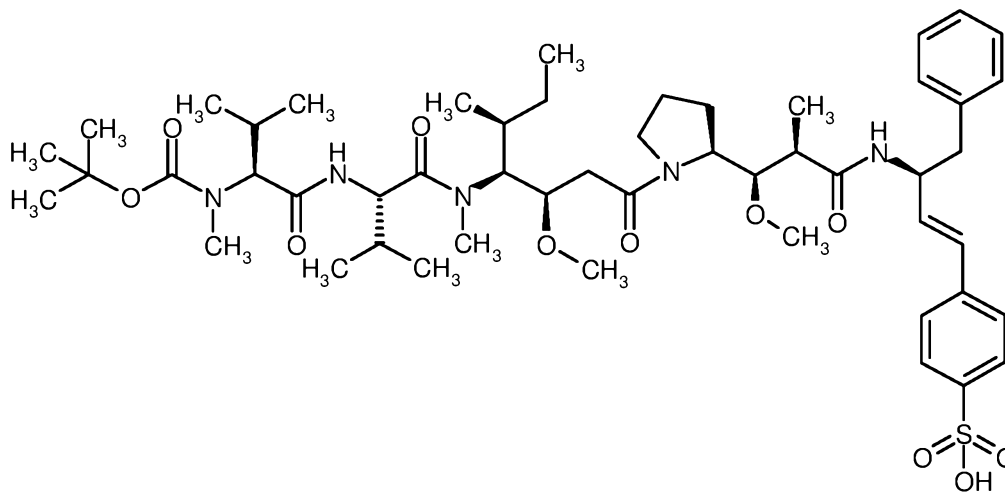
100 mg (0.25 mmol) of 4-[(1E,3S)-3-amino-4-phenylbut-1-en-1-yl]benzenesulphonic acid trifluoroacetate were suspended in 10 ml of acetic acid and a few drops of DMF and water, admixed with 70 mg (0.07 mmol) of palladium on carbon (10%) and hydrogenated at hydrogen pressure 2.2 bar for 24 h. The solution was filtered and the filtrate purified by means of
 20 preparative HPLC.

29 mg (76% purity, 21% of theory) of product were obtained.

LC-MS (Method 1): $R_t = 0.46$ min; MS (ESIpos): $m/z = 306$ ($M+H$)⁺.

Intermediate 181

N-(tert-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

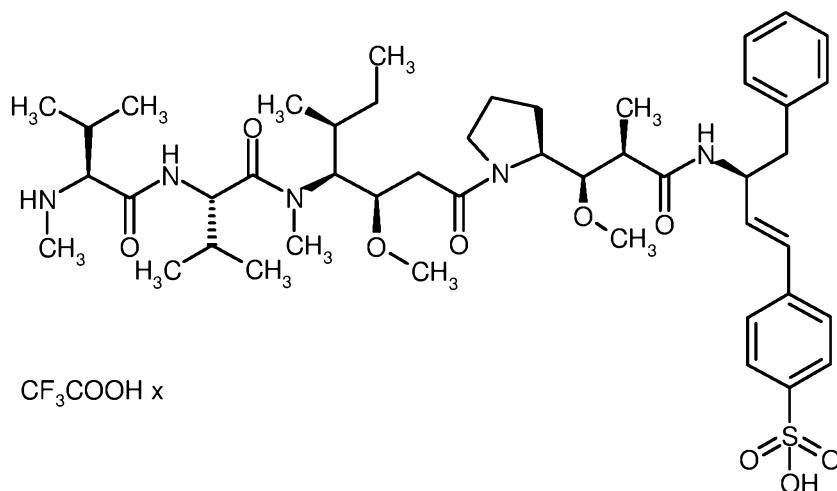


To a solution of 90 mg (0.13 mmol) of N-(tert-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 4 ml of DMF were added 60 mg (0.16 mmol) of HATU and 69 μ l of (0.39 mmol) Hünig's base. The reaction mixture was stirred at RT for 30 min and then admixed with 60 mg (0.15 mmol) 60.3 mg (0.13 mmol) of 4-[(1E,3S)-3-amino-4-phenylbut-1-en-1-yl]benzenesulphonic acid trifluoroacetate. After stirring overnight, the reaction mixture was purified by means of preparative HPLC. This yielded 127 mg of a 44:56 mixture of the title compound and of the already deprotected amine.

LC-MS (Method 1): $R_t = 1.21$ min; MS (ESIpos): $m/z = 971$ ($M+H$)⁺; $R_t = 0.84$ min; MS (ESIpos): $m/z = 871$ ($M+H$)⁺ for the deprotected compound.

Intermediate 182

N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



90 mg of Intermediate 180 were dissolved in 4.6 ml of dichloromethane, and 0.92 ml of trifluoroacetic acid were added. The reaction mixture was stirred at RT for 30 min and then concentrated. The obtained crude product was purified by means of preparative HPLC.

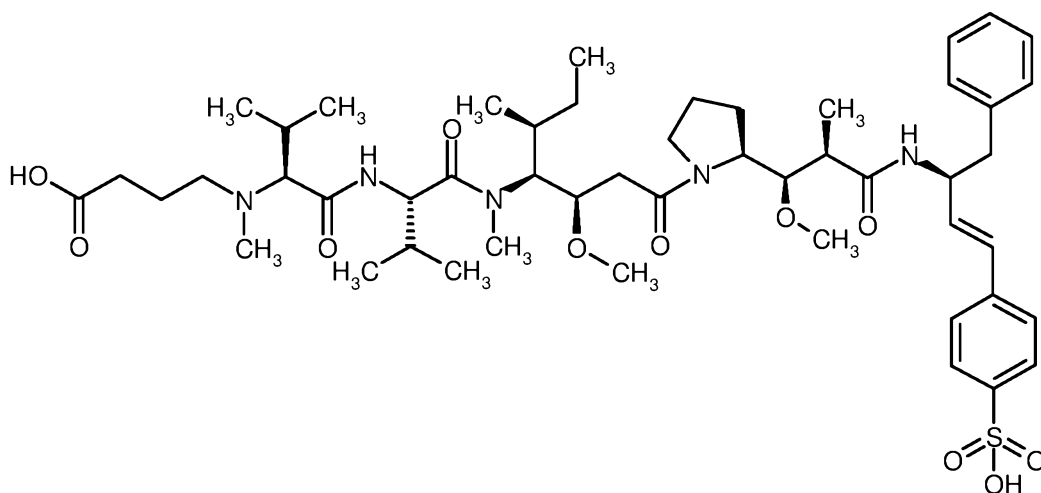
- 5 91 mg (98% of theory) of the target compound were obtained.

LC-MS (Method 1): $R_t = 0.85$ min; MS (ESIpos): $m/z = 871$ ($M+H$)⁺

Intermediate 183

N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-

- 10 yl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



16.7 μl (0.03 mmol) of a 15% aqueous succinaldehyde solution were initially provided in 943 μl of methanol and admixed with 17 mg (0.02 mmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-3-

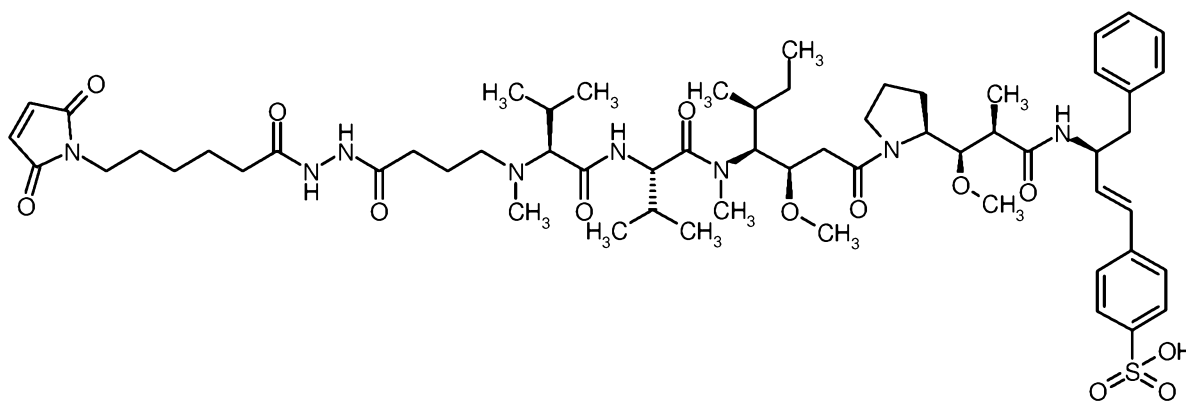
methoxy-1-((2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl)-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate (Intermediate 181) and 1.1 µl (0.02 mmol) of acetic acid. The reaction mixture was stirred for 5 min at RT, and then 2.9 µl (0.02 mmol) of borane-pyridine complex were added. After 1 h, a further 2 equivalents each of succinaldehyde, acetic acid and borane-pyridine complex were added, and the mixture was stirred at RT for 20 h. The reaction mixture was then purified by means of preparative HPLC.

This yielded 20 mg (83% purity, 80% of theory) of the title compound.

LC-MS (Method 1): $R_t = 0.87$ min; MS (ESIpos): $m/z = 957$ (M+H)⁺

10 Intermediate 184

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-((2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl)-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



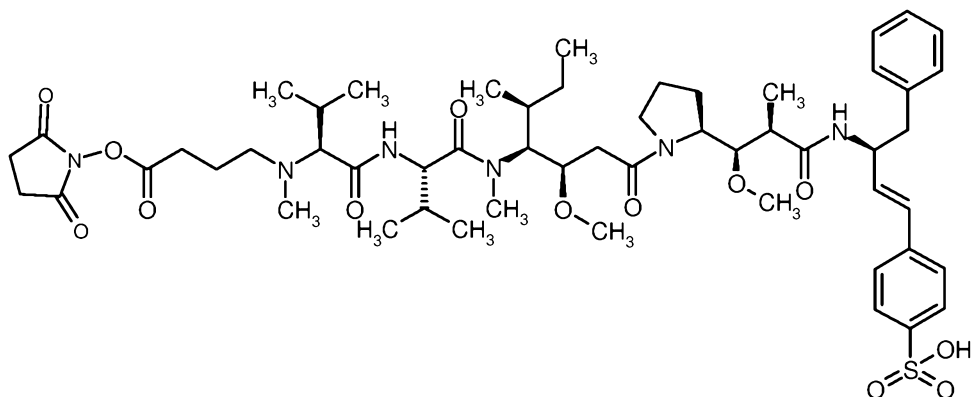
15 8 mg (7.5 µmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-((2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl)-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide, 2.8 mg (8.2 µmol) of 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanehydrazide trifluoroacetate, 3.4 mg (9 µmol) of HATU and 3.9 µl of Hünig's base were stirred in 0.77 ml of DMF at RT for 20 h. Subsequently, the reaction mixture was purified by means of preparative HPLC.

3 mg (31% of theory) of the title compound were obtained.

LC-MS (Method 1): $R_t = 0.90$ min; MS (ESIpos): $m/z = 1164$ (M+H)⁺

Intermediate 185

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



5

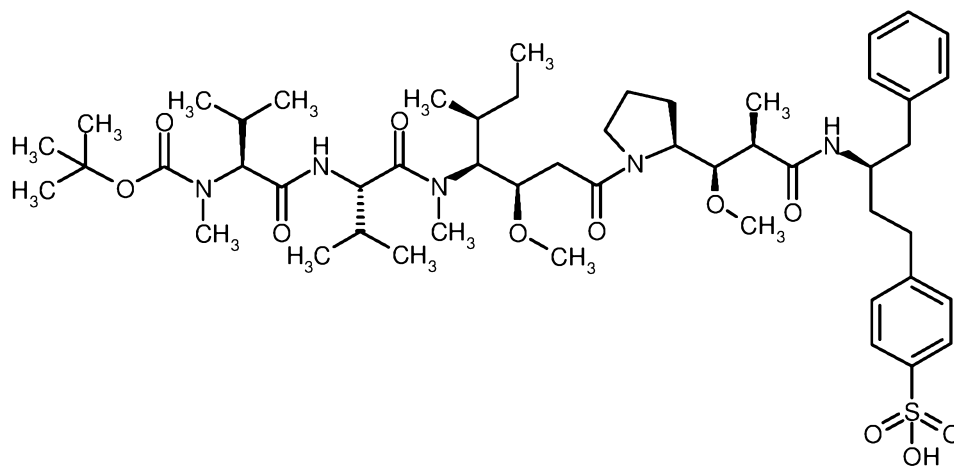
To a solution of 8 mg (7.5 μ mol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 2 ml of DMF were added 8.6 mg (74.8 μ mol) of N-hydroxysuccinimide, 8.5 mg (22.4 μ mol) of EDCI and 0.1 mg (0.75 μ mol) of DMAP. The reaction mixture was stirred at RT for 20 h. Subsequently, 1.3 μ l (7.5 μ mol) of Hünig's base were added, and the mixture was stirred for another 1 h. The reaction mixture was then purified by means of preparative HPLC. 2.6 mg (72% purity, 21% of theory) of the title compound were obtained.

10

LC-MS (Method 1): R_t = 0.89 min; MS (ESIpos): m/z = 1054 ($M+H$)⁺

15 Intermediate 186

N-(tert-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

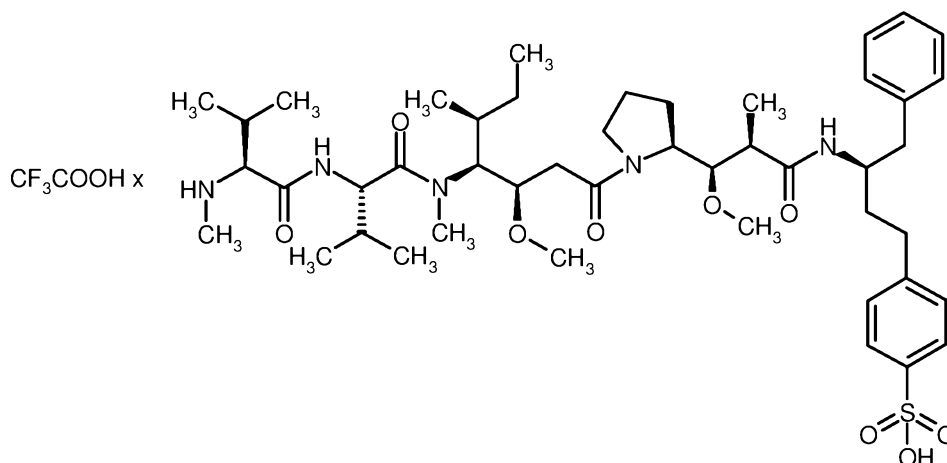


To a solution of 43 mg (0.06 mmol) of N-(tert-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 1.9 ml of DMF were added 29 mg (0.07 mmol) of HATU and 33 μ l (0.19 mmol) of Hünig's base. The reaction mixture was stirred at RT for 30 min and then admixed with 29 mg (0.07 mmol) of 4-[(3R)-3-amino-4-phenylbutyl]benzenesulphonic acid trifluoroacetate. After stirring overnight, the reaction mixture was purified by means of preparative HPLC. This yielded 58 mg of a 45:55 mixture of the title compound and of the already deprotected amine.

LC-MS (Method 1): R_t = 1.09 min; MS (ESIpos): m/z = 973 ($M+H$)⁺; R_t = 0.87 min; MS (ESIpos): m/z = 873 ($M+H$)⁺ for the deprotected compound.

Intermediate 187

N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



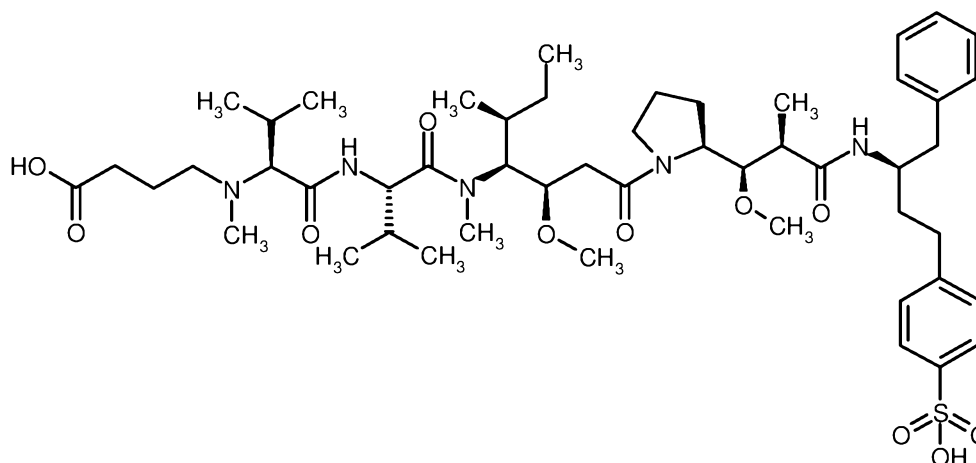
58 mg of Intermediate 186 were dissolved in 4.1 ml of dichloromethane, 0.41 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. After concentration *in vacuo*, the crude product was purified by means of preparative HPLC.

50 mg (90% purity, 85% of theory) of the title compound were obtained.

- 5 LC-MS (Method 1): $R_t = 0.87$ min; MS (ESIpos): $m/z = 873$ (M+H)⁺

Intermediate 188

N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



10

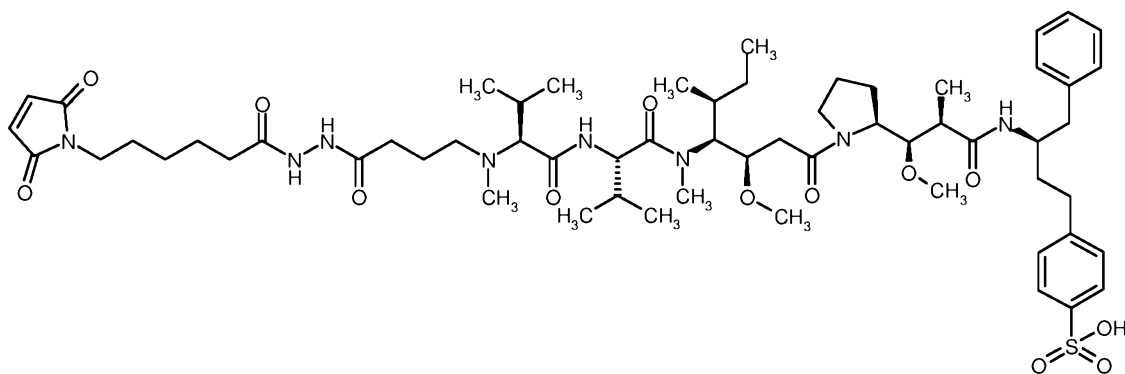
171 μ l (0.26 mmol) of a 15% aqueous succinaldehyde solution were initially provided in 2.5 ml of methanol and admixed with 50 mg (0.05 mmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate and 11.6 μ l (0.2 mmol) of acetic acid. The reaction mixture was stirred for 5 min at RT, and then 30 μ l (0.24 mmol) of borane-pyridine complex were added. After stirring for 24 hours, another equivalent of borane-pyridine complex was added, and the mixture was stirred for another 2 h. The reaction mixture was then purified by means of preparative HPLC.

- 20 40 mg (90% purity, 66% of theory) of the title compound were obtained.

LC-MS (Method 1): $R_t = 0.91$ min; MS (ESIpos): $m/z = 959$ (M+H)⁺

Intermediate 189

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



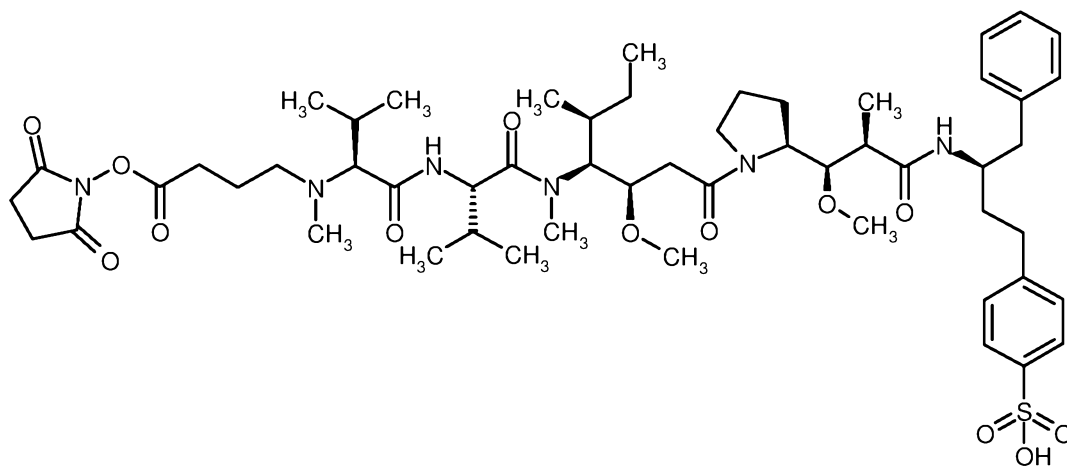
10 mg (9.3 μmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide, 3.5 mg (10.3 μmol) of 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanehydrazide trifluoroacetate, 4.3 mg (11.2 μmol) of HATU and 4.9 μl (28 μmol) of Hünig's base were stirred in 1 ml of DMF at RT for 20 h. Subsequently, the reaction mixture was purified by means of preparative HPLC.

4.2 mg (92% purity, 33% of theory) of the title compound were obtained.

LC-MS (Method 1): $R_t = 0.91$ min; MS (ESIpos): $m/z = 1166$ ($M+H$)⁺

15 Intermediate 190

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



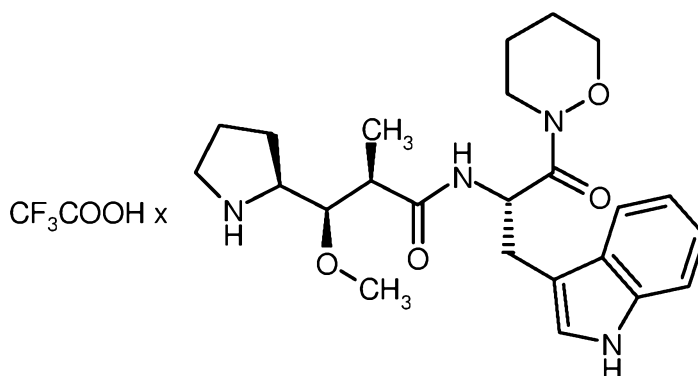
To a solution of 10 mg (9.3 μmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino]propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 2.5 ml of DMF were added 10.7 mg (93 μmol) of N-hydroxysuccinimide, 10.6 mg (28 μmol) of EDCI and 0.12 mg (0.9 μmol) of DMAP. The reaction mixture was stirred at RT for 20 h and then purified by means of preparative HPLC.

3.8 mg (72% purity, 25% of theory) of the title compound were obtained.

LC-MS (Method 1): $R_t = 0.90$ min; MS (ESIpos): $m/z = 1055$ ($M+H$)⁺

10 Intermediate 191

(2R,3R)-N-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]-3-methoxy-2-methyl-3-[(2S)-pyrrolidin-2-yl]propanamide trifluoroacetate



The title compound was prepared in analogy to the synthesis of Intermediate 7 over two stages from Starting Compound 1 and (2S)-2-amino-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)propan-1-one trifluoroacetate (Intermediate 99).

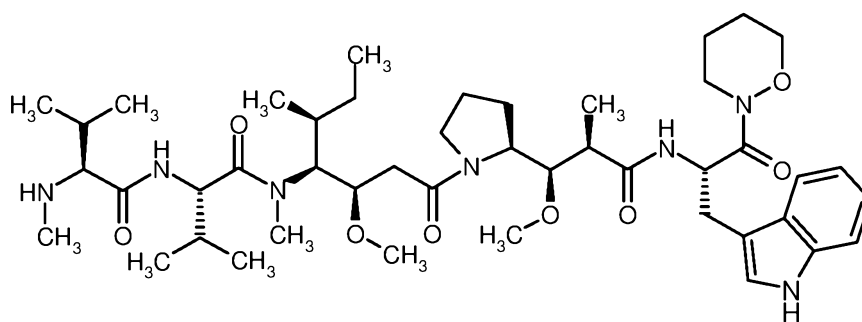
Yield over 2 stages: 62 mg (67% of theory)

HPLC (Method 6): $R_t = 1.65$ min;

LC-MS (Method 1): $R_t = 0.7$ min; MS (ESIpos): $m/z = 443$ ($M+H$)⁺.

Intermediate 192

- 5 N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



- 1015 mg (1.59 mmol) of *N*-(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-
 10 [(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4)
 were taken up in 50 ml of DMF, admixed with 654 mg (2.39 mmol) of 2-bromo-1-
 ethylpyridinium tetrafluoroborate (BEP) and 2.8 ml of *N,N*-diisopropylethylamine, then stirred at
 RT for 10 min. Then 1083 mg (1.75 mmol) of (2*R*,3*R*)-*N*-[(2*S*)-3-(1*H*-indol-3-yl)-1-(1,2-
 oxazinan-2-yl)-1-oxopropan-2-yl]-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanamide
 15 trifluoroacetate (Intermediate 191) were added, and then the mixture was treated in an ultrasound
 bath at RT for 30 min. The reaction mixture was then concentrated *in vacuo*, and the residue was
 taken up in 300 ml of ethyl acetate. The organic phase was washed successively with 5%
 aqueous citric acid solution and 5% aqueous sodium hydrogencarbonate solution, dried over
 magnesium sulphate, filtered and concentrated. The crude product thus obtained (1684 mg) was,
 20 without further purification, taken up in 20 ml of acetonitrile, 2 ml of piperidine were added to
 this, and the reaction mixture was then stirred at RT for 10 min. Then the mixture was
 concentrated *in vacuo*, and the residue was admixed with diethyl ether. The solvent was again
 concentrated by evaporation, and the residue was purified by flash chromatography on silica gel
 (eluent: 15:1:0.1 → 15:2:0.2 dichloromethane/methanol/17% aqueous ammonia solution). The
 25 corresponding fractions were combined, the solvent was removed *in vacuo*, and the residue was
 lyophilized from acetonitrile/water. Thus, 895 mg (67% over 2 stages) of the title compound
 were obtained.

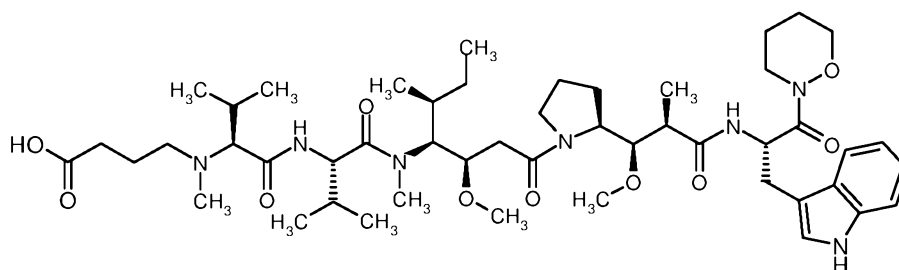
HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.84$ min; MS (ESIpos): $m/z = 840$ (M+H)⁺.

¹H NMR (500 MHz, DMSO-d₆): $\delta = 10.8$ (d, 1H), 8.3 and 8.05 (2d, 1H), 8.0 (d, 1H), 7.5 (m, 1H), 7.3 (m, 1H), 7.15 and 7.08 (2s, 1H) 7.05-6.9 (m, 2H), 5.12 and 4.95 (2m, 1H), 4.65 (m, 1H),
 5 4.55 (m, 1H), 4.1-3.8 (m, 4H), 3.75 (d, 1H), 3.23, 3.18, 3.17, 3.12, 2.95 and 2.88 (6s, 9H), 3.1-3.0 and 2.85 (2m, 2H), 2.65 (d, 1H), 2.4-2.2 (m, 3H), 2.15 (m, 3H), 1.95 (br. m, 2H), 1.85-0.8 (br. m, 11H), 1.08 and 1.04 (2d, 3H), 0.9-0.75 (m, 15H), 0.75-0.65 (dd, 3H) [further signals hidden under H₂O peak].

Intermediate 193

10 N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



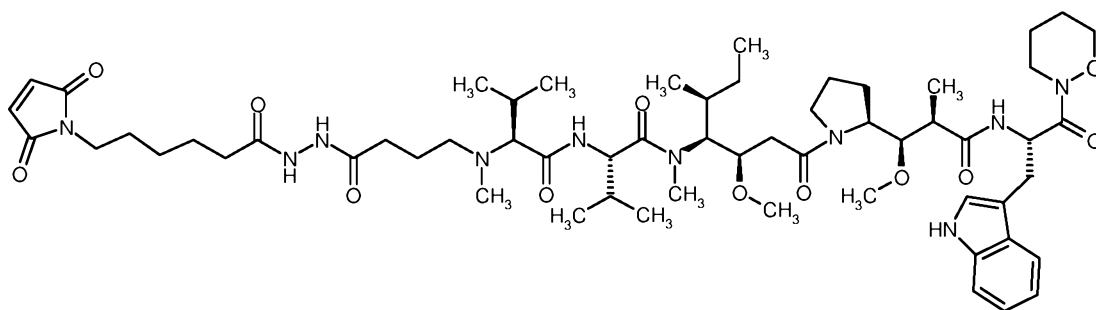
50 mg (0.052 mmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide
 15 (Intermediate 192) and 204 μ l of a 15% aqueous solution of 4-oxobutanoic acid were combined in 2 ml of methanol and admixed with 23.4 mg (0.252 mmol) of borane-pyridine complex and 6 μ l of acetic acid. The reaction mixture was stirred at RT overnight. This was
 20 followed by concentration *in vacuo*, and the residue was purified by means of preparative HPLC. After concentration of the corresponding fractions, 38 mg (78% of theory) of the title compound were obtained.

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 9): $R_t = 4.7$ min; MS (ESIpos): $m/z = 926$ (M+H)⁺.

Intermediate 194

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



This compound was prepared in analogy to the synthesis described in Intermediate 157 from 10 mg (11 μ mol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanehydrazide.

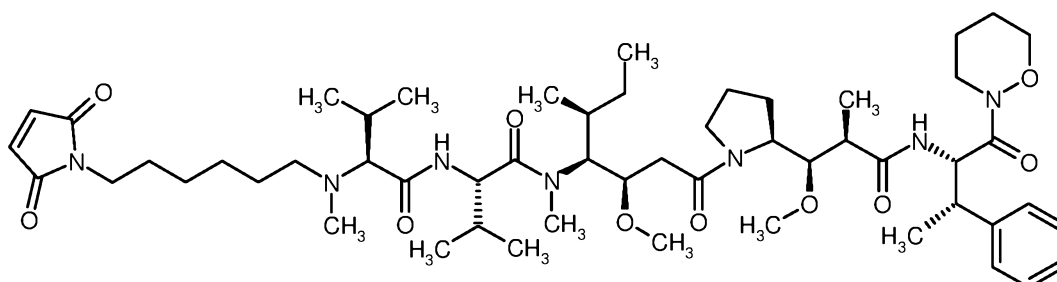
Yield: 4.4 mg (35% of theory)

HPLC (Method 5): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.90 min; MS (ESIpos): m/z = 1133 ($M+H$)⁺.

Intermediate 195

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S,3S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 166 starting from 9 mg (0.010 mmol) of Intermediate 170.

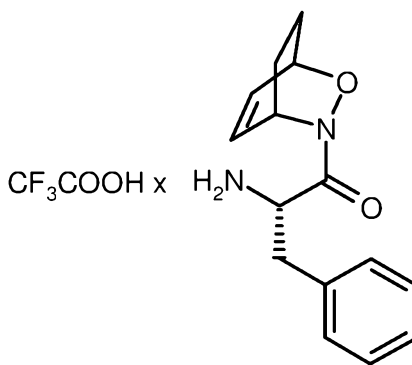
Yield: 1.1 mg (10% of theory)

HPLC (Method 12): $R_t = 2.0$ min;

- 5 LC-MS (Method 1): $R_t = 0.99$ min; MS (ESIpos): $m/z = 994$ (M+H)⁺.

Intermediate 196

(2*S*)-2-amino-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-3-phenylpropan-1-one trifluoroacetate



- 41 mg (0.37 mmol) of 2,5-dioxopyrrolidin-1-yl *N*-(*tert*-butoxycarbonyl)-L-phenylalaninate were
10 taken up in 10 ml of DMF and admixed with 149 mg (0.41 mmol) of 2-oxa-3-azabicyclo[2.2.2]oct-5-ene (Starting Compound 6) and 72 μ l (0.41 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 1 h. The solvent was then removed *in vacuo*, and the residue was taken up in ethyl acetate and extracted by shaking with 5% aqueous citric acid solution and then with 5% aqueous sodium hydrogencarbonate solution. The organic
15 phase was concentrated, and the residue was purified by flash chromatography on silica gel with 10:1 toluene/ethanol as the eluent. The corresponding fractions were combined, and the solvent was removed *in vacuo*. After the residue had been dried under high vacuum, 69 mg (47% of theory) of the Boc-protected intermediate *tert*-butyl-[(2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]carbamate were thus obtained as a diastereomer mixture.

- 20 LC-MS (Method 1): $R_t = 1.1$ min; MS (ESIpos): $m/z = 359$ (M+H)⁺.

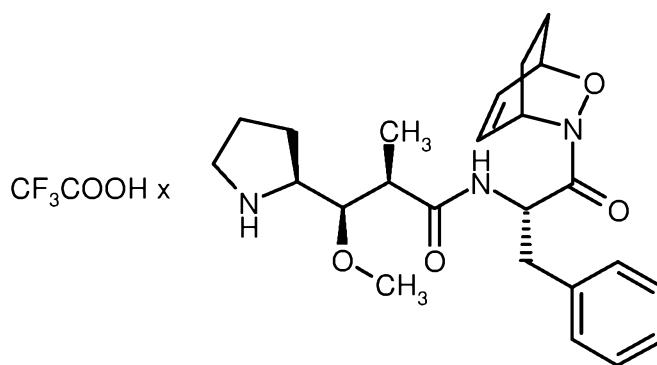
64 mg (0.18 mmol) of this intermediate were taken up in 10 ml of dichloromethane, 1 ml of trifluoroacetic acid was added, and the mixture was stirred at RT for 30 min. This was followed by concentration *in vacuo* and lyophilization of the remaining residue from water/dioxane. In this way, 66 mg (quant.) of the title compound were obtained as a foam.

HPLC (Method 6): $R_t = 1.45$ min;

LC-MS (Method 3): $R_t = 1.12$ min; MS (ESIpos): $m/z = 259$ (M+H)⁺.

Intermediate 197

(2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate



First, (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1) was released from 83 mg (0.18 mmol) of its dicyclohexylamine salt by taking it up in ethyl acetate and extractive shaking with 5% aqueous potassium hydrogensulphate solution. The organic phase was dried over magnesium sulphate, filtered and concentrated. The residue was taken up in 10 ml of DMF and admixed successively with 66 mg (0.18 mmol) of (2*S*)-2-amino-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-3-phenylpropan-1-one trifluoroacetate (Intermediate 196), 101 mg (0.266 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 93 μ l (0.53 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 30 min. The reaction mixture was then concentrated, and the residue was purified by means of preparative HPLC. This yielded 52 mg (56% of theory) of the Boc-protected intermediate *tert*-butyl-(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-[(2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidine-1-carboxylate.

HPLC (Method 6): $R_t = 2.13$ min;

LC-MS (Method 1): $R_t = 1.13$ min; MS (ESIpos): $m/z = 528$ (M+H)⁺.

52 mg (0.1 mmol) of this intermediate were taken up in 10 ml of dichloromethane, 1 ml of trifluoroacetic acid was added, and the mixture was stirred at RT for 20 min. This was followed by concentration *in vacuo* and stirring of the remaining residue with 20 ml of diethyl ether. After

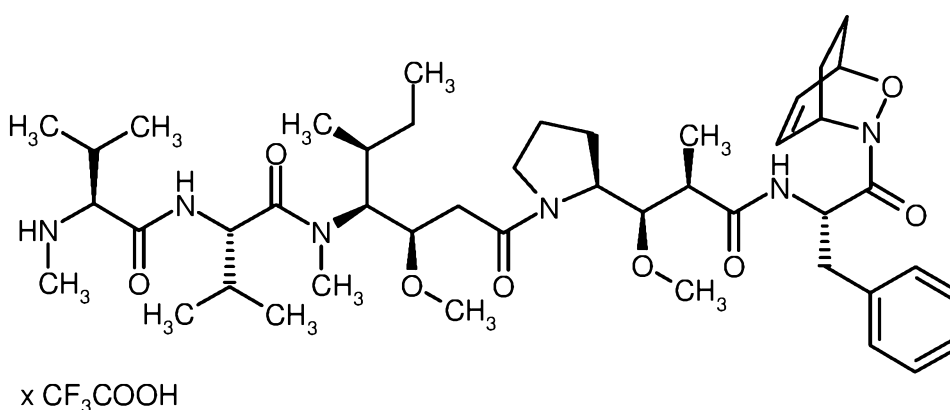
10 min, the mixture was filtered, and the filter residue was dried under high vacuum. In this way, 39 mg (72% of theory) of the title compound were obtained.

HPLC (Method 6): $R_t = 1.62$ min;

LC-MS (Method 1): $R_t = 0.68$ min; MS (ESIpos): $m/z = 428$ ($M+H$)⁺.

5 Intermediate 198

N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate



- 10 44.5 mg (0.071 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were taken up in 10 ml of DMF and admixed successively with 38.6 mg (0.071 mmol) of (2*R*,3*R*)-3-methoxy-2-methyl-*N*-[(2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]-3-[(2*S*)-pyrrolidin-2-yl]propanamide trifluoroacetate (Intermediate 197),
- 15 32.5 mg (0.086 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 41 μ l (0.235 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT for 1 h. The reaction mixture was then concentrated *in vacuo*, and the residue was taken up in ethyl acetate. The organic phase was washed successively with 5% aqueous citric acid solution and 5% aqueous sodium hydrogencarbonate solution, dried over
- 20 magnesium sulphate, filtered and concentrated. This yielded 73 mg (98% of theory) of the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

HPLC (Method 6): $R_t = 2.78$ min;

LC-MS (Method 3): $R_t = 2.96$ min; MS (ESIpos): $m/z = 1047$ (M+H)⁺.

73 mg (0.071 mmol) of this intermediate were dissolved in 5 ml of DMF. After adding 0.5 ml of piperidine, the reaction mixture was stirred at RT for 10 min. This was followed by concentration *in vacuo*, and the residue was digested repeatedly with diethyl ether. After the diethyl ether had been decanted off, the residue was purified by means of preparative HPLC (eluent: acetonitrile / 0.1% aq. TFA). 16 mg (26% of theory) of the title compound were obtained as a foam.

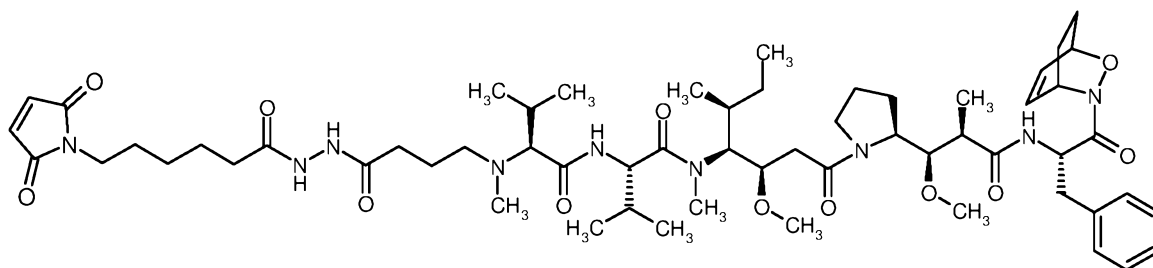
HPLC (Method 6): $R_t = 1.94$ min;

LC-MS (Method 3): $R_t = 1.71$ min; MS (ESIpos): $m/z = 825$ (M+H)⁺

¹H NMR (400 MHz, DMSO-*d*₆): $\delta = 8.9$ -8.6 (m, 3H), 8.4, 8.3, 8.1 and 8.0 (4d, 1H), 7.3-7.1 (m, 5H), 6.7-6.5 (m, 2H), 5.2-4.8 (m, 3H), 4.75-4.55 (m, 3H), 4.05-3.95 (m, 1H), 3.7-3.4 (m, 4H), 3.22, 3.17, 3.15, 3.05, 3.02 and 2.95 (6s, 9H), 3.0 and 2.7 (2 br. m, 2H), 2.46 (m, 3H), 2.4-1.2 (br. m, 13H), 1.1-0.85 (m, 18H), 0.75 (m, 3H) [further signals hidden under H₂O peak].

Intermediate 199

N-(4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-[(2S)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



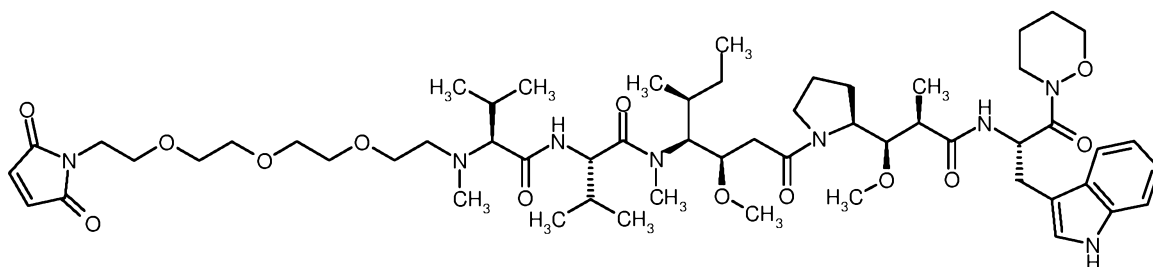
The title compound was prepared in analogy to Intermediates 193 and 194 starting from 23 mg (24 μ mol) of N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-[(2S)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate (Intermediate 198).

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 2): $R_t = 2.1$ min; MS (ESIpos): $m/z = 1118$ (M+H)+.

Intermediate 200

N-[2-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy]ethoxy}ethoxy)ethyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



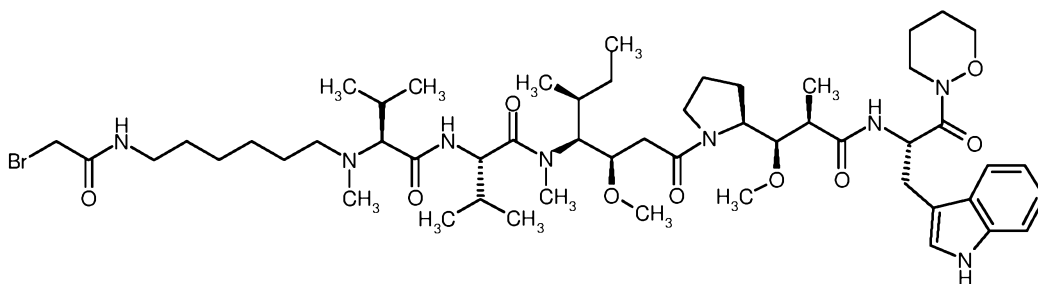
The preparation was done in analogy to Intermediates 174 and 175 starting with the reductive alkylation of Intermediate 192 with Intermediate 172, subsequent deprotection and formation of the maleimide.

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 1025$ (M+H)+.

Intermediate 201

N-{6-[(bromoacetyl)amino]hexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



22 mg (0.023 mmol) of N-(6-aminoheptyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

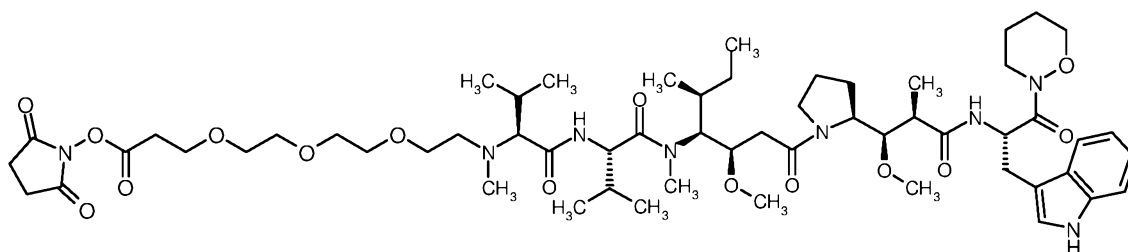
methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 101) were dissolved in 9.5 ml of THF and admixed at 0°C with 4.2 µl of triethylamine. A solution of bromoacetyl chloride in THF was added dropwise, and the reaction mixture was stirred at 0°C for 30 min. The reaction mixture was concentrated and the residue was purified by means of preparative HPLC. Thus, 6.9 mg (26% of theory) of the title compound were obtained as a foam.

HPLC (Method 5): $R_t = 1.8$ min;

LC-MS (Method 11): $R_t = 0.9$ min; MS (ESIpos): $m/z = 1059$ and 1061 ($M+H$)⁺.

Intermediate 202

N-{2-[2-(2-{3-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-oxopropoxy}ethoxy)ethoxy]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



The preparation was at first done in analogy to Intermediate 168 starting with the reductive alkylation of Intermediate 192 with Intermediate 167 and subsequent hydrogenolytic cleavage of the benzyl ester of N-(2-[2-(2-(2-carboxyethoxy)ethoxy)ethoxy]ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide.

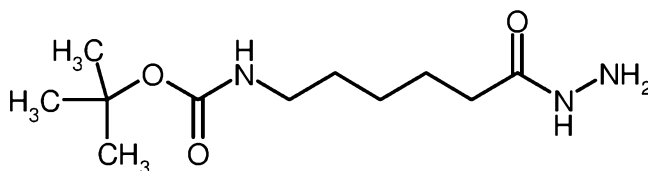
13 mg (10 µmol) of this intermediate were dissolved in 5 ml of DMF and admixed with 2.1 mg (20 mmol) of 1-hydroxypyrrolidine-2,5-dione, 6.5 µl of *N,N*-diisopropylethylamine and 7.1 mg (0.02 mmol) of HATU. The reaction mixture was stirred at RT overnight and then concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization from acetonitrile/water, 9.2 mg (62% of theory) of the title compound were obtained.

HPLC (Method 12): $R_t = 2.0$ min;

LC-MS (Method 2): $R_t = 2.1$ min; MS (ESIpos): $m/z = 1141$ ($M+H$)⁺.

Intermediate 203

tert-butyl-(6-hydrazino-6-oxohexyl)carbamate

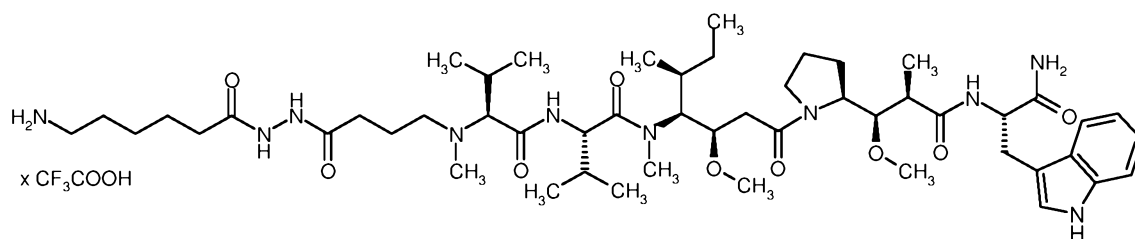


- 5 This compound was prepared by standard peptide chemistry methods by coupling of 6-[(*tert*-butoxycarbonyl)amino]hexanoic acid with benzyl hydrazinecarboxylate in the presence of EDCI and HOBT and subsequent hydrogenolytic cleavage of the benzyloxycarbonyl protecting group.

LC-MS (Method 11): $R_t = 0.59$ min; MS (ESIpos): $m/z = 246$ (M+H)⁺.

Intermediate 204

- 10 N-{4-[2-(6-aminohexanoyl)hydrazino]-4-oxobutyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-
[(1R,2R)-3-[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-
oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide
trifluoroacetate



- 15 146 mg (50 μ mol) of (N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-
[(1R,2R)-3-[[[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-
oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide
were dissolved in 5 ml of DMF and then admixed with 30.6 mg (80 μ mol) of O-(7-
azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate, 19 μ l of N,N-
20 diisopropylethylamine and with 22.4 mg (60 μ mol) of *tert*-butyl-(6-hydrazino-6-
oxohexyl)carbamate. The reaction mixture was stirred at RT for 1.5 h. This was followed by
concentration under high vacuum and purification of the remaining residue by means of
preparative HPLC. Thus, 43 mg (68% of theory) of the protected intermediate were obtained,
which were then taken up in 10 ml of dichloromethane and deprotected with 1 ml of
25 trifluoroacetic acid. The reaction mixture was concentrated, and the residue was stirred in with

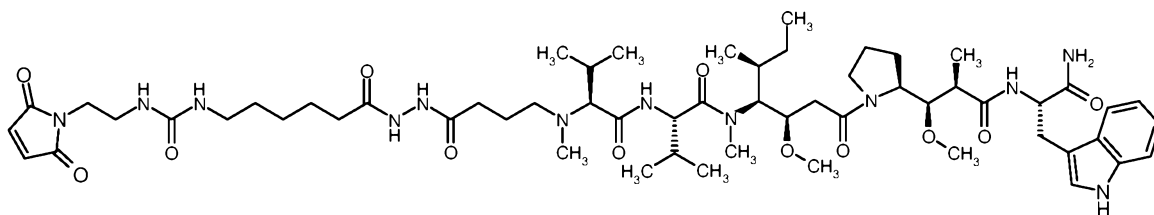
dichloromethane, and the solvent was removed again *in vacuo*. Thus, 45 mg (68% of theory over 2 stages) of the title compound were obtained.

HPLC (Method 12): $R_t = 1.6$ min;

LC-MS (Method 11): $R_t = 0.66$ min; MS (ESIpos): $m/z = 983$ ($M+H$)⁺.

5 Intermediate 205

N-(4-{2-[6-({2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl}carbamoyl)amino]hexanoyl}hydrazino)-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



10

This compound was prepared in analogy to Intermediate 114 starting from Intermediates 50 and 204.

Yield: 4 mg (78% of theory)

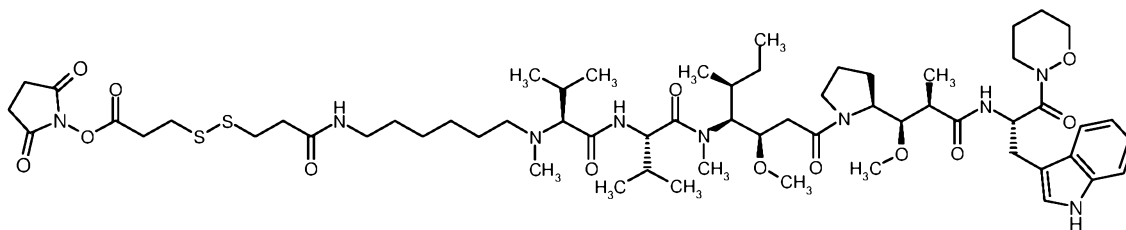
15 HPLC (Method 12): $R_t = 1.7$ min;

LC-MS (Method 11): $R_t = 0.73$ min; MS (ESIpos): $m/z = 1149$ ($M+H$)⁺.

Intermediate 206

N-(6-{[3-({3-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-oxopropyl}disulphonyl)propanoyl]amino}hexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

20



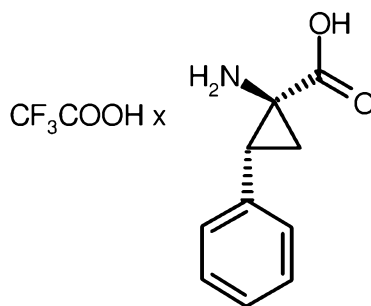
8 mg (10 μ mol) of Intermediate 101 were dissolved in 2 ml of DMF and admixed with 8.6 mg (20 μ mol) of 1,1'-[disulphanediylbis[(1-oxopropane-3,1-diyl)oxy]]dipyrrolidine-2,5-dione and 3.7 μ l of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT for 2 h, and then the solvent was evaporated off *in vacuo*, and the residue was purified by means of preparative HPLC. 7.2 mg (68% of theory) of the title compound were obtained.

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 11): R_t = 0.94 min; MS (ESIpos): m/z = 615 [$\frac{1}{2}$ ($M+2H^+$)]

Intermediate 207

(1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylic acid trifluoroacetate

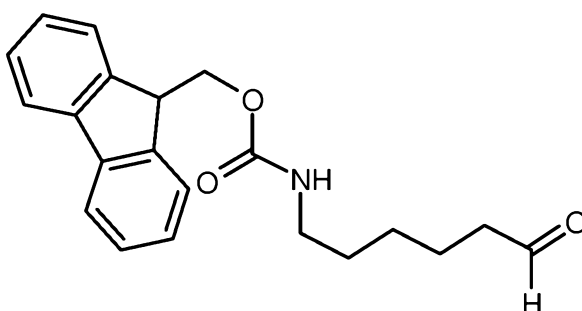


The title compound was obtained in quantitative yield by deprotecting 210 mg (0.76 mmol) of commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with trifluoroacetic acid.

LC-MS (Method 1): R_t = 0.23 min; MS (ESIpos): m/z = 178 ($M+H^+$).

Intermediate 208

9H-fluoren-9-ylmethyl-(6-oxohexyl)carbamate



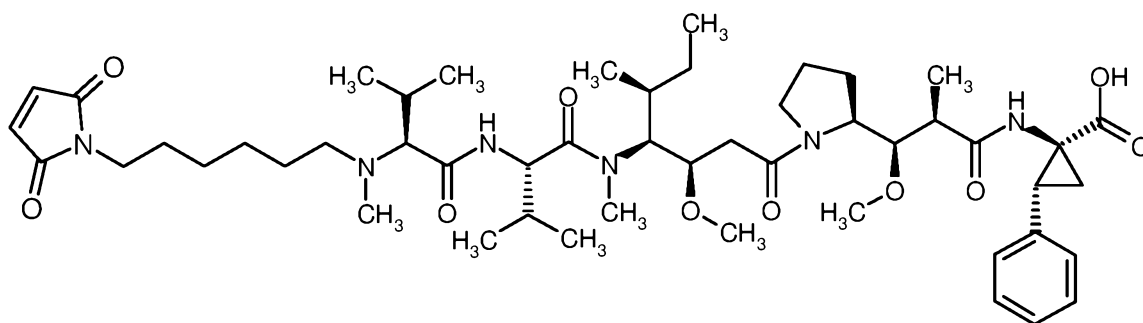
The title compound was prepared from 1 g (2.95 mmol) of commercially available 9H-fluoren-9-ylmethyl-(6-hydroxyhexyl)carbamate under standard conditions, by oxidation with sulphur trioxide-pyridine complex. 840 mg (85% of theory) of the title compound were obtained.

5 HPLC (Method 12): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 1.1$ min; MS (ESIpos): $m/z = 338$ ($M+H$)⁺.

Intermediate 209

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-
 [(1R,2R)-3-[(1S,2R)-1-carboxy-2-phenylcyclopropyl]amino]-1-methoxy-2-methyl-3-
 10 oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



First prepared was, in analogy to the synthesis described in Intermediate 75, by coupling of N-(*tert*-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-2-carboxy-1-
 15 methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide
 (Intermediate 26) and (1S,2R)-1-amino-2-phenylcyclopropanecarboxylic acid trifluoroacetate
 (Intermediate 207) in the presence of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium
 hexafluorophosphate and the subsequent cleaving of the Boc protecting group by means of
 trifluoroacetic acid, the amine compound N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-
 20 3-[(1S,2R)-1-carboxy-2-phenylcyclopropyl]amino]-1-methoxy-2-methyl-3-

oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide as the trifluoroacetate.

To 22 mg (0.026 mmol) of this compound in 10 ml of methanol were then added 17 mg (0.05 mmol) of 9H-fluoren-9-ylmethyl-(6-oxohexyl)carbamate (Intermediate 208) and 2.3 mg of acetic acid, and also 11.4 mg (0.12 mmol) of borane-pyridine complex. The reaction mixture was stirred at RT overnight. Then the same amounts of borane-pyridine complex and acetic acid, and also 8 mg of fluoren-9-ylmethyl-(6-oxohexyl)carbamate were added once again, and the reaction mixture was stirred at RT for a further 24 h. This was followed by concentration *in vacuo*, and the residue was purified by means of preparative HPLC. After concentration of the corresponding fractions, the product was used immediately in the next stage.

33 mg of the still contaminated intermediate were taken up in 5 ml of DMF, and 1 ml of piperidine was added. After stirring at RT for 15 min, the reaction mixture was concentrated, and the obtained residue was purified by means of preparative HPLC. Thus, 11 mg (55% of theory over 2 stages) of the aminocarboxylic acid intermediate were obtained.

HPLC (Method 12): $R_t = 1.7$ min;

LC-MS (Method 11): $R_t = 0.7$ min; MS (ESIpos): $m/z = 843$ (M+H)⁺.

6 mg (7.12 μ mol) of this intermediate were taken up in 1 ml of dioxane and then admixed with 6.6 mg (42.7 μ mol) of methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate and with 5 μ l of saturated aqueous sodium hydrogencarbonate solution. The reaction mixture was stirred at RT for 1 h. Then another 3 portions each of 50 μ l of the saturated aqueous sodium hydrogencarbonate solution were added, and the reaction mixture was stirred at RT for another 30 min. Then the reaction mixture was acidified to pH 2 with trifluoroacetic acid and subsequently concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization from acetonitrile/water, 4 mg (60% of theory) of the title compound were obtained as a foam.

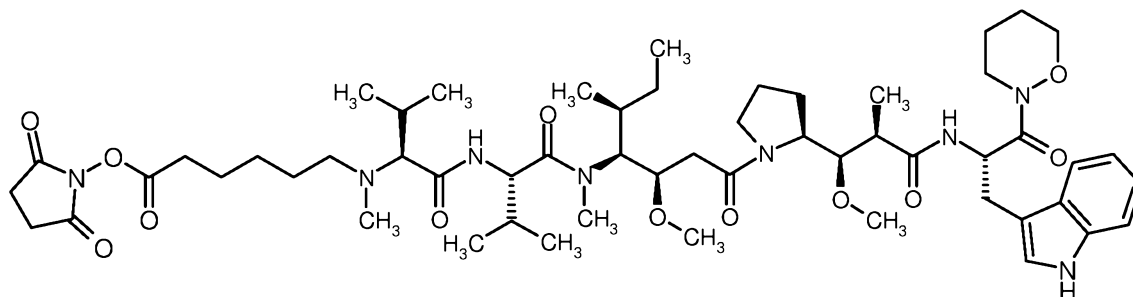
HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 11): $R_t = 0.88$ min; MS (ESIpos): $m/z = 923$ (M+H)⁺.

Intermediate 210

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-

methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



- 5 First, 6-oxohexanoic acid was prepared by a literature method (J. Org. Chem. 58, 1993, 2196-2200).

80 mg (0.08 mmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-1-((2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

- 10 (Intermediate 192) and 65.4 mg (0.5 mmol) of 6-oxohexanoic acid were combined in 9 ml of methanol and admixed with 10 μ l of acetic acid and 37.4 mg (0.4 mmol) of borane-pyridine complex. The reaction mixture was stirred at RT overnight. This was followed by concentration *in vacuo*, and the residue was taken up in 1:1 acetonitrile/water and adjusted to pH 2 with trifluoroacetic acid. The reaction mixture was concentrated again, and the residue was purified by
- 15 means of preparative HPLC. After concentration of the corresponding fractions, 70 mg (86% of theory) of N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-((2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide were obtained as the trifluoroacetate.

- 20 HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 955 ($M+H$)⁺.

- ¹H NMR (500 MHz, DMSO- d_6 , characteristic signals): δ = 12.0 (br. M, 1H), 10.8 (s, 1H), 9.4 (m, 1H), 8.9 and 8.8 (2d, 1H), 8.3 and 8.02 (2d, 1H), 7.5 (m, 1H), 7.3 (m, 1H), 7.15 and 7.1 (2s, 1H), 7.05-6.9 (m, 2H), 5.12 and 4.95 (2m, 1H), 4.7-4.5 (m, 2H), 4.1-3.8 (m, 4H), 3.75 (d, 1H), 3.25, 3.2, 3.18, 3.13, 2.98 and 2.88 (6s, 9H), 2.8 (m, 3H), 1.08 and 1.04 (2d, 3H), 0.95-0.8 (m, 15H), 0.8-0.65 (dd, 3H).
- 25

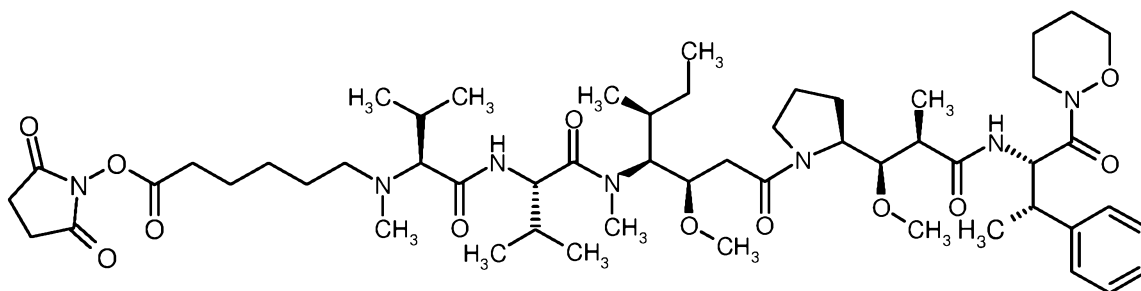
22 mg (23 μ mol) of this intermediate were dissolved in 1.8 ml of dichloromethane and admixed with 13.2 mg (70 μ mol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 26.5 mg (230 μ mol) of 1-hydroxypyrrolidine-2,5-dione and 0.28 mg (2 μ mol) of dimethylaminopyridine, and the reaction mixture was stirred at RT for 2 h. Subsequently, the reaction mixture was concentrated *in vacuo* and the remaining residue was purified by means of preparative HPLC. After lyophilization from acetonitrile/water, 21.3 mg (88% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.94 min; MS (ESIpos): m/z = 1052 ($M+H$)⁺.

10 Intermediate 211

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*,3*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



15

15 mg (20 μ mol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*,3*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate (Intermediate 15) were reductively alkylated with 6-oxohexanoic acid, in analogy to Intermediate 210.

20

Yield: 9.2 mg (61% of theory)

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 929 ($M+H$)⁺.

9 mg (10 μ mol) of this intermediate were dissolved in 3 ml of DMF and admixed with 5.6 mg (48 μ mol) of 1-hydroxypyrrolidine-2,5-dione, 5 μ l of *N,N*-diisopropylethylamine and 5.5 mg

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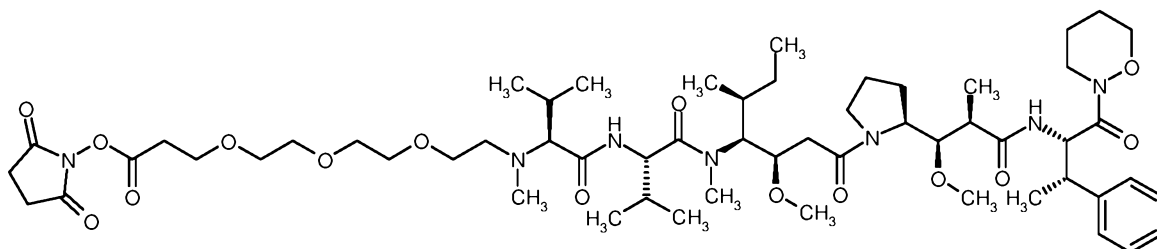
(0.015 mmol) of HATU, and the reaction mixture was treated in an ultrasound bath for 6 h. In the course of this, 5.5 mg of HATU were re-added every hour. Subsequently, the reaction mixture was concentrated *in vacuo*, and the residue was taken up in acetonitrile/water and adjusted to pH 2 with trifluoroacetic acid. After concentrating again *in vacuo*, the remaining residue was purified by means of preparative HPLC. After lyophilization from acetonitrile/water, 5.8 mg (57% of theory) of the title compound were obtained.

HPLC (Method 12): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 0.95$ min; MS (ESIpos): $m/z = 1027$ (M+H)⁺.

Intermediate 212

N-{2-[2-(2-{3-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-oxopropoxy}ethoxy)ethoxy]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S,3S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



15

The preparation was at first done in analogy to Intermediate 168 starting with the reductive alkylation of Intermediate 15 with Intermediate 167 and subsequent hydrogenolytic cleavage of the benzyl ester of N-(2-[2-(2-(2-carboxyethoxy)ethoxy)ethoxy]ethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S,3S)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylbutan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide.

8.4 mg (8 μ mol) of this intermediate were dissolved in 3 ml of DMF and admixed with 9.5 mg (80 μ mol) of 1-hydroxypyrrolidine-2,5-dione, 10 μ l of *N,N*-diisopropylethylamine and 9.4 mg (25 μ mol) of HATU, and the reaction mixture was stirred at RT overnight and then concentrated *in vacuo*. Subsequently, the reaction mixture was concentrated *in vacuo*, and the residue was taken up in acetonitrile/water and adjusted to pH 2 with trifluoroacetic acid. After concentrating again *in vacuo*, the remaining residue was purified by means of preparative HPLC. After

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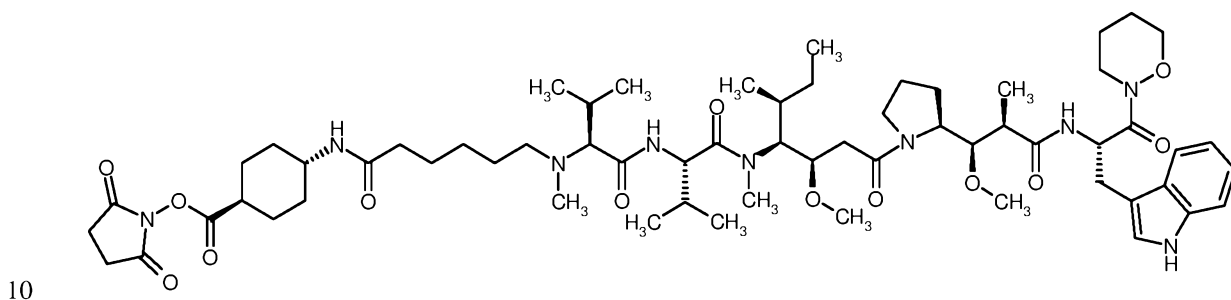
lyophilization from acetonitrile/water, 4 mg (32% of theory) of the title compound were obtained.

HPLC (Method 12): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 0.96$ min; MS (ESIpos): $m/z = 1117$ ($M+H$)⁺.

5 Intermediate 213

N-{6-[(trans-4-[[[2,5-dioxopyrrolidin-1-yl]oxy]carbonyl]cyclohexyl)amino]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[[[2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



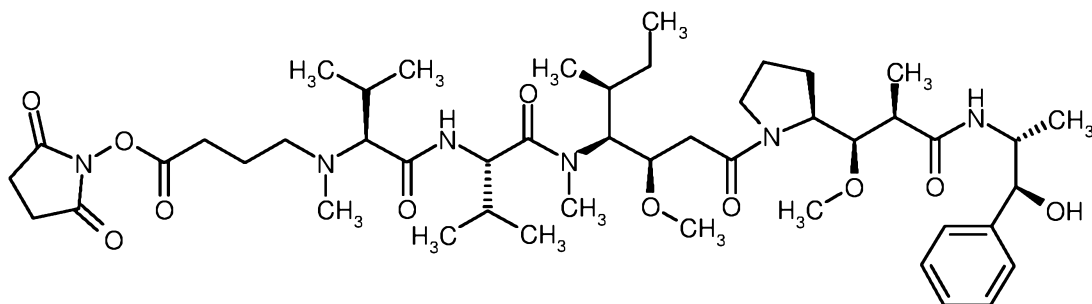
This compound was prepared in analogy to Intermediate 104 starting from N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[[[2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide, the synthesis of which was described under Intermediate 210. 9.3 mg of the title compound (37% of theory over 3 stages) were obtained.

15 HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.9$ min; MS (ESIpos): $m/z = 1177$ ($M+H$)⁺.

20 Intermediate 214

N-{4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[[[1S,2R)-1-hydroxy-1-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



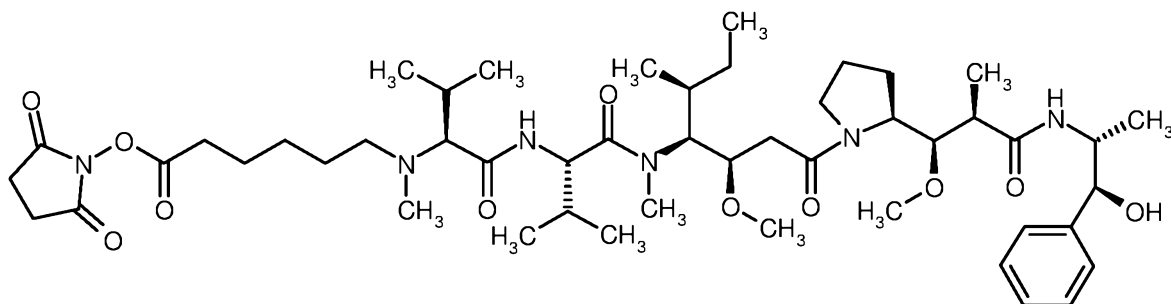
This compound was prepared in analogy to Intermediate 210 by conversion of Intermediate 92 to the active ester.

HPLC (Method 5): $R_t = 1.6$ min;

- 5 LC-MS (Method 11): $R_t = 0.82$ min; MS (ESIpos): $m/z = 901$ ($M+H$)⁺.

Intermediate 215

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(1S,2R)-1-hydroxy-1-phenylpropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



10

First, from Intermediate 40, in analogy to Intermediate 183 with borane-pyridine complex, was prepared N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(1S,2R)-1-hydroxy-1-phenylpropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-

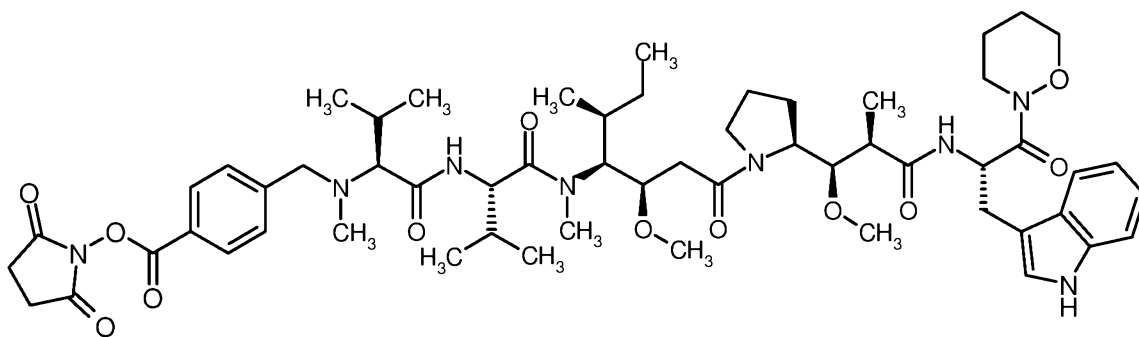
- 15 1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide. From this compound, in analogy to Intermediate 210, the active ester was then generated. 34 mg (36% of theory over 2 stages) of the title compound were obtained.

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.85$ min; MS (ESIpos): $m/z = 930$ ($M+H$)⁺.

Intermediate 216

N-(4-[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl)benzyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-
 {(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-
 methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-
 5 methyl-L-valinamide



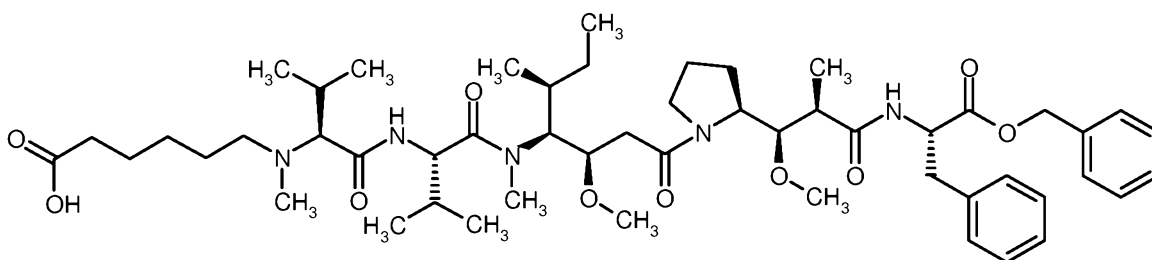
First, in analogy to the preparation of Intermediate 183, Intermediate 192 was reacted with 4-
 formylbenzoic acid with borane-pyridine complex to yield N-(4-carboxybenzyl)-N-methyl-L-
 10 valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-
 oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-
 methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide. This compound was then used, in analogy to
 Intermediate 210, to generate 11 mg (68% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.8$ min;

15 LC-MS (Method 1): $R_t = 1.13$ min; MS (ESIpos): $m/z = 1072$ ($M+H$)⁺.

Intermediate 217

N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-[(2S)-1-
 (benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-
 yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



53 mg (84 μ mol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) and 45 mg (84 μ mol) of benzyl-*N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-phenylalaninate trifluoroacetate (Intermediate 12) were taken up in 2 ml of DMF, 19 μ l of *N,N*-diisopropylethylamine, 14 mg (92 μ mol) of HOBt and 17.6 mg (92 μ mol) of EDC were added, and then the mixture was stirred at RT overnight. Subsequently, the reaction mixture was concentrated and the residue was purified by means of preparative HPLC. This yielded 59 mg (68% of theory) of the Fmoc-protected intermediate *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide.

LC-MS (Method 1): R_t = 1.55 min; m/z = 1044 ($M+H$)⁺.

57 mg (0.055 mmol) of this intermediate were treated with 1.2 ml of piperidine in 5 ml of DMF to cleave the Fmoc protecting group. After concentration and purification by means of preparative HPLC, 39 mg (76% of theory) of the free amine intermediate *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were obtained as the trifluoroacetate.

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 1.01 min; m/z = 822 ($M+H$)⁺.

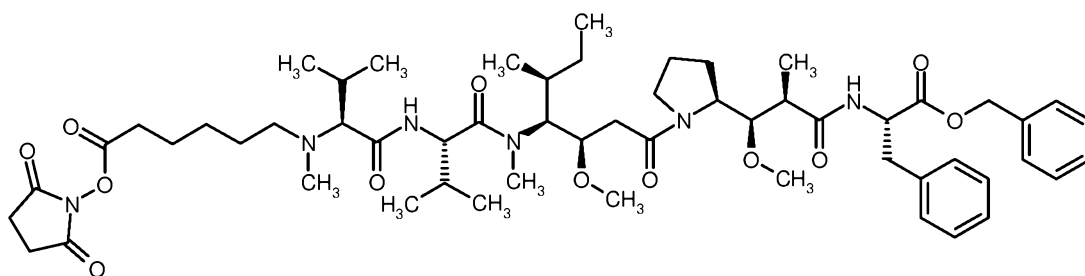
60 mg (0.06 mmol) of this intermediate were reacted, in analogy to Intermediate 210, with 6-oxohexanoic acid in the presence of borane-pyridine complex. 45 mg (75% of theory) of the title compound were obtained as a foam.

HPLC (Method 5): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 9936 ($M+H$)⁺.

Intermediate 218

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared by conversion of 42 mg (0.05 mmol) of Intermediate 217 to the active ester.

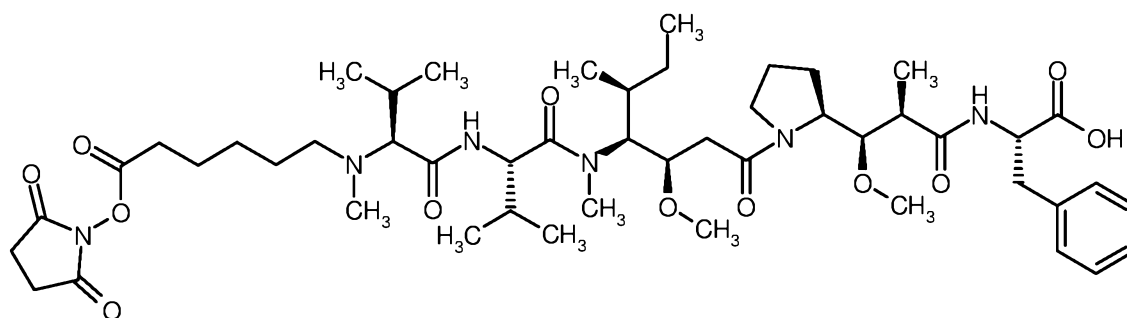
Yield: 26 mg (54%)

5 HPLC (Method 5): $R_t = 2.1$ min;

LC-MS (Method 1): $R_t = 1.01$ min; MS (ESIpos): $m/z = 1034$ (M+H)⁺.

Intermediate 219

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-
 10 [(1R,2R)-3-{[(1S)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



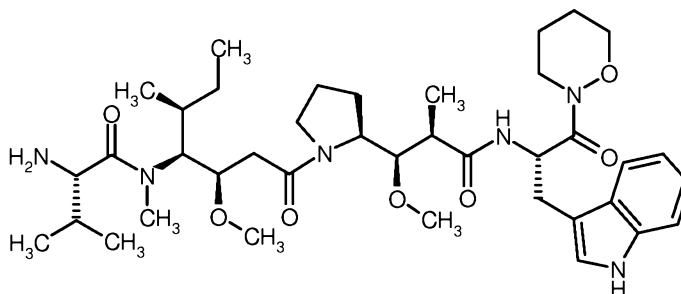
20 mg (0.02 mol) of the compound from Intermediate 218 were taken up in 2.4 ml of methanol and hydrogenated over 5% palladium on activated carbon under standard hydrogen pressure at
 15 RT for 30 min. The catalyst was then filtered off, and the solvent was removed *in vacuo*. The residue was lyophilized from 1:1 acetonitrile/water. This yielded 14 mg (92% of theory) of the title compound as a colourless foam.

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 944$ (M+H)⁺.

Intermediate 220

N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



5

0.5 g (1.01 mmol) of Intermediate 1 were admixed in 10 ml of dichloromethane with 1 ml of trifluoroacetic acid. After treatment in an ultrasound bath for 30 min, the batch was concentrated and redistilled first with DCM and then with diethyl ether, then dried under high vacuum. The oily residue was used without further purification in the next stage.

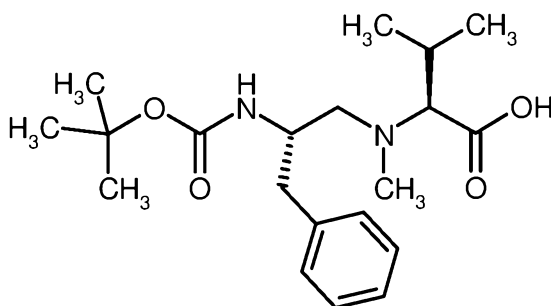
- 10 500 mg of this intermediate were dissolved in 20 ml of DMF and admixed with 466 mg (3.8 mmol) of Intermediate 191, 382 mg (1.01 mmol) of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) and 440 μ l (2.5 mmol) of N,N-diisopropylethylamine. The mixture was stirred at RT for 1 h and then concentrated. The residue was taken up in dichloromethane and extracted by shaking first twice with 5% aqueous citric acid solution and then with saturated aqueous sodium hydrogencarbonate solution. The organic phase was concentrated, and the residue was purified by flash chromatography on silica gel with 95:5 dichloromethane/methanol as the eluent. The corresponding fractions were combined, and the solvent was removed *in vacuo*. After the residue had been dried under high vacuum, 562 mg (65% of theory over both stages) of the Z-protected intermediate were obtained.
- 15
- 20 562 mg (0.57 mmol) of this intermediate were taken up in 50 ml of methanol and hydrogenated with 155 mg of 10% palladium on activated carbon under standard hydrogen pressure at RT for 20 min. The catalyst was then filtered off, and the solvent was removed *in vacuo*. The residue was purified by means of preparative HPLC. The corresponding fractions were combined, the solvent was evaporated *in vacuo*, and the residue was lyophilized from dioxane. This yielded
- 25 361 mg (87% of theory) of the title compound as a foam.

HPLC (Method 5): double peak with R_t = 1.75 and 1.86 min;

LC-MS (Method 1): double peak at $R_t = 0.84$ min and 0.91 min with the same mass; MS (ESIpos): $m/z = 944$ (M+H)⁺.

Intermediate 221

N-[(2S)-2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropyl]-N-methyl-L-valine



5

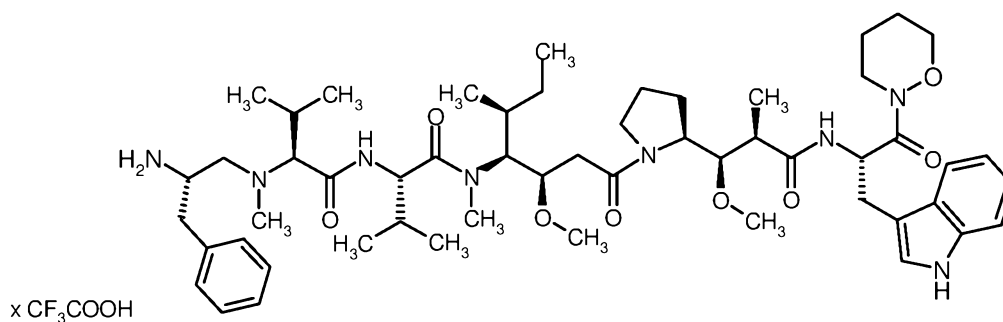
100 mg (0.76 mmol) of commercially available N-methyl-L-valine and 285 mg (1.14 mmol) of commercially available *tert*-butyl (2S)-1-oxo-3-phenylpropan-2-yl carbamate were combined in 22 ml of methanol and admixed with 340 mg (3.66 mmol) of borane-pyridine complex and 70 μ l of acetic acid. The reaction mixture was stirred at RT overnight. This was followed by concentration *in vacuo*, and the residue was purified by flash chromatography on silica gel with dichloromethane/methanol/17% aqueous ammonia solution as the eluent. After concentration of the corresponding fractions and lyophilization from 1:1 dioxane/water, 259 mg (93% of theory) of the title compound were obtained.

HPLC (Method 12): $R_t = 1.6$ min;

15 LC-MS (Method 11): $R_t = 0.76$ min; MS (ESIpos): $m/z = 365$ (M+H)⁺.

Intermediate 222

20 N-[(2S)-2-amino-3-phenylpropyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[[[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



40 mg (0.11 mmol) of N-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-phenylpropyl]-N-methyl-L-valine (Intermediate 221) were dissolved in 5 ml of DMF and admixed with 80 mg (0.11 mmol) of N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 220), 50 mg (0.13 mmol) of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) and 57 μ l (2.5 mmol) of N,N-diisopropylethylamine. The mixture was stirred at RT for 1 h and then concentrated. The residue was taken up in ethyl acetate and washed first with 5% aqueous citric acid solution and then with water. The organic phase was concentrated, and the residue was purified by means of preparative HPLC. The corresponding fractions were combined, and the solvent was removed *in vacuo*. After lyophilization from dioxane, 60 mg (50% of theory) of the protected intermediate were obtained.

HPLC (Method 12): R_t = 2.2 min;

LC-MS (Method 1): R_t = 1.17 min; MS (ESIpos): m/z = 1073 (M+H)⁺.

60 mg (0.05 mmol) of this intermediate were taken up in 10 ml of dichloromethane, 2 ml of trifluoroacetic acid were added, and the reaction mixture was stirred at RT for 1.5 h. Subsequently, the reaction mixture was concentrated *in vacuo*, and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were combined, the solvent was removed *in vacuo*, and the residue was lyophilized from dioxane/water. In this way, 25 mg (42% of theory) of the title compound were obtained as a foam.

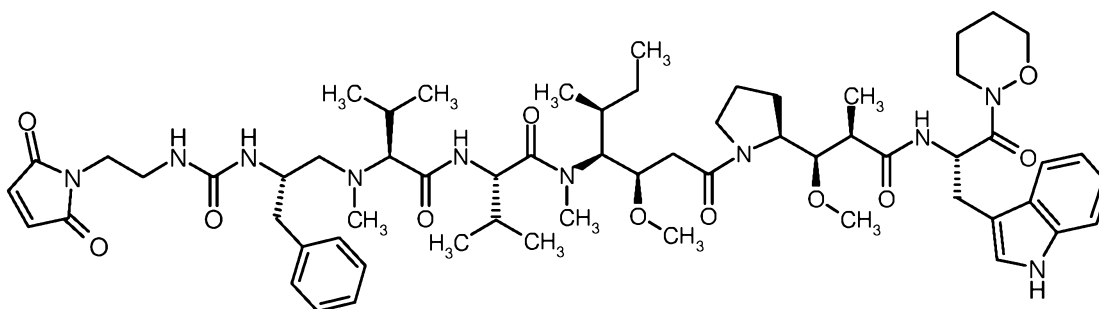
HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.95 min; MS (ESIpos): m/z = 974 (M+H)⁺.

Intermediate 223

N-[(2S)-2-([2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbamoyl)amino]-3-phenylpropyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-

1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



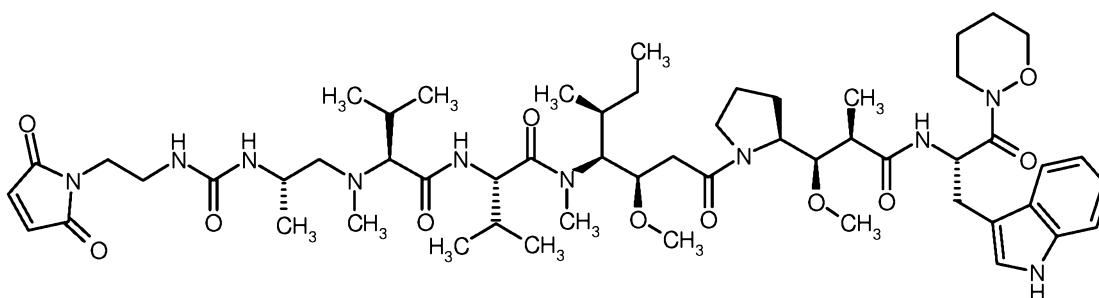
The preparation was done in analogy to Intermediate 134 starting from 5 mg (4.6 μ mol) of Intermediate 222. 3.4 mg (65% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.99 min; MS (ESIpos): m/z = 1140 ($M+H$)⁺.

Intermediate 224

N-[(2S)-2-({[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbonyl}amino)propyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



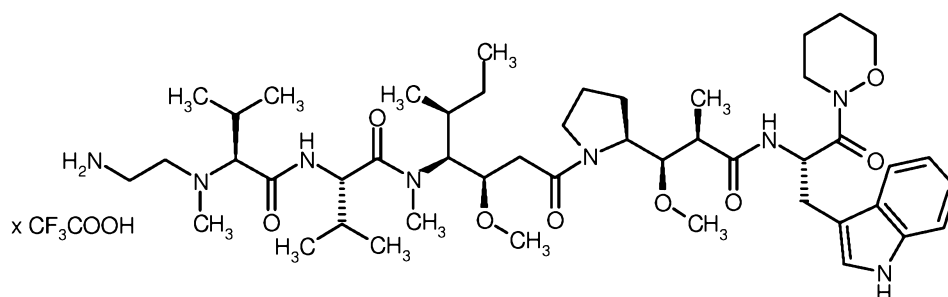
The preparation was done in analogy to the synthesis of Intermediate 223.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 1064 ($M+H$)⁺.

Intermediate 225

N-(2-aminoethyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



100 mg (0.76 mmol) of commercially available N-methyl-L-valine and 182 mg (1.14 mmol) of commercially available *tert*-butyl 2-oxoethyl carbamate were combined in 20 ml of methanol and admixed with 340 mg (3.66 mmol) of borane-pyridine complex and 65 μ l of acetic acid. The reaction mixture was stirred at RT overnight. This was followed by concentration under reduced pressure, and the residue was purified by flash chromatography on silica gel with dichloromethane/methanol/17% aqueous ammonia solution (15/4/0.5) as the eluent. After concentration of the corresponding fractions and lyophilization from 1:1 dioxane/water, 190 mg in 39% purity (35% of theory) of the intermediate were obtained, which were converted further without further purification.

50 mg (0.07 mmol) of this intermediate were dissolved in 10 ml of DMF and admixed with 52 mg (0.07 mmol) of N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 220), 32 mg (0.09 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and 37 μ l (0.2 mmol) of *N,N*-diisopropylethylamine. The mixture was stirred at RT overnight and then concentrated. The residue was taken up in ethyl acetate and extracted by shaking first with 5% aqueous citric acid solution and then with water. The organic phase was concentrated and the residue was purified by means of preparative HPLC. The corresponding fractions were combined, and the solvent was removed *in vacuo*. After lyophilization from dioxane, 53 mg (76% of theory) of the protected intermediate were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 1.02 min; MS (ESIpos): m/z = 984 ($M+H$)⁺.

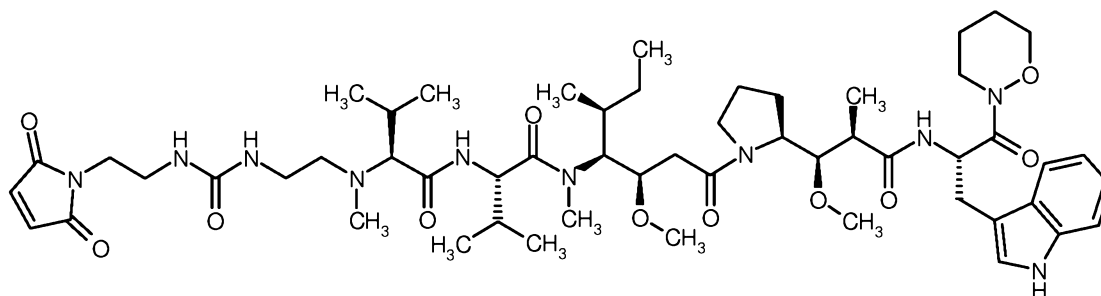
53 mg (0.05 mmol) of this intermediate were taken up in 10 ml of dichloromethane, 2 ml of trifluoroacetic acid were added, and the reaction mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated *in vacuo* and the remaining residue was purified by means of preparative HPLC. The corresponding fractions were combined, the solvent was removed *in vacuo*, and the residue was lyophilized from dioxane/water. In this way, 21 mg (40% of theory) of the title compound were obtained with 65% purity.

HPLC (Method 12): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.87$ min; MS (ESIpos): $m/z = 884$ (M+H)⁺.

Intermediate 226

- 10 N-[2-([2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbamoyl)amino)ethyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



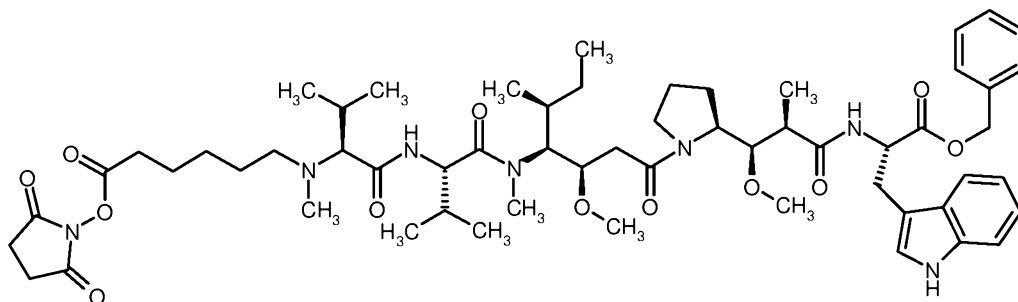
- 15 The preparation was done starting from Intermediate 225 in analogy to the synthesis of Intermediate 134. 11.6 mg (59% of theory) of the title compound were obtained.

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.90$ min; MS (ESIpos): $m/z = 1050$ (M+H)⁺.

Intermediate 227

- 20 N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-1-(benzyloxy)-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



This compound was prepared analogously to Intermediate 218 by conversion to the active ester.

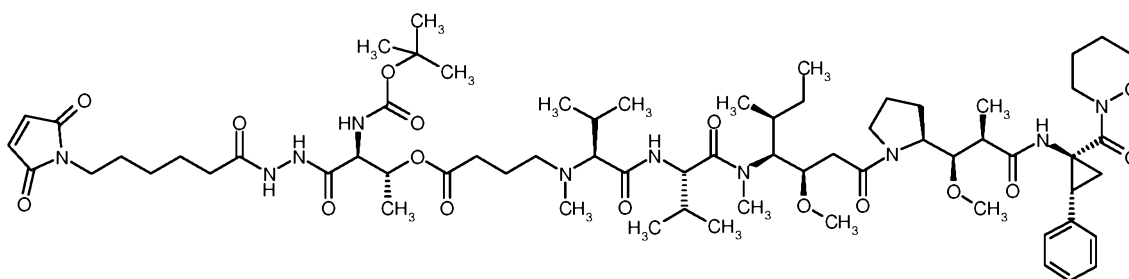
Yield: 18 mg (51% of theory)

HPLC (Method 5): $R_t = 2.1$ min;

- 5 LC-MS (Method 1): $R_t = 0.98$ min; MS (ESIpos): $m/z = 1073$ ($M+H$)⁺.

Intermediate 228

- (2R,3S)-3-[(*tert*-butoxycarbonyl)amino]-4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutan-2-yl(3R,4S,7S,10S)-4-[(2S)-butan-2-yl]-7,10-diisopropyl-3-(2-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



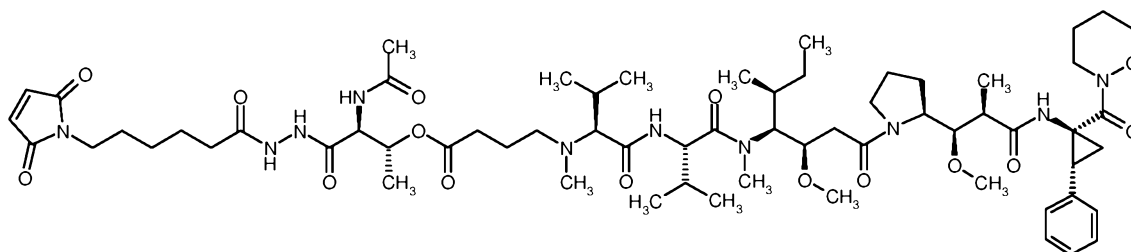
- The title compound was prepared by coupling the Boc-protected intermediate obtained from the synthesis of Intermediate 154 with commercially available 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanehydrazide.

HPLC (Method 12): $R_t = 2.1$ min;

LC-MS (Method 1): $R_t = 0.97$ min; MS (ESIpos): $m/z = 1308$ ($M+H$)⁺.

Intermediate 229

(2R,3S)-3-acetamido-4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutan-2-yl (3R,4S,7S,10S)-4-[(2S)-butan-2-yl]-7,10-diisopropyl-3-(2-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



The title compound was prepared from 7.5 mg (2.5 μ mol) of Intermediate 154 by acetylation with 2.3 μ l of acetic anhydride in 1 ml of DMF in the presence of 0.4 μ l of *N,N*-diisopropylethylamine.

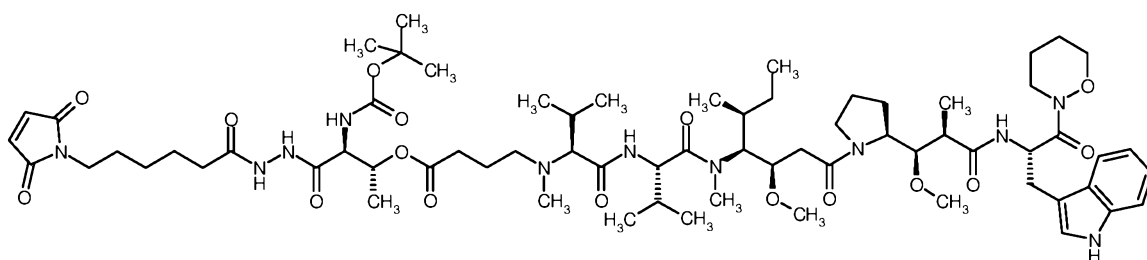
Yield: 1.4 mg (40% of theory)

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1250 ($M+H$)⁺.

Intermediate 230

(2R,3S)-3-[(*tert*-butoxycarbonyl)amino]-4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl] hydrazino}-4-oxobutan-2-yl (3R,4S,7S,10S)-4-[(2S)-butan-2-yl]-3-(2-{(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl] pyrrolidin-1-yl}-2-oxoethyl)-7,10-diisopropyl-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



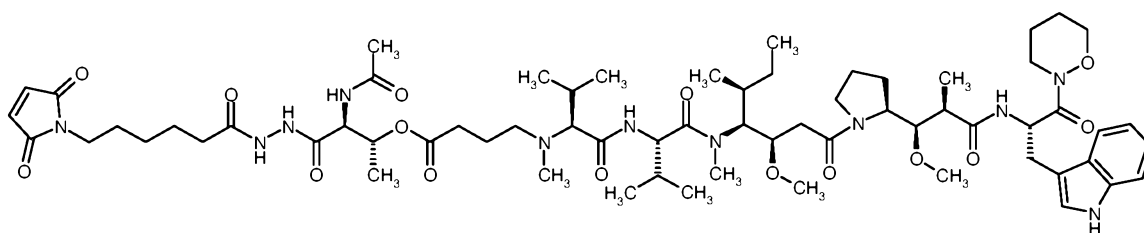
This compound was prepared in analogy to Intermediate 228 starting from Intermediate 193. 16 mg (30% of theory over 3 stages) of the title compound were obtained.

HPLC (Method 12): $R_t = 2.0$ min;

LC-MS (Method 1): $R_t = 1.02$ min; MS (ESIpos): $m/z = 1335$ ($M+H$)⁺.

5 Intermediate 231

(2R,3S)-3-acetamido-4-{2-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]hydrazino}-4-oxobutan-2-yl (3R,4S,7S,10S)-4-[(2S)-butan-2-yl]-3-(2-{(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-7,10-diisopropyl-5,11-dimethyl-6,9-dioxo-2-oxa-5,8,11-triazapentadecan-15-oate



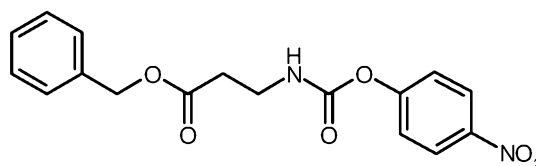
This compound was prepared from 8 mg (6 μ mol) of Intermediate 230, first by deprotection with trifluoroacetic acid and subsequent acetylation with acetic anhydride in DMF in the presence of *N,N*-diisopropylethylamine. 2 mg (37% of theory over 2 stages) of the title compound were obtained.

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.88$ min; MS (ESIpos): $m/z = 1277$ ($M+H$)⁺.

Intermediate 232

20 Benzyl-N-[(4-nitrophenoxy)carbonyl]-beta-alaninate



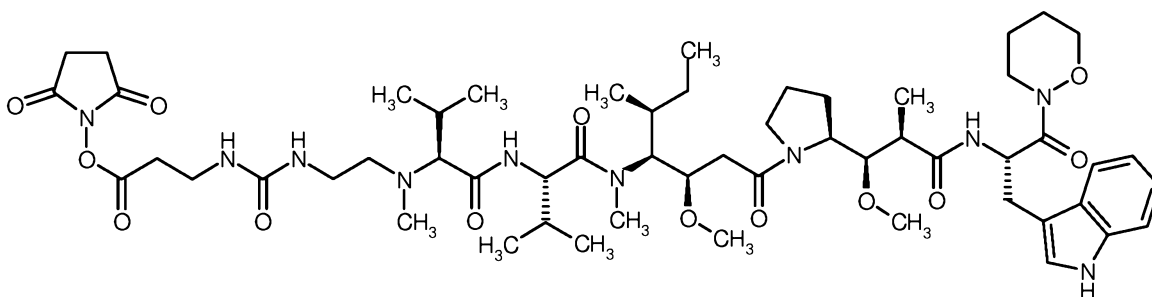
200 mg (0.57 mmol) of commercially available 4-methylbenzenesulphonic acid benzyl beta-alaninate and 229 mg (1.14 mmol) of 4-nitrophenyl chlorocarbonate were taken up in 15 ml of tetrahydrofuran, and the reaction mixture was then heated to reflux for 30 min. Subsequently, the reaction mixture was concentrated *in vacuo*, and the residue was purified by means of preparative HPLC. After concentration of the corresponding fractions and drying of the residue under high vacuum, 86 mg (44% of theory) of the title compound were obtained.

HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 1.07$ min; MS (ESIpos): $m/z = 345$ (M+H)⁺.

Intermediate 233

N-{2-[(3-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-oxopropyl)carbamoyl]amino]ethyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



13 mg (10 μ mol) of Intermediate 225 and 6.7 mg (20 μ mol) of Intermediate 232 were dissolved in 3 ml of DMF, and then 7 μ l of N,N-diisopropylethylamine were added. The mixture was stirred at RT overnight and then concentrated under high vacuum. The remaining residue was purified by means of preparative HPLC. After concentration of the corresponding fractions and drying of the residue under high vacuum, 5.4 mg (38% of theory) of the protected intermediate were obtained.

HPLC (Method 5): $R_t = 2.1$ min;

LC-MS (Method 1): $R_t = 0.6$ min; MS (ESIpos): $m/z = 1089$ (M+H)⁺.

5.4 mg (5 μ mol) of this intermediate were dissolved in 5 ml of methanol and, after adding 2 mg of 10% palladium on activated carbon, hydrogenated under standard hydrogen pressure at RT for 20 min. The catalyst was then filtered off, and the solvent was removed *in vacuo*. After drying of the residue under high vacuum, 5 mg (quant.) of the acid intermediate were obtained.

HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.84$ min; MS (ESIpos): $m/z = 999$ (M+H)⁺.

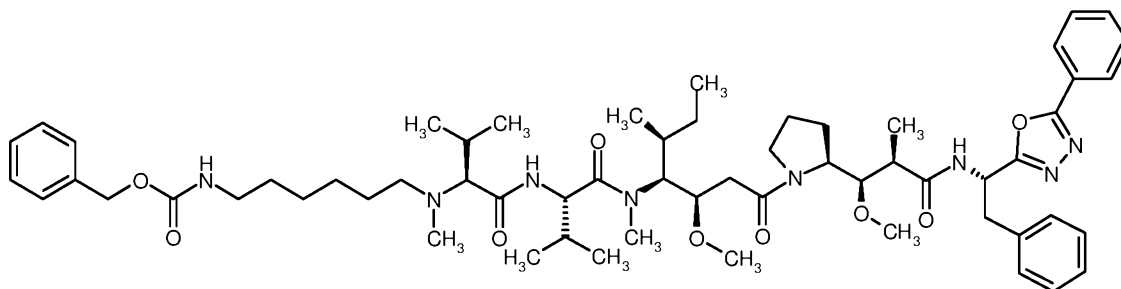
5 mg (10 μ mol) of this intermediate were dissolved in 1 ml of DMF and admixed with 5.8 mg (50 mmol) of 1-hydroxypyrrolidine-2,5-dione and then with 2.6 μ l of *N,N*-diisopropylethylamine and 3.8 mg (10 μ mol) of HATU. After stirring at RT for 20 h, the reaction mixture was concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization from 1:1 dioxane/water, 1.1 mg (20% of theory) of the title compound were obtained.

HPLC (Method 12): $R_t = 1.9$ min;

10 LC-MS (Method 1): $R_t = 0.87$ min; MS (ESIpos): $m/z = 1096$ (M+H)⁺.

Intermediate 234

N-(6-{[(benzyloxy)carbonyl]amino}hexyl)-*N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide



15

25 mg (30 μ mol) of *N*-methyl-*L*-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-*L*-valinamide (Intermediate 55) and 45 mg (180 μ mol) of benzyl-(6-oxohexyl)carbamate were taken up in 3 ml of methanol and acidified with acetic acid. At room temperature, 15 μ l (144 μ mol; 9.4M) of borane-pyridine complex were subsequently added. The batch was subsequently stirred at RT for 24 h, and acetic acid and 15 μ l (144 μ mol; 9.4M) of borane-pyridine complex were added again after 8 h. The reaction mixture was subsequently adjusted to pH 2 with TFA and purified by means of preparative HPLC. The product fractions were combined and concentrated, and the residue was dried under high vacuum. This gave 15 mg (46% of theory) of the title compound as a foam.

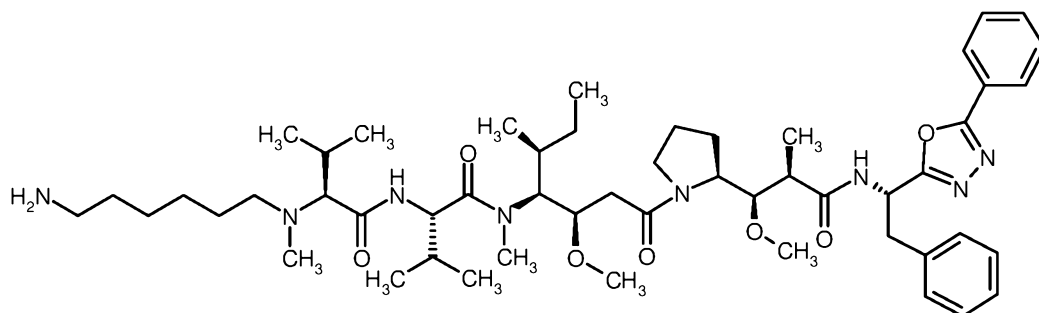
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LC-MS (Method 1): $R_t = 1.03$ min; $m/z = 1066$ ($M+H$)⁺.

Intermediate 235

N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

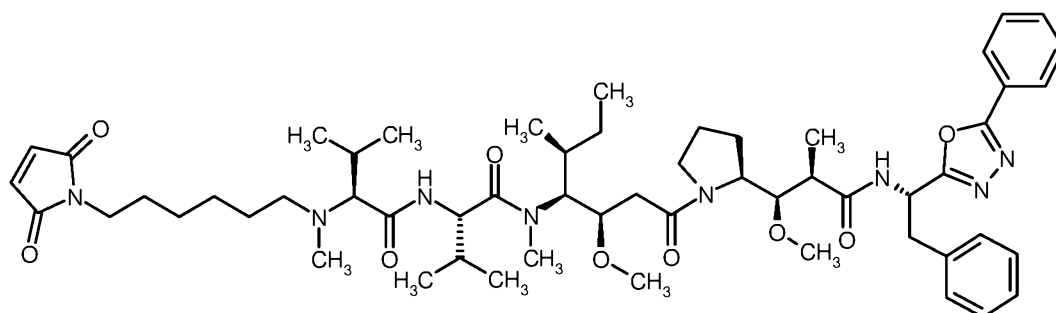


15 mg (14 μ mol) of N-(6-[(benzyloxy)carbonyl]amino)hexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 234) were taken up in 3 ml of methanol, and 1.8 mg of palladium on carbon (5%) were added. The reaction mixture was subsequently hydrogenated under standard hydrogen pressure at RT for 2 h. The catalyst was then filtered off, and the solvent was removed *in vacuo*. The residue was lyophilized from 1:1 acetonitrile/water. 11 mg (86% of theory) of the title compound were obtained as a foam.

LC-MS (Method 1): $R_t = 0.81$ min; $m/z = 932$ ($M+H$)⁺.

Intermediate 236

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

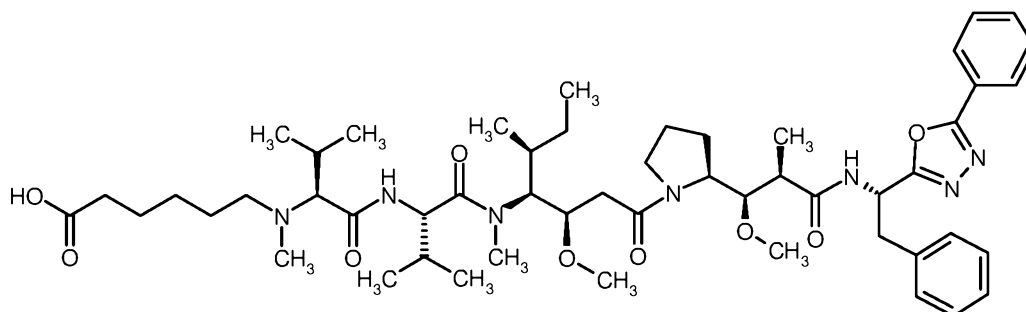


11 mg (12 μ mol) of N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-
 [(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-
 yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide
 5 (Intermediate 235) were taken up in 500 μ l of 1:1 dioxane/water and admixed with 253 μ l of 1M
 aqueous sodium hydrogencarbonate solution and then with 2.8 mg (18 μ mol) of methyl 2,5-
 dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate. The reaction mixture was stirred at RT for 30 min
 and then acidified with trifluoroacetic acid. The reaction mixture was purified by means of
 preparative HPLC. After lyophilization, 0.8 mg (7% of theory) of the title compound was
 10 obtained.

LC-MS (Method 1): R_t = 1.01 min; m/z = 1012 ($M+H$)⁺.

Intermediate 237

N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-
 methoxy-2-methyl-3-oxo-3-[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-
 15 yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



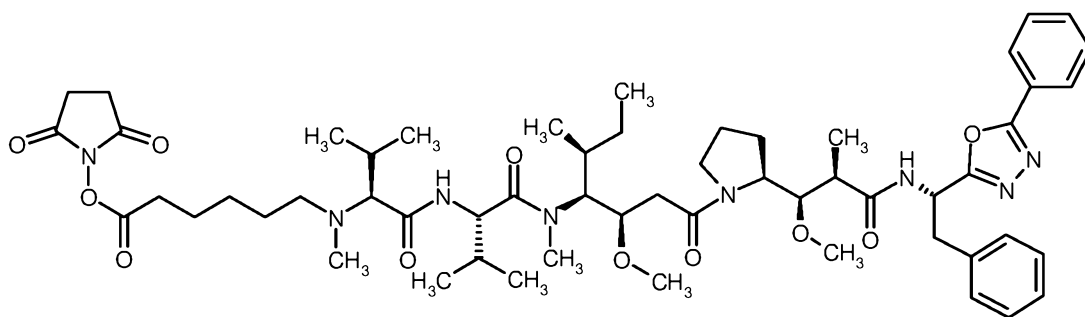
25 mg (30 μ mol) of N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-
 2-methyl-3-oxo-3-[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-
 yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide
 20 (Intermediate 55) and 23 mg (180 μ mol) of 6-oxohexanoic acid were taken up in 3 ml of
 methanol and acidified with acetic acid. At room temperature, 15 μ l (144 μ mol; 9.4M) of borane-

pyridine complex were subsequently added. The reaction mixture was subsequently stirred at RT for 20 h, and acetic acid and 15 μ l (144 μ mol; 9.4M) of borane-pyridine complex were added again after 8 h. The reaction mixture was subsequently adjusted to pH 2 with trifluoroacetic acid and purified by means of preparative HPLC. The product fractions were combined and concentrated, and the residue was lyophilized. 21 mg (74% of theory) of the title compound were thus obtained as a foam.

LC-MS (Method 1): R_t = 0.91 min; m/z = 947 (M+H)⁺.

Intermediate 238

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino]propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

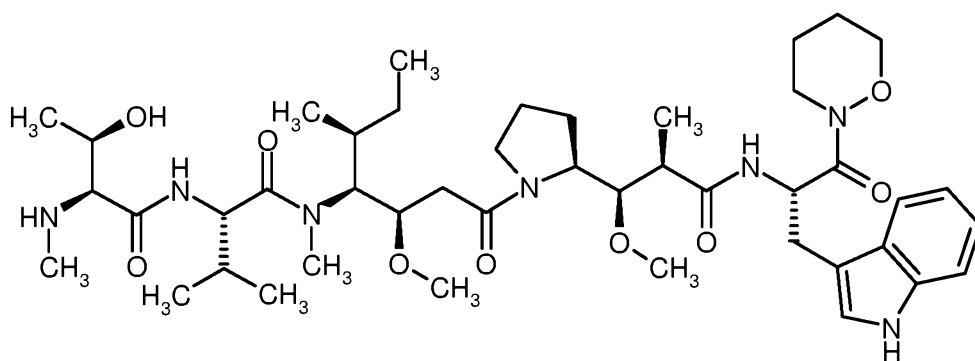


21 mg (22 μ mol) of Intermediate 237 were dissolved in 1 ml of DMF and admixed with 38 mg (333 μ mol) of 1-hydroxypyrrolidine-2,5-dione and then with 2.4 mg (10 μ mol) of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) and 19 μ l of N,N-diisopropylethylamine. After stirring at RT for 2 h, the reaction mixture was purified by means of preparative HPLC. After lyophilization from dioxane, 22 mg (96% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.95 min; m/z = 1044 (M+H)⁺.

Intermediate 239

N-methyl-L-threonyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



First, *N*-[(benzyloxy)carbonyl]-*N*-methyl-L-threonine was released from 237 mg (0.887 mmol) of its dicyclohexylamine salt by taking it up in ethyl acetate and extractive shaking with 5% aqueous sulphuric acid. The organic phase was dried over magnesium sulphate, filtered and concentrated. 14.7 mg (0.055 mmol) of *N*-[(benzyloxy)carbonyl]-*N*-methyl-L-threonine were taken up in 3 ml of DMF and admixed successively with 40 mg (0.055 mmol) of Intermediate 220, 12.7 mg (0.066 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 10 mg (0.066 mmol) of 1-hydroxy-1*H*-benzotriazole hydrate. The mixture was subsequently stirred at RT for 2 h. The solvent was then removed *in vacuo*, and the residue purified by means of preparative HPLC. 29 mg (54% of theory) of the *Z*-protected intermediate were thus obtained.

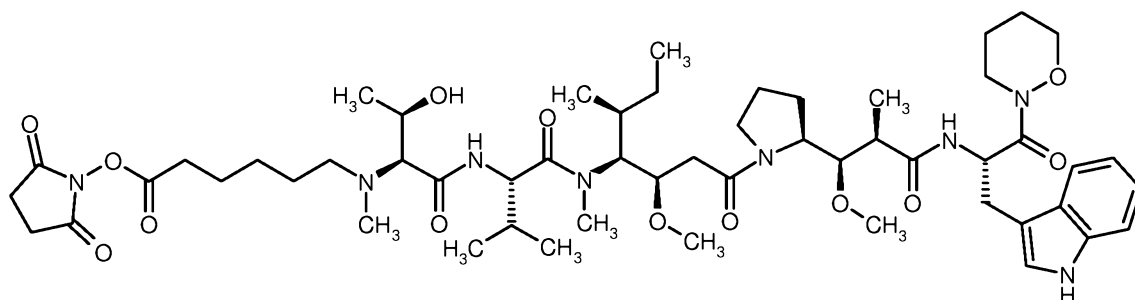
LC-MS (Method 1): $R_t = 1.15$ min; MS (ESIpos): $m/z = 976$ ($M+H$)⁺.

29 mg (0.003 mmol) of this intermediate were dissolved in 5 ml of methanol and hydrogenated over 5 mg of 5% palladium/carbon at RT and standard pressure for 1 h. The catalyst was subsequently filtered off and the solvent evaporated. The remaining residue was purified by means of preparative HPLC. 17 mg (54% of theory) of the title compound were obtained.

LC-MS (Method 1): $R_t = 0.77$ min; MS (ESIpos): $m/z = 842$ ($M+H$)⁺.

Intermediate 240

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-*N*-methyl-L-threonyl-*N*-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[[[(2*S*)-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



This compound was prepared in analogy to Intermediate 210 from 15.6 mg (0.016 mmol) of Intermediate 239. 10.8 mg (67% of theory over 2 stages) of the title compound were obtained.

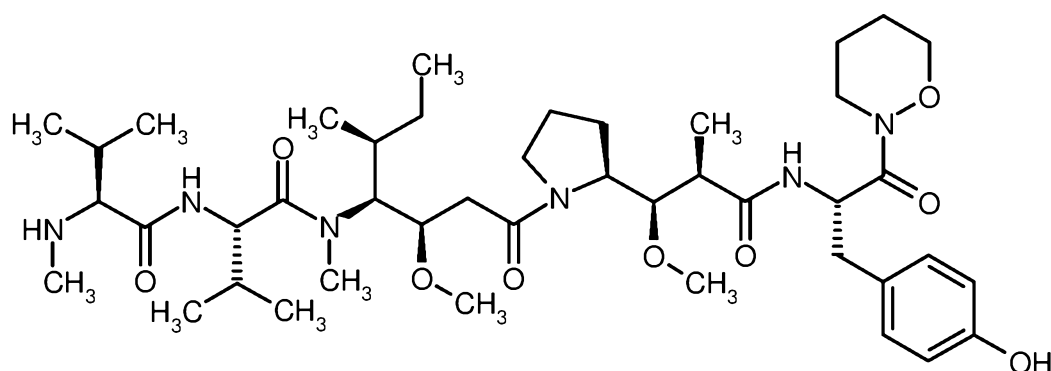
5 HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.85$ min; MS (ESIpos): $m/z = 1053$ ($M+H$)⁺.

Intermediate 241

N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate

10



First, in analogy to Intermediate 5, trifluoroacetic acid-(2S)-2-amino-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)propan-1-one (1:1) was prepared. This component was then used to obtain the title compound, in analogy to the synthesis described in Intermediate 75, by coupling with N-(tert-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 26) in the presence of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid.

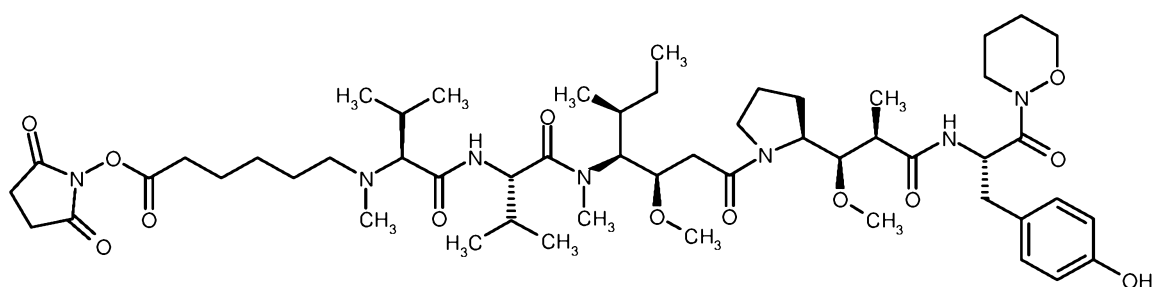
15

HPLC (Method 12): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.85$ min; MS (ESIpos): $m/z = 817$ ($M+H$)⁺.

Intermediate 242

5 N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



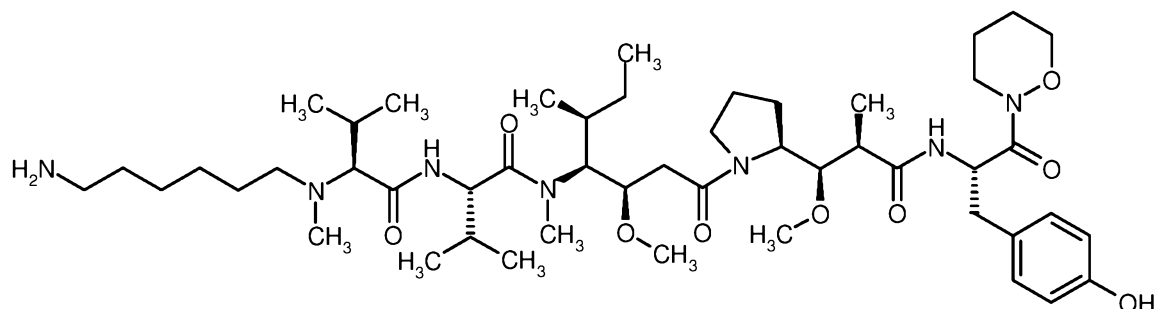
10 50 mg (0.05 mmol) of Intermediate 241 were reacted, in analogy to Intermediate 210, with 6-oxohexanoic acid in the presence of borane-pyridine complex. Subsequently, 22.5 mg (0.02 mmol) of the obtained acid were converted to the activated ester. 13.5 mg (36% of theory over 2 stages) of the title compound were obtained.

HPLC (Method 12): $R_t = 1.8$ min;

15 LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 1028$ ($M+H$)⁺.

Intermediate 243

N-(6-aminoheptyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



The preparation was done, in analogy to Intermediate 78, by reductive alkylation of Intermediate 241 with benzyl-(6-oxohexyl)carbamate and borane-pyridine complex and subsequent
5 hydrogenation in methanol as the solvent.

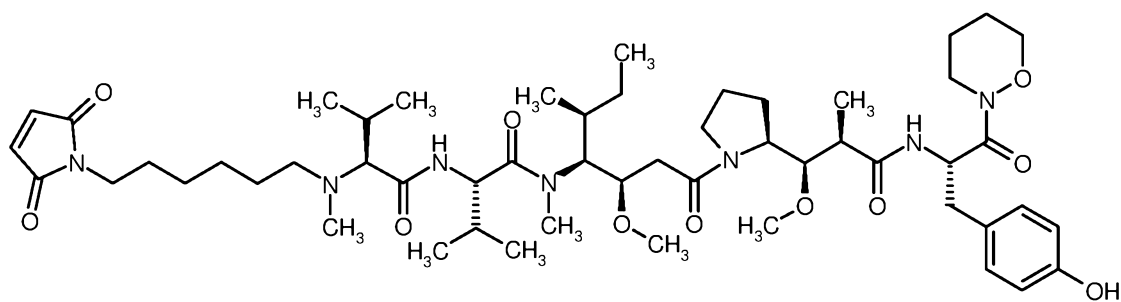
Yield: 17.5 mg (34% of theory over 2 stages)

HPLC (Method 12): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.63$ min; MS (ESIpos): $m/z = 916$ ($M+H$)⁺.

Intermediate 244

10 N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



15 The preparation was done in analogy to Intermediate 166 starting from Intermediate 243.

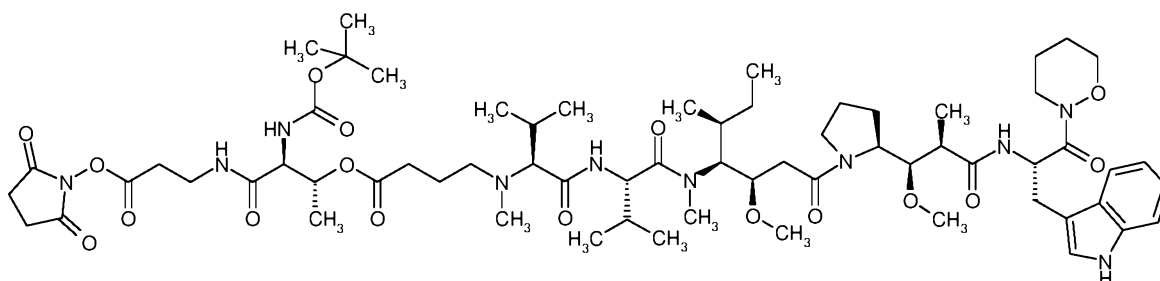
Yield: 1.3 mg (12% of theory)

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.89$ min; MS (ESIpos): $m/z = 996$ ($M+H$)⁺.

Intermediate 245

2,5-dioxopyrrolidin-1-yl O-[(3R,4S,7S,10S)-4-[(2S)-butan-2-yl]-3-(2-{(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-2-oxoethyl)-7,10-diisopropyl-5,11-dimethyl-6,9,15-trioxo-2-oxa-5,8,11-triazapentadecan-15-yl]-N-(tert-butoxycarbonyl)-L-threonyl-beta-alaninate



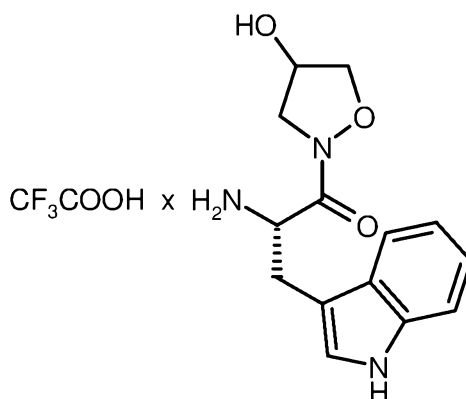
First, Intermediate 193, as described for Intermediate 154, was reacted with benzyl N-(tert-butoxycarbonyl)-L-threoninate, and then the benzyl ester was removed by hydrogenolytic means. 30 mg (0.027 mmol) of the thus obtained N-[4-[(1S,2R)-1-[(tert-butoxycarbonyl)amino]-1-carboxypropan-2-yl]oxy)-4-oxobutyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide were then coupled with 4-methylbenzenesulphonic acid benzyl-beta-alaninate in the presence of HATU, and the benzyl ester was removed again by hydrogenolysis (yield: 24 mg (71% of theory over 2 stages)). Finally, 10 mg (0.008 mmol) of the obtained acid were converted to the activated ester. After HPLC purification, 2.7 mg (23% of theory) of the title compound were obtained.

HPLC (Method 5): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 1.01$ min; MS (ESIpos): $m/z = 1295$ ($M+H$)⁺

20 Intermediate 246a

(2S)-2-amino-1-(4-hydroxy-1,2-oxazolidin-2-yl)-3-(1H-indol-3-yl)propan-1-one trifluoroacetate
(Diastereomer 1)



1.6 g (3.982 mmol) of 2,5-dioxopyrrolidin-1-yl N-(tert-butoxycarbonyl)-L-tryptophanate were dissolved in 15 ml of DMF and admixed with 500 mg (3.982 mmol) of 1,2-oxazolidin-4-ol and 100 µl of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT overnight. Then
 5 another 100 µl of *N,N*-diisopropylethylamine were added, and the mixture was first treated in an ultrasound bath for 5 h, then stirred at RT overnight and subsequently concentrated *in vacuo*. The remaining residue was taken up in ethyl acetate and extracted first twice with 5% aqueous citric acid solution, then with saturated aqueous sodium hydrogencarbonate solution and finally with water. The organic phase was concentrated and the residue separated into the diastereomers by
 10 means of flash chromatography on silica gel with 95:5 dichloromethane/methanol as the eluent. The corresponding fractions of both diastereomers were combined and the solvent was removed *in vacuo*. After drying of the residues under high vacuum, 272 mg (18% of theory) of Diastereomer 1 ($R_f = 0.18$ (95:5 dichloromethane/methanol) and 236 mg (16% of theory) of Diastereomer 2 ($R_f = 0.13$ (95:5 dichloromethane/methanol) as wells as 333 mg (22% of theory)
 15 of a mixed fraction of the Boc-protected intermediates were obtained.

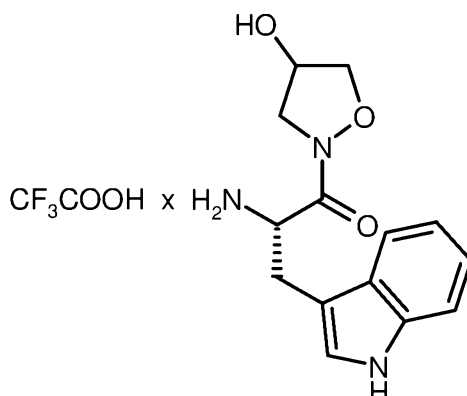
5 ml of trifluoroacetic acid in 20 ml of dichloromethane were used under standard conditions for cleaving the Boc protecting group from 272 mg (725 µmol) of Diastereomer 1 of this intermediate and, after lyophilization from dioxane/water, 290 mg (quant) of the title compound were obtained in 75% purity and used without further purification in the next stage.

20 HPLC (Method 12): $R_t = 1.1$ min;

LC-MS (Method 13): $R_t = 1.80$ min; MS (ESIpos): $m/z = 276$ ($M+H$)⁺

Intermediate 246b

(2S)-2-amino-1-(4-hydroxy-1,2-oxazolidin-2-yl)-3-(1H-indol-3-yl)propan-1-one trifluoroacetate
 (Diastereomer 2)

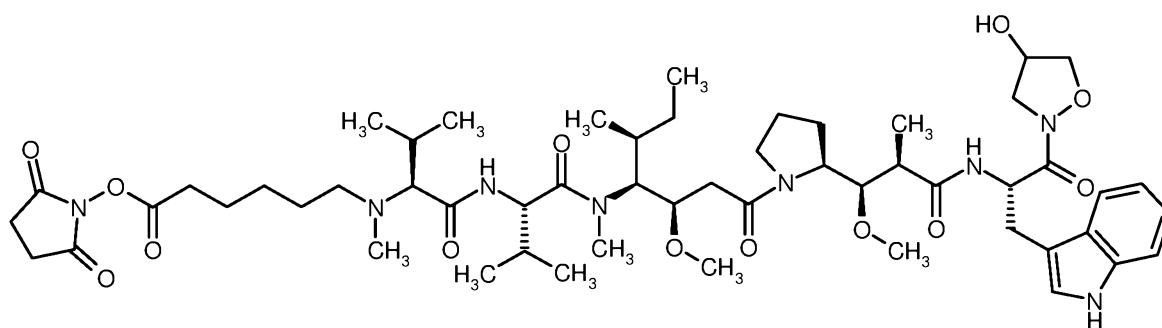


5 ml of trifluoroacetic acid in 20 ml of dichloromethane were used under standard conditions for cleaving the Boc protecting group from 236 mg (630 μ mol) of Diastereomer 2 of the intermediate described in 246a and, after concentration, stirring with diethyl ether and drying of the residue under high vacuum, 214 mg (76%) of the title compound were obtained.

LC-MS (Method 13): R_t = 1.84 min; MS (ESIpos): m/z = 276 ($M+H$)⁺

Intermediate 247a

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-
10 [(1R,2R)-3-{[(2S)-1-(4-hydroxy-1,2-oxazolidin-2-yl)-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-
1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-
methyl-L-valinamide (Diastereomer 1)



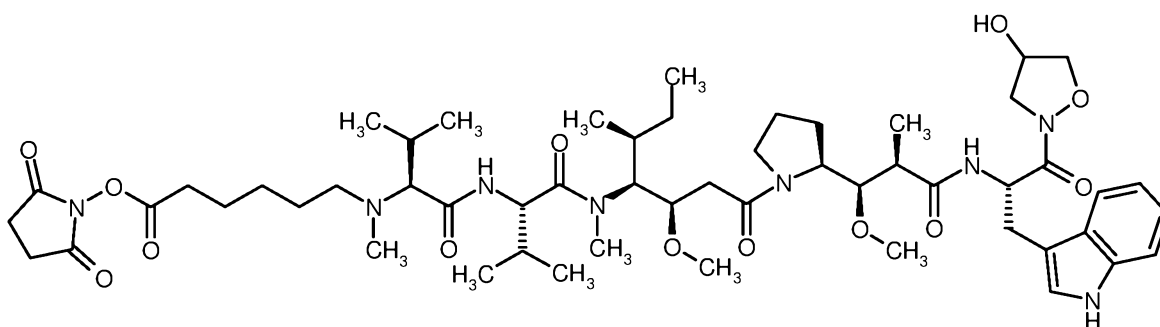
To synthesize this compound, the coupling of Intermediates 26 and 246a with subsequent
15 cleaving of the Boc protecting group was first performed as described for Intermediate 74. Subsequently, the alkylation with 6-oxohexanoic acid in the presence of borane-pyridine complex and subsequent conversion of the acid to the active ester were performed, as described for Intermediate 210. The title compound was purified by means of preparative HPLC.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 1053$ ($M+H$)⁺

Intermediate 247b

N-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-(4-hydroxy-1,2-oxazolidin-2-yl)-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Diastereomer 2)



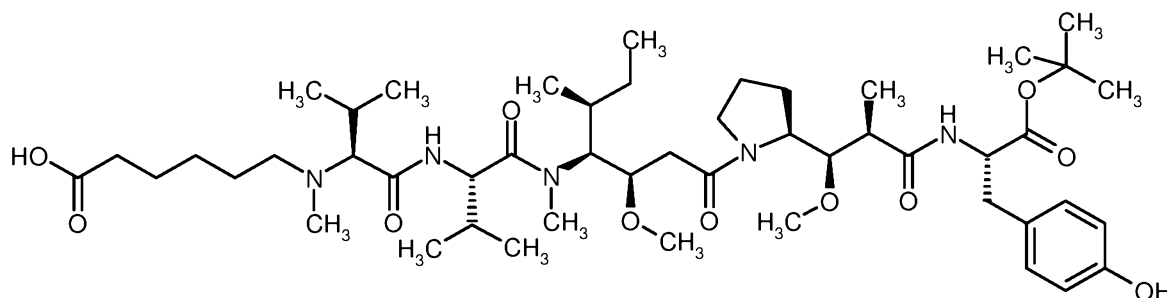
To synthesize this compound, the coupling of Intermediates 26 and 246b with subsequent cleaving of the Boc protecting group was first performed as described for Intermediate 74. Subsequently, the alkylation with 6-oxohexanoic acid in the presence of borane-pyridine complex and subsequent conversion of the acid to the active ester were performed, as described for Intermediate 210. The title compound was purified by means of preparative HPLC.

HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 1053$ ($M+H$)⁺

Intermediate 248

N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-tert-butoxy-3-(4-hydroxyphenyl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



First, in analogy to the synthesis described in Intermediate 86, the amine compound tert-butyl N-[(2R,3R)-3-methoxy-3-{(2S)-1-[(3R,4S,5S)-3-methoxy-5-methyl-4-(methyl{(2S)-3-methyl-2-[(N-methyl-L-valyl)amino]butyl}amino)heptanoyl] pyrrolidin-2-yl}-2-methylpropanoyl]-L-tyrosinate was prepared as the trifluoroacetate by coupling *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and tert-butyl-L-tyrosinate in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid to obtain the tert-butyl ester (stirring with trifluoroacetic acid in dichloromethane for 40 min). 38 mg (0.04 mmol) of this compound were then used to obtain 31 mg (99% of theory) of the title compound, in analogy to the preparation of Intermediate 210, by reaction with 6-oxohexanoic acid in the presence of borane-pyridine complex.

HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.88$ min; MS (ESIpos): $m/z = 918$ (M+H)⁺.

Exemplary Embodiments:

Anti-EGFR1 antibodies used

Cetuximab (INN No. 7906)

- 5 Additional names: IMC-225, C225, EMR-62202, BMS-564717, Fab C225

Cetuximab (DrugBank Accession No. DB00002) is a chimeric anti-EGFR1-antibody that is produced in SP2/0 mouse myeloma cells and is distributed by ImClone Systems Inc./Merck KGaA/Bristol-Myers Squibb Co.

- 10 Cetuximab is indicated for treatment of metastatic EGFR-expressing colorectal carcinoma with the wild-type K-Ras gene. It has an affinity of 10^{-10} M.

Sequence:

Light chain (kappa):

- DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQR TNGSPRLLIKYASESISGIPS
RFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPP
15 SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTL
T LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

- QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGGNTDYN
TPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTSAA
20 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQ
25 QGNVFSCSVMHEALHNHYTQKSLSLSPGK

Panitumumab (INN No. 8499)

Panitumumab (additional names: ABX-EGF, E7.6.3) (Drug Bank Accession No. DB01269) is a recombinant monoclonal human IgG2 antibody that binds specifically to the human EGF receptor 1 and is distributed by Abgenix / Amgen.

Panitumumab originates from the immunization of transgenic mice (XenoMouse). These mice are capable of producing human immunoglobulins (light chains and heavy chains). A special B cell clone was selected which produces antibodies to EGFR and it was immortalized with CHO cells (Chinese hamster ovary cells). These cells are now used for the production of a 100% human antibody.

Panitumumab is indicated for treatment of an EGFR-expressing metastatic colorectal carcinoma that is refractory to a chemotherapeutic treatment with fluoropyrimidine, oxaliplatin and irinotecan. It has an affinity of 10^{-11} M.

Sequence:

10 Light chain (kappa):

DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPS
RFSGSGSGTDFTFTISSLPEDIATYFCQHFDHLPLAFGGGKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTL
T LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

15 Heavy chain:

QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKGLEWIGHIYYSGNTN
YNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTMTVSSA
STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVTVTPSSNFGTQTYTCNVDPHKPSNTKVDKTVERKCCVECPPCAPPVAGPSVFL
20 FPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV
VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSV MHEALHNHYTQKSLSLSPG

Nimotuzumab (INN No. 8545)

25 Nimotuzumab (additional names: TheraCIM-h-R3; h-R3; Theraloc; BioMAb; BIOMAb-EGFR; Vecthix; KI-0501) (patents EP 00586002, EP 00712863) is a humanized monoclonal IgG1 antibody that binds specifically to human EGF receptor 1 and is produced by YM BioSciences Inc. (Mississauga, Canada). It is produced in non-secreting NSO cells (mammalian line).

30 Nimotuzumab is approved for treatment of head and neck tumors, highly malignant astrocytomas and glioblastoma multiform (not in the EU and US) and pancreatic cancer (orphan drug, EMA). It has an affinity of 10^{-8} M.

B. Production of ADCs

The intermediates described above can be linked to the anti-EGF receptor antibodies cetuximab, nimotuzumab or panitumumab, for example, as well as additional antibodies listed below, and such linkages may optionally be via cysteine or lysine side chains of the antibody protein according to the methods described below.

B-1.1 Workup of the EGFR antibodies before conjugation

Erbix commercial product (Erbix® 5 mg/mL infusion solution 100 mL, PZN 0493540, N1, 500 mg, Merck), Vectibix commercial product (Vectibix® 20 mg/mL concentrate for preparing an infusion solution, one puncturable vial (N1) 100 mg, 20 mL, PZN 6078606, Amgen) or CIMAh commercial product (CIMAh® 50 mg AMP 4 × 10 mL, imported from Cuba, YMBioSciences Inc. (Mississauga, Canada) were obtained commercially from a pharmacy.

To remove the polysorbate 80 contained in the formulation, it was bound to protein A (MabSelectSure) and rinsed with 15% isopropanol. After elution with acidic acetate buffer, the mixture was rebuffed after gel filtration on D-PBS, and the resulting material was coupled to the respective toxophores.

B-1.2 General procedure for expression of antibodies in mammalian cells

The antibodies, e.g., anti-PDL1 or other antibodies to the various targets are produced in mammalian cell culture by transfecting HEK293 6E cells transiently with a suitable CMV promoter-based expression plasmid. The light and heavy chains of the antibodies were cloned either together in a single-vector system or separately in a two-vector system. The cell culture standard was up to 1.5 L in an agitated flask or 10 L in the "Wave Bag." The expression occurred at 37°C for 5-6 days in F17 medium (Invitrogen) supplemented with tryptone TN1 (Organotechnie) with 1% "FCS ultra-low IgG" (Invitrogen) and 0.5 mM valproic acid. The expression yields were between 7 and 310 mg/L.

B-1.3 General method for purifying antibodies from cell supernatants

The antibodies, e.g., PDL1 or other antibodies to the various targets were obtained from the cell culture supernatants. The cell culture supernatants were clarified by centrifugation of cells. Then the supernatant was purified by affinity chromatography on a MabSelectSure (GE Healthcare) chromatography column. The column was therefore equilibrated in DPBS, pH 7.4 (Sigma/Aldrich), the cell supernatant was applied and the column was washed with approx. 10 column volumes of DPBS, pH 7.4, + 500 mM sodium chloride. The antibodies were eluted in

50 mM sodium acetate, pH 3.5, + 500 mM sodium chloride and then purified further by gel filtration chromatography on a Superdex 200 column (GE Healthcare) in DPBS, pH 7.4.

B-1.4 General method for coupling to cysteine side chains

The following antibodies were used in the coupling reaction:

5 *Anti-EGFR1 antibodies:*

cetuximab

nimotuzumab

panitumumab

Other antibodies:

10 anti-PDL1

anti-ICOSLG

anti-FGFR3

herceptin

anti-TYRP1

15 anti-glypican-3

To a solution of the corresponding antibody in PBS buffer in the concentration range between 1 mg/mL and 10 mg/mL, 3 eq. of tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) dissolved in PBS buffer were added and stirred for one hour at RT. Then, depending on the desired load, between two and ten equivalents of the maleimide precursor compound to be
20 coupled or the halide precursor compound (intermediates 102, 103, 105-109, 111-114, 117-126, 128, 129, 132-146, 148-155, 157, 159-161, 166, 171, 175-177, 184, 189, 194-195, 199-201, 205, 209, 223-224, 226, 228-231, 236 and 244) were added as a solution in DMSO. The amount of DMSO should not exceed 10% of the total volume. The batch was stirred for 60-120 minutes at RT and then applied to PD10 columns (Sephadex® G-25, GE Healthcare) equilibrated in PBS
25 and then eluted with PBS buffer. If necessary, the concentration was increased further by ultracentrifugation.

Unless otherwise indicated, 5 mg of the corresponding antibody was generally used in PBS buffer for reduction and the following coupling. After purification on the PD10 column, the solutions of the corresponding ADC in 3.5 mL PBS buffer were each obtained. The protein concentration indicated in each case was then determined for these solutions. In addition, the

5 load of the antibody (drug/mAb ratio) was determined by the methods described below.

According to this method, the immunoconjugates synthesized in Examples 1-34, 36-37, 39-41, 43-44, 52-53, 55, 338-339, 341-344, 349, 351-352, 354, 356-358 and 374 were prepared.

In the structural formulas presented, AK_{IA} - AK_{IJ} have the following meanings:

AK_{IA} = cetuximab (partially reduced)- $S\text{\S}^1$

10 AK_{IB} = nimotuzumab (partially reduced)- $S\text{\S}^1$

AK_{IC} = panitumumab (partially reduced)- $S\text{\S}^1$

AK_{ID} = anti-PDL1 (partially reduced)- $S\text{\S}^1$

AK_{IE} = anti-ICOSLG (partially reduced)- $S\text{\S}^1$

AK_{IF} = anti-FGFR3 (partially reduced)- $S\text{\S}^1$

15 AK_{IG} = herceptin (partially reduced)- $S\text{\S}^1$

AK_{IH} = anti-TYRP1 (partially reduced)- $S\text{\S}^1$

AK_{IJ} = anti-glypican-3 (partially reduced)- $S\text{\S}^1$

wherein

$\text{\S}^1$ denotes the linkage to the succinimide group

20 and

S stands for the sulfur atom of a cysteine radical of the partially reduced antibody.

B-1.5 General method for coupling to lysine side chains

The following antibodies were used in the coupling reactions:

Anti-EGFR1 antibodies:

cetuximab

nimotuzumab

panitumumab

Other antibodies:

5 anti-PDL1

anti-ICOSLG

anti-FGFR3

herceptin

anti-TYRP1 hIgG1-kapp

10 anti-glypican-3

Between 2 and 5 eq. of the precursor compound to be coupled from the intermediates 104, 110, 115, 116, 127, 130, 131, 147, 156, 158, 162, 169, 178, 185, 190, 202, 206, 210-216, 218, 219, 227, 233, 238, 240, 242, 245, 247a and 247b were added as a solution in DMSO to a solution of the corresponding antibody in PBS buffer in the concentration range between 1 mg/mL and 15 10 mg/mL, depending on the desired load. After stirring for 30 minutes at RT, the same amount of precursor compound in DMSO was again added. The amount of DMSO should not exceed 10% of the total volume. After stirring for 30 minutes more at RT the batch was poured over PD10 columns (Sephadex[®] G-25) and then eluted with PBS buffer. Another concentration step was optionally performed by ultrafiltration. If necessary, for better separation of low-molecular 20 components, the concentration step by ultrafiltration was repeated after diluting again with PBS buffer.

Unless otherwise indicated, 5 mg of the corresponding antibody in PBS buffer was generally used for coupling. After purification on the PD10 column, solutions of the corresponding ADCs in 3.5 mL PBS buffer were obtained. For these solutions, the respective protein concentrations 25 then given were determined, and the antibody load (drug/mAb ratio) was determined according to the methods described below.

According to this method, the immunoconjugates described in Examples 35, 38, 42, 54, 337, 340, 345-348, 350, 353, 355, 359, 363, 375 and 376 were prepared.

In the structural formulas shown here, AK_{2A}, AK_{2B}, AK_{2C}, AK_{2D}, AK_{2E}, AK_{2F}, AK_{2G}, AK_{2H} and AK_{2J} have the following meanings:

AK_{2A} = cetuximab-NH§²

AK_{2B} = nimotuzumab-NH§²

5 AK_{2C} = panitumumab-NH§²

AK_{2D} = anti-PDL1- NH§²

AK_{1E} = anti-ICOSLG- NH§²

AK_{2F} = anti-FGFR3- NH§²

AK_{2G} = herceptin-NH§²

10 AK_{2H} = anti-TYRP1- NH§²

AK_{2J} = anti-glypican-3- NH§²

wherein

§² denotes the linkage to the carbonyl group

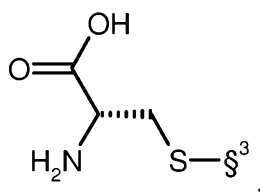
and

15 NH stands for the side chain amino group of a lysine radical of the antibody.

B-1.6a General method for preparing cysteine adducts

10 µmol of the maleimide precursor compounds described above was placed in 3 mL DMF and mixed with 2.1 mg (20 µmol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by preparative HPLC.

20 In the structural formulas shown here, Cys has the following meaning:



wherein

§³ denotes the linkage to the linker toxophore unit.

B-1.6b General method for preparing lysine adducts:

10 μmol of the active ester precursor compounds described above was placed in 5 mL DMF and mixed with α-amino-protected L-lysine in the presence of 30 μmol N,N-diisopropylethylamine.

- 5 The reaction mixture was stirred for 2 hours at RT and then concentrated *in vacuo* and next was purified by preparative HPLC. Then the protective group was removed by known methods.

Further purification and characterization of the conjugates according to the invention

- After the reaction was successful, the reaction mixture was concentrated in some cases by ultracentrifugation, for example, and then was desalinated and purified by chromatography using a Sephadex® G-25 column, for example. Elution was performed using phosphate-buffered saline solution (PBS), for example. Then the solution was sterile filtered and frozen. Alternatively, the conjugate may be lyophilized.

B-1.7 Determining the toxophore load

- The toxophore load was determined as follows on the resulting solutions of the conjugates in PBS buffer as described in the exemplary embodiments:

- The toxophore load of lysine-linked ADCs was determined by mass spectrometric determination of the molecular weights of the individual conjugated species. The antibody conjugates were first deglycosylated by PNGaseF, the sample was acidified and next, after HPLC separation, the sample was analyzed by mass spectrometry using ESI MicroTof_Q (Bruker Daltonik). All the spectra over the signal in the TIC (total ion chromatogram) were added up and the molecular weights of the various conjugate species were calculated on the basis of MaxEnt deconvolution. After signal integration of the various species, the DAR (drug/antibody ratio) was calculated.

- For protein identification, after deglycosylation and/or denaturing, in addition to determination of the molecular weight, tryptic digestion was performed, confirming the identity of the protein on the basis of the tryptic peptides identified after denaturing, reduction and derivatization.

- The toxophore load of cysteine-linked conjugates was determined by reversed-phase chromatography of the reduced and denatured ADCs. Guanidinium hydrochloride (GuHCl, 28.6 mg) and a solution of DL-dithiothreitol (DTT, 500 mM, 3 μL) were added to the ADC solution (1 mg/mL, 50 μL). The mixture was incubated for one hour at 55°C and then analyzed by HPLC.

HPLC analysis was performed on an adjutant 1260 HPLC system with detection at 220 nm, using a Polymer Laboratories PLRP-S polymeric reversed-phase column (catalog number PL1912-3802) (2.1 × 150 mm, 8 µm particle size, 1000 Å) at a flow rate of 1 mL/min with the following gradient: 0 min, 25% B; 3 min, 25% B; 28 min, 50% B. Eluent A consisted of 0.05% trifluoroacetic acid (TFA) in water, and eluent B consisted of 0.05% trifluoroacetic acid in acetonitrile.

The peaks detected were assigned based on a comparison of the retention times with the light chain (L0) and the heavy chain (H0) of the unconjugated antibody. Peaks detected exclusively in the conjugated sample were assigned to the light chain with one toxophore (L1) and to the heavy chains with one, two and three toxophores (H1, H2, H3).

The average toxophore load of the antibody was calculated from the peak areas determined by integration as the sum of the integration results of all peaks times 2, weighted by the number of toxophores, divided by the total of the integration results of all peaks with simple weighting. In isolated cases, it may happen that the toxophore load cannot be determined accurately due to co-elution of some peaks.

B-1.8 Testing the antigen binding of the ADC

The ability of the binder to bind to the target molecule was tested after successful coupling. Those skilled in the art are familiar with a variety of methods for doing so; for example, the affinity of the conjugate can be tested by means of ELISA technology or surface plasmon resonance analysis (BIAcore™ measurements). The skilled person can measure the conjugate concentration using conventional methods, e.g., protein assay for antibody conjugates (see also Doronina et al., *Nature Biotechnol.* 2003; **21**:778-784 and Polson et al., *Blood* 2007, **1102**:616-623).

B2 Producing antibody-drug conjugates (ADCs)

The intermediates described above were linked to the anti-mesothelin antibody MF-Ta, for example, with the linkage optionally taking place via the cysteine or lysine side chains of the antibody protein using the methods described below. The anti-mesothelin antibody MF-Ta was produced by methods like those described in WO 2009/068204 A1. The antibody MF-Ta was expressed in eukaryotic CHO cells (stable cell line) and purified by protein A and gel filtration before being subjected to conjugation in DPBS buffer.

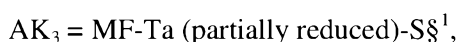
B-2.1 General working procedure 1 (coupling via cysteine):

To a solution of the corresponding antibody in PBS buffer in the concentration range between 1 mg/mL and 10 mg/mL, 3 eq. of *tris*-(2-carboxyethyl)phosphine hydrochloride (TCEP) dissolved in PBS buffer were added and stirred for one hour at RT. Next, depending on the desired load, between 2 and 10 eq. of the maleimide precursor compound or the halide precursor compound to be coupled (intermediates 128, 129, 132-146, 148-155, 157, 159-161, 171, 175-177, 184, 189, 194-195, 199-201, 205, 209, 223-224, 226, 228-231, 236 and 244) were added as a solution in DMSO. The amount of DMSO should not exceed 10% of the total volume. The reaction mixture was stirred for 60-120 minutes at RT and then applied to PD10 columns (Sephadex® G-25) and eluted with PBS buffer. The solution was then optionally concentrated by ultrafiltration. Concentration by ultrafiltration was repeated, if necessary, after diluting again with PBS buffer to achieve a better separation of low-molecular components.

Unless otherwise indicated, 5 mg of the corresponding antibody in PBS buffer was generally used for reduction and the subsequent coupling. After purification over the PD10 column, solutions of the corresponding ADCs in 3.5 mL PBS buffer were thus obtained. Then the respective protein concentration was determined for each of these solutions. In addition, the antibody load (drug/mAb ratio) was determined by the methods described in B-4.

The immunoconjugates synthesized in Examples 56, 57, 60-74, 76-83, 85, 86, 88-92, 94-101, 103, 106-112, 114, 115, 126, 128-131, 133-135, 137-139, 141-142, 151, 153-154, 366 and 377 were prepared by this method.

In the structural formulas given, AK₃ has the meaning



wherein

$\text{\S}^1$ denotes the linkage to the succinimide group,
 MF-Ta (partially reduced) stands for the partially reduced MF-Ta antibody (heavy chain SEQ ID NO: 408 and light chain SEQ ID NO: 409),

and

S stands for the sulfur atom of a cysteine radical of the partially reduced antibody.

B-2.2 General working procedure 2 (coupling via lysine side chains):

Between 2 and 5 eq. of the precursor compound to be coupled (intermediates 104, 110, 115, 116, 127, 130, 131, 147, 156, 158, 162, 169, 178, 185, 190, 202, 206, 210-216, 218, 219, 227, 233, 238, 240, 242, 245, 247a and 247b) were added as a solution in DMSO to a solution of the corresponding antibody in PBS buffer in the concentration range between 1 mg/mL and 10 mg/mL, depending on the desired load. After stirring for 30 minutes at RT, the same amount of precursor compound in DMSO was added. The amount of DMSO should not exceed 10% of the total volume. After stirring for 30 minutes more at RT, the batch was poured over PD10 columns (Sephadex[®] G-25) and eluted with PBS buffer. Further concentration by ultrafiltration was optionally also performed. Concentration was repeated by ultrafiltration, if necessary, after diluting again with PBS buffer to improve the separation of low-molecular components.

Unless otherwise indicated, 5 mg of the corresponding antibody in PBS buffer was generally used for coupling. After purification over the PD10 column, solutions of the corresponding ADC in 3.5 mL PBS buffer were obtained. Then the protein concentration indicated was determined for these solutions and the load of the antibody (drug/mAb ratio) was determined by the methods described under B-4.

Following this method, the immunoconjugates synthesized in Examples 58, 59, 75, 84, 87, 93, 102, 104, 105, 113, 116, 127, 132, 136, 140, 143- 150, 152, 367-369 and 378-380 were prepared.

In the structural formulas given, AK₄ has the meaning



wherein

§² denotes the linkage to the carbonyl group

MF-Ta stands for the unreduced MF-Ta antibody (heavy chain SEQ ID NO: 408 and light chain SEQ ID NO: 409)

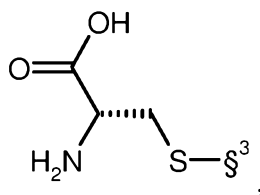
and

NH stands for the side chain amino group of a lysine radical of the antibody.

B-2.3a General method for synthesis of cysteine adducts:

10 µmol of the maleimide precursor compounds described above was dissolved in 3 mL DMF and mixed with 2.1 mg (10 µmol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and purified by preparative HPLC.

Cys in the structural formulas given has the meaning



wherein

$\S^3$ denotes the linkage to the linker toxophore unit.

5 **B-2.3b** General method for preparing lysine adducts:

10 μmol of the active ester precursor compounds described above was placed in 5 mL DMF and mixed with α -amino-protected L-lysine in the presence of 30 μmol N,N-diisopropylethylamine. The reaction mixture was stirred for 2 hours at RT and then concentrated *in vacuo* and next was purified by preparative HPLC. Then the protective group was removed by known methods.

10 Further purification and characterization of the conjugates according to the invention

After a successful reaction, the reaction mixture was concentrated by ultracentrifugation, for example, in some cases and then was desalinated and purified by chromatography using a Sephadex® G-25 column, for example. Elution was performed using phosphate-buffered saline solution (PBS), for example. Then the solution was sterile filtered and frozen. Alternatively, the
15 conjugate may be lyophilized.

B-2.4 Determining the toxophore load

The toxophore load was determined as follows on the resulting solutions of the conjugates in PBS buffer as described in the exemplary embodiments:

20 The toxophore load of lysine-linked ADCs was determined by mass spectrometric determination of the molecular weights of the individual conjugated species. First, the antibody conjugates were deglycosylated by PNGaseF, then the sample was acidified; next, after HPLC separation, the sample was analyzed by mass spectrometry using ESI MicroTof_Q (Bruker Daltonik). All the spectra over the signal in the TIC (total ion chromatogram) were added up and the molecular weights of the various conjugate species were calculated based on MaxEnt deconvolution. The
25 DAR (drug/antibody ratio) was calculated after signal integration of the various species.

For protein identification, after deglycosylation and/or denaturing, tryptic digestion was performed, in addition to determination of the molecular weight, the identity of the protein being confirmed on the basis of the tryptic peptides identified after denaturing, reduction and derivatization.

- 5 The toxophore load of cysteine-linked conjugates was determined by reversed-phase chromatography of the reduced and denatured ADCs. Guanidinium hydrochloride (GuHCl, 28.6 mg) and a solution of DL-dithiothreitol (DTT, 500 mM, 3 μ L) were added to the ADC solution (1 mg/mL, 50 μ L). The mixture was then incubated for one hour at 55°C and analyzed by HPLC.

10 The HPLC analysis was performed on an adjutant 1260 HPLC system with detection at 220 nm, using a Polymer Laboratories PLRP-S polymeric reversed-phase column (catalog no. PL1912-3802) (2.1 $\times$ 150 mm, 8 μ m particle size, 1000 Å) at a flow rate of 1 mL/min with the following gradient: 0 min, 25% B; 3 min, 25% B; 28 min, 50% B. Eluent A was 0.05% trifluoroacetic acid (TFA) in water, and eluent B was 0.05% trifluoroacetic acid in acetonitrile.

15 The peaks detected were assigned based on a comparison of the retention times with the light chain (L0) and the heavy chain (H0) of the unconjugated antibody. Peaks detected exclusively in the conjugated sample were assigned to the light chain with one toxophore (L1) and to the heavy chains with one, two and three toxophores (H1, H2, H3).

20 The average toxophore load of the antibody was calculated from the peak areas determined by integration as two times the sum of the integration results of all peaks, weighted by the number of toxophores, divided by the total of the integration results of all peaks with simple weighting. In isolated cases, it may happen that the toxophore load cannot be determined accurately due to co-elution of some peaks.

B-2.5 Testing the antigen binding of the ADC

25 The ability of the binder to bind to the target molecule was tested after successful coupling. Those skilled in the art are familiar with a variety of methods for doing so; for example, the affinity of the conjugate can be tested by ELISA technology or surface plasmon resonance analysis (BIAcore™ measurements). The skilled person can measure the conjugate concentration using conventional methods, e.g., by protein determination for antibody conjugates (see also Doronina et al., *Nature Biotechnol.* 2003; **21**:778-784 and Polson et al., *Blood* 2007, **110**:616-30 623).

B3 Synthesis of antibody-drug conjugates (ADCs)

B-3.1 General method for generating anti-C4.4a antibodies

The anti-C4.4a antibodies described by the sequences according to Table 1 and Table 2 were generated by screening of a phage display library for recombinant human C4.4a SEQ ID NO: 1 and murine C4.4a SEQ ID NO: 2 and for C4.4a-expressing cells. The antibodies thereby
5 obtained were reformatted into the human IgG1 format and used for the exemplary embodiments described here.

B-3.2 General method for expression of anti-C4.4a antibodies in mammalian cells

The antibodies, e.g., M31-B01 (light chain SEQ ID NO: 346 and heavy chain SEQ ID NO: 347) or other antibodies according to Table 2, were produced in mammalian cell culture. To do so,
10 HEK293 6E cells were transfected transiently using a suitable CMV promoter-based expression plasmid. The heavy and light chains of the antibodies were cloned either together in a single-vector system or separately in a two-vector system. This cell culture standard was up to 1.5 L in a shaken flask or 10 L in a Wave Bag. The cells were expressed for 5-6 days at 37°C in F17 medium (Invitrogen) supplemented with tryptone TN1 (Organotechnie) with 1% FCS ultralow
15 IgG (Invitrogen) and 0.5 mM valproic acid. Expression yields were between 100 mg/L and 600 mg/L.

B-3.3 General method for purification of antibodies from cell supernatants

The antibodies, e.g., M31-B01 (light chain SEQ ID NO: 346 and heavy chain SEQ ID NO: 347) or additional antibodies according to Table 2 were obtained from the cell culture supernatants.
20 The cell supernatants were clarified of cells by centrifugation. Then the cell supernatant was purified by affinity chromatography on a MabSelectSure (GE Healthcare) chromatography column. To do so, the column was equilibrated in DPBS, pH 7.4 (Sigma/Aldrich), the cell supernatant was applied and the column was washed with approx. 10 column volumes of DPBS, pH 7.4, + 500 mM NaCl. The antibodies were eluted in 50 mM sodium acetate, pH 3.5, +
25 500 mM NaCl and then purified further by gel filtration chromatography on a Superdex 200 column (GE Healthcare) in DPBS, pH 7.4.

B-3.4 General method for coupling to cysteine side chains

The following antibodies were used in the coupling reactions:

anti-C4.4a M31-B01

30 anti-C4.4a B01-3

anti-C4.4a B01-10

anti-C4.4a B01-7

anti-C4.4a D02-4

anti-C4.4a D02-6

5 anti-C4.4a D02-7

Three equivalents of tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) dissolved in PBS buffer were added to a solution of the corresponding antibody in PBS buffer in the concentration range between 1 mg/mL and 10 mg/mL and stirred for one hour at RT. Next, between 2 and 10 eq. of the maleimide precursor compound from intermediates 128, 129, 132-146, 148-155, 10 157, 159-161, 166, 171, 175-177, 184, 188, 190, 194-195, 199-201, 205, 209, 223-224, 226, 228-231, 236 and 244 to be coupled, depending on the desired load, were added as a solution in DMSO. The amount of DMSO should not exceed 10% of the total volume. The batch was stirred for 60-120 minutes at RT and then applied to PD10 columns (Sephadex® G-25, GE Healthcare) equilibrated in PBS and eluted with PBS buffer. If necessary, further concentration was 15 performed by ultracentrifugation.

Unless otherwise indicated, 5 mg of the corresponding antibody in PBS buffer was generally used for reduction and for the subsequent coupling. After purification on the PD10 column, solutions of the corresponding ADC in 3.5 mL PBS buffer were obtained. The protein concentration indicated was then determined for each of these solutions. In addition, the load of 20 the antibody (drug/mAb ratio) was determined according to the methods described below.

The immunoconjugates prepared in Examples 163-165, 167-192, 194-198, 200-221, 223-228, 230-232, 242, 244-247, 249, 250, 254-257, 259-260, 269, 271-275, 371 and 385 were produced by this method.

In the structural formulas shown, AK_{5A} through AK_{5G} have the meanings given below:

25 AK_{5A} = anti-C4.4a antibody M31-B01 (partially reduced)-S§¹

AK_{5B} = anti-C4.4a antibody B01-3 (partially reduced)-S§¹

AK_{5C} = anti-C4.4a antibody B01-10 (partially reduced)-S§¹

AK_{5D} = anti-C4.4a antibody B01-7 (partially reduced)-S§¹

AK_{SE} = anti-C4.4a antibody D02-4 (partially reduced)-S§¹

AK_{SF} = anti-C4.4a antibody D02-6 (partially reduced)-S§¹

AK_{SG} = anti-C4.4a antibody D02-7 (partially reduced)-S§¹

wherein

5 §¹ denotes the linkage to the succinimide group

and

S stands for the sulfur atom of a cysteine radical of the partially reduced antibody.

B-3.5 General method for coupling to lysine side chains:

The following antibodies were used in the coupling reactions:

10 anti-C4.4a antibody M31-B01

anti-C4.4a antibody B01-3

To a solution of the corresponding antibody in PBS buffer in the concentration range between 1 mg/mL and 10 mg/mL, between 2 and 5 eq. of the precursor compound to be coupled, depending on the desired load, from the intermediates 104, 110, 115, 116, 127, 130, 131, 147, 156, 158, 162, 169, 178, 185, 190, 202, 206, 210-216, 218-219, 227, 233, 238, 240, 242, 245, 247a and 247b were added as a solution in DMSO. After stirring for 30 minutes at RT, the same amount of precursor compound in DMSO was added again. In doing so, the amount of DMSO should not exceed 10% of the total volume. After stirring for 30 minutes more at RT, the batch was applied to PD10 columns (Sephadex[®] G-25) and eluted with PBS buffer. The batch was optionally concentrated further by ultrafiltration. For better separation of low-molecular components, the ultrafiltration concentration step was repeated after diluting with PBS buffer again, if necessary.

Usually, unless otherwise indicated, 5 mg of the corresponding antibody in PBS buffer was used for coupling. After purification on the PD10 column, solutions of the corresponding ADC in 3.5 mL PBS buffer were thus obtained. Then the specific protein concentration indicated was determined for these solutions, and the antibody load (drug/mAb ratio) was determined according to the methods described below.

The immunoconjugates synthesized in Examples 166, 193, 199, 222, 229, 243, 248, 251- 253, 258, 261-268, 270, 276, 370, 372-373 and 386-388 were prepared by this method.

In the structural formulas given, AK_{6A} and AK_{6B} have the following meanings

AK_{6A} = anti-C4.4a antibody M31-B01-NH§²

5 AK_{6B} = anti-C4.4a antibody B01-3-NH§²

wherein

§² denotes the linkage to the carbonyl group

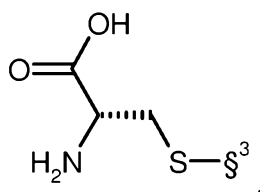
and

NH stands for the side chain amino group of a lysine radical of the antibody.

10 **B-3.6** General method for synthesis of cysteine adducts:

10 µmol of the maleimide precursor compounds described above was placed in 3 mL DMF and mixed with 2.1 mg (10 µmol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and purified by preparative HPLC.

Cys in the structural formulas given has the meaning



15

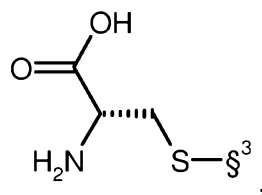
wherein

§³ denotes the linkage to the linker toxophore unit.

B-3.6 General method 2.3a for synthesis of cysteine adducts:

20 10 µmol of the maleimide precursor compounds described above was placed in 3 mL DMF and mixed with 2.1 mg (10 µmol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and purified by preparative HPLC.

Cys in the structural formulas given has the meaning



wherein

§³ denotes the linkage to the linker toxophore unit.

5 Further purification and characterization of the conjugates according to the invention

After successful reaction, the reaction mixture was concentrated further in some cases by ultracentrifugation, for example, and then was desalinated and purified by chromatography, for example, using a Sephadex® G-25 column. Then elution was performed using phosphate-buffered saline solution (PBS), for example. Then the solution was sterile filtered and frozen.

10 Alternatively, the conjugate may be lyophilized.

B-3.7 Determining the toxophore load

The toxophore load was determined as follows on the resulting solutions of the conjugates in PBS buffer, as described in the exemplary embodiments:

The toxophore load of lysine-linked ADCs was determined by mass spectrometric determination
 15 of the molecular weights of the individual conjugated species. The antibody conjugates were first deglycosylated by PNGaseF, the sample was acidified and then, after HPLC separation, the sample was analyzed by mass spectrometry using ESI-MicroTofQ (Bruker Daltonik). All the spectra over the signal in the TIC (total ion chromatogram) were added up and the molecular weights of the various conjugate species were calculated based on MaxEnt deconvolution. The
 20 DAR (drug/antibody ratio) was then calculated after signal integration of the various species.

For protein identification, after deglycosylation and/or denaturing, in addition to determination of the molecular weight, tryptic digestion was performed, confirming the identity of the protein on the basis of the tryptic peptides identified after denaturing, reduction and derivatization.

The toxophore load of cysteine-linked conjugates was determined by reversed-phase
 25 chromatography of the reduced and denatured ADCs. Guanidinium hydrochloride (GuHCl, 28.6 mg) and a solution of DL-dithiothreitol (DTT, 500 mM, 3 µL) were added to the ADC

solution (1 mg/mL, 50 µL). The mixture was then incubated for one hour at 55°C and then analyzed by HPLC.

The HPLC analysis was performed on an adjutant 1260 HPLC system with detection at 220 nm, using a Polymer Laboratories PLRP-S polymeric reversed-phase column (catalog number
5 PL1912-3802) (2.1 × 150 mm, 8 µm particle size, 1000 Å) at a flow rate of 1 mL/min with the following gradient: 0 min, 25% B; 3 min, 25% B; 28 min, 50% B. Eluent A consisted of 0.05% trifluoroacetic acid (TFA) in water; eluent B consisted of 0.05% trifluoroacetic acid in acetonitrile.

The peaks detected were assigned on the basis of a comparison of the retention times with the
10 light chain (L0) and the heavy chain (H0) of the unconjugated antibody. Peaks detected exclusively in the conjugated sample were assigned to the light chain with one toxophore (L1) and to the heavy chains with one, two and three toxophores (H1, H2, H3). The average toxophore load of the antibody was calculated from the peak areas determined by integration as two times the sum of the integration results of all peaks, weighted by the number of toxophores, dividing by
15 the total of the integration results of all peaks with simple weighting. In isolated cases, it may happen that the toxophore load cannot be determined accurately due to co-elution of some peaks.

B-3.8 Testing the antigen binding of the ADC

The ability of the binder to bind to the target molecule was tested after successful coupling. Those skilled in the art are familiar with a variety of methods for doing so. For example, the
20 affinity of the conjugate can be tested by means of ELISA technology or surface plasmon resonance analysis (BIAcore™ measurements). The skilled person can measure the conjugate concentration using conventional methods, e.g., by protein determination for antibody conjugates (see also Doronina et al., *Nature Biotechnol.* 2003; **21**:778-784 and Polson et al., *Blood* 2007, **110**:616-623).

B4 Producing antibody-drug conjugates (ADCs)

The intermediates described above were linked to the anti-CA9 antibody (3ee9), for example, with the linkage taking place optionally by way of the cysteine or lysine side chains of the antibody protein using the methods described below.

B-4.1 General method for generating anti-CA9 antibodies

The antibodies to CA9, e.g., the antibody 3ee9, were obtained by panning the HuCAL GOLD phage display library for recombinant antigen. The Fab antibody fragments thereby isolated were recloned to the IgG format (WO 2007/070538 A2).

5 **B-4.2** General method for expression of anti-CA9 antibodies in mammalian cells and for purification

The anti-CA9 IgG antibodies, e.g., 3ee9, were expressed by transient transfection of HEK 293 and purified from their cell supernatants by methods familiar to those skilled in the art. These methods are described in WO 2007/070538 A2.

B-4.3 General method for coupling to cysteine side chains

10 To a solution of the corresponding anti-CA9 antibody, e.g., 3ee9, which may be present in PBS buffer or in Tris buffer in the concentration range between 1 mg/mL and 10 mg/mL, for example, three equivalents of *tris*-(2-carboxyethyl)phosphine hydrochloride (TCEP) dissolved in PBS buffer were added and stirred for one hour at RT. Then, depending on the desired load, between 2 and 10 eq. of the maleimide precursor compound or the halide precursor compound from
15 intermediates 102, 103, 105-109, 111-114, 117-126, 128, 129, 132-146, 148-155, 157, 159-161, 166, 171, 175-177, 184, 189, 194-195, 199-201, 205, 209, 223-224, 226, 228-231, 236 and 244 to be coupled were added as a solution in DMSO. The amount of DMSO should not exceed 10% of the total volume. The batch was stirred for 60-120 minutes at RT, then applied to PD10 columns (Sephadex® G-25, GE Healthcare) and eluted with PBS buffer. A further concentration
20 was optionally performed by ultracentrifugation. If necessary, for better separation of low-molecular components, the concentration step by ultrafiltration was repeated after diluting again with PBS buffer.

Unless otherwise indicated, 5 mg of the corresponding antibody was generally used in PBS buffer for reduction and subsequent coupling. After purification on the PD10 column, solutions
25 of the corresponding ADC in 3.5 mL PBS buffer were obtained. Then the respective protein concentration given was determined for each of these solutions. In addition, the antibody load (drug/mAb ratio) was determined by the methods described below.

According to this method the immunoconjugates synthesized in Examples 280-289, 291-302, 304-305, 313, 315-318, 320-321, 324-325, 327-328 and 330-331 were prepared.

30 In the structural formulas shown, AK₁ has the meaning



wherein

§¹ denotes the linkage to the succinimide group

3ee9 (partially reduced) stands for the partially reduced anti-CA9 3ee9 antibody,

and

5 S stands for the sulfur atom of a cysteine radical of the partially reduced antibody.

B-4.4 General method for coupling to lysine side chains

To a solution of the corresponding anti-CA antibody 3ee9 in PBS buffer in the concentration range between 1 mg/mL and 10 mg/mL, between two and five equivalents, depending on the
10 desired load, of the precursor compound of the intermediates 104, 110, 115, 116, 127, 130, 131, 147, 156, 158, 162, 169, 178, 185, 190, 202, 206, 210-216, 218, 219, 227, 233, 238, 240, 242, 245, 247a and 247b to be coupled were added as a solution in DMSO. After stirring for 30 minutes at RT, the same amount of precursor compound in DMSO was added again. The amount of DMSO should not exceed 10% of the total volume. After stirring for 30 minutes more
15 at RT, the batch was applied to PD10 columns (Sephadex[®] G-25) and eluted with PBS buffer. A further concentration step by ultrafiltration was optionally performed. Concentration by ultrafiltration was repeated, if necessary, after diluting with PBS buffer again for better separation of the low-molecular components.

Unless otherwise indicated, 5 mg of the corresponding antibody in PBS buffer was generally
20 used for coupling. After purification on the PD10 column, solutions of the corresponding ADC in 3.5 mL PBS buffer were obtained. Then the respective protein concentration indicated was determined for each of these solutions, and the antibody load (drug/mAb ratio) by the methods described below.

According to this method the immunoconjugates synthesized in Examples 290, 303, 306, 314,
25 319, 322-323, 326, 329, 332-333 and 384 were prepared.

In the structural formulas given, AK₂ has the meaning



wherein

§² denotes the linkage to the carbonyl group

anti-CA9 stands for the unreduced CA9 antibody 3ee9,

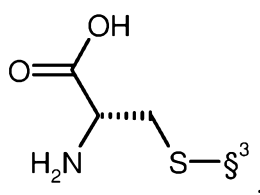
and

NH stands for the side chain amino group of a lysine radical of the antibody.

B-4.5a General method for synthesis of cysteine adducts

- 5 10 μmol of the maleimide precursor compounds described above was placed in 3 mL DMF and mixed with 2.1 mg (20 μmol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and purified by preparative HPLC.

Cys in the structural formulas given has the following meaning:



- 10 wherein

$\S^3$ denotes the linkage to the linker toxophore unit.

B-4.5b General method for synthesis of lysine adducts:

- 10 μmol of the active ester precursor compounds described above was placed in 5 mL DMF and mixed with α -amino-protected L-lysine in the presence of 30 μmol N,N-diisopropylethylamine.
- 15 The reaction mixture was stirred for 2 hours at RT and then concentrated *in vacuo* and next was purified by preparative HPLC. Then the protective group was removed by known methods.

Further purification and characterization of the conjugates according to the invention

- After successful reaction, in some cases the reaction mixture was concentrated by ultracentrifugation, for example, and then was desalinated and purified by chromatography for
- 20 example, using a Sephadex® G-25 column. Elution was performed using phosphate-buffered saline solution (PBS), for example. Then the solution was sterile filtered and frozen. Alternatively, the conjugate may be lyophilized.

B-4.6 Determining the toxophore load

The toxophore load was determined as follows on the resulting solutions of the conjugates in PBS buffer as described in the exemplary embodiments:

5 The toxophore load of lysine-linked ADCs was determined by mass spectrometric determination of the molecular weights of the individual conjugated species. The antibody conjugates were first deglycosylated by PNGaseF, the sample was acidified and then, after HPLC separation, the sample was analyzed by mass spectrometry using ESI MicroTof_Q (Bruker Daltonik). All the spectra over the signal in the TIC (total ion chromatogram) were added up and the molecular weights of the various conjugate species were calculated on the basis of MaxEnt deconvolution. After signal integration of the various species, the DAR (drug/antibody ratio) was calculated.

10 For protein identification, after deglycosylation and/or denaturing, tryptic digestion was performed in addition to determination of the molecular weight, with the identity of the protein being confirmed on the basis of the tryptic peptides identified after denaturing, reduction and derivatization.

15 The toxophore load of cysteine-linked conjugates was determined by reversed-phase chromatography of the reduced and denatured ADCs. Guanidinium hydrochloride (GuHCl, 28.6 mg) and a solution of DL-dithiothreitol (DTT, 500 mM, 3 µL) were added to the ADC solution (1 mg/mL, 50 µL). The mixture was then incubated for one hour at 55°C and then analyzed by HPLC.

20 HPLC analysis was performed on an adjutant 1260 HPLC system with detection at 220 nm, using a Polymer Laboratories PLRP-S polymeric reversed-phase column (catalog number PL1912-3802) (2.1 × 150 mm, 8 µm particle size, 1000 Å) at a flow rate of 1 mL/min with the following gradient: 0 min, 25% B; 3 min, 25% B; 28 min, 50% B. Eluent A was 0.05% trifluoroacetic acid (TFA) in water, and eluent B was 0.05% trifluoroacetic acid in acetonitrile.

25 The peaks detected were assigned based on a comparison of the retention times with the light chain (L0) and the heavy chain (H0) of the unconjugated antibody. Peaks detected exclusively in the conjugated sample were assigned to the light chain with one toxophore (L1) and to the heavy chains with one, two and three toxophores (H1, H2, H3).

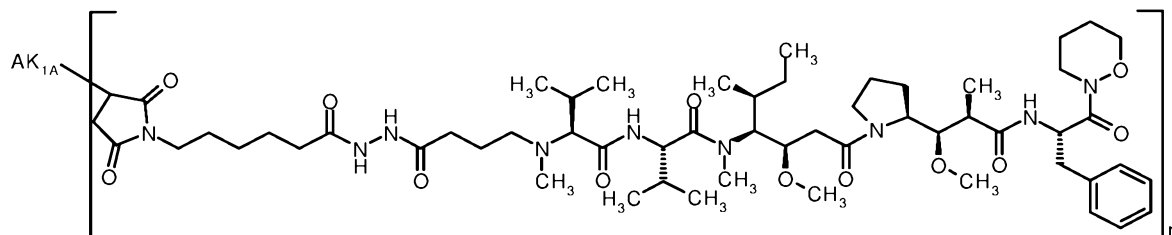
30 The average toxophore load of the antibody was calculated from the peak areas determined by integration as two times the sum of the integration results of all peaks, weighted by the number of toxophores, divided by the total of the integration results of all peaks with simple weighting. In isolated cases it may occur that the toxophore load cannot be determined accurately due to co-elution of some peaks.

B-4.7 Testing the antigen binding of the ADC

5 The ability of the binder to bind to the target molecule was tested after successful coupling. Those skilled in the art are familiar with a variety of methods for doing so; for example, the affinity of the conjugate can be tested by means of ELISA technology or surface plasmon resonance analysis (BIAcore™ measurements). The skilled person can measure the conjugate concentration using conventional methods, for example, by protein determination for antibody conjugates (see also Doronina et al., *Nature Biotechnol.* 2003; **21**:778-784 and Polson et al., *Blood* 2007, **1102**:616-623).

Embodiments

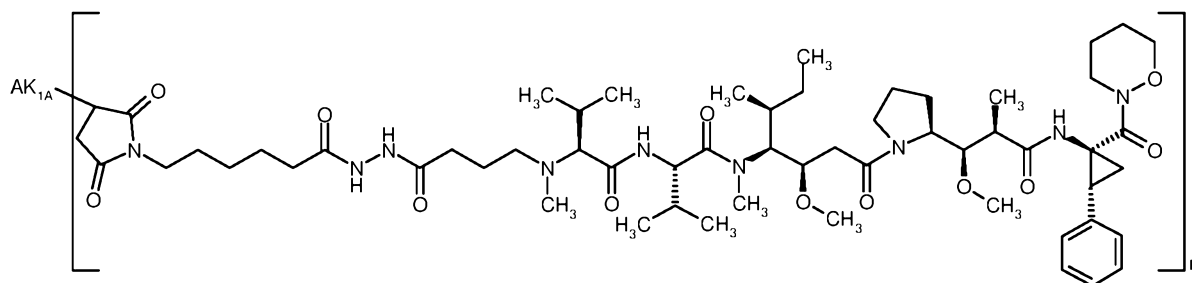
Example 1



5 Protein concentration: 1.27 mg/ml

Drug/mAb ratio: 1.5

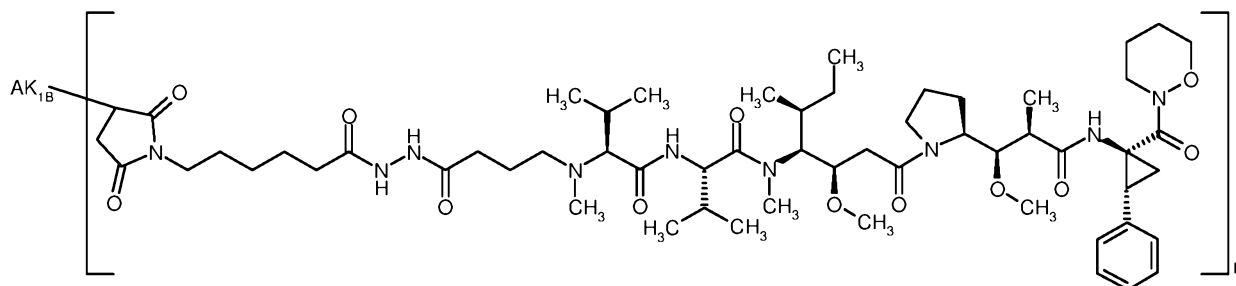
Example 2



10 Protein concentration: 0.64 mg/ml

Drug/mAb ratio: 3.3

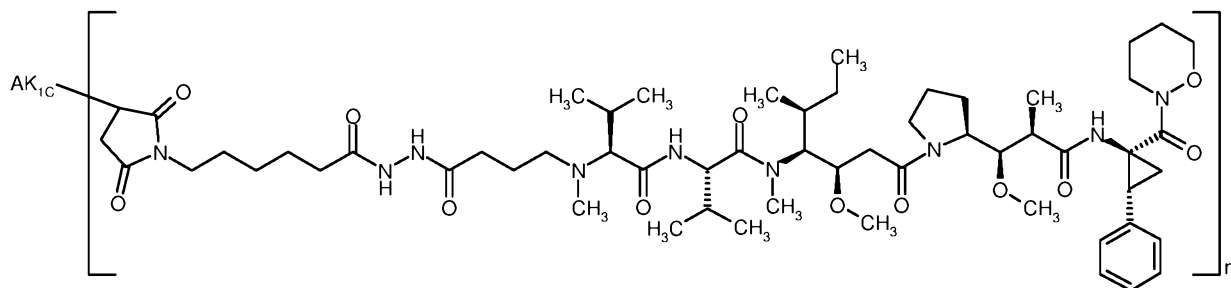
Example 3



Protein concentration: 0.61 mg/ml

Drug/mAb ratio: 5.1

Example 4

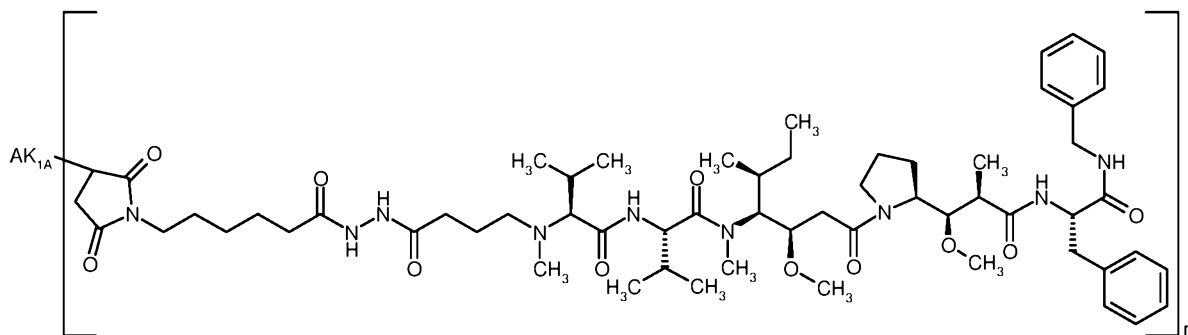


5

Protein concentration: 0.61 mg/ml

Drug/mAb ratio: not detectable

Example 5

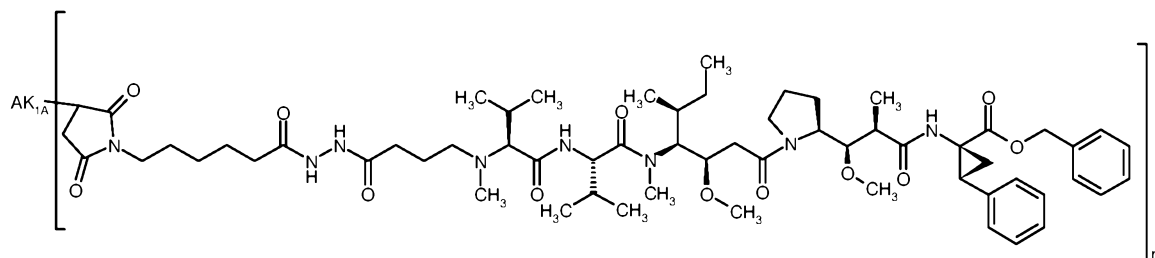


10

Protein concentration: 1.37 mg/ml

Drug/mAb ratio: 1.9

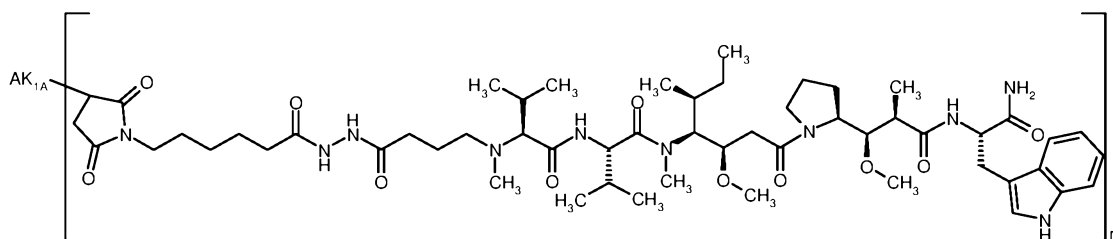
Example 6



Protein concentration: 1.12 mg/ml

Drug/mAb ratio: 1.3

5 Example 7

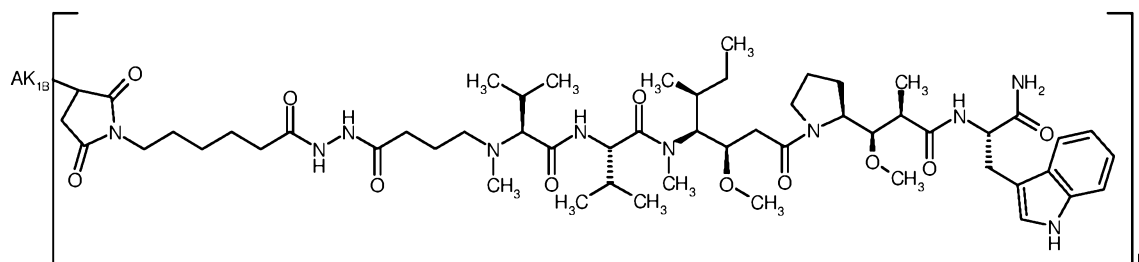


270 mg cetuximab in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation

Protein concentration: 10.46 mg/ml

10 Drug/mAb ratio: 2.8

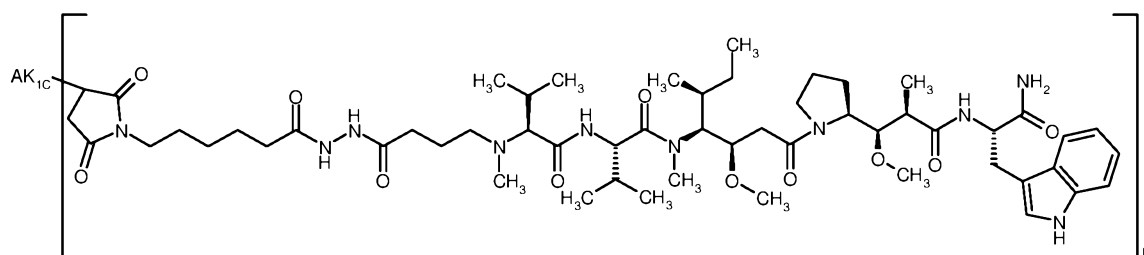
Example 8



Protein concentration: 1.86 mg/ml

Drug/mAb ratio: 2.4

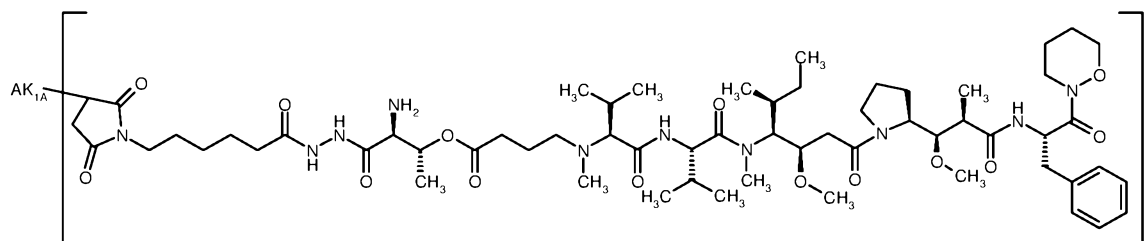
Example 9



Protein concentration: 1.39 mg/ml

5 Drug/mAb ratio: not detectable

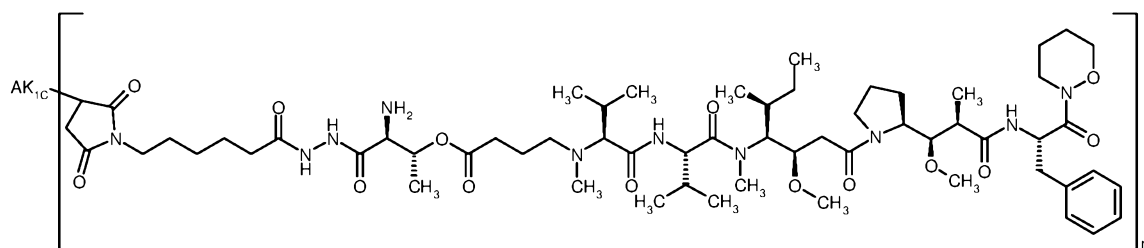
Example 10



Protein concentration: 0.66mg/ml

10 Drug/mAb ratio: 2.4

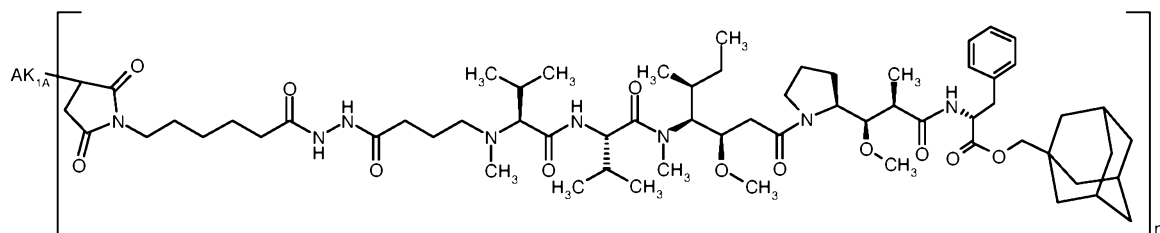
Example 11



Protein concentration: 0.66 mg/ml

Drug/mAb ratio: not detectable

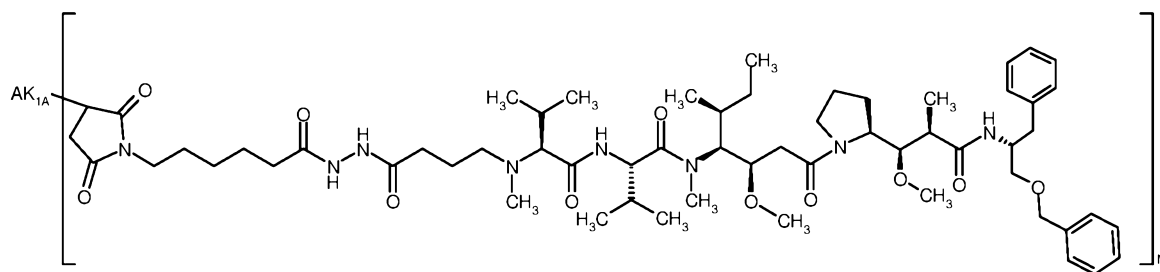
Example 12



Protein concentration: 0.9 mg/ml

5 Drug/mAb ratio: 1.1

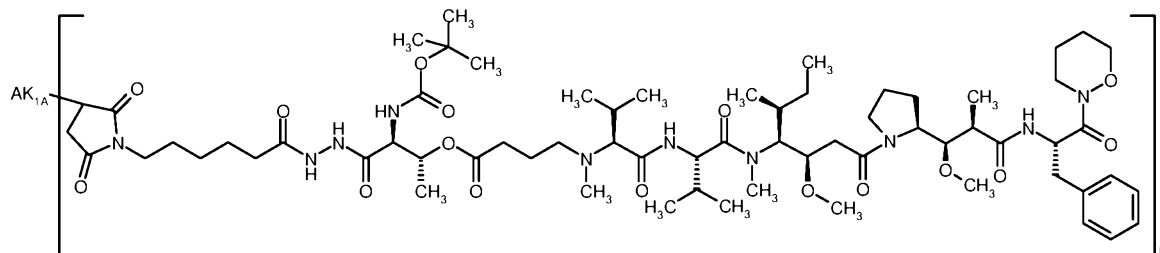
Example 13



Protein concentration: 1.52 mg/ml

10 Drug/mAb ratio: not detectable

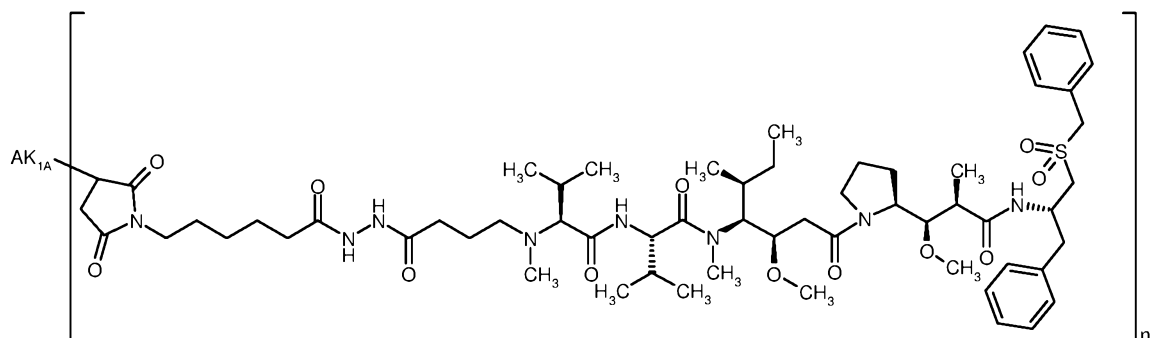
Example 14



Protein concentration: 1.44 mg/ml

Drug/mAb ratio:3.2

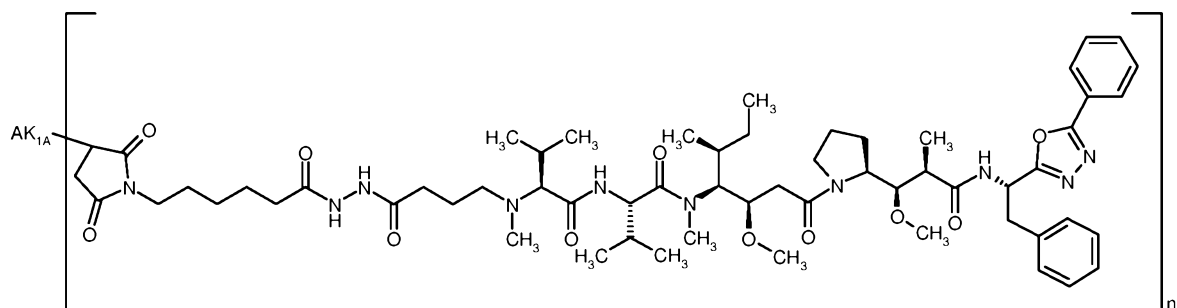
Example 15



5 Protein concentration: 1.23 mg/ml

Drug/mAb ratio:2.7

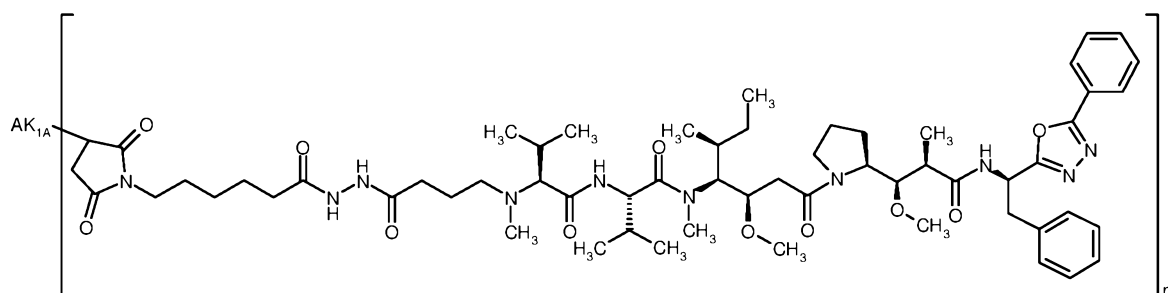
Example 16



10 Protein concentration: 1.27 mg/ml

Drug/mAb ratio:1.3

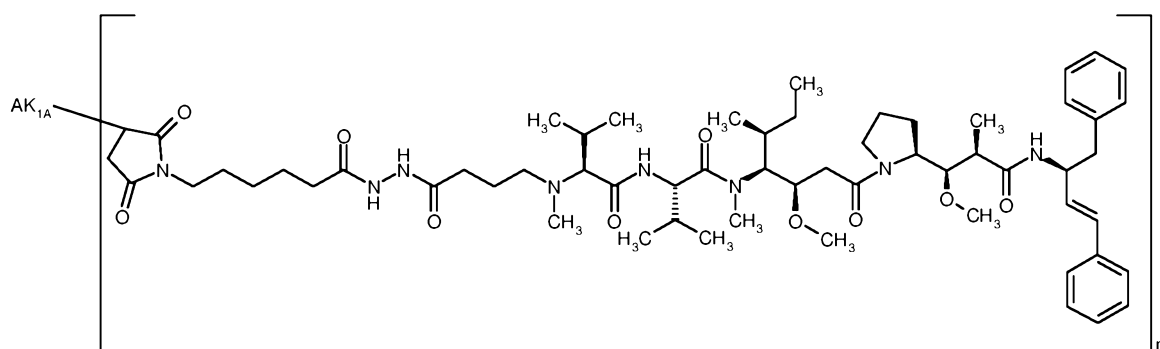
Example 17



Protein concentration: 1.61 mg/ml

5 Drug/mAb ratio:4.7

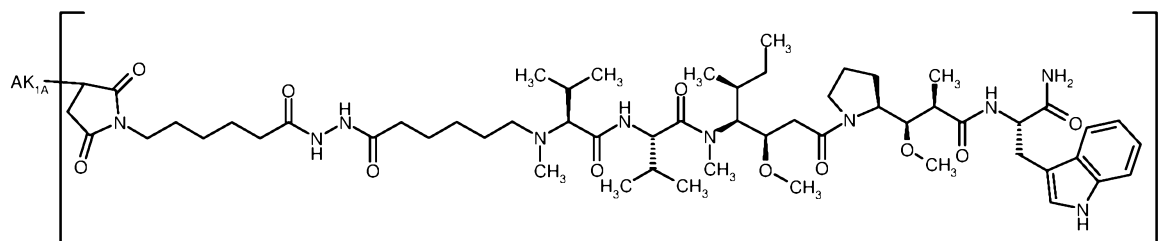
Example 18



Protein concentration: 1.24 mg/ml

10 Drug/mAb ratio:2.4

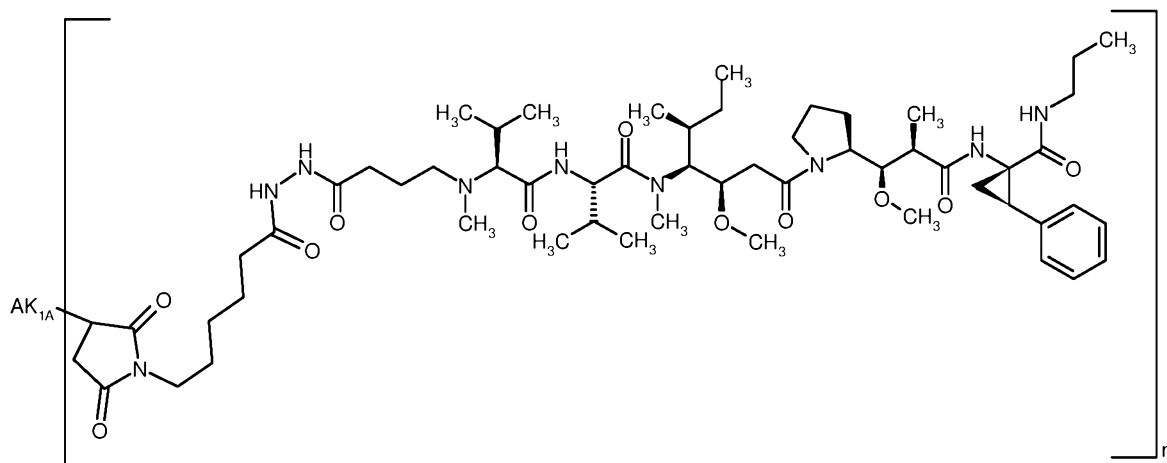
Example 19



Protein concentration: 1.49 mg/ml

Drug/mAb ratio: 1.9

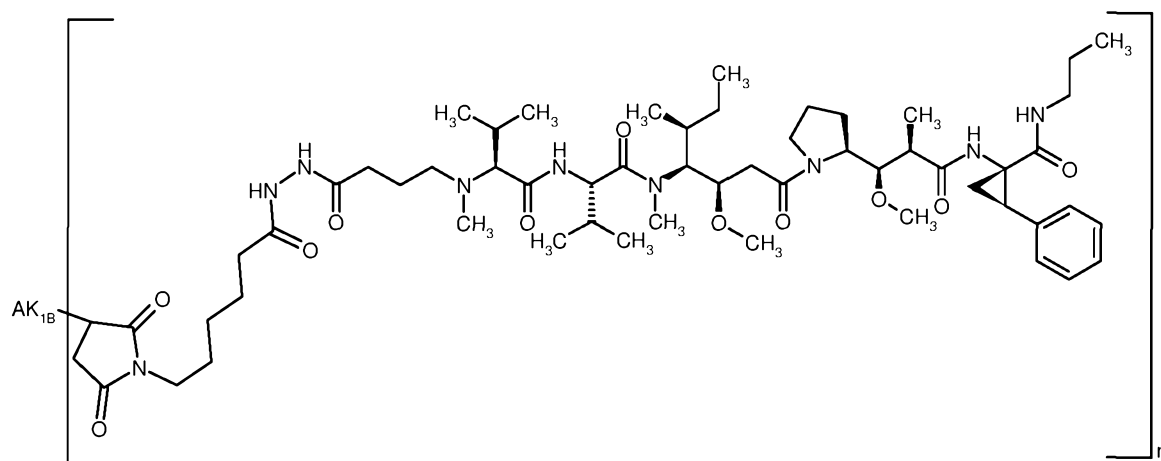
Example 20



5

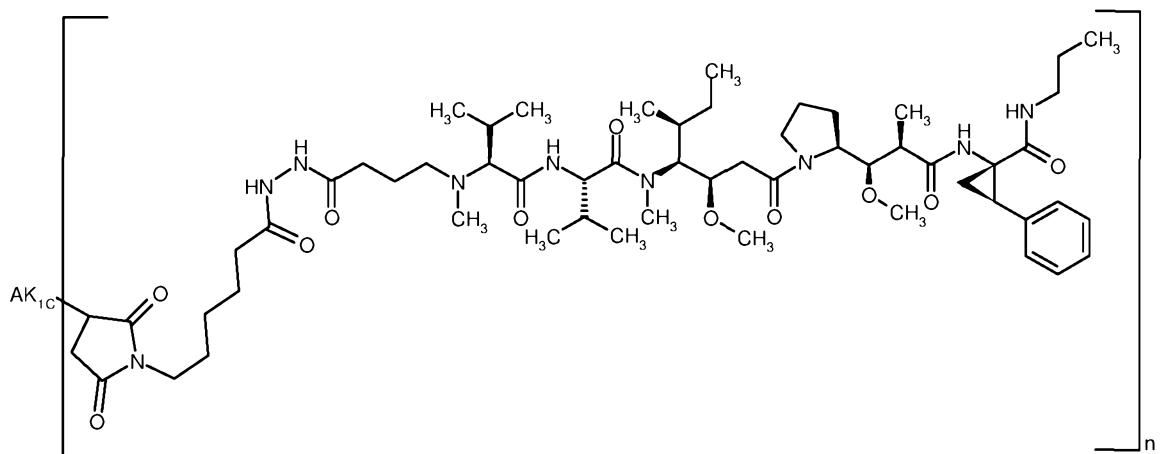
Protein concentration: 1.49 mg/ml

Drug/mAb ratio: 2.0

Example 21

Protein concentration: 1.46 mg/ml

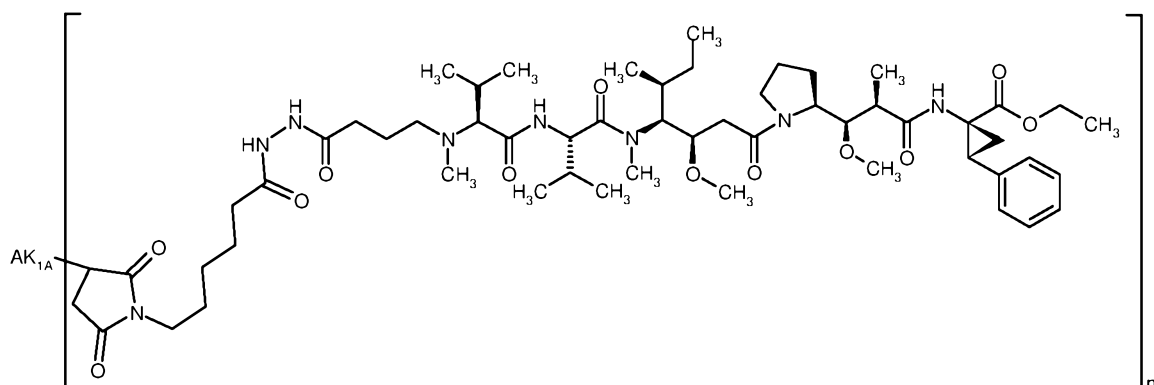
- 5 Drug/mAb ratio: >0.9

Example 22

Protein concentration: 1.28 mg/ml

- 10 Drug/mAb ratio: not detectable

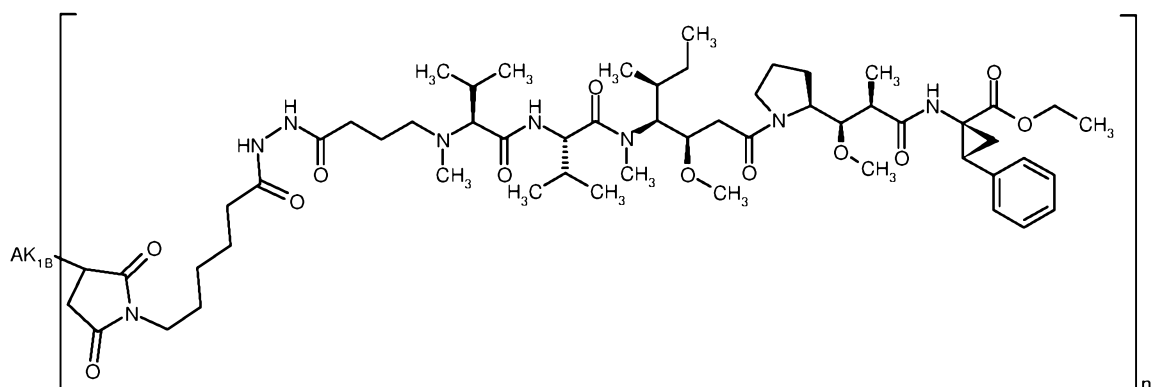
Example 23



Protein concentration: 1.33 mg/ml

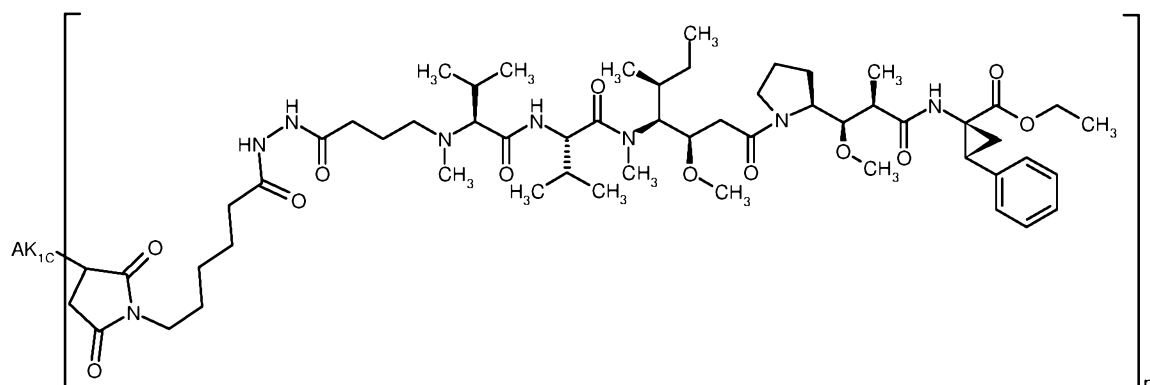
5 Drug/mAb ratio: 1.8

Example 24



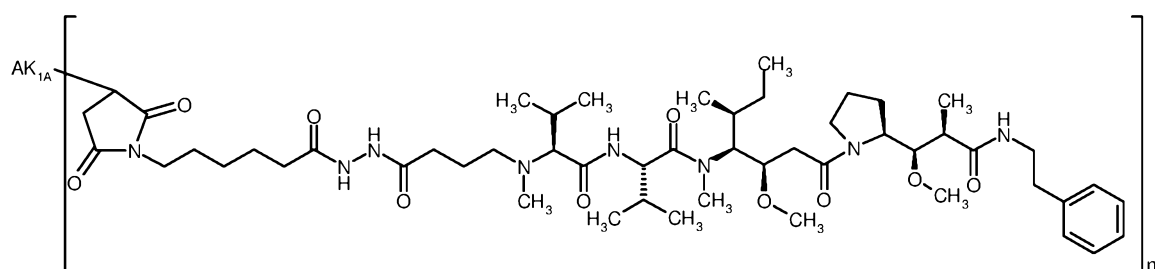
Protein concentration: 1.39 mg/ml

10 Drug/mAb ratio: >0.8

Example 25

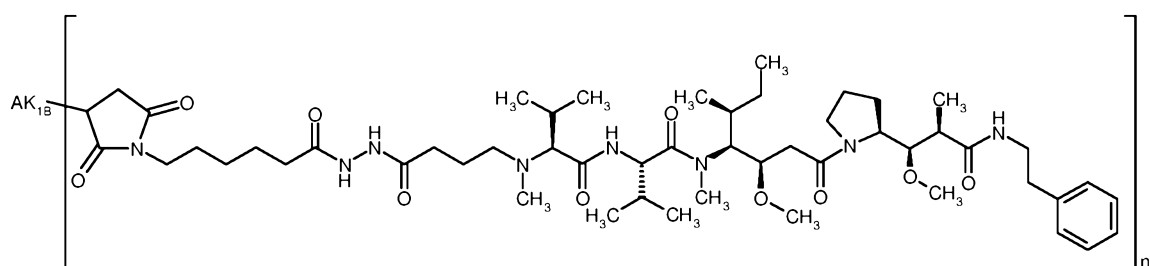
Protein concentration: 1.26 mg/ml

5 Drug/mAb ratio: not detectable

Example 26

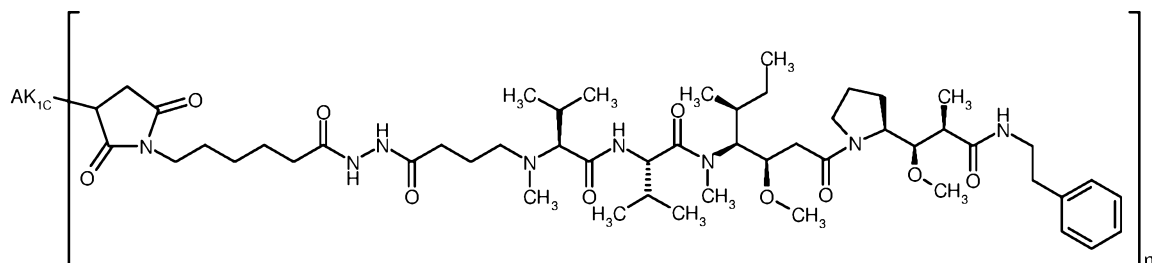
Protein concentration: 1.51 mg/ml

10 Drug/mAb ratio: 1.8

Example 27

Drug/mAb ratio: 1.8

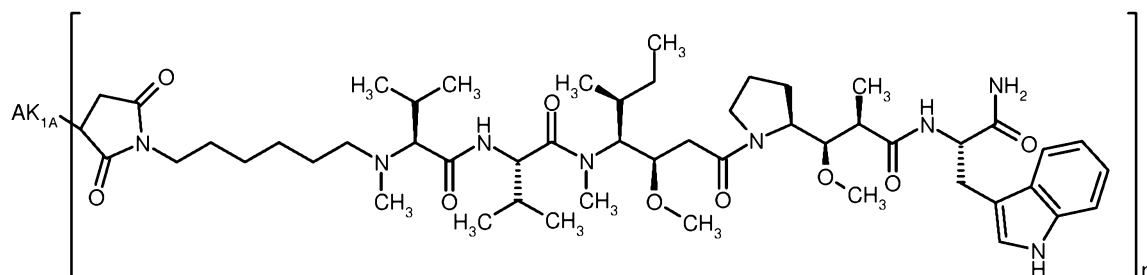
Example 28



5

Drug/mAb ratio: not detectable

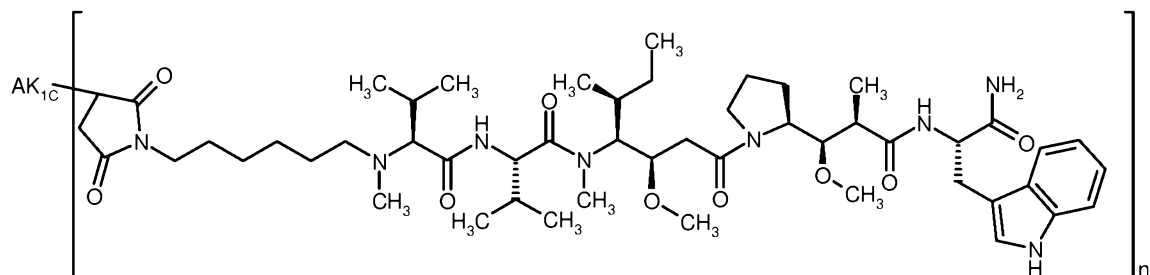
Example 29



10

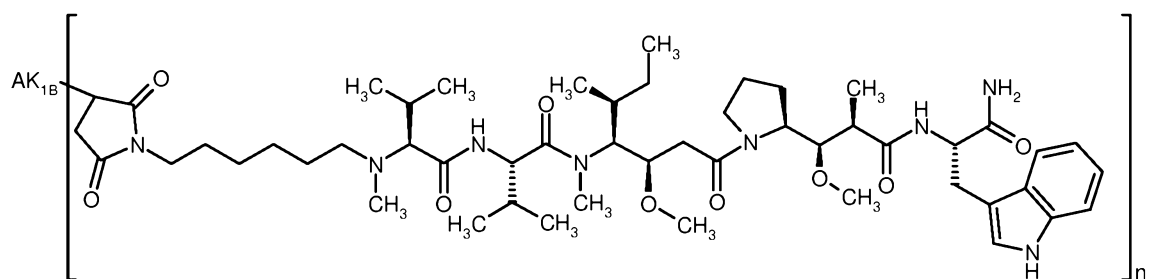
Drug/mAb ratio: 2.7

Example 30



Protein concentration: 1.4 mg/ml

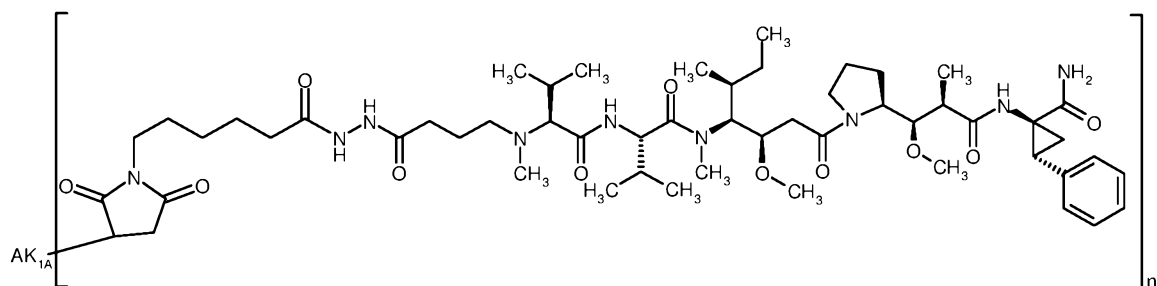
5 Drug/mAb ratio: not detectable

Example 31

Protein concentration: 1.66 mg/ml

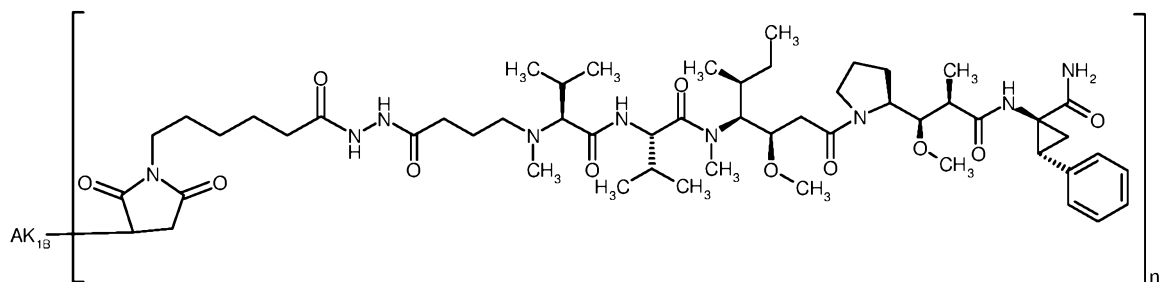
Drug/mAb ratio: 2.2

10 Example 32



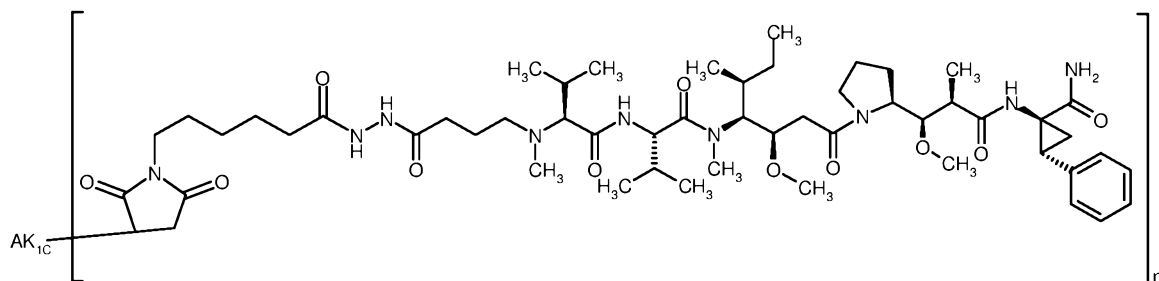
Protein concentration: 1.21 mg/ml

Example 33



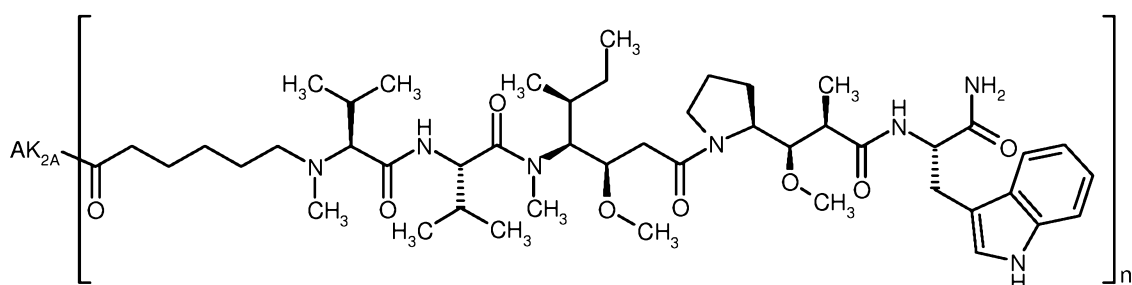
Drug/mAb ratio: 2

Example 34



Drug/mAb ratio: not detectable

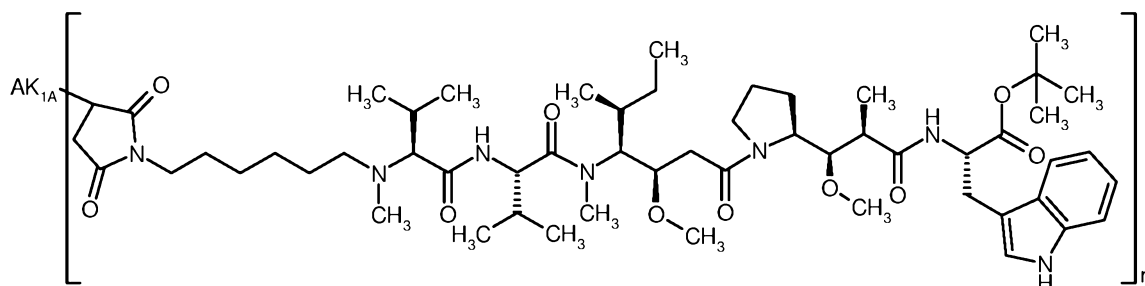
Example 35



Protein concentration: 1.66 mg/ml

Drug/mAb ratio: not detectable

Example 36

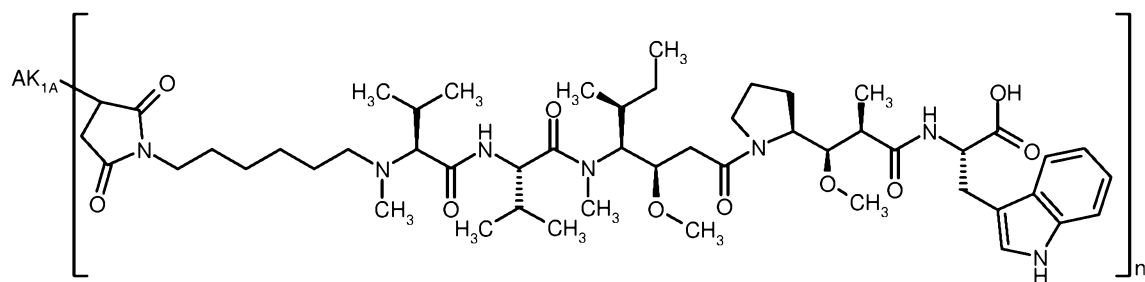


5

Protein concentration: 1.48 mg/ml

Drug/mAb ratio: 2.2

Example 37

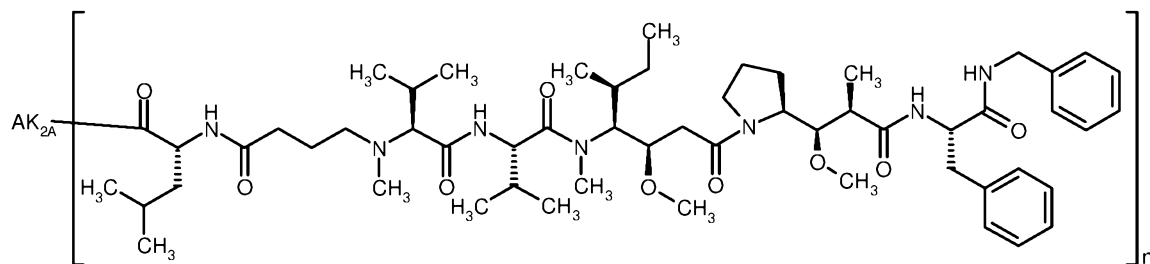


10

Protein concentration: 1.45 mg/ml

Drug/mAb ratio: 2.7

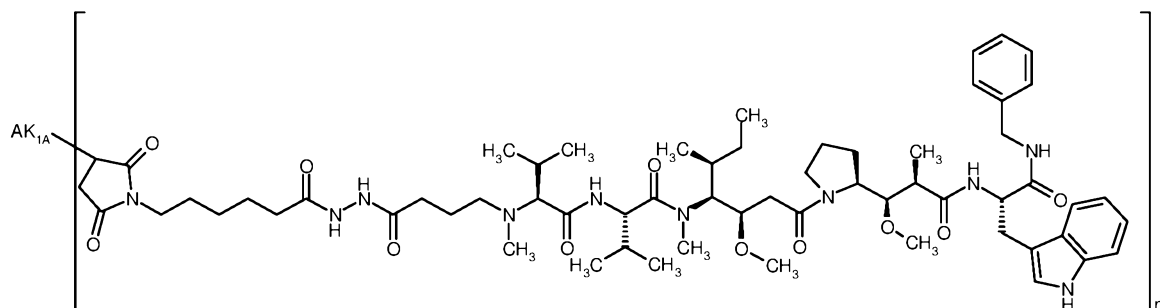
Example 38



Protein concentration: 1.5 mg/ml

5 Drug/mAb ratio: 0.15

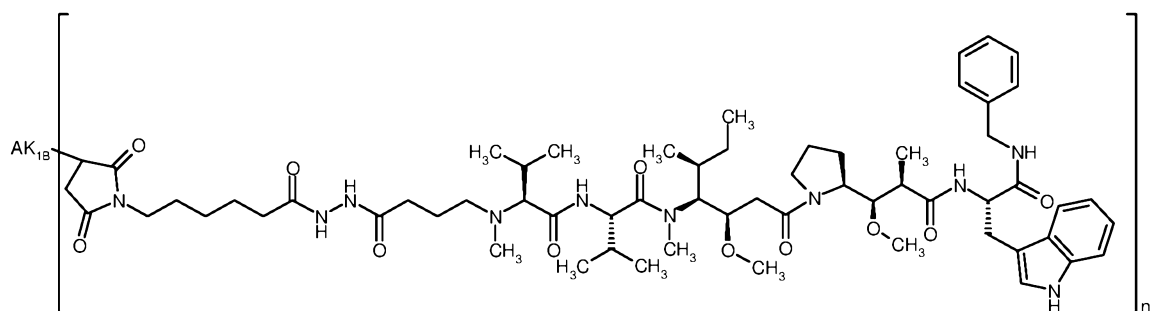
Example 39



Protein concentration: 1.5 mg/ml

10 Drug/mAb ratio: 2.1

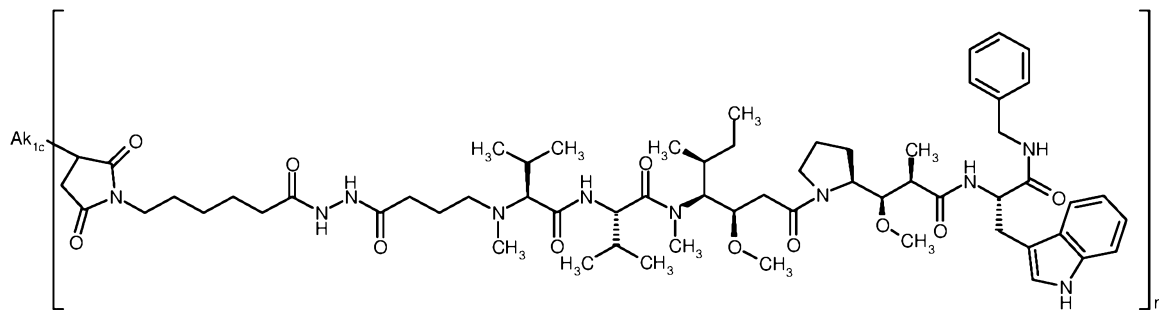
Example 40



Protein concentration: 1.54 mg/ml

Drug/mAb ratio:>0.9

Example 41

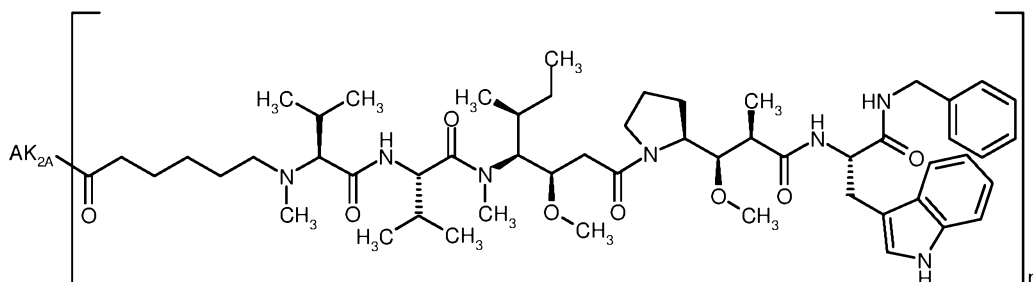


5

Protein concentration: 1.39 mg/ml

Drug/mAb ratio: not detectable

Example 42



10 Protein concentration: 1.52 mg/ml

Drug/mAb ratio: 1.5

[illegible]

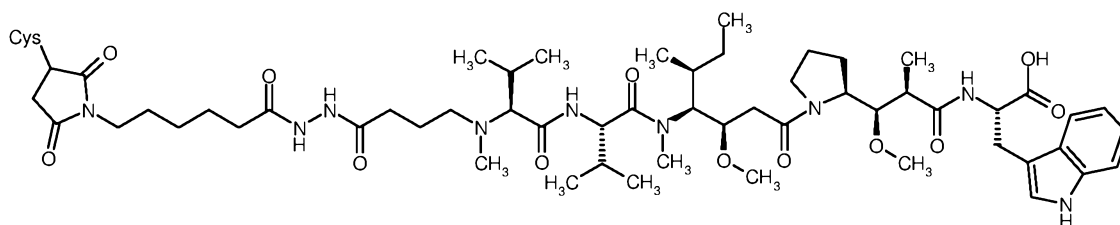
Drug/mAb ratio: 2.6

[illegible]

Drug/mAb ratio: 1.9

Example 45

N-(4-{2-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



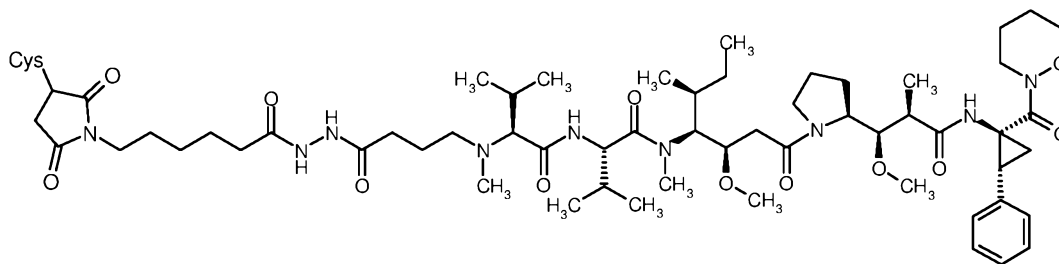
10 mg (10 μ mol) of Intermediate 113 weretaken up in 5.2 ml DMF and mixed with 2.28 mg (20 μ mol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 6 mg (54% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.5$ min;

LC-MS (Method 1): $R_t = 0.77$ min; MS (ESIpos): $m/z = 1185$ ($M+H$)⁺.

Example 46

N-(4-{2-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



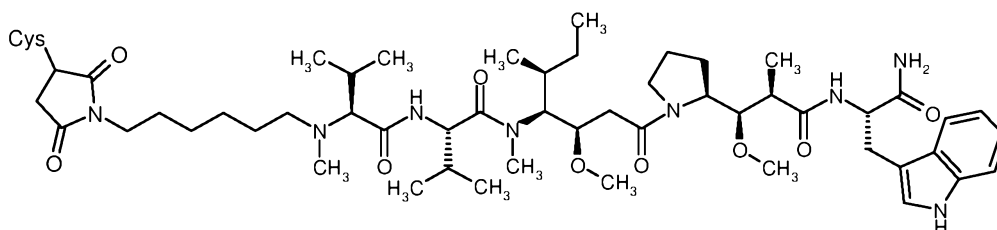
9 mg (8.3 μ mol) of Intermediate 132 weretaken up in 4 ml DMF and mixed with 3 mg (24.4 μ mol) L-cysteine. The batch was stirred overnight at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 6.8 mg (68% of theory) of the title compound.

HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.78$ min; MS (ESIpos): $m/z = 1227$ ($M+H$)⁺.

Example 47

N-[6-(3-[[[(2R)-2-amino-2-carboxyethyl]sulfanyl]-2,5-dioxopyrrolidin-1-yl]hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[[[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



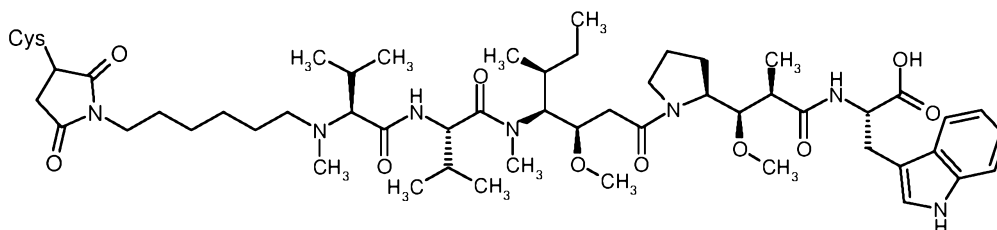
10 mg (10 μ mol) of Intermediate 106 weretaken up in 5.8 ml DMF and mixed with 2.5 mg (20 μ mol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 5.2 mg (46% of theory) of the title compound.

HPLC (Method 5): R_t = 1.5 min;

LC-MS (Method 11): R_t = 0.71 min; MS (ESIpos): m/z = 1070 ($M+H$)⁺.

Example 48

N-[6-(3-[[[(2R)-2-amino-2-carboxyethyl]sulfanyl]-2,5-dioxopyrrolidin-1-yl]hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[[[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



10 mg (10 μ mol) of Intermediate 124 weretaken up in 4 ml DMF and mixed with 2.5 mg (20 μ mol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next

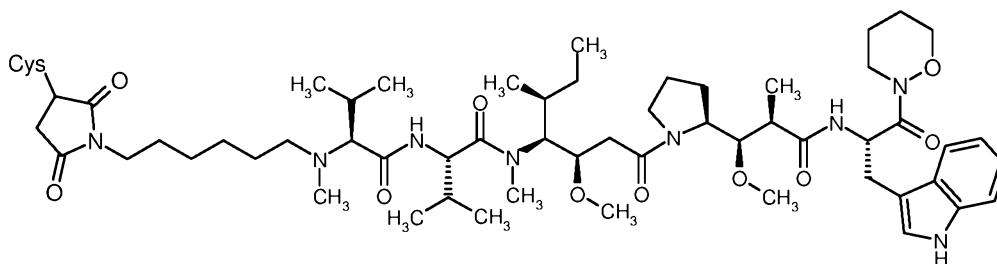
purified by means of preparative HPLC. The yield was 7.2 mg (64% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.8$ min; MS (ESIpos): $m/z = 1071$ ($M+H$)⁺.

5 Example 49

N-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



10

10 mg (10 μ mol) of Intermediate 125 were taken up in 4 ml DMF and mixed with 2.4 mg (20 μ mol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 7.7 mg (69% of theory) of the title compound.

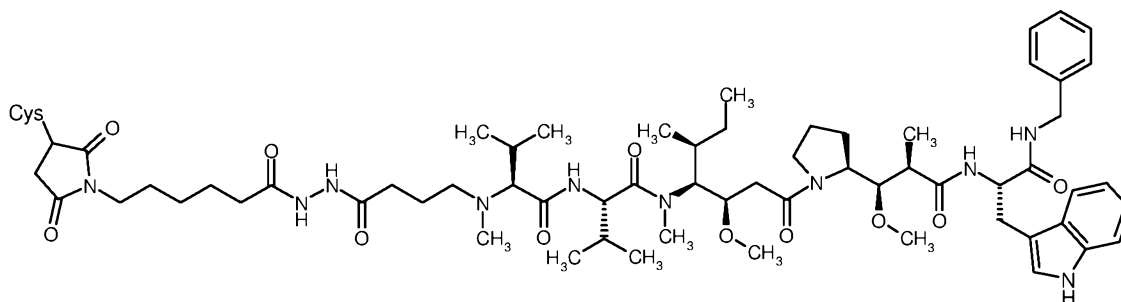
15 HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 2): $R_t = 1.91$ min; MS (ESIpos): $m/z = 1140$ ($M+H$)⁺.

Example 50

N-(4-{2-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-(benzylamino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

20



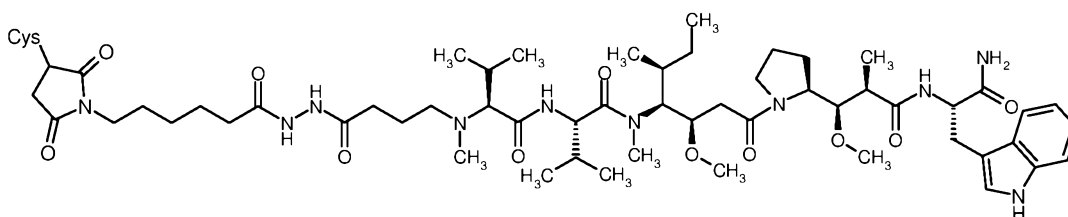
10 mg (10 μ mol) of Intermediate 160 weretaken up in 3 ml DMF and mixed with 2.1 mg (20 μ mol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 8.1 mg (73% of theory) of the title compound.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1274 ($M+H$)⁺.

Example 51

N-(4-{2-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

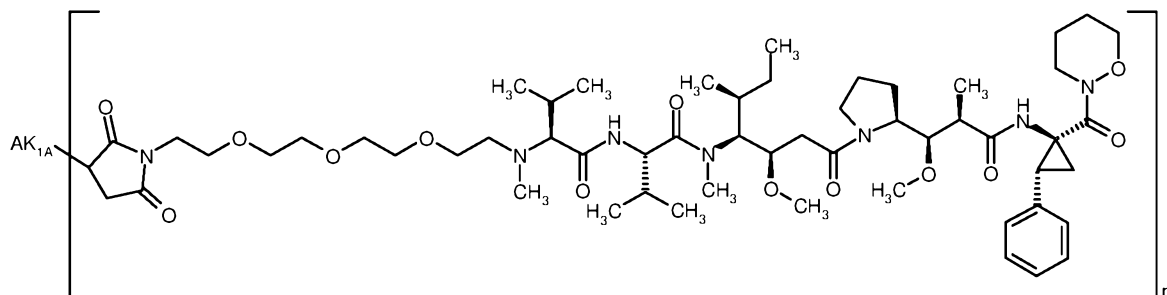


10 mg (10 μ mol) of Intermediate 157 weretaken up in 5.2 ml DMF and mixed with 2.28 mg (20 μ mol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 5.8 mg (48% of theory) of the title compound.

HPLC (Method 5): R_t = 1.45 min;

LC-MS (Method 1): R_t = 0.74 min; MS (ESIpos): m/z = 1184 ($M+H$)⁺.

Example 52

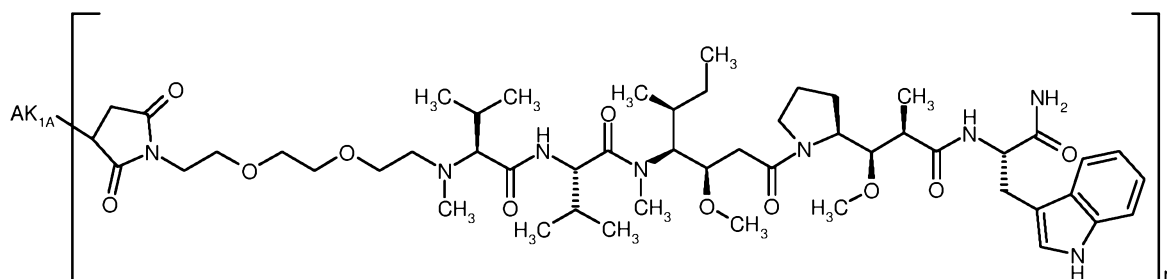


5 mg cetuximab in PBS were presently used for the coupling and, after purification on a
5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.73 mg/ml

Drug/mAb ratio: 2.8

Example 53



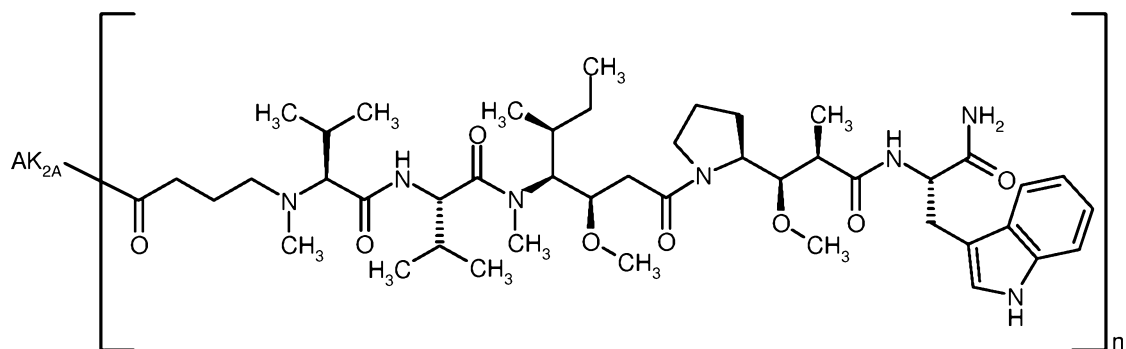
10

5 mg cetuximab in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 0.86 mg/ml

Drug/mAb ratio: 4.9

Example 54

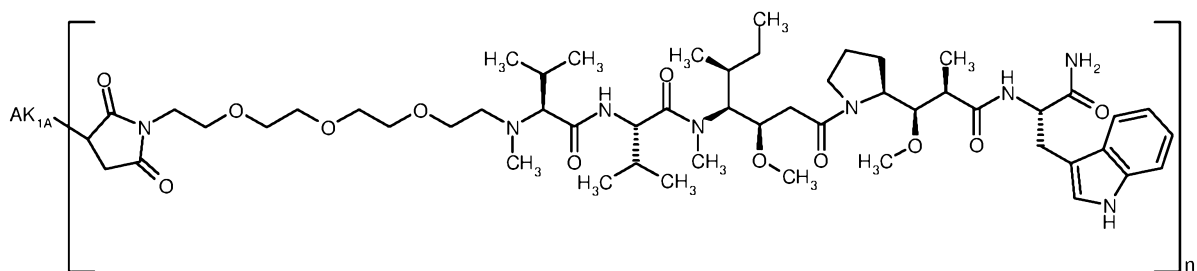


5 mg cetuximab in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

5 Protein concentration: 1.64 mg/ml

Drug/mAb ratio: 0.7

Example 55

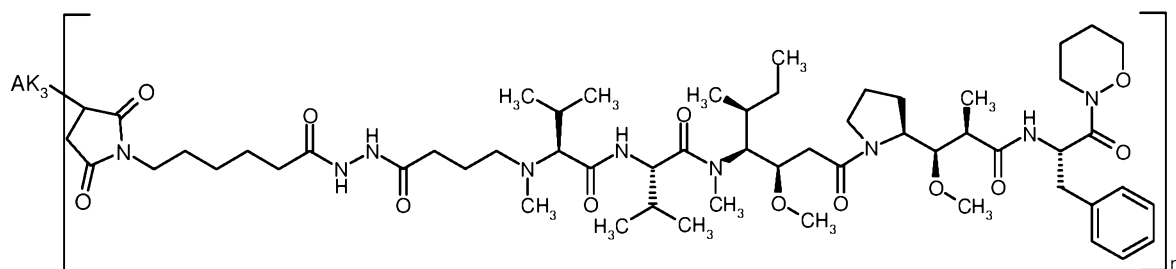


5 mg cetuximab in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.43 mg/ml

Drug/mAb ratio: 3.2

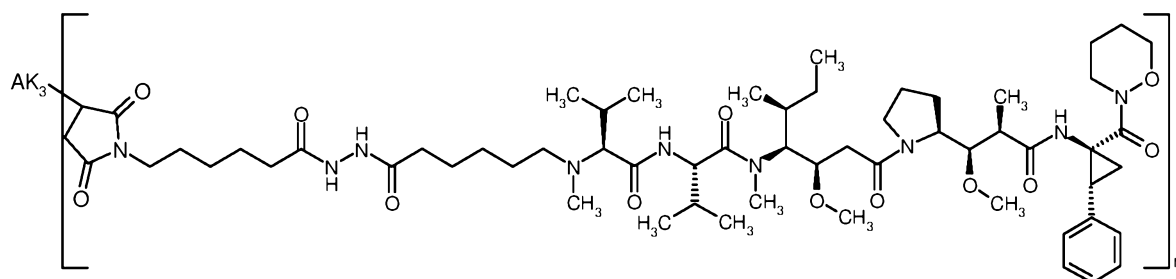
Example 56



Protein concentration: 0.96 mg/ml

5 Drug/mAb ratio: 3.1

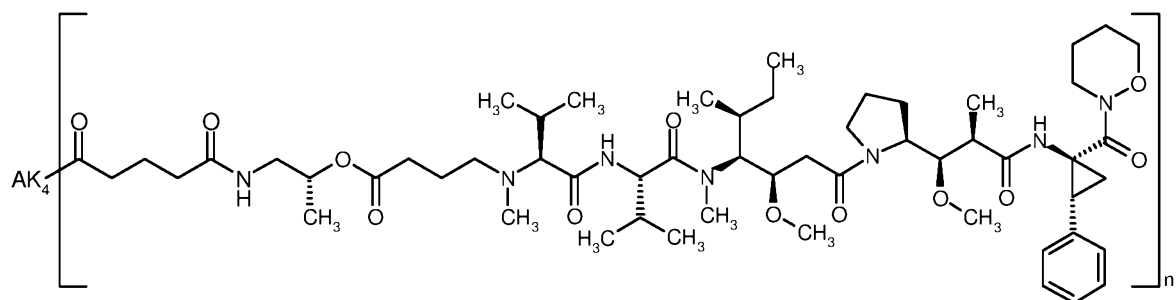
Example 57



Protein concentration: 0.44 mg/ml

10 Drug/mAb ratio: 4.6

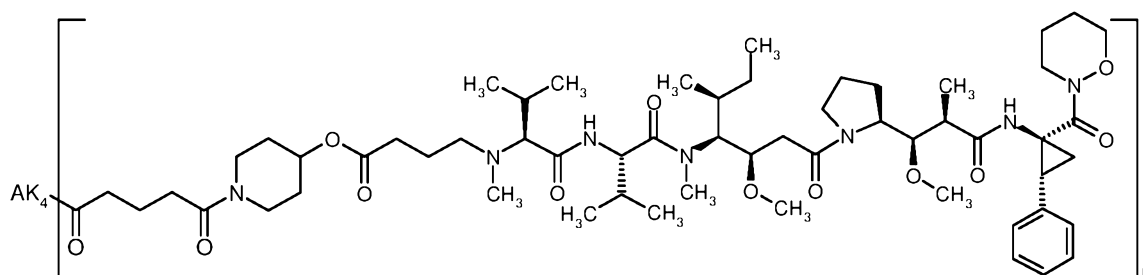
Example 58



Protein concentration: 1.09 mg/ml

Drug/mAb ratio: 2.1

Example 59

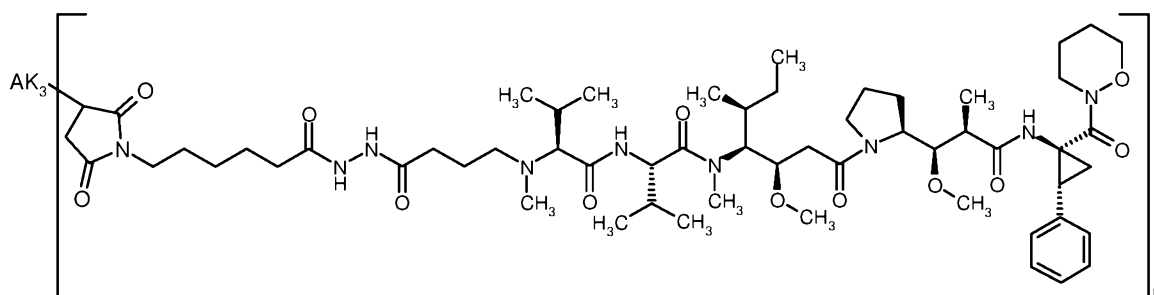


5

Protein concentration: 0.87 mg/ml

Drug/mAb ratio: 3.8

Example 60

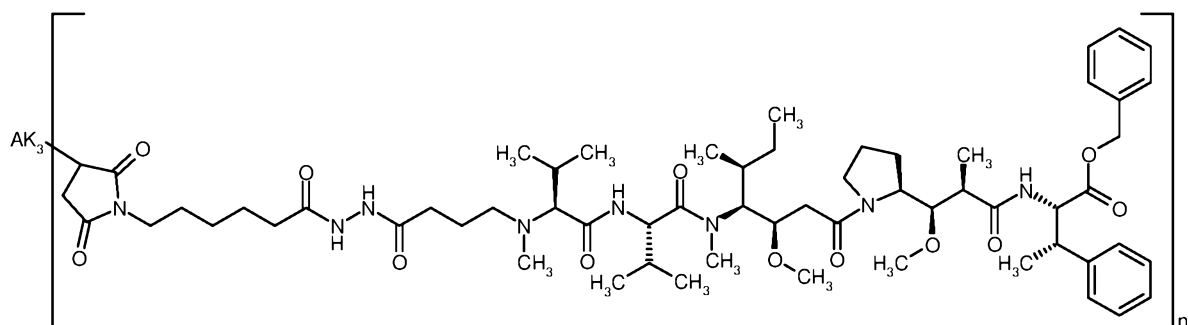


10

Protein concentration: 0.45 mg/ml

Drug/mAb ratio: 6.5

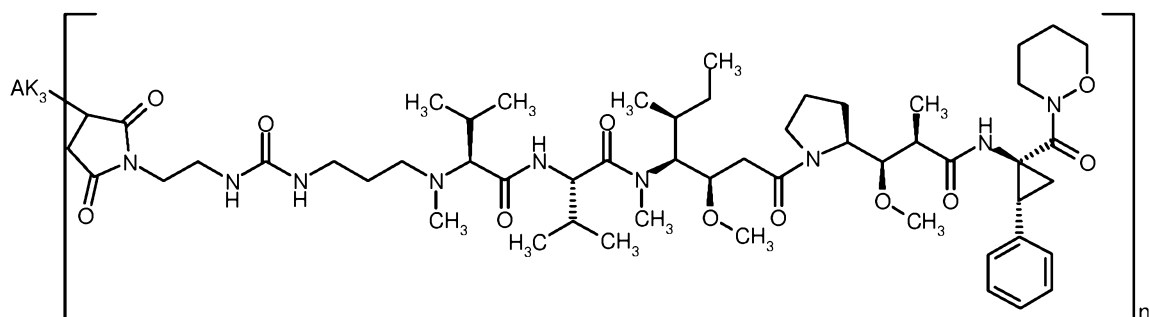
Example 61



Protein concentration: 0.15 mg/ml

5 Drug/mAb ratio: 3.1

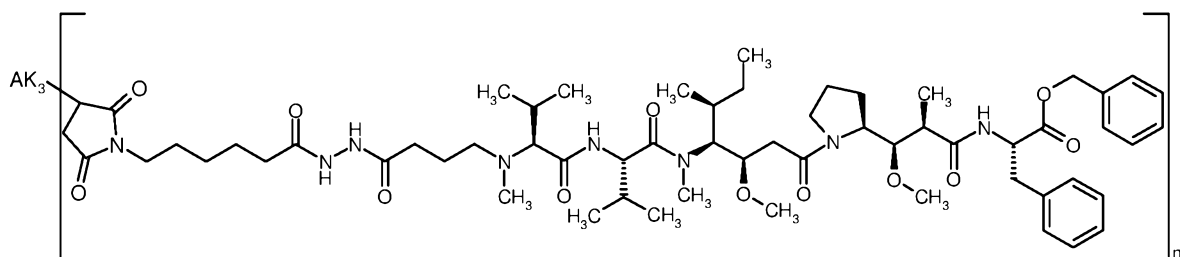
Example 62



Protein concentration: 0.94 mg/ml

10 Drug/mAb ratio: 2.8

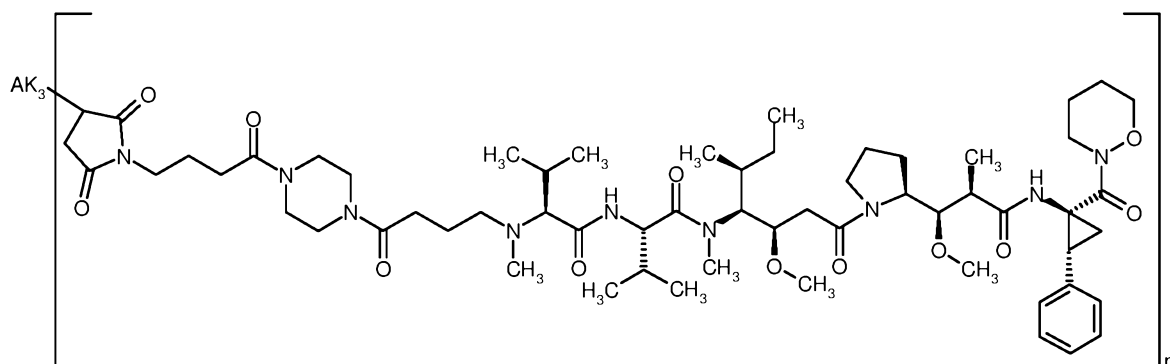
Example 63



Protein concentration: 0.45 mg/ml

Drug/mAb ratio: 0.9

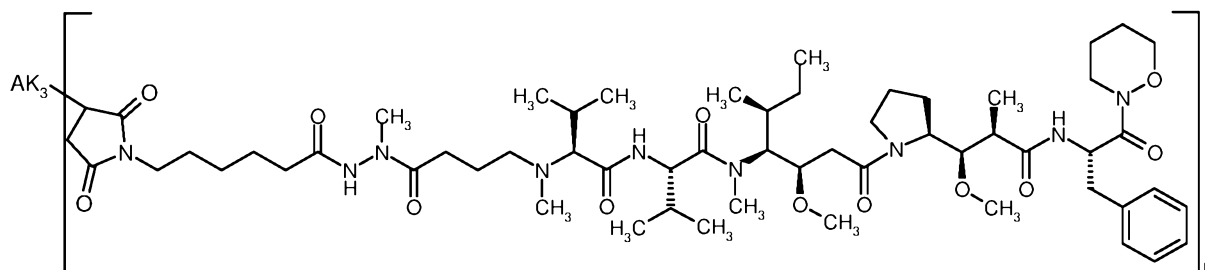
Example 64



Protein concentration: 0.51 mg/ml

5 Drug/mAb ratio: 6.6

Example 65



Protein concentration: 0.47 mg/ml

10 Drug/mAb ratio: 4.2

[illegible]

5 Drug/mAb ratio: 5.9

[illegible]

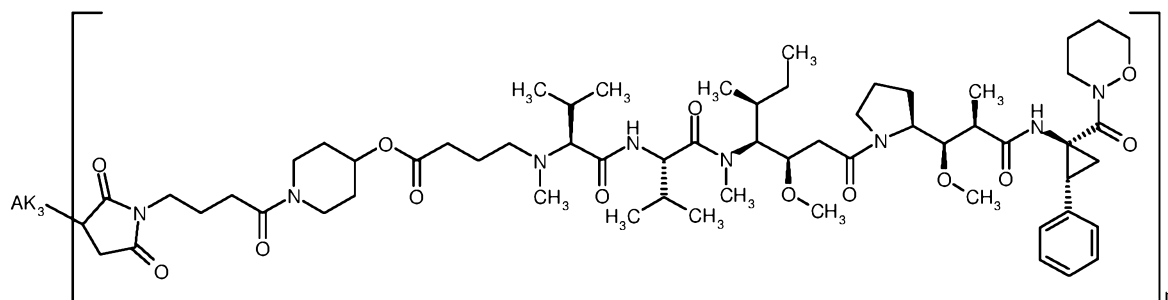
10 Drug/mAb ratio: 3.3

[illegible]

Protein concentration: 0.53 mg/ml

Drug/mAb ratio: 2.8

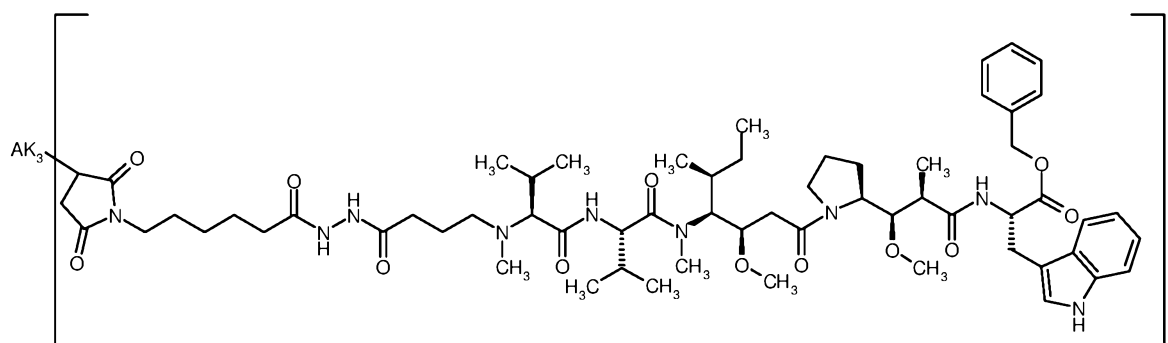
Example 69



5 Protein concentration: 0.92 mg/ml

Drug/mAb ratio: 3.5

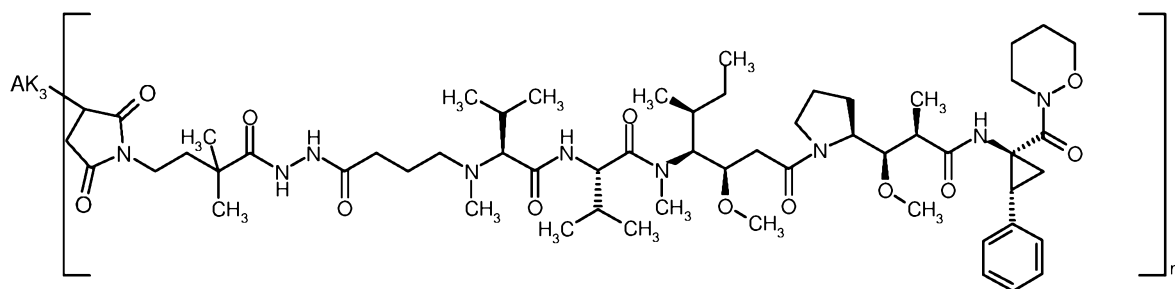
Example 70



10 Protein concentration: 0.09 mg/ml

Drug/mAb ratio: nd

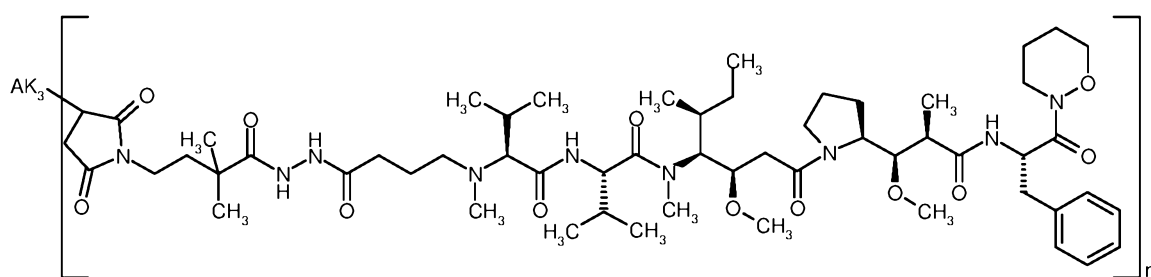
Example 71



Protein concentration: 0.62 mg/ml

5 Drug/mAb ratio: 1.8

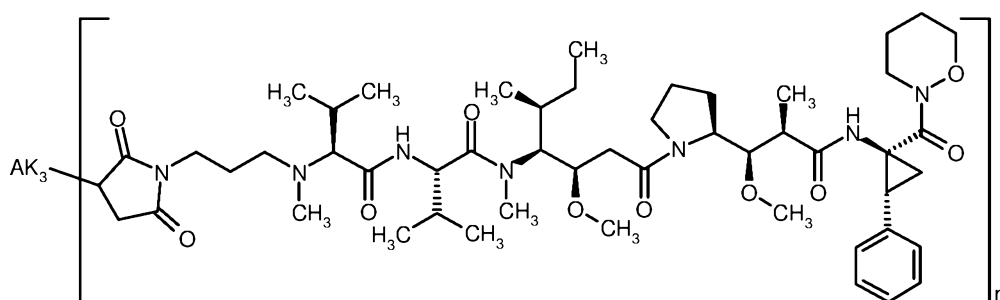
Example 72



Protein concentration: 0.55 mg/ml

10 Drug/mAb ratio: 3.8

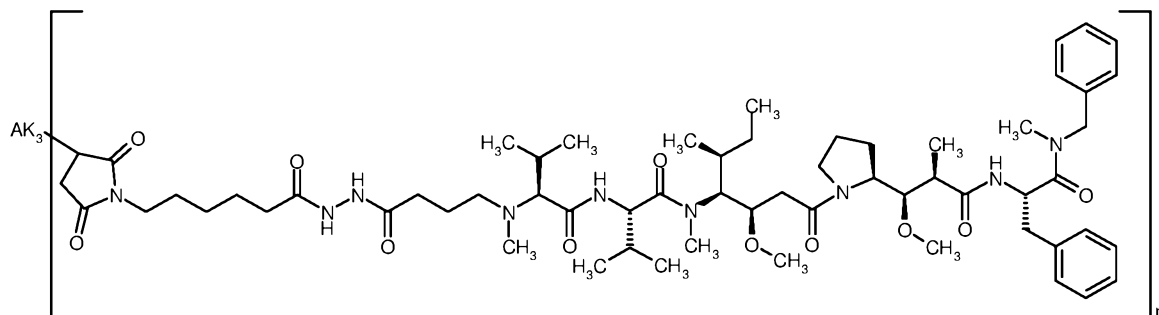
Example 73



Protein concentration: 0.54 mg/ml

Drug/mAb ratio: 4.4

Example 74

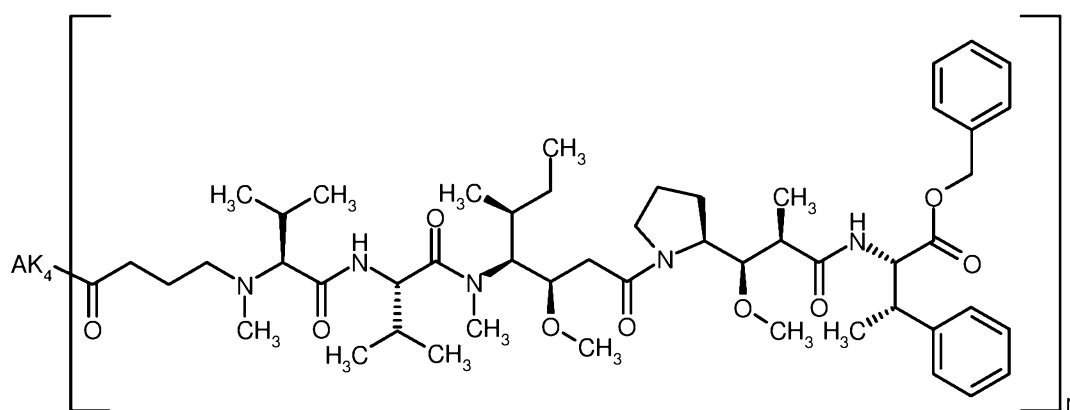


5

Protein concentration: 0.56 mg/ml

Drug/mAb ratio: 4.0

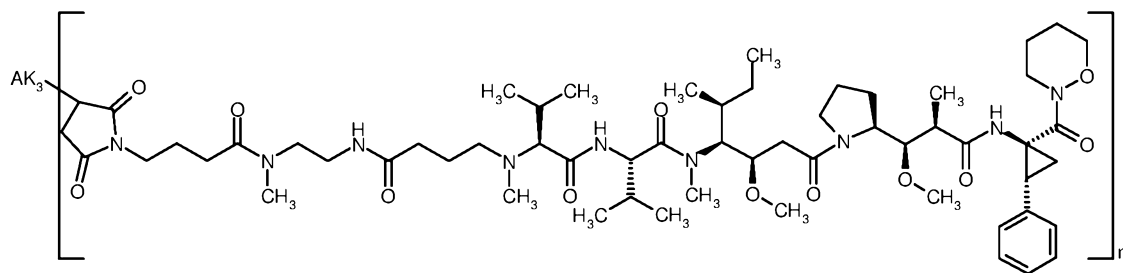
Example 75



10 Protein concentration: 1.1 mg/ml

Drug/mAb ratio: 0.3

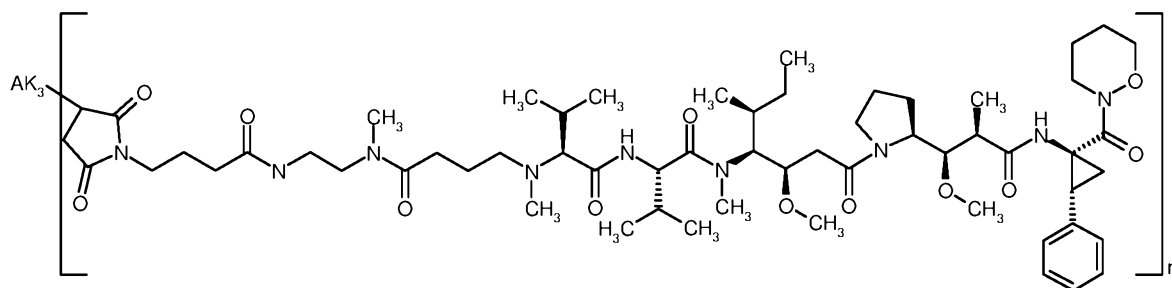
Example 76



Protein concentration: 0.61 mg/ml

Drug/mAb ratio: 0.9

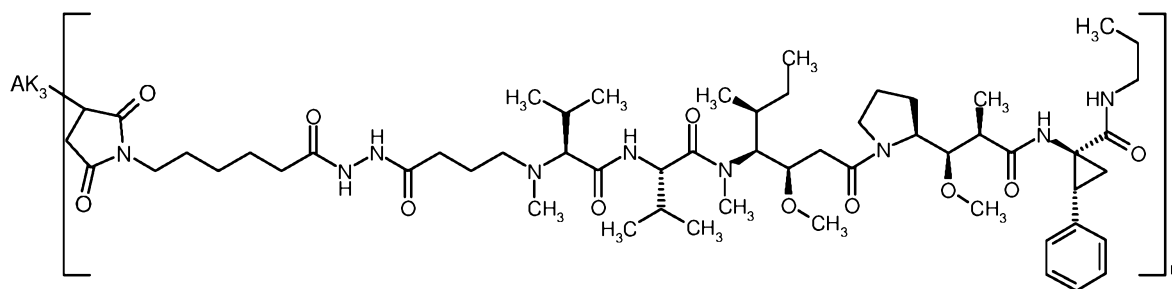
5 Example 77



Protein concentration: 0.57 mg/ml

Drug/mAb ratio: 1.2

10 Example 78

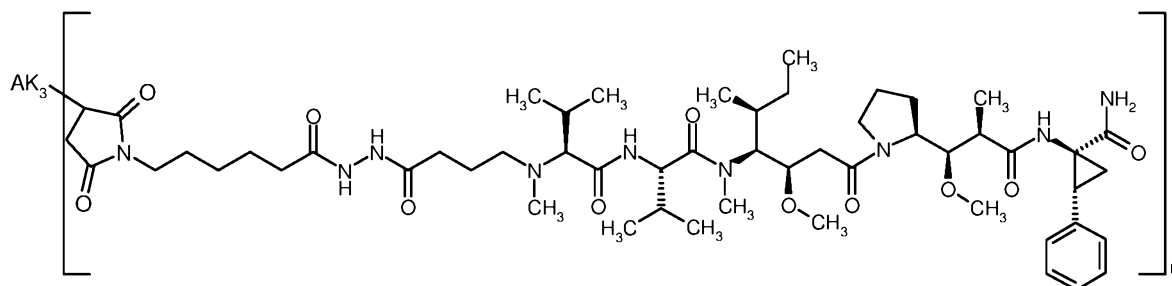


100mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation.

Protein concentration: 11.2 mg/ml

Drug/mAb ratio: 3.4

Example 79

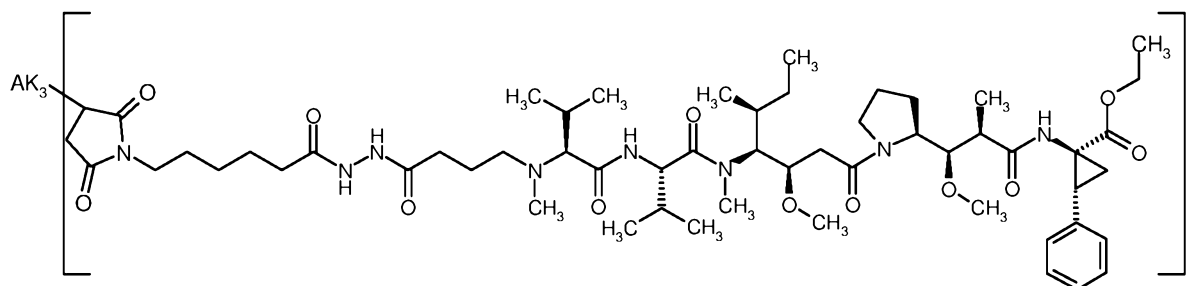


5

Protein concentration: 1.56 mg/ml

Drug/mAb ratio: 2.8

Example 80



10

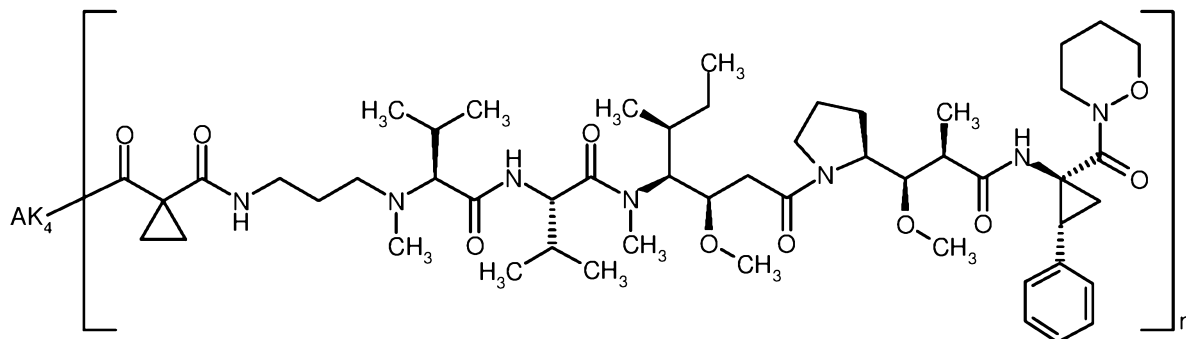
Protein concentration: 0.60 mg/ml

Drug/mAb ratio: 2.4

Protein concentration: 13.2 mg/ml

Drug/mAb ratio: 4.6

Example 84

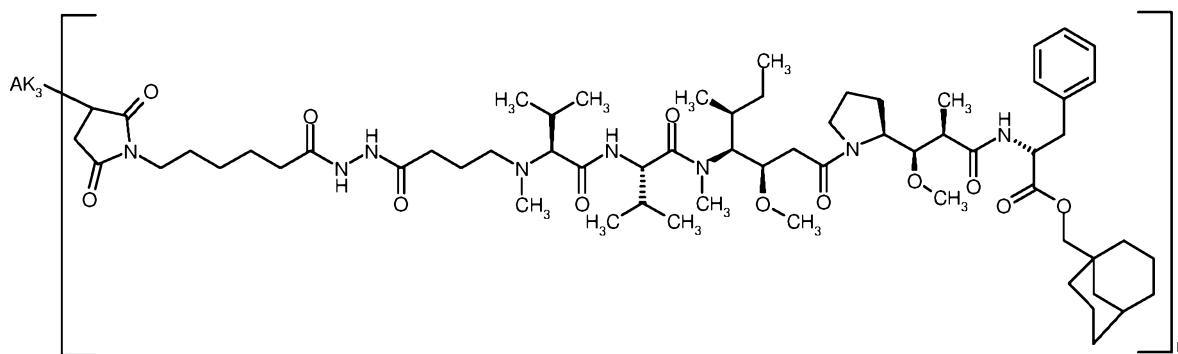


5

Protein concentration: 0.98ml

Drug/mAb ratio: 1.1

Example 85

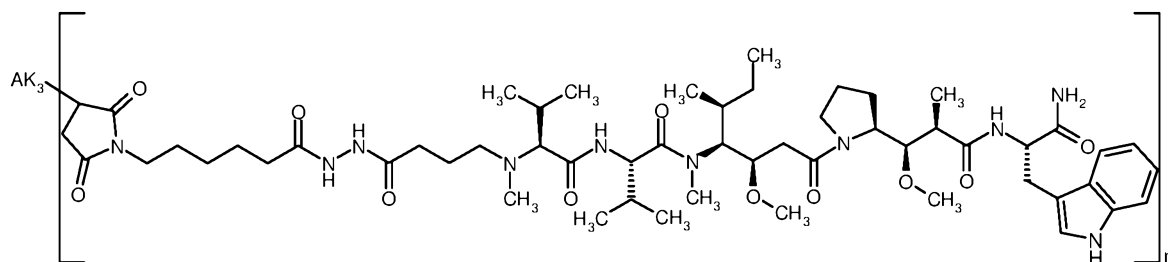


10

Protein concentration: 0.55 mg/ml

Drug/mAb ratio: not detectable

Example 86

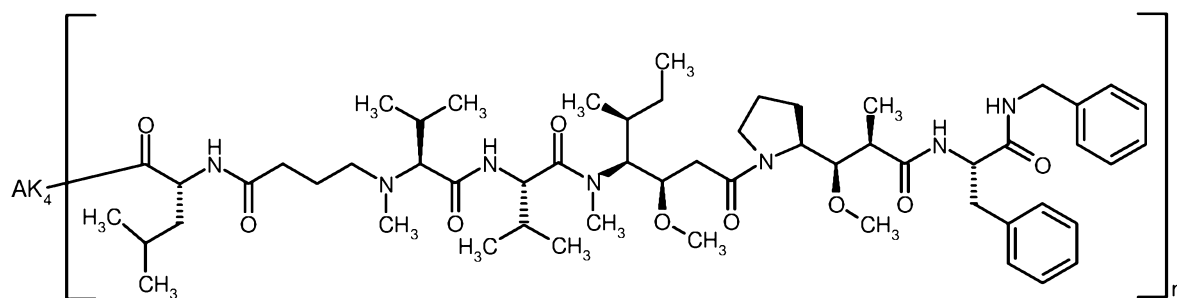


40mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
5 column, the reaction mixture was concentrated by ultracentrifugation.

Protein concentration: 10.6 mg/ml

Drug/mAb ratio: 4.1

Example 87

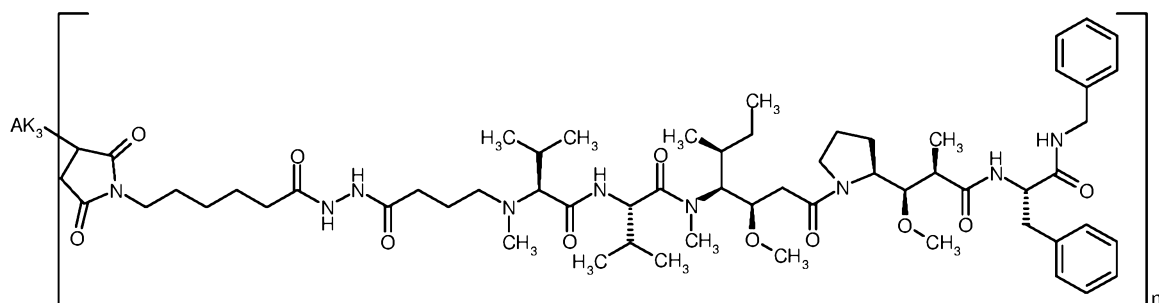


10

Protein concentration: 0.96 mg/ml

Drug/mAb ratio: 0.4

Example 88

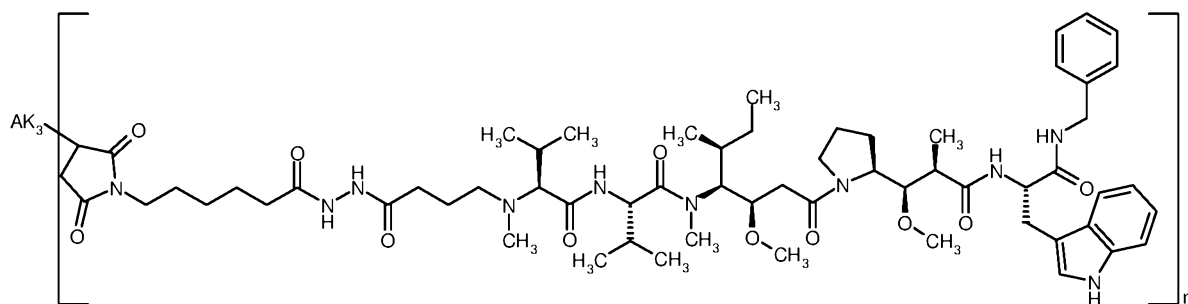


70mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
5 column, the reaction mixture was concentrated by ultracentrifugation.

Protein concentration: 12.7 mg/ml

Drug/mAb ratio: 3.6

Example 89



10

Protein concentration: 1.1 mg/ml

Drug/mAb ratio: 2.7

[illegible]

5 Drug/mAb ratio: 2.6

[illegible]

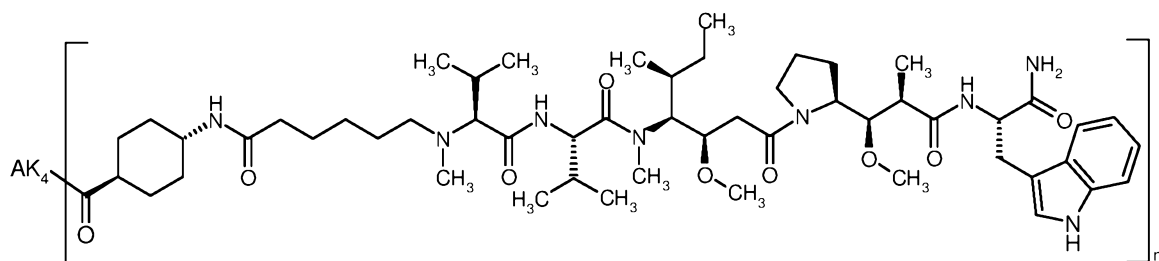
10 Drug/mAb ratio: 2.3

[illegible]

Protein concentration: 1.22 mg/ml

Drug/mAb ratio: 3.3

Example 93

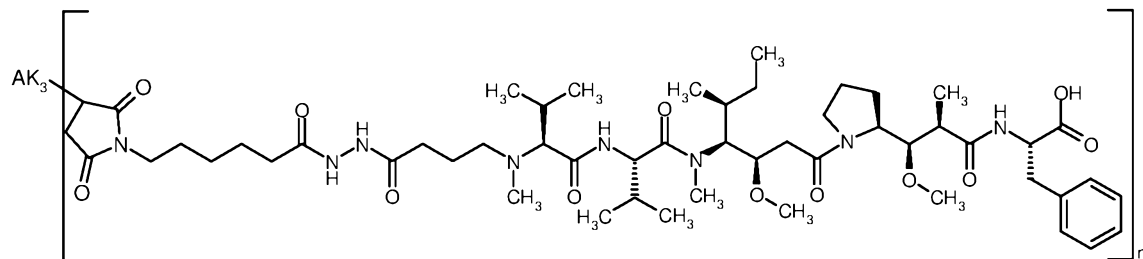


5

Protein concentration: 1.34 mg/ml

Drug/mAb ratio: 1.2

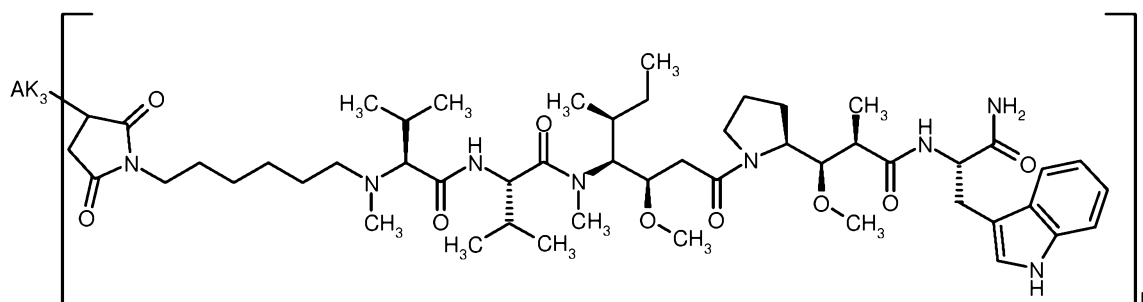
Example 94



10 Protein concentration: 1.28 mg/ml

Drug/mAb ratio: 3.2

Example 95

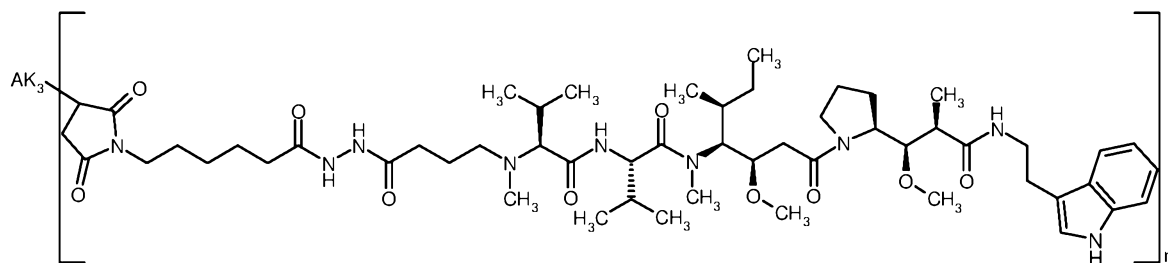


70mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation.

Protein concentration: 10.9 mg/ml

Drug/mAb ratio: 5.1

5 Example 96

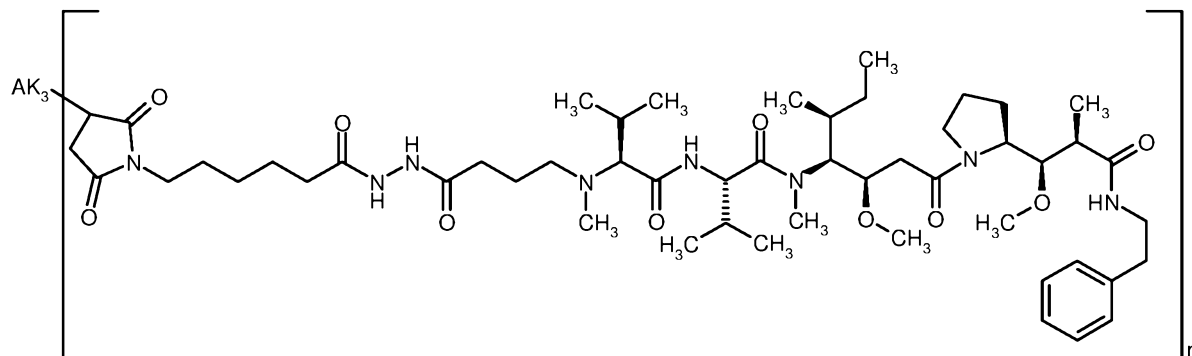


100mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation.

10 Protein concentration: 10.3 mg/ml

Drug/mAb ratio: 4.3

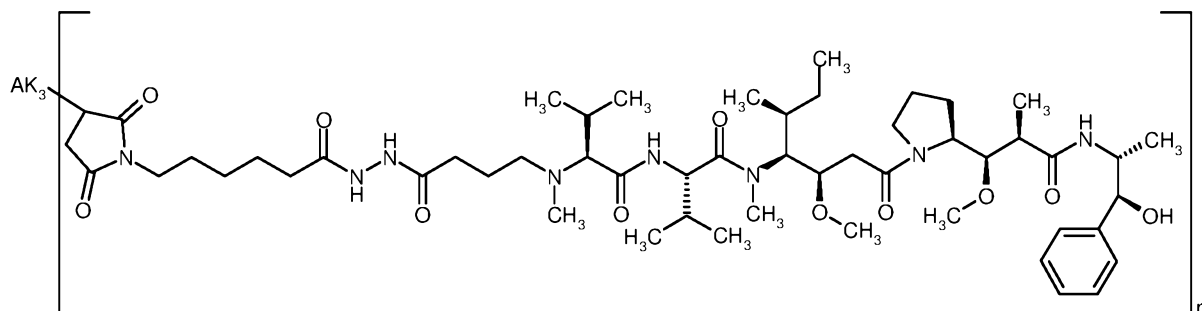
Example 97



15 Protein concentration: 1.08 mg/ml

Drug/mAb ratio: 2.8

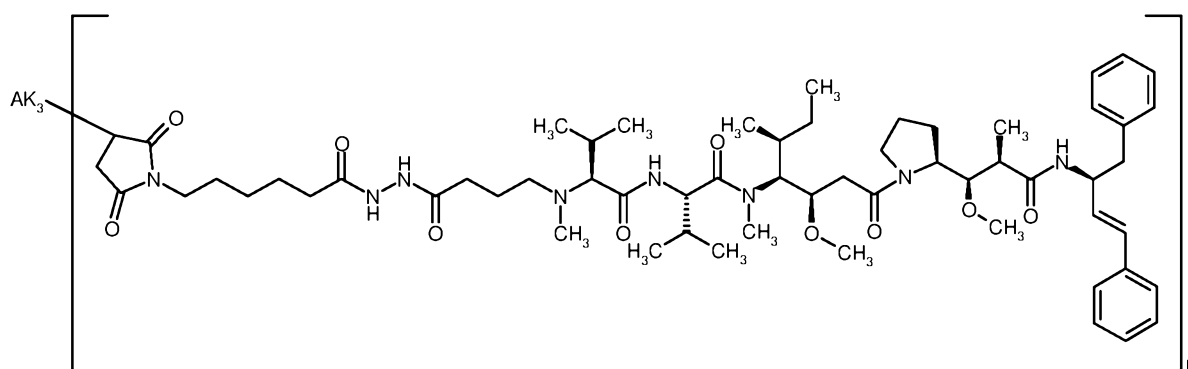
Example 98



Protein concentration: 1.24 mg/ml

5 Drug/mAb ratio: 2.8

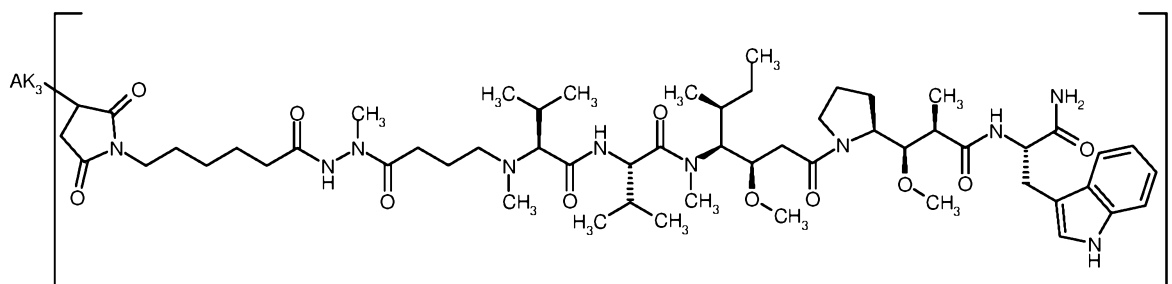
Example 99



Protein concentration: 1.28 mg/ml

10 Drug/mAb ratio: 3.8

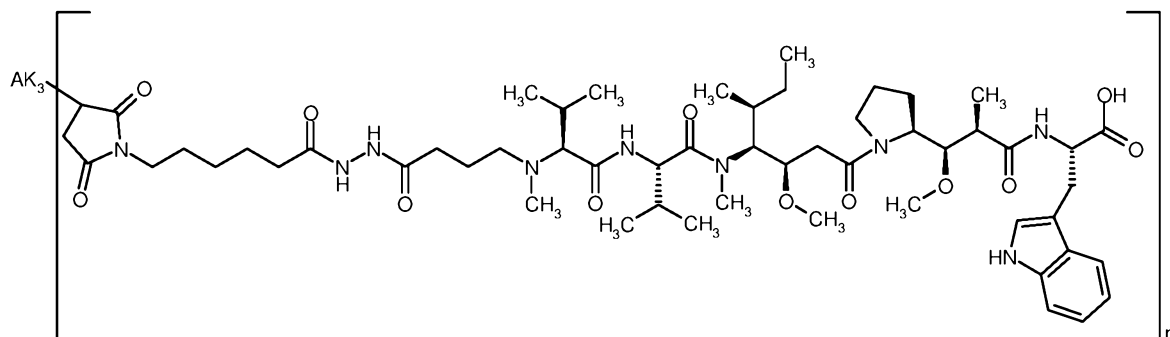
Example 100



Protein concentration: 1.07 mg/ml

Drug/mAb ratio: 3.0

Example 101

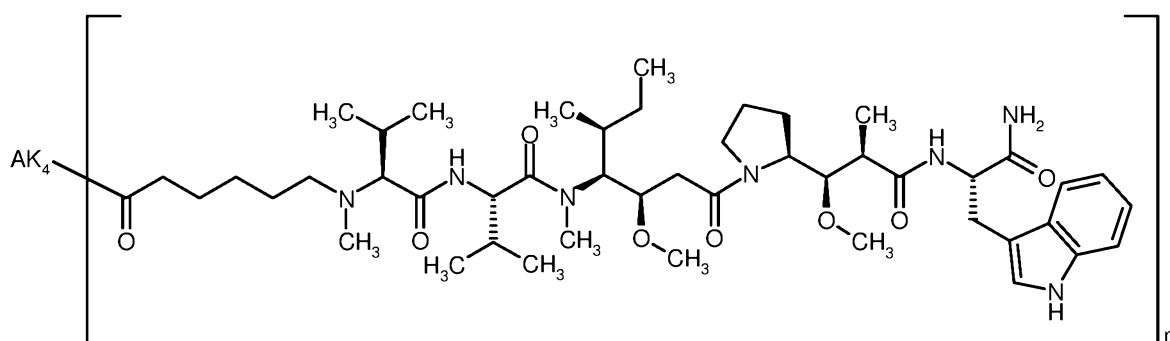


5

Protein concentration: 1.35 mg/ml

Drug/mAb ratio: 4.0

Example 102



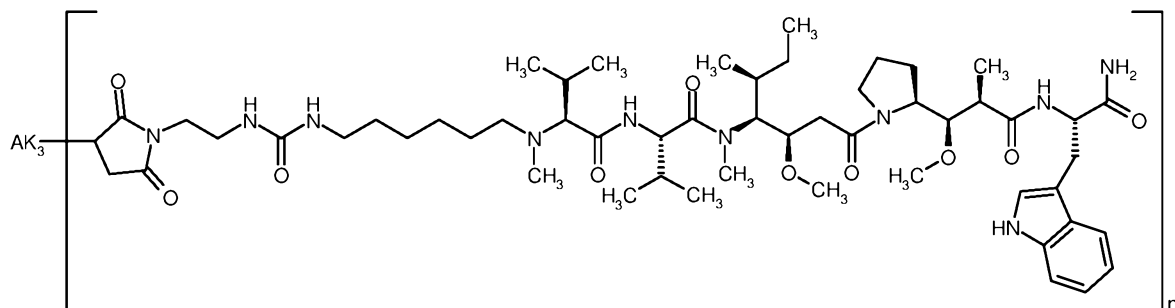
10

100mg MF-Ta in PBS werepresently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation.

Protein concentration: 12.2 mg/ml

15 Drug/mAb ratio: 5.6

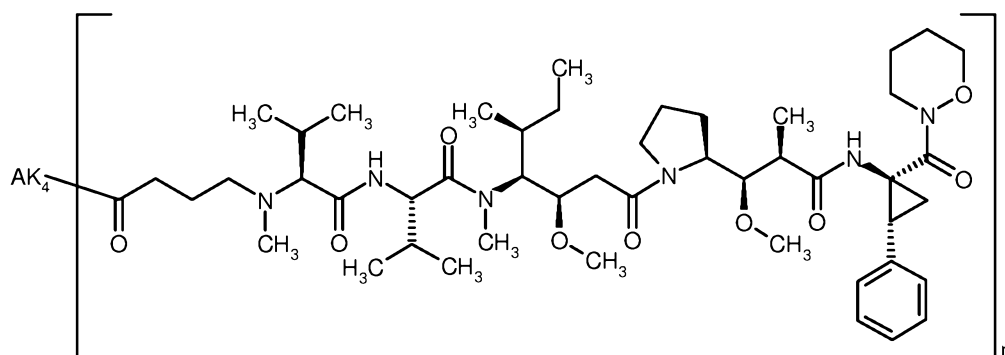
Example 103



Protein concentration: 1.32 mg/ml

5 Drug/mAb ratio: 3.2

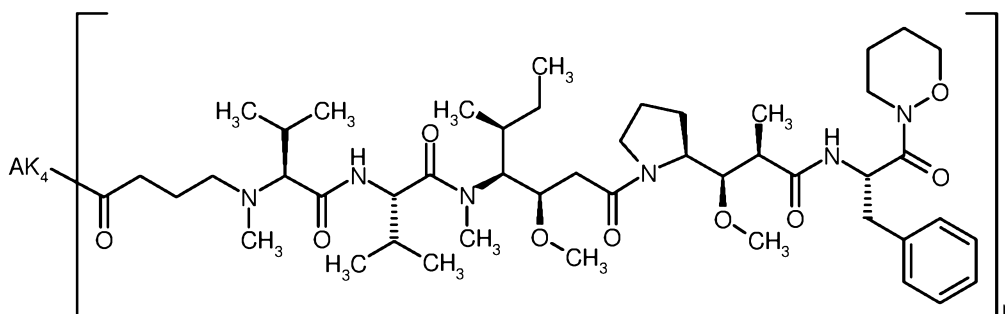
Example 104



Protein concentration: 1.01 mg/ml

Drug/mAb ratio: 0.9

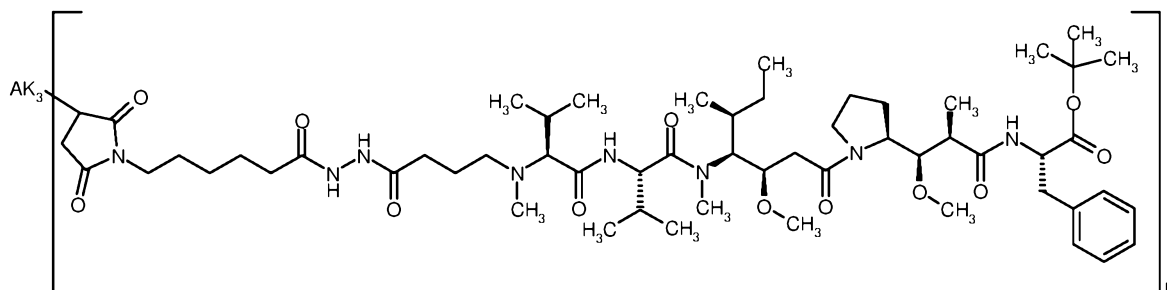
10 Example 105



Protein concentration: 1.03 mg/ml

Drug/mAb ratio: 0.3

Example 106



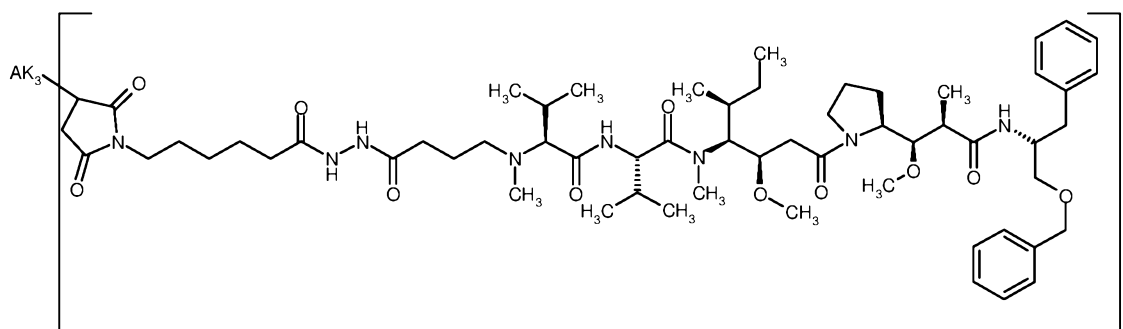
5

Protein concentration: 0.62 mg/ml

Drug/mAb ratio: 3.1

This ADC was concentrated by Vivaspin centrifugation and diluted again, followed by another concentration and dilution.

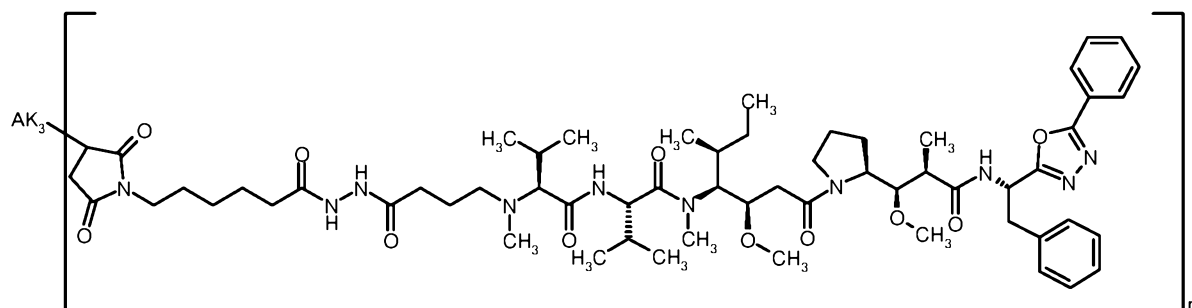
10 Example 107



Protein concentration: 1.26 mg/ml

Drug/mAb ratio: not detectable

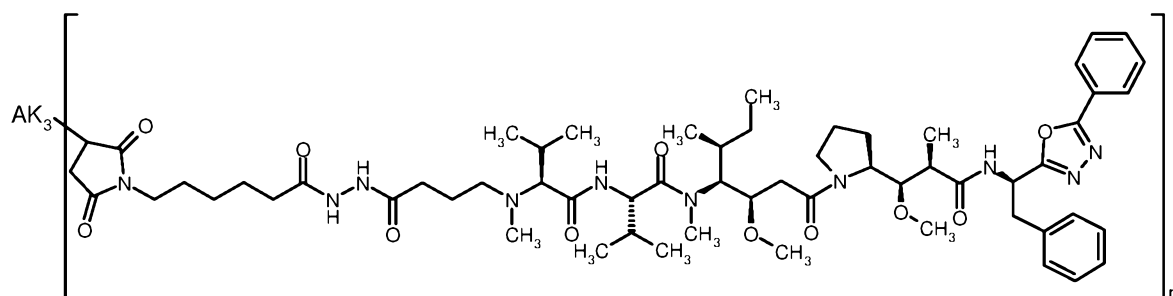
Example 108



Protein concentration: 1.55 mg/ml

5 Drug/mAb ratio: not detectable

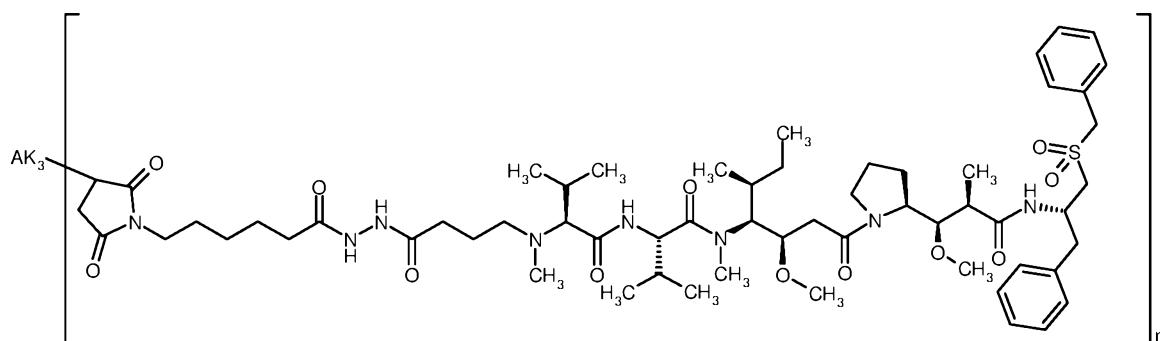
Example 109



Protein concentration: 1.23 mg/ml

10 Drug/mAb ratio: 3.5

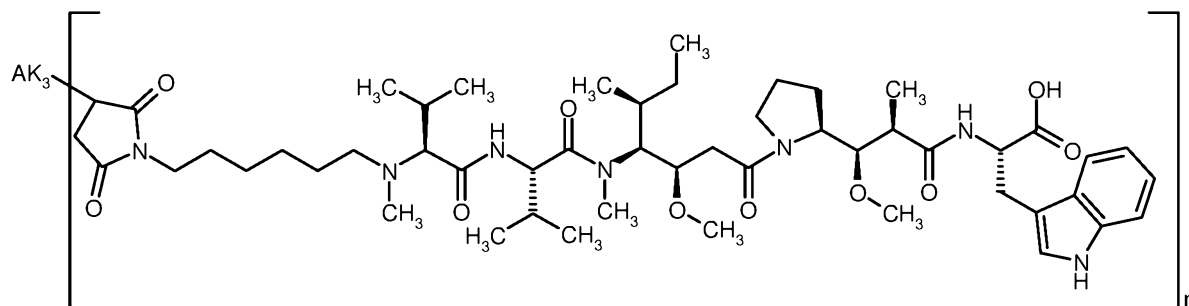
Example 110



Protein concentration: 1.44 mg/ml

Drug/mAb ratio:4.1

Example 111

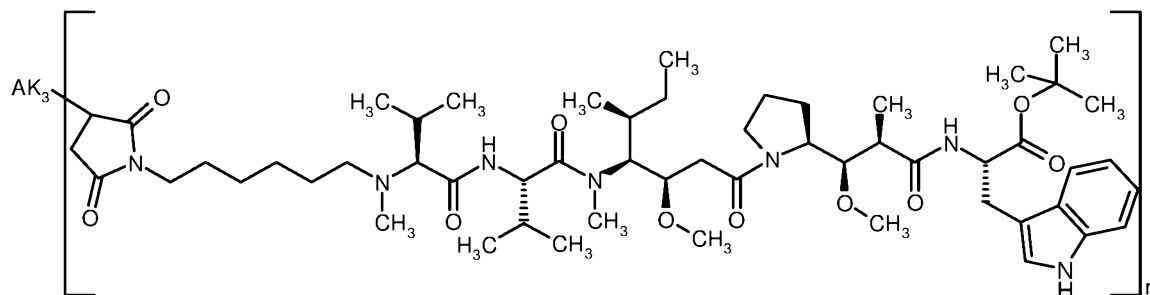


5

Protein concentration: 0.92 mg/ml

Drug/mAb ratio: 3.5

Example 112



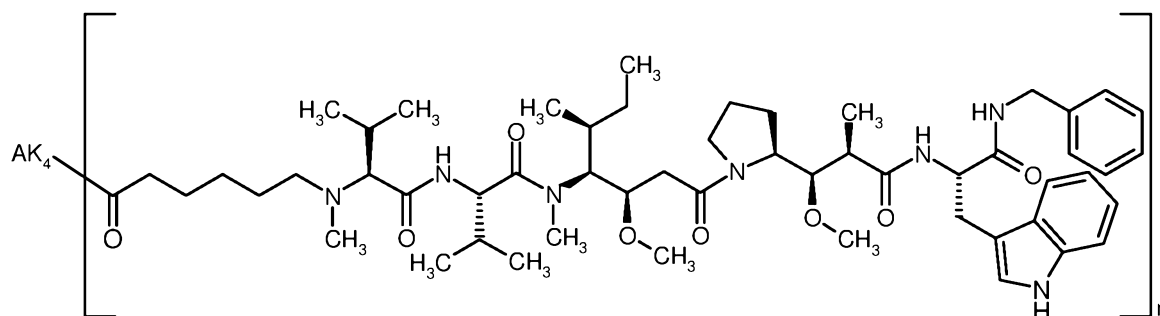
10

5 mg MF-T in PBS werepresently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 0.77 mg/ml

Drug/mAb ratio: >1.5 (not exactly detectable)

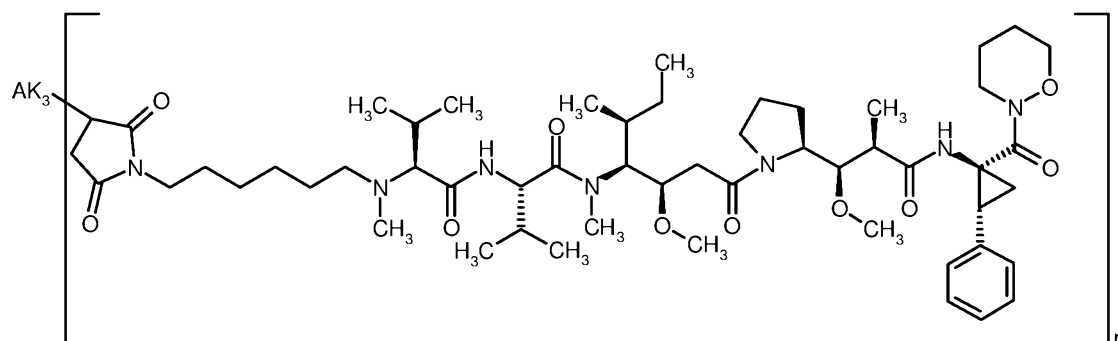
Example 113



Protein concentration: 1.3 mg/ml

5 Drug/mAb ratio: 2.0

Example 114

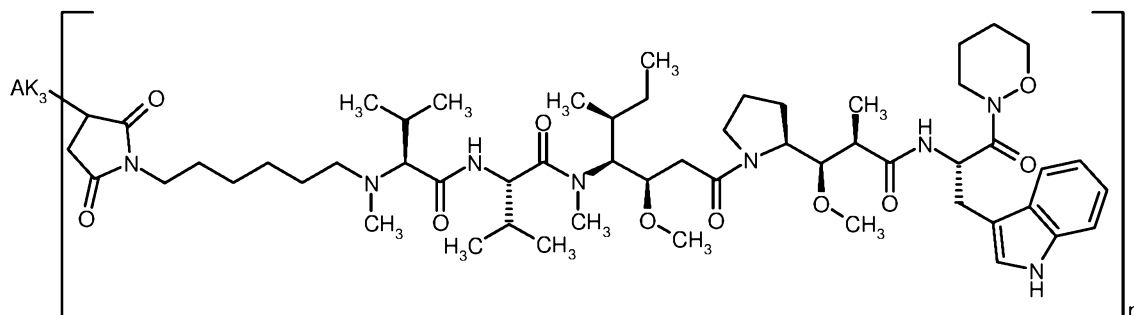


500mg MF-Ta in PBS werepresently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation.

10 Protein concentration: 11.2 mg/ml

Drug/mAb ratio: 3.7

Example 115

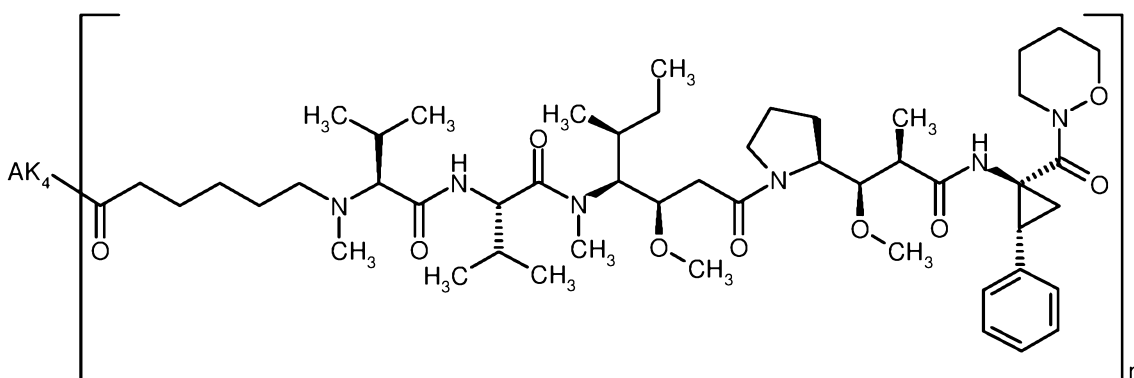


100mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation.

5 Protein concentration: 11.4 mg/ml

Drug/mAb ratio: 3.9

Example 116



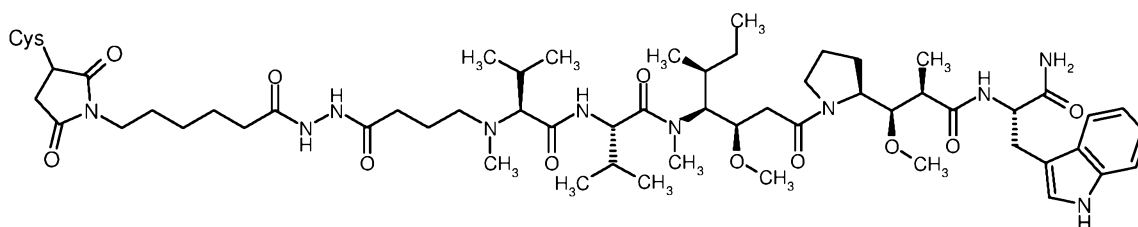
60mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation.

10 Protein concentration: 10.5 mg/ml

Drug/mAb ratio: 4.4

Example 117

N-(4-{2-[6-(3-{[(2*R*)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



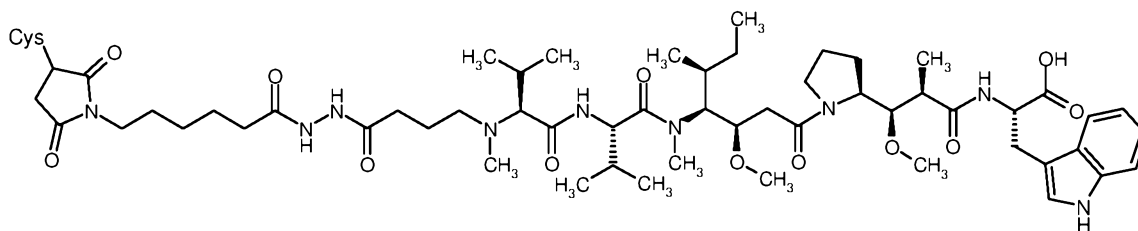
10 mg (10 μ mol) of Intermediate 157 were taken up in 5.2 ml DMF and mixed with 2.28 mg (20 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 5.8 mg (48% of theory) of the title compound.

HPLC (Method 5): R_t = 1.45 min;

LC-MS (Method 1): R_t = 0.74 min; MS (ESIpos): m/z = 1184 ($M+H$)⁺.

Example 118

N-(4-{2-[6-(3-{[(2*R*)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



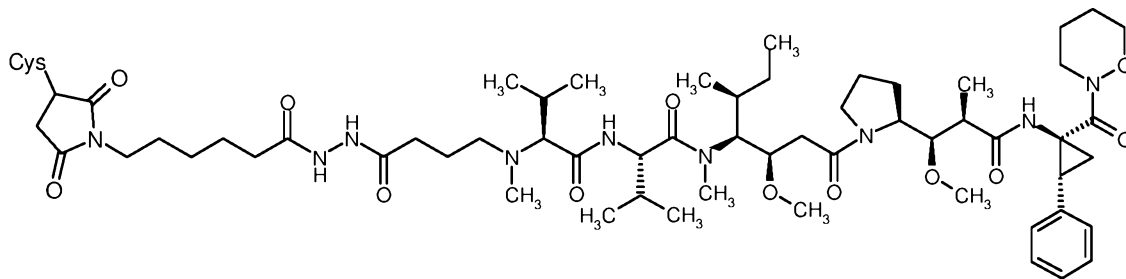
10 mg (10 μ mol) of Intermediate 113 weretaken up in 5.2 ml DMF and mixed with 2.28 mg (20 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 6 mg (54% of theory) of the title compound.

5 HPLC (Method 5): $R_t = 1.5$ min;

LC-MS (Method 1): $R_t = 0.77$ min; MS (ESIpos): $m/z = 1185$ (M+H)⁺.

Example 119

N-(4-{2-[6-(3-{[(2*R*)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl)-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



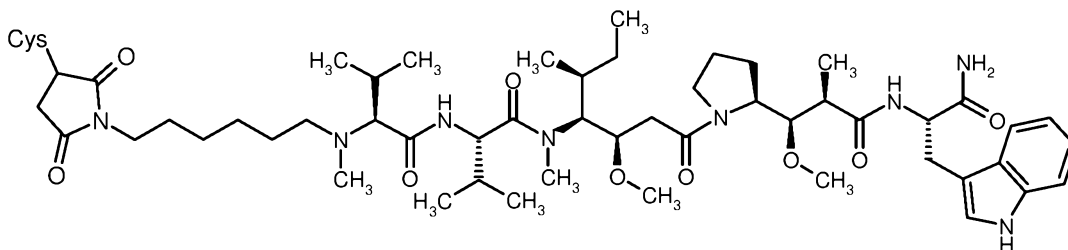
9 mg (8.3 μ mol) of Intermediate 132 weretaken up in 4 ml DMF and mixed with 3 mg (24.4 μ mol) L-cysteine. The reaction mixture was stirred overnight at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 6.8 mg (68% of theory) of the title compound.

HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.78$ min; MS (ESIpos): $m/z = 1227$ (M+H)⁺.

Example 120

20 *N*-[6-(3-{[(2*R*)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl)-*N*-methyl-L-valyl)-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



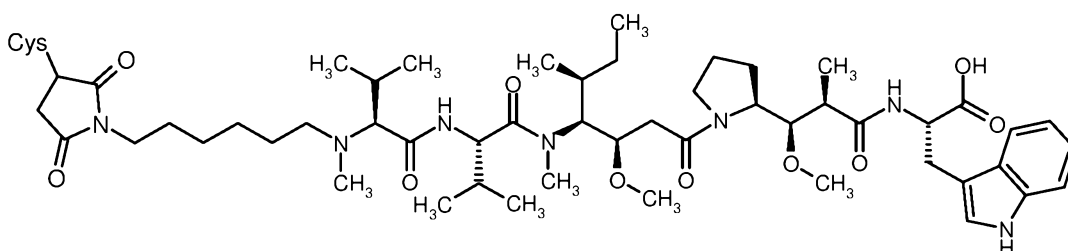
10 mg (10 μ mol) of Intermediate 106 weretaken up in 5.8 ml DMF and mixed with 2.5 mg (20 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 5.2 mg (46% of theory) of the
5 title compound.

HPLC (Method 5): $R_t = 1.5$ min;

LC-MS (Method 11): $R_t = 0.71$ min; MS (ESIpos): $m/z = 1070$ (M+H)⁺.

Example 121

N-[6-(3-{[(2*R*)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-*N*-methyl-L-
10 valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



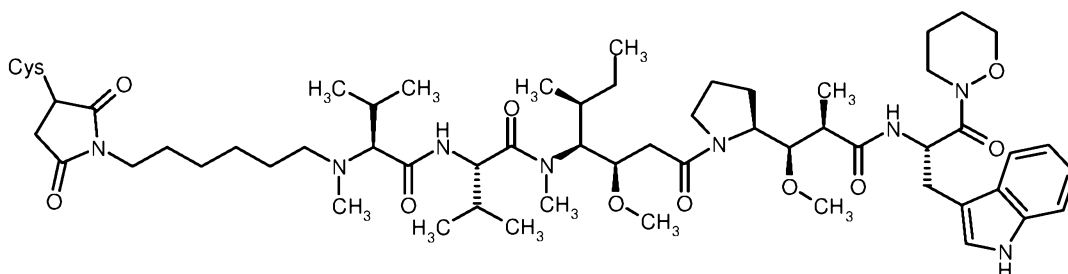
10 mg (10 μ mol) of Intermediate 124 weretaken up in 4 ml DMF and mixed with 2.5 mg (20 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 7.2 mg (64% of theory) of the
15 title compound.

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.8$ min; MS (ESIpos): $m/z = 1071$ (M+H)⁺.

Example 122

N-[6-(3-[(*2R*)-2-amino-2-carboxyethyl]sulfanyl)-2,5-dioxopyrrolidin-1-yl]hexyl]-*N*-methyl-L-valyl-*N*-[(*3R,4S,5S*)-1-[(*2S*)-2-[(*1R,2R*)-3-[(*2S*)-3-(*1H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



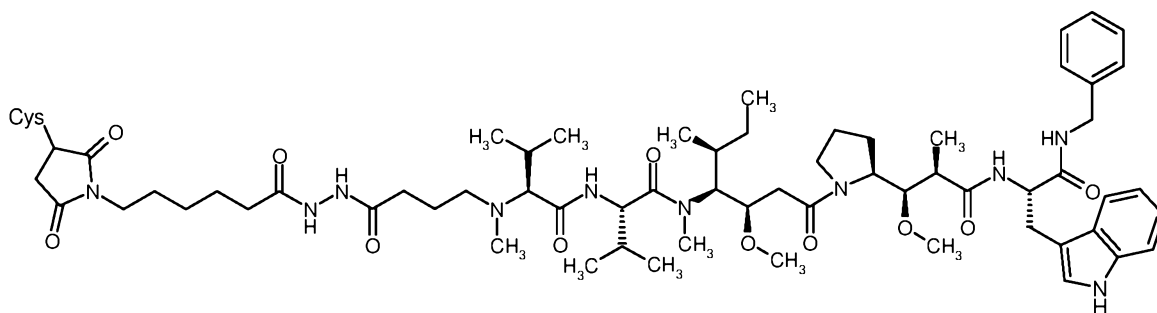
10 mg (10 μ mol) of Intermediate 125 were taken up in 4 ml DMF and mixed with 2.4 mg (20 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 7.7 mg (69% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 2): $R_t = 1.91$ min; MS (ESIpos): $m/z = 1140$ ($M+H$)⁺.

Example 123

N-(4-{2-[6-(3-[(*2R*)-2-amino-2-carboxyethyl]sulfanyl)-2,5-dioxopyrrolidin-1-yl]hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(*3R,4S,5S*)-1-[(*2S*)-2-[(*1R,2R*)-3-[(*2S*)-1-(benzylamino)-3-(*1H*-indol-3-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



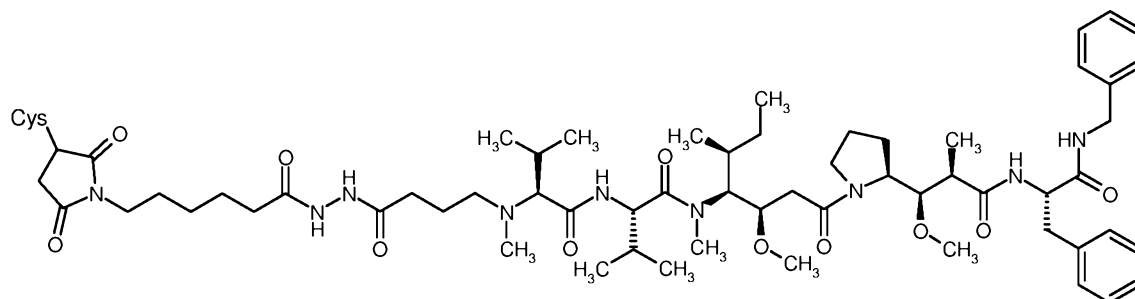
10 mg (10 μ mol) of Intermediate 160 weretaken up in 3 ml DMF and mixed with 2.1 mg (20 μ mol) L-cysteinine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 8.1 mg (73% of theory) of the title compound.

5 HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 1274$ (M+H)⁺.

Example 124

N-(4-{2-[6-(3-{[(2*R*)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



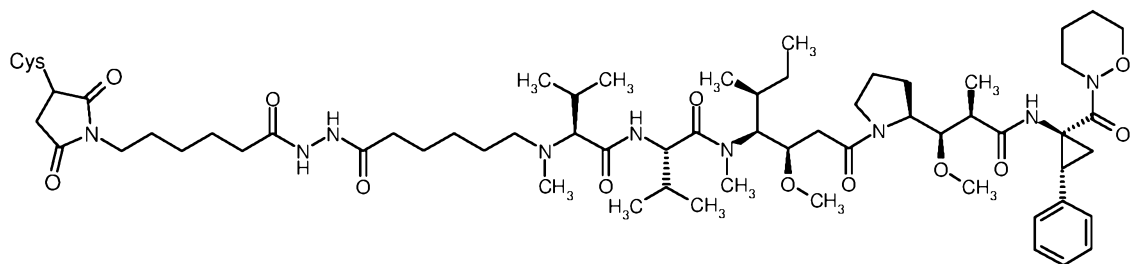
3.5 mg (3 μ mol) of Intermediate 159 weretaken up in 1 ml DMF and mixed with 0.76 mg (6 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 2.6 mg (65% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.75$ min;

LC-MS (Method 1): $R_t = 0.85$ min; MS (ESIpos): $m/z = 1235$ (M+H)⁺.

Example 125

N-(6-{2-[6-(3-{[(2*R*)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-6-oxohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide

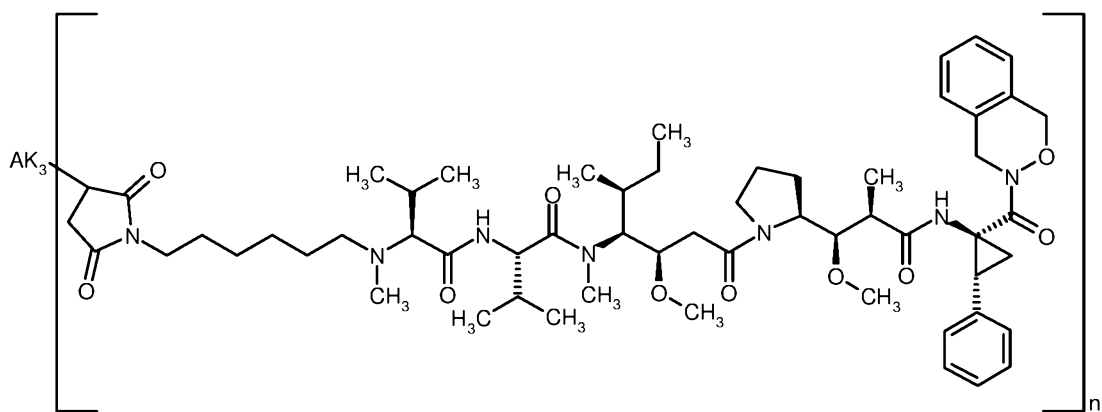


3.6 mg (3 μ mol) of Intermediate 129 were taken up in 1 ml DMF and mixed with 0.77 mg (6 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 1.55 mg (39% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.87$ min; MS (ESIpos): $m/z = 1255$ ($M+H$) $^+$.

Example 126

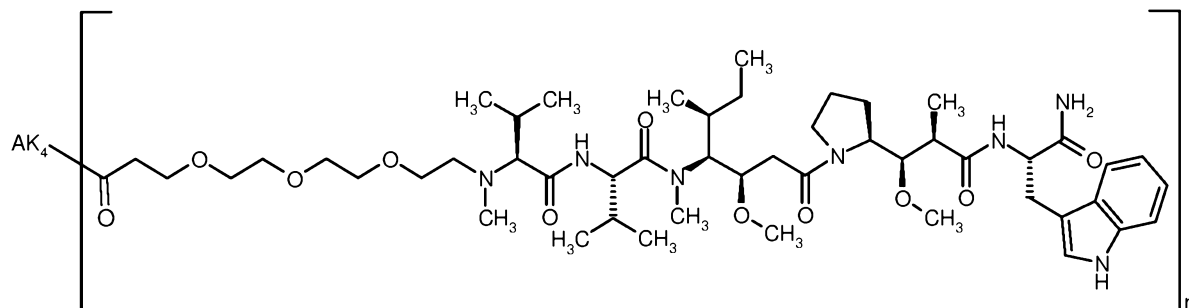


5 mg MF-T[a] in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 0.9 mg/ml

Drug/mAb ratio: 1

Example 127

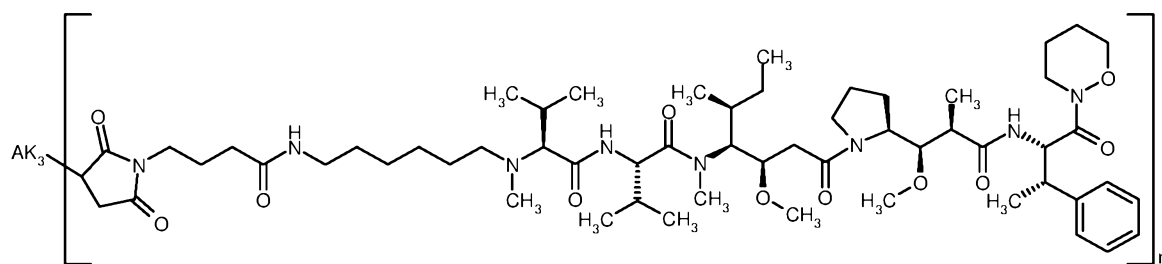


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
5 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.86 mg/ml

Drug/mAb ratio: 2.9

Example 128



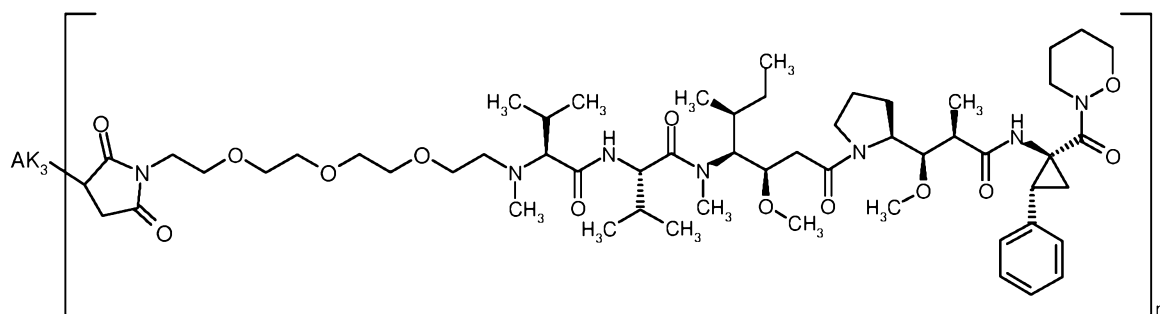
10

5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.05 mg/ml

Drug/mAb ratio: 4.4

Example 129

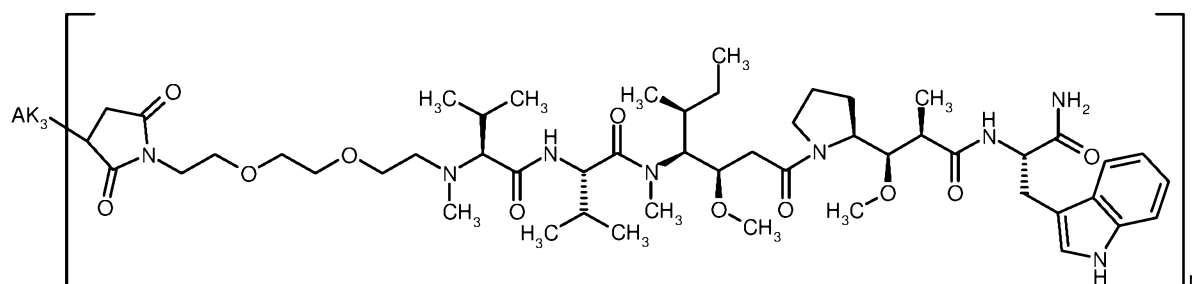


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
 5 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.13 mg/ml

Drug/mAb ratio: 2.8

Example 130



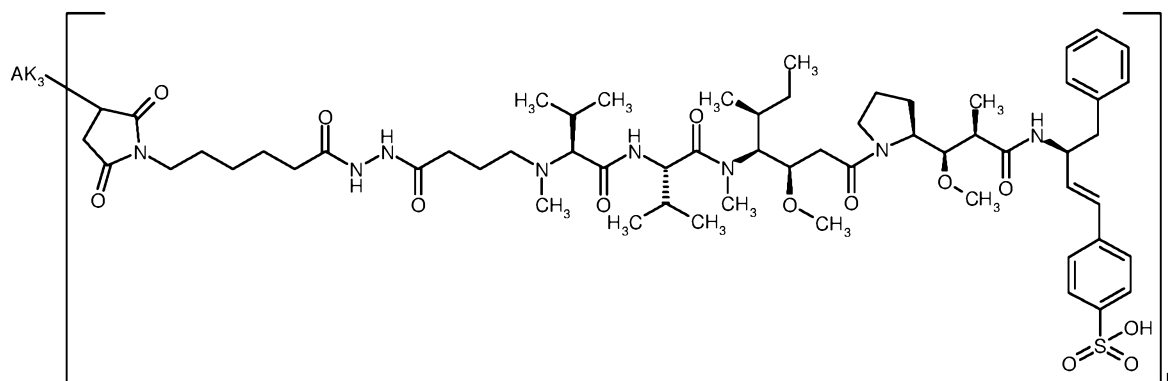
10

5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.41 mg/ml

Drug/mAb ratio: 3.9

Example 133

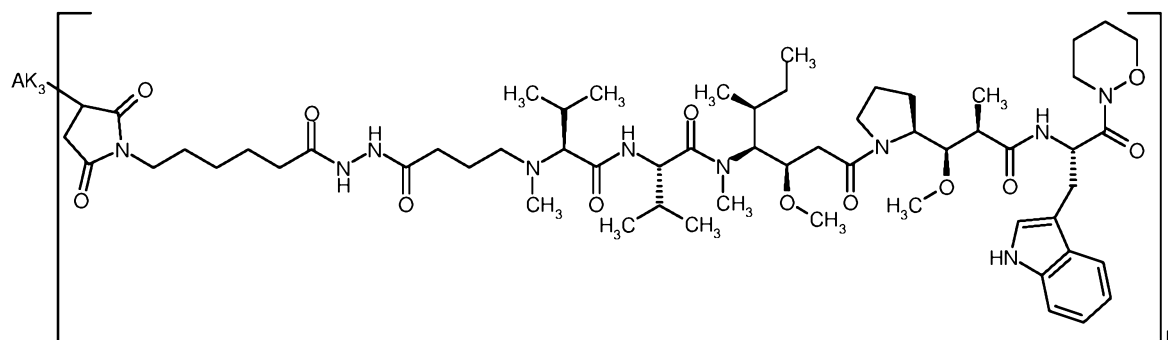


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
 5 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.14 mg/ml

Drug/mAb ratio: 5.3

Example 134



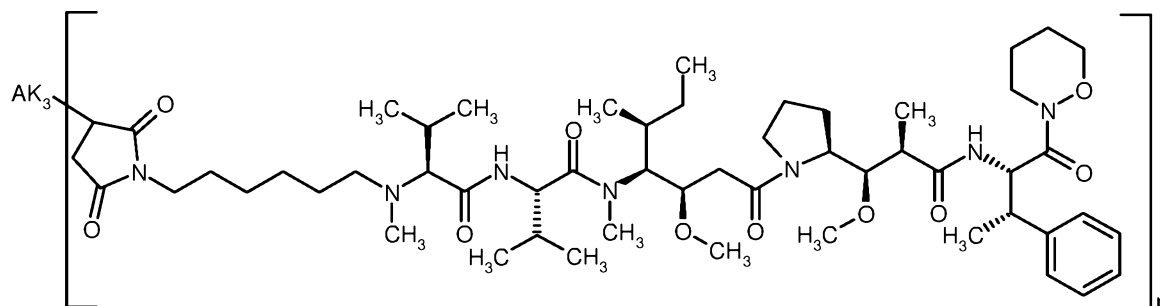
10

5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.25 mg/ml

Drug/mAb ratio: 4.8

Example 135

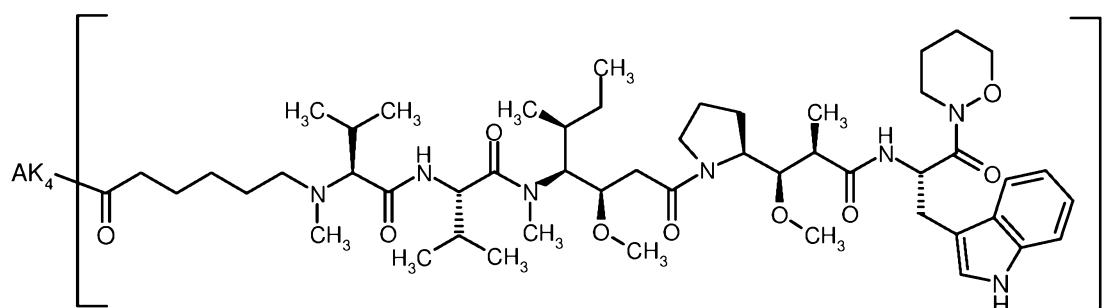


5 5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.12 mg/ml

Drug/mAb ratio: 1.7

Example 136

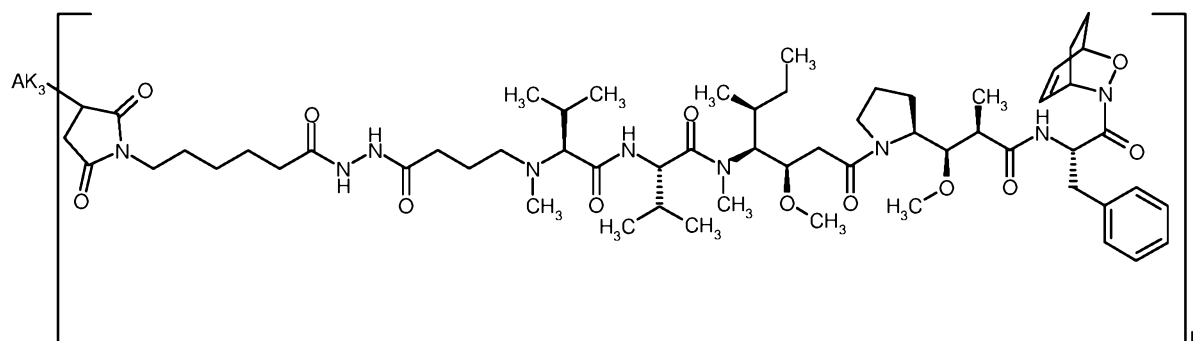


10 150 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation, diluted again with PBS and concentrated again.

Protein concentration: 12.2 mg/ml

Drug/mAb ratio: 4.1

Example 137

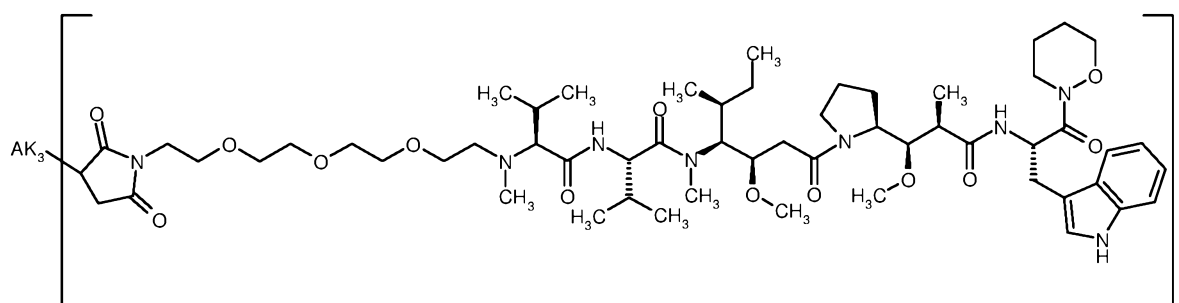


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
5 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 0.86 mg/ml

Drug/mAb ratio: 3.4

Example 138



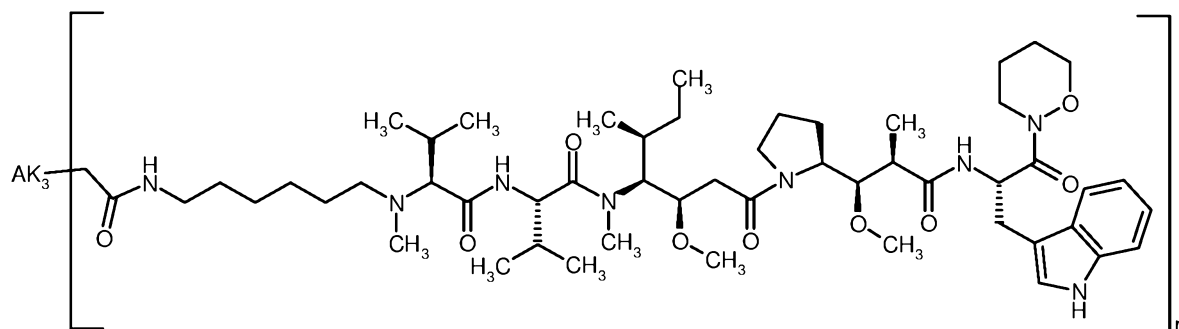
10

5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.43 mg/ml

Drug/mAb ratio: 3.7

Example 139

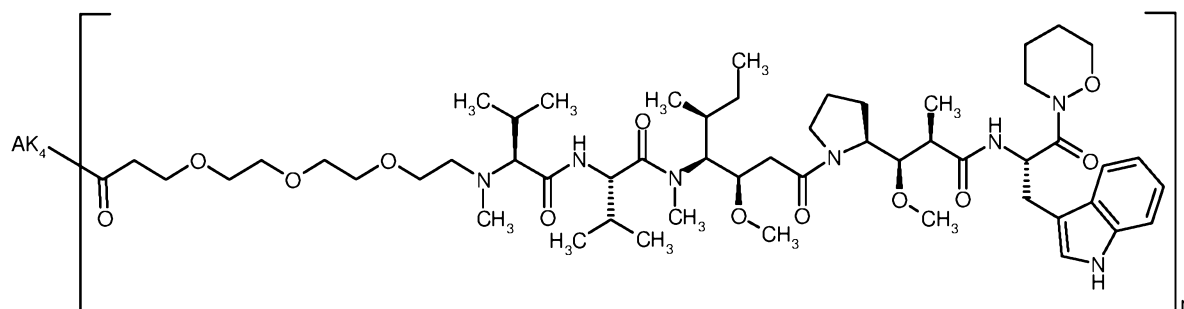


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
 5 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 0.8 mg/ml

Drug/mAb ratio: 0.7

Example 140



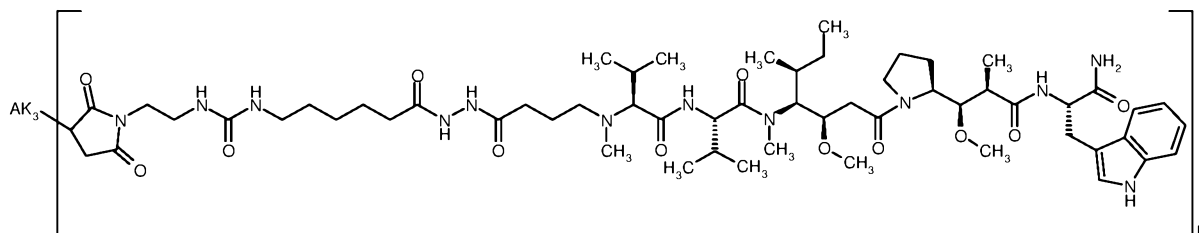
10

50 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
 column, the reaction mixture was concentrated by ultracentrifugation, diluted again with PBS
 and reconcentrated.

Protein concentration: 9.5 mg/ml

15 Drug/mAb ratio: 2.9

Example 141

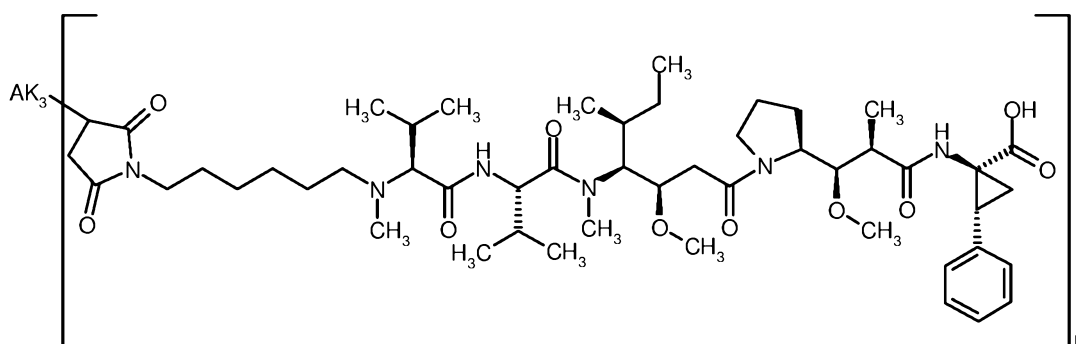


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
5 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.52 mg/ml

Drug/mAb ratio: 3.2

Example 142

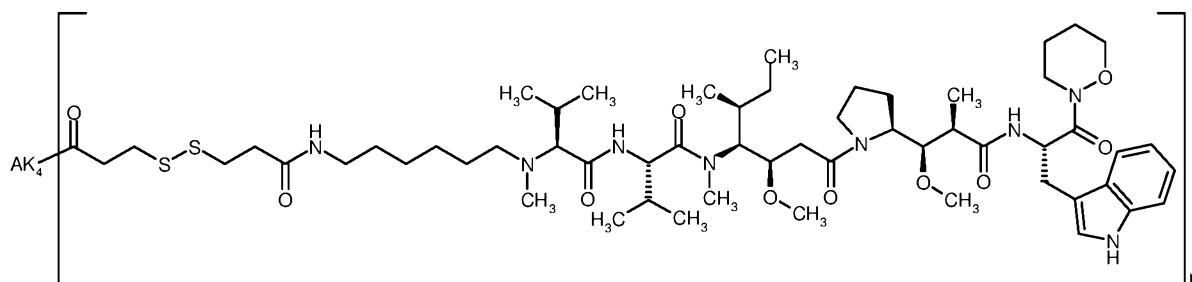


10 5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.25 mg/ml

Drug/mAb ratio: 4.6

Example 143

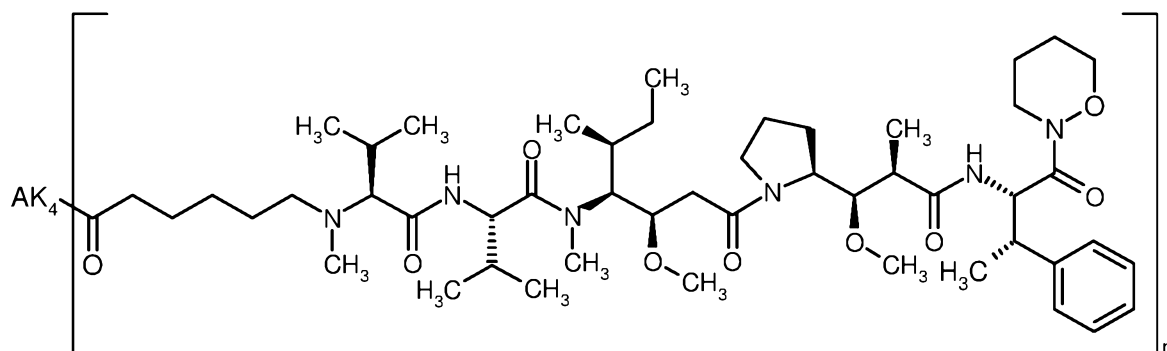


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
5 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.47 mg/ml

Drug/mAb ratio: 1.6

Example 144



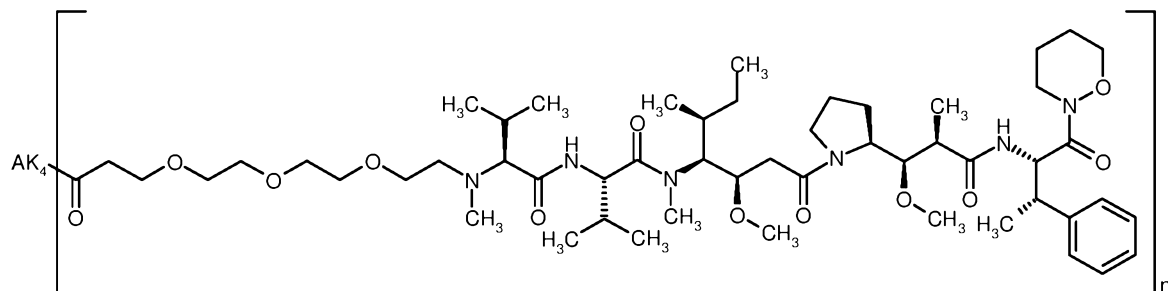
10

5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 0.99 mg/ml

Drug/mAb ratio: 5.5

Example 145

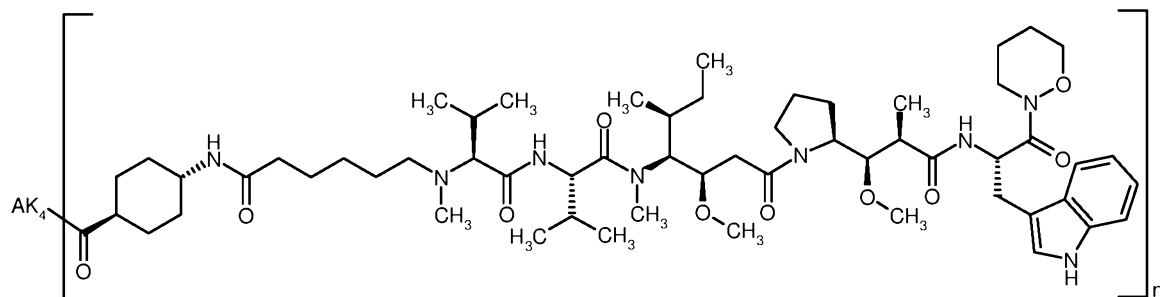


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
 5 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.02 mg/ml

Drug/mAb ratio: 4.0

Example 146



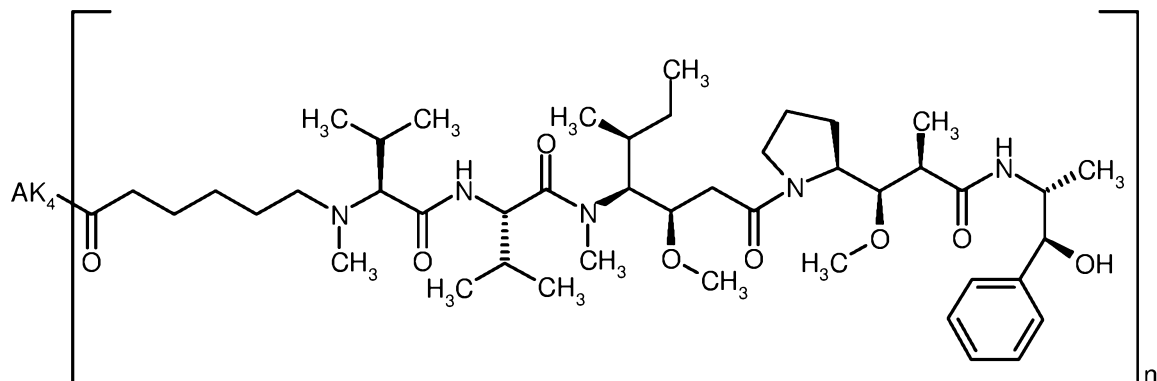
10

5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.63 mg/ml

Drug/mAb ratio: 3.8

Example 149

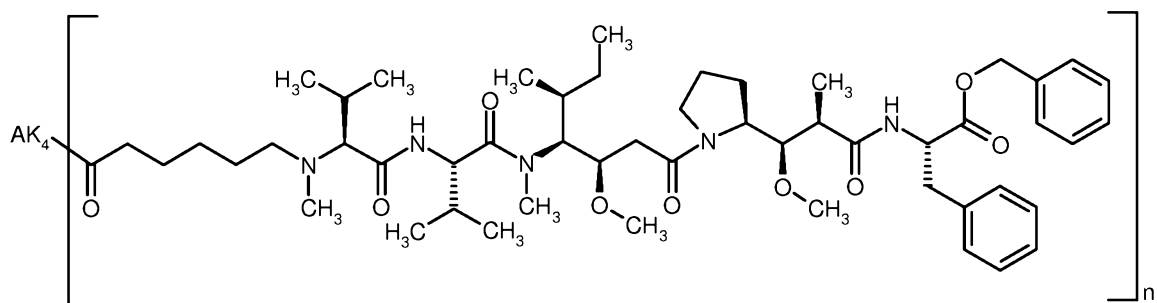


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
5 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.31 mg/ml

Drug/mAb ratio: 6.6

Example 150



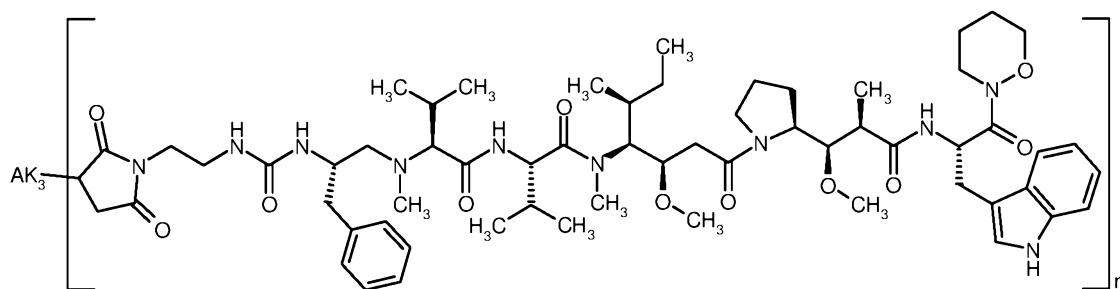
10

5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.75 mg/ml

Drug/mAb ratio: 1.8

Example 151

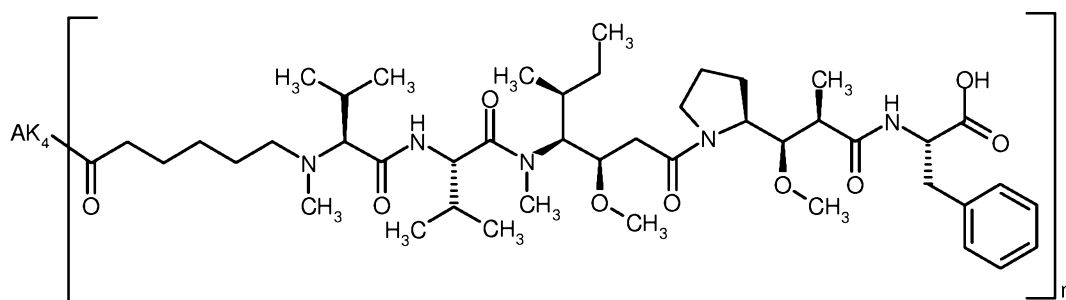


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

5 Protein concentration: 1.44 mg/ml

Drug/mAb ratio: 2.5

Example 152

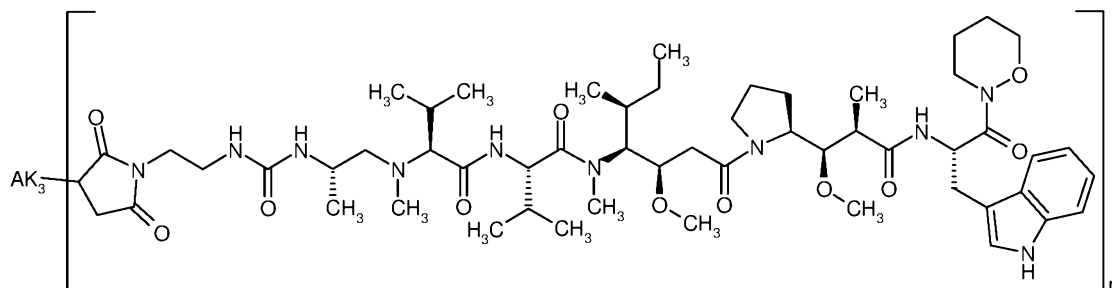


10 5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.96 mg/ml

Drug/mAb ratio: 5.6

Example 153

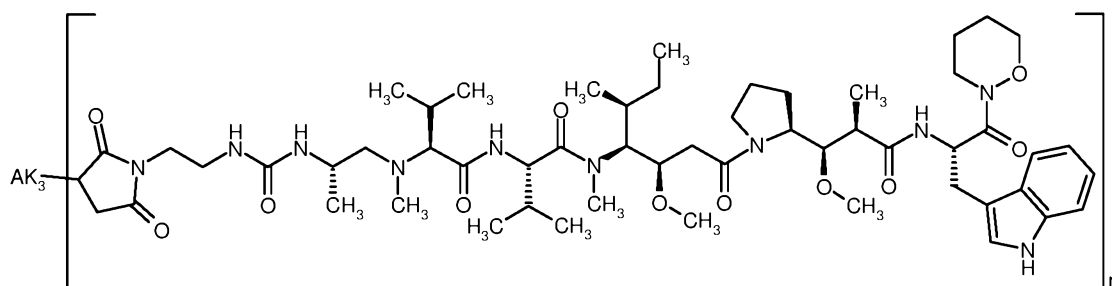


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

5 Protein concentration: 1.58 mg/ml

Drug/mAb ratio: 4.2

Example 154

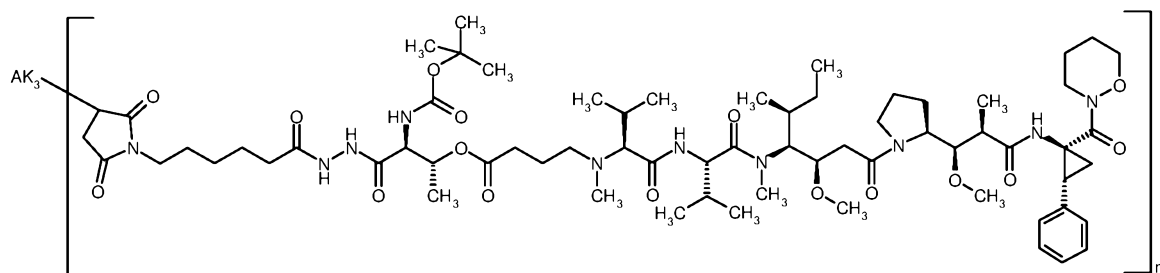


10 5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.48 mg/ml

Drug/mAb ratio: 4.6

Example 155

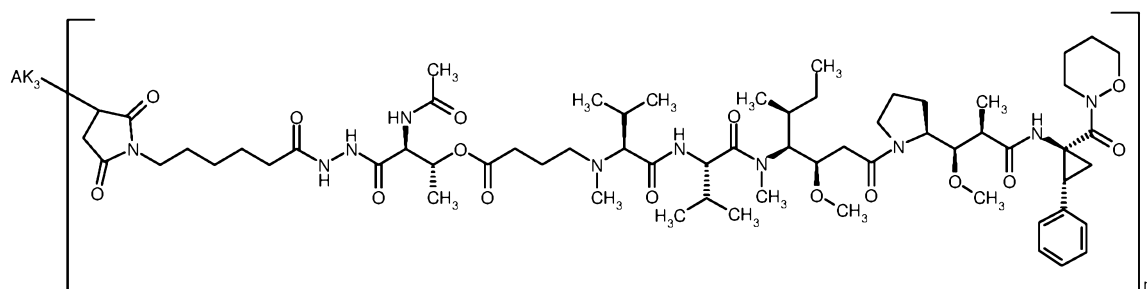


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
5 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.5 mg/ml

Drug/mAb ratio: 3.1

Example 156



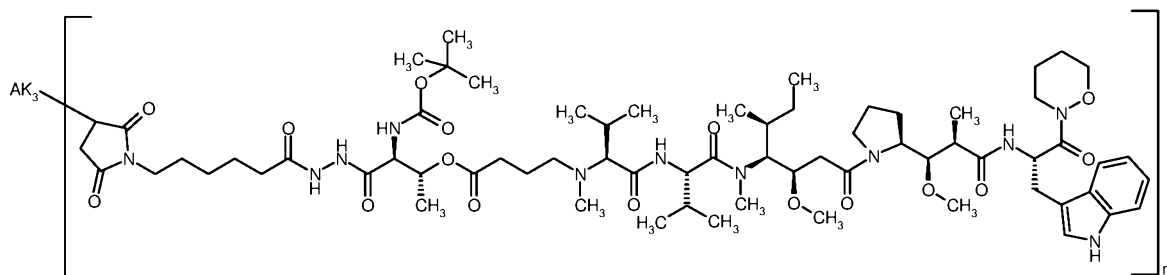
10

5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: mg/ml

Drug/mAb ratio:

Example 157

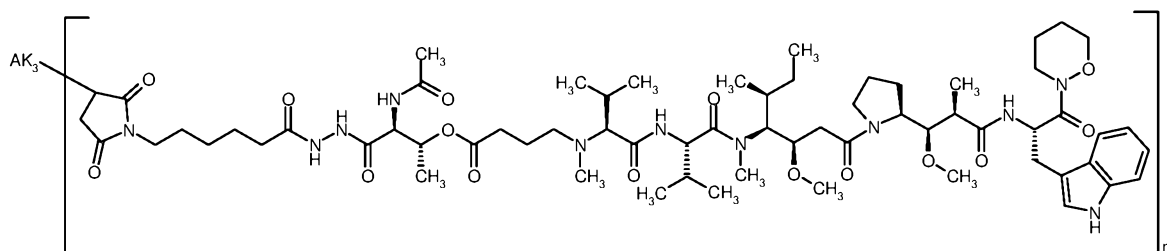


5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
5 column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.62 mg/ml

Drug/mAb ratio: 2.2

Example 158



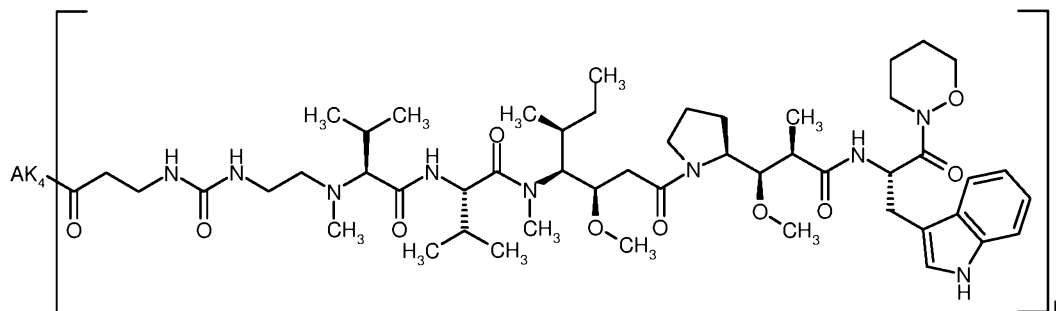
10

5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex
column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.37 mg/ml

Drug/mAb ratio: 2.8

Example 159



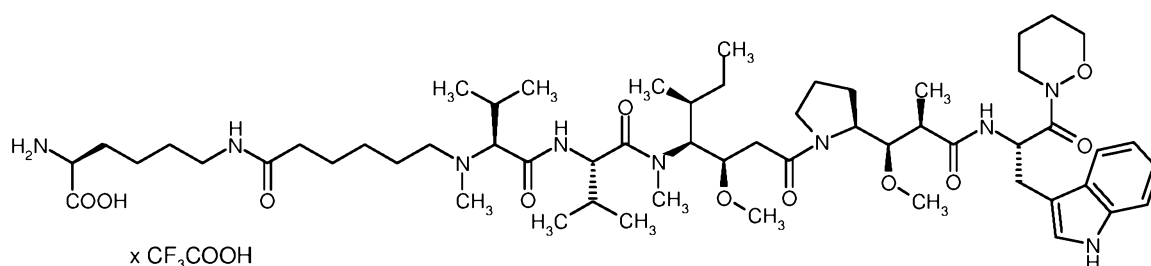
5 mg MF-Ta in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

5 Protein concentration: 1.43 mg/ml

Drug/mAb ratio: 4.0

Example 160 **

N-(6-([(5S)-5-amino-5-carboxypentyl]amino)-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-
 {(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-
 10 methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-
 methyl-L-valinamide trifluoroacetate



15.5 mg (15 μ mol) of Intermediate 210 weretaken up in 5 ml DMF and mixed with 4.4 mg (18 μ mol) N^2 -(*tert*.-butoxycarbonyl)-L-lysine and 7.7 μ L (44 μ mol) *N,N*-diisopropylethylamine. The reaction mixture was stirred overnight at RT, then concentrated *in vacuo*. The residue was purified by means of preparative HPLC. The yield was 14 mg (81% of theory) of the protected intermediate of the title compound, which were then taken up in 1 mldichloromethane and deprotected with 1 mltrifluoroascetic acid. The reaction mixture was concentrated and, after

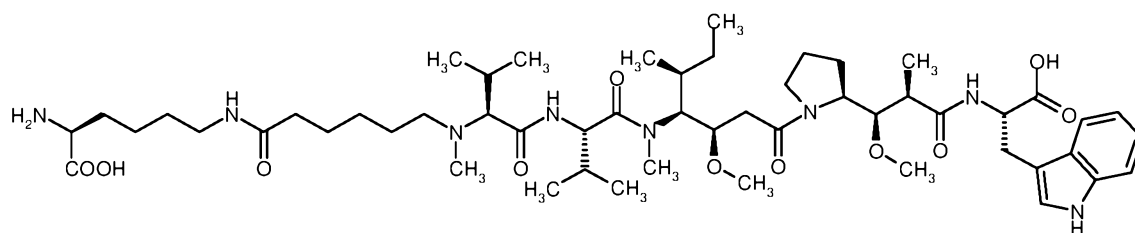
lyophilization of the residue from acetonitrile/wasser1:1, 15 mg (97% of theory) of the title compound were obtained.

HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.79$ min; MS (ESIpos): $m/z = 1083$ (M+H)⁺.

5 Example 161

N-(6-{[(5S)-5-amino-5-carboxypentyl]amino}-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



10

40 mg (40 μ mol) of Intermediate 226 were taken up in 5 ml DMF and mixed with 11.5 mg (40 μ mol) N²-[(benzyloxy)carbonyl]-L-lysine and 13 μ l (80 μ mol) *N,N*-diisopropylethylamine. The reaction mixture was stirred overnight at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 32.5 mg (70% of theory) of the protected intermediate of the title compound.

15

These 32.5 mg of the intermediate were dissolved in 10 ml methanol and, after adding 2 mg 10% palladium on activated carbon, hydrogenated for 30 min at RT under standard hydrogen pressure. The catalyst was then filtered off and the solvent removed *in vacuo*. Following lyophilization of the residue from dioxane/water 1:1, 26 mg (99% of theory) of the title compound were obtained.

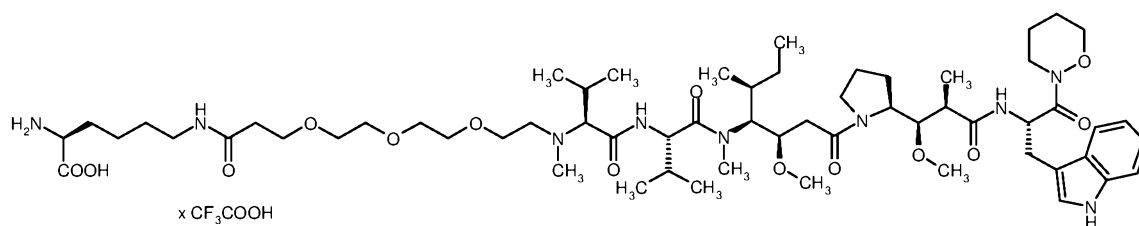
20 HPLC (Method 12): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.76$ min; MS (ESIpos): $m/z = 1014$ (M+H)⁺.

Example 162

N-[(18S)-18-amino-18-carboxy-12-oxo-3,6,9-trioxa-13-azaotadec-1-yl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-

2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate

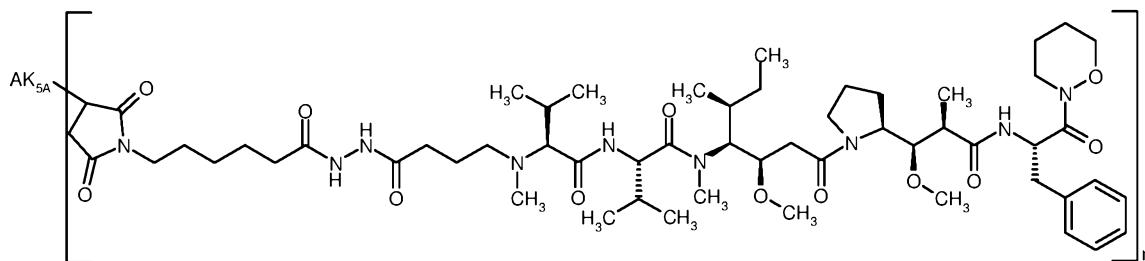


- 5 3.5 mg (3 μ mol) of Intermediate 202 were taken up in 2 ml DMF and mixed with 0.8 mg (3 μ mol) N^2 -(tert-butoxycarbonyl)-L-lysine and 1.6 μ l (10 μ mol) N,N -diisopropylethylamine. The reaction mixture was stirred overnight at RT and then concentrated *in vacuo*. The residue was taken up in acetonitrile/water 1:1, adjusted to pH 2 with trifluoroacetic acid and then purified by means of preparative HPLC. The yield was 1 mg (25% of theory) of the protected intermediate of
- 10 the title compound that was then taken up in 500 μ l dichloromethane and deprotected with 500 μ l trifluoroacetic acid. The reaction mixture was concentrated and, following lyophilization of the residue from acetonitrile/water 1:1, 1 mg (89% of theory) of the title compound was obtained.

HPLC (Method 12): $R_t = 1.9$ min;

- 15 LC-MS (Method 1): $R_t = 0.82$ min; MS (ESIpos): $m/z = 1173$ ($M+H$) $^+$.

Example 163

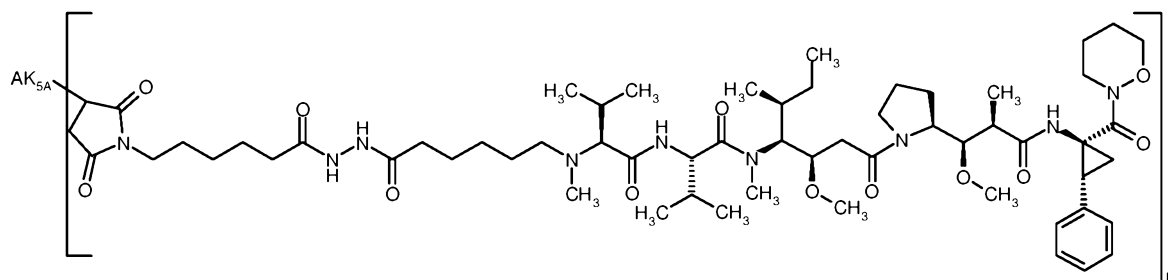


- 70 mg anti-C4.4a M31-B01 in DPBS pH 7.4 were presently used for the coupling and, after
- 20 purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 12.2 mg/ml

Drug/mAb ratio: 1.5

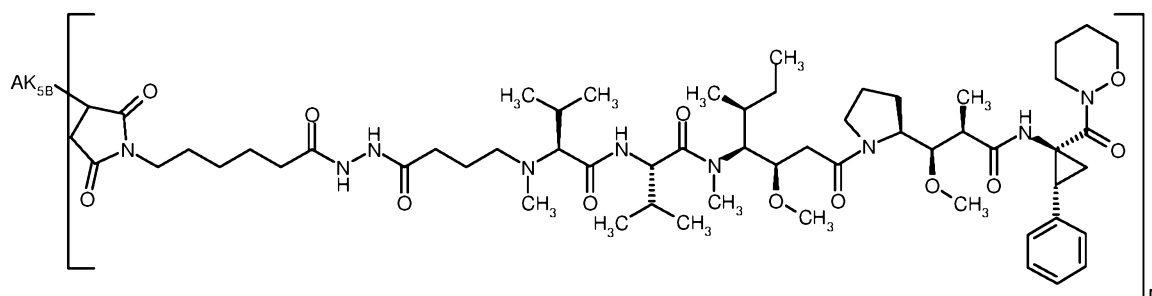
Example 164



5 Protein concentration: 0.87 mg/ml

Drug/mAb ratio: 5.8

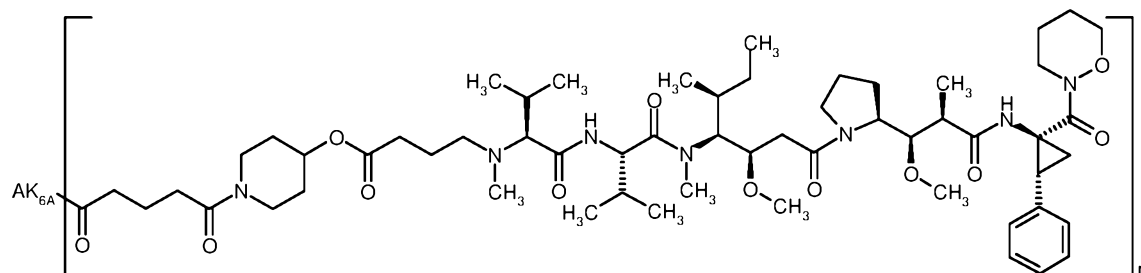
Example 165



10 Protein concentration: 1.16 mg/ml

Drug/mAb ratio: 3.1

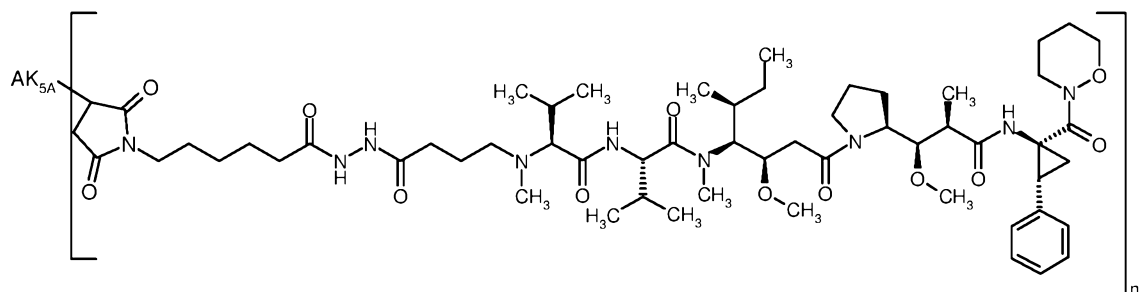
Example 166



Protein concentration: 1.24 mg/ml

Drug/mAb ratio: 1.6

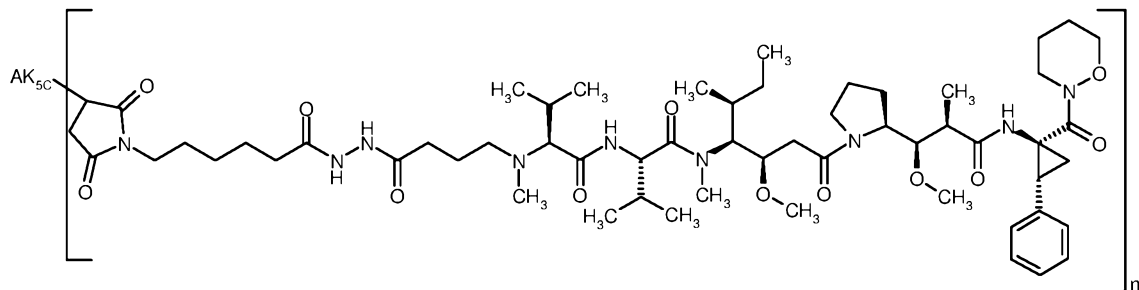
Example 167



5 Protein concentration: 0.88 mg/ml

Drug/mAb ratio: 6.9

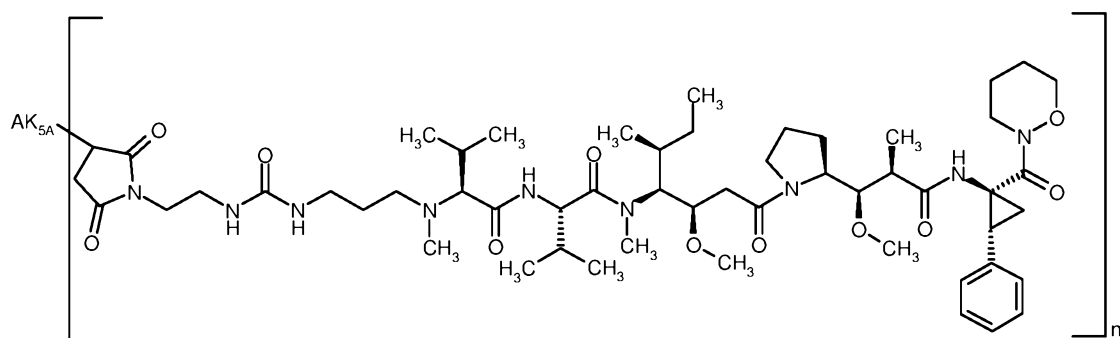
Example 168



Protein concentration: 1.2 mg/ml

10 Drug/mAb ratio: 2.8

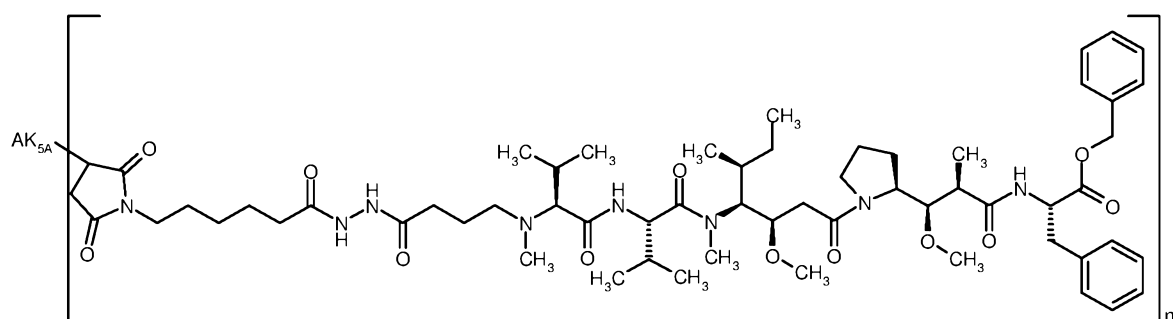
Example 169



Protein concentration: 0.9 mg/ml

Drug/mAb ratio: 3.9

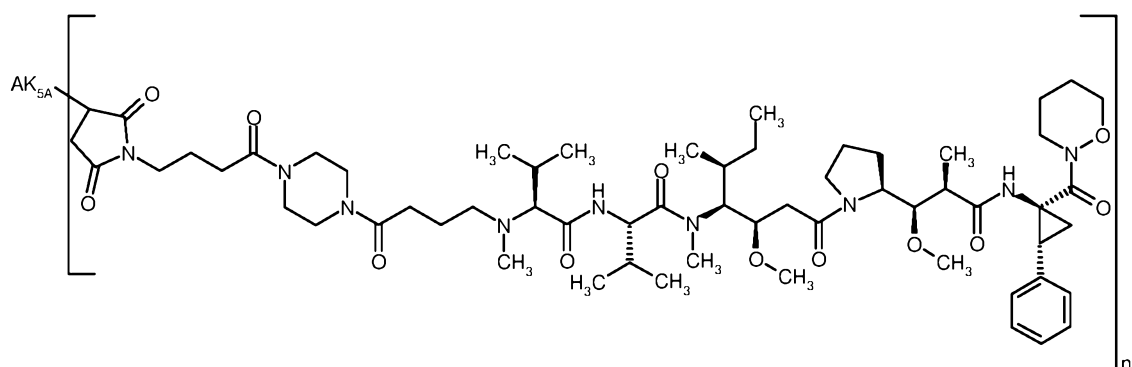
5 Example 170



Protein concentration: 0.52 mg/ml

Drug/mAb ratio: 1.6

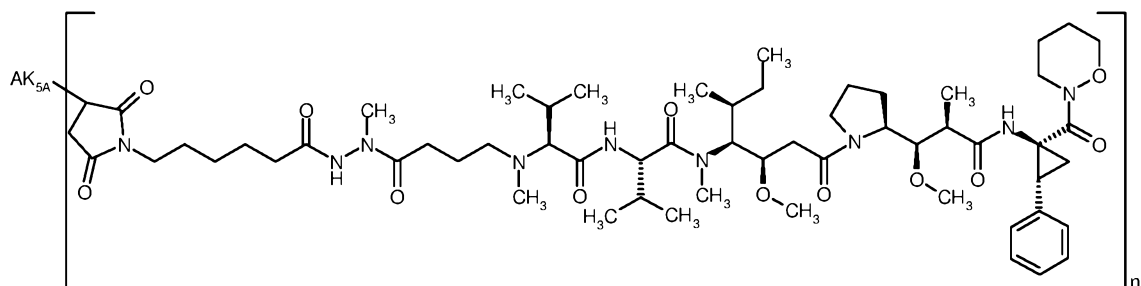
10 Example 171



Protein concentration: 1.46 mg/ml

Drug/mAb ratio: 2.5

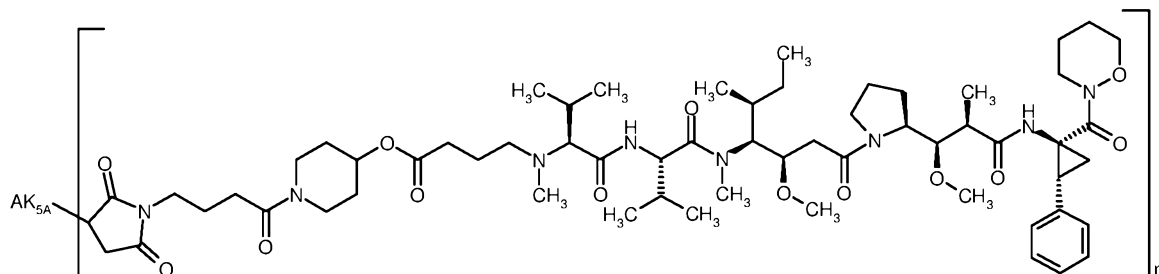
Example 175



5 Protein concentration: 0.45 mg/ml

Drug/mAb ratio: 3.3

Example 176



10 Protein concentration: 0.98 mg/ml

Drug/mAb ratio: 3.6

CN(C)CCCC(=O)NNC(=O)CCCCN(C)[C@H](C)C(=O)N[C@@H](C)C(=O)N(C)[C@H](C)[C@H](OC)CC(=O)N1CC[C@H]1[C@H](OC)C(=O)N[C@@H](Cc2c[nH]c3ccccc23)C(=O)OCc4ccccc4

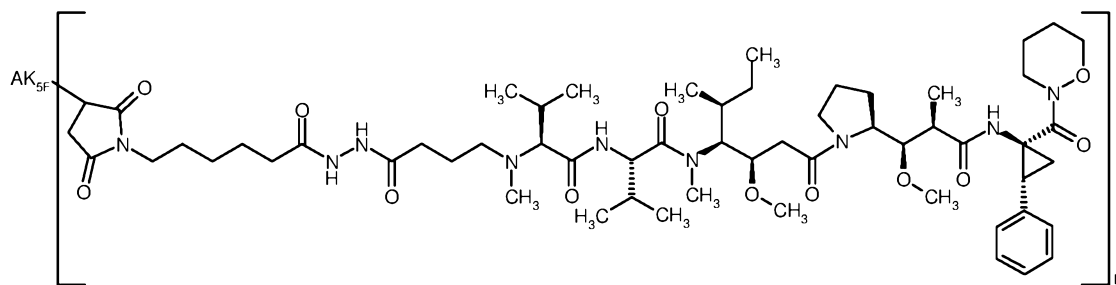
Protein concentration: 9.42 mg/ml

Drug/mAb ratio: 4.1

[illegible]

Drug/mAb ratio: 1.8

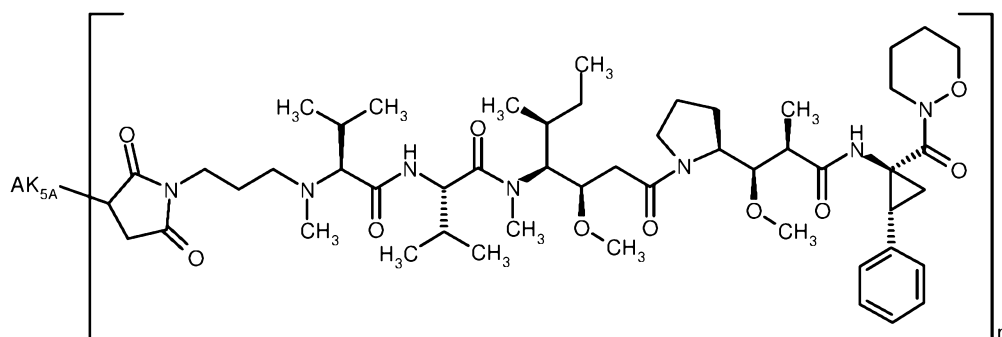
Example 179



Protein concentration: 1.07 mg/ml

Drug/mAb ratio: not detectable

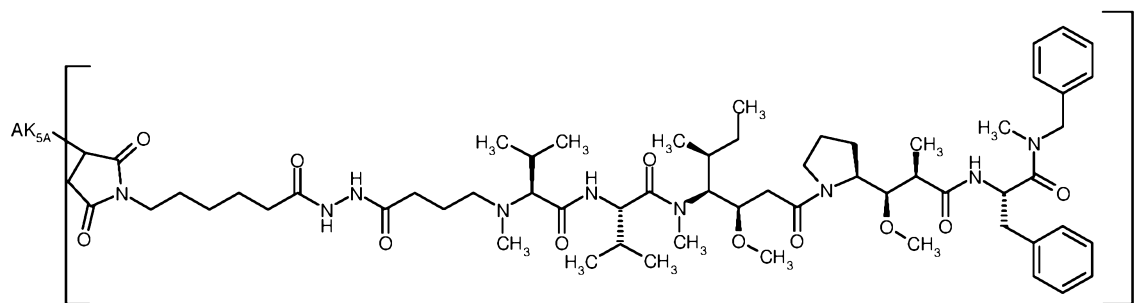
5 Example 180



Protein concentration: 0.47 mg/ml

Drug/mAb ratio: 4.4

Example 181

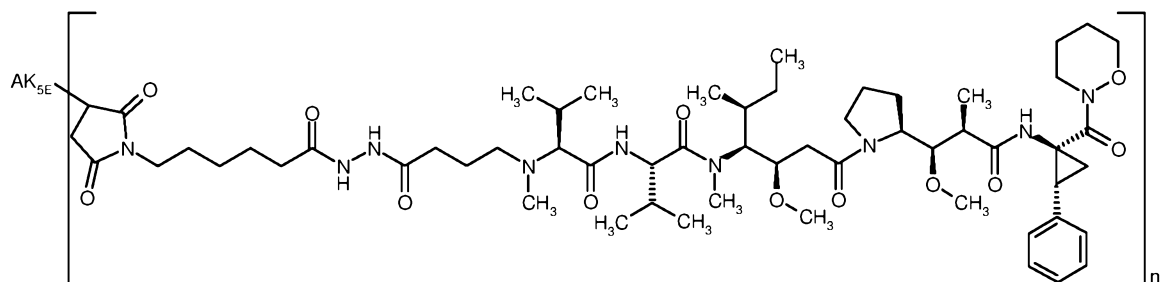


10

Protein concentration: 0.43 mg/ml

Drug/mAb ratio: 4.8

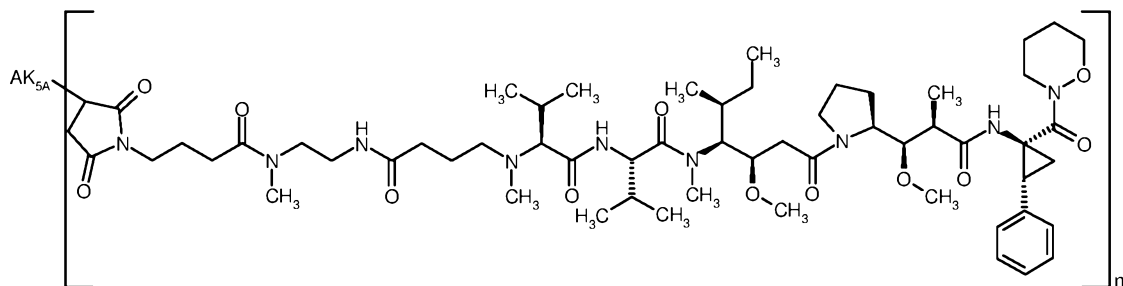
Example 182



5 Protein concentration: 1.01 mg/ml

Drug/mAb ratio: 2.6

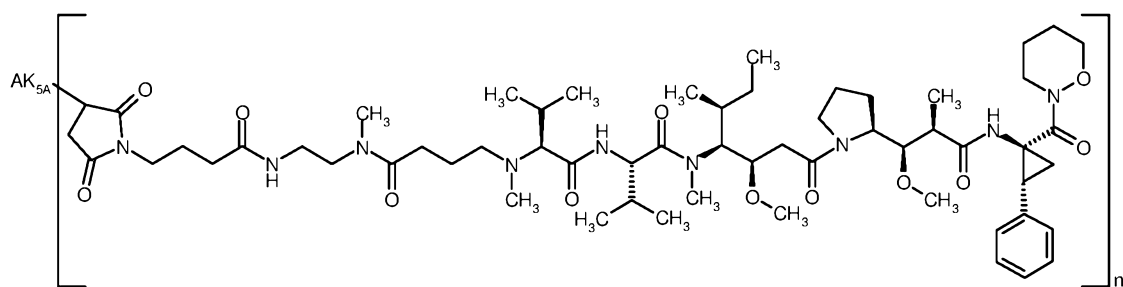
Example 183



Protein concentration: 0.53 mg/ml

10 Drug/mAb ratio: 0.6

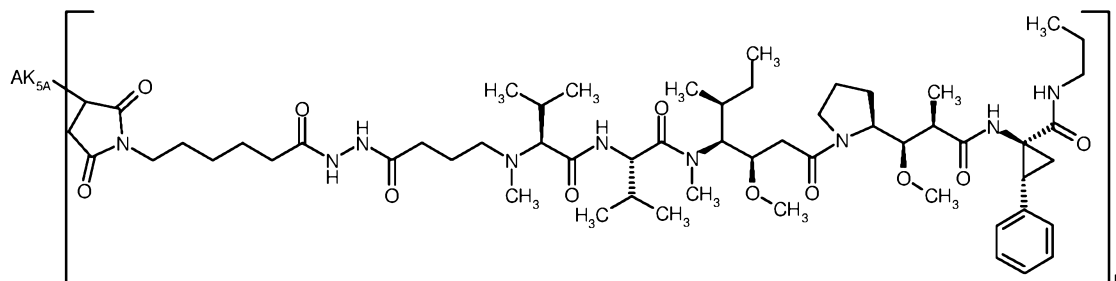
Example 184



Protein concentration: 0.55 mg/ml

Drug/mAb ratio: 1.3

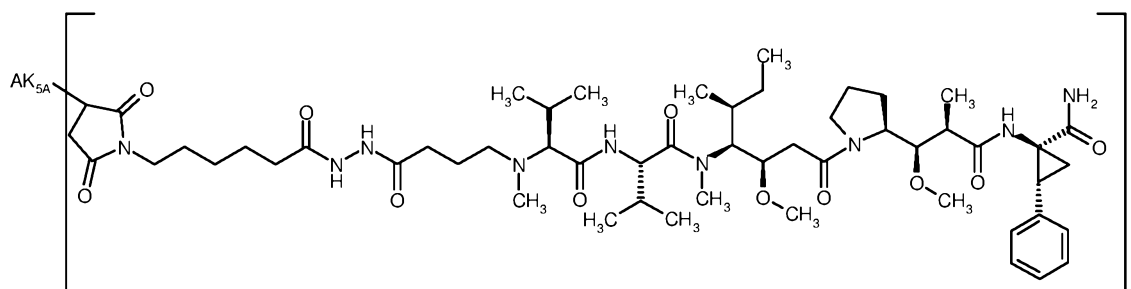
Example 185



5 Protein concentration: 0.65 mg/ml

Drug/mAb ratio: 1.1

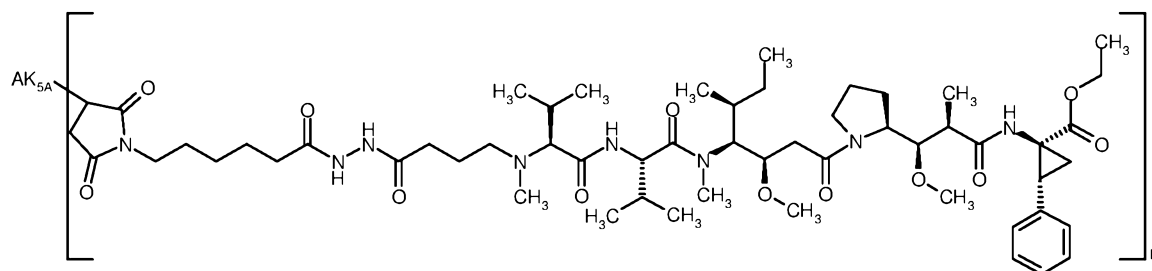
Example 186



Protein concentration: 1.04

10 Drug/mAb ratio: 3.5

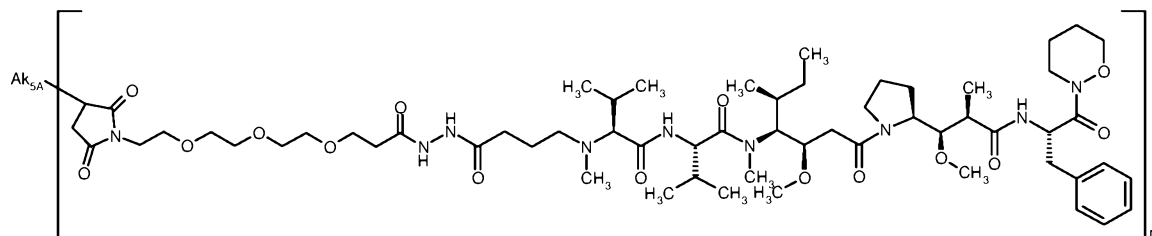
Example 187



Protein concentration: 0.62 mg/ml

5 Drug/mAb ratio: 2.4

Example 188

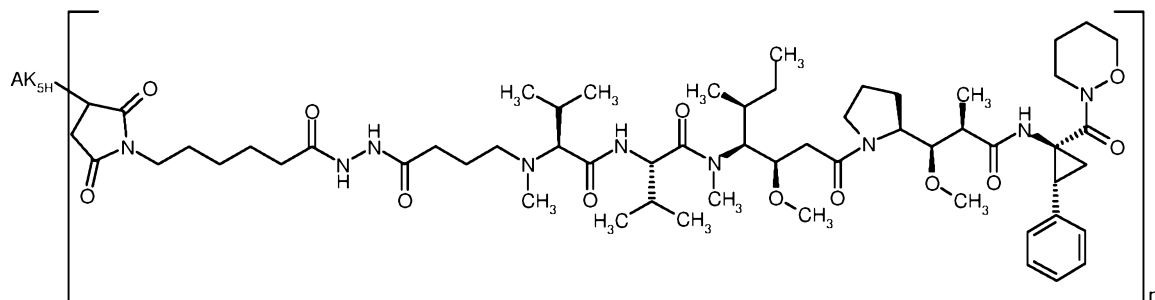


90 mg anti-C4.4a M31-B01 in DPBS pH 7.4 were presently used for the coupling and, after
 10 purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 11.2 mg/ml

Drug/mAb-Ratio: 2.3

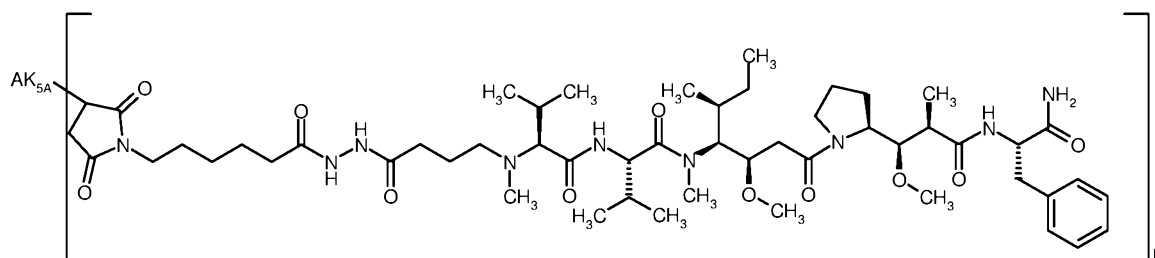
Example 189



Protein concentration: 1.11 mg/ml

5 Drug/mAb-Ratio: 2.4

Example 190

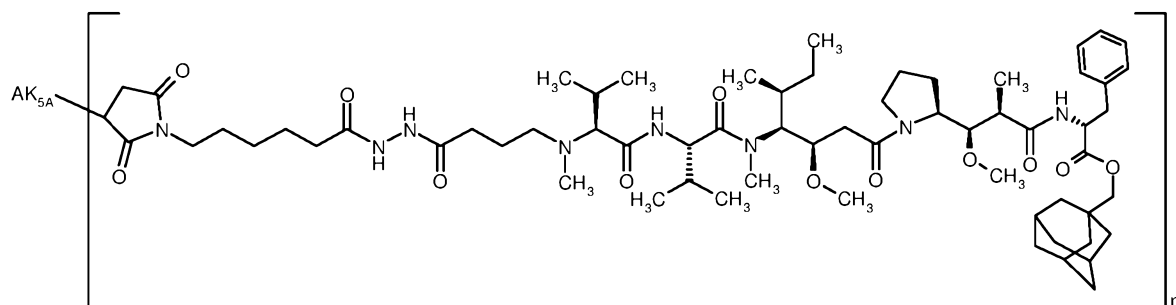


70 mg anti-C4.4a M31-B01 in DPBS pH 7.4 were presently used for the coupling and, after
 10 purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 10.7 mg/ml

Drug/mAb ratio: 2.2

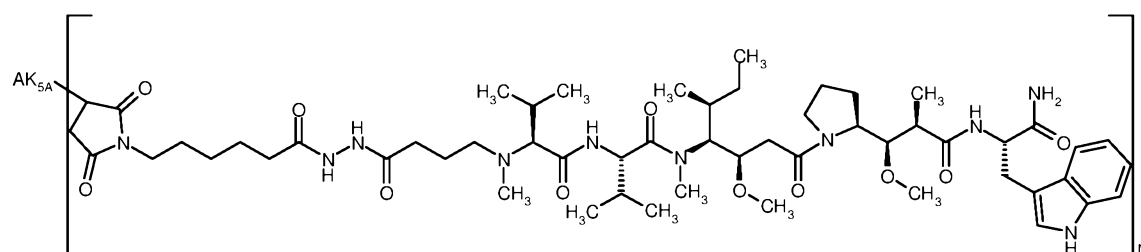
Example 191



Protein concentration: 0.87 mg/ml

5 Drug/mAb ratio: 1.8

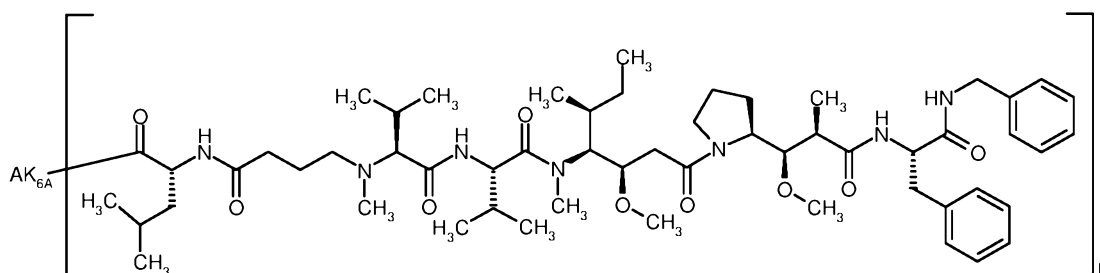
Example 192



Protein concentration: 1.3 mg/ml

Drug/mAb ratio: 2.1

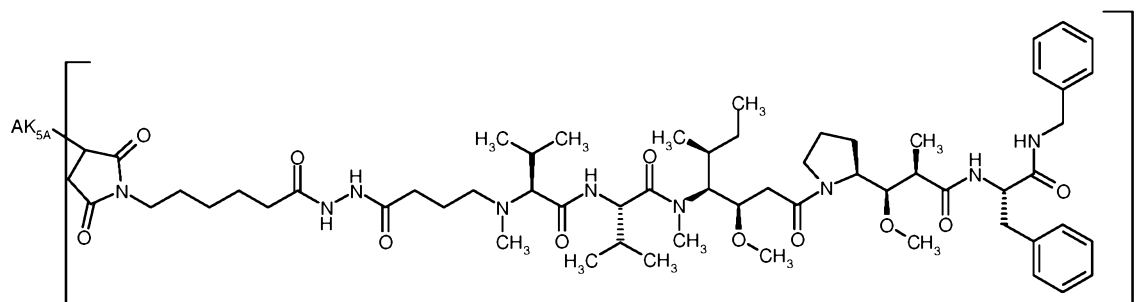
10 Example 193



Protein concentration: 1.3 mg/ml

Drug/mAb ratio: 0.3

Example 194

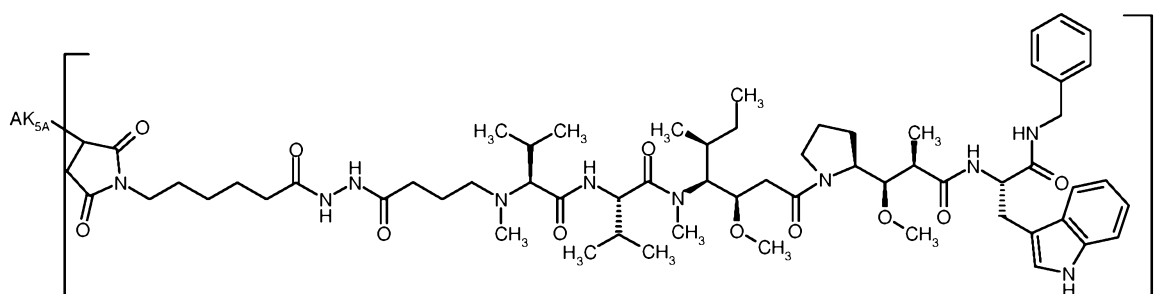


70 mg anti-C4.4a M31-B01 in DPBS pH 7.4 were presently used for the coupling and, after
5 purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 12.0 mg/ml

Drug/mAb ratio: 3.2

Example 195



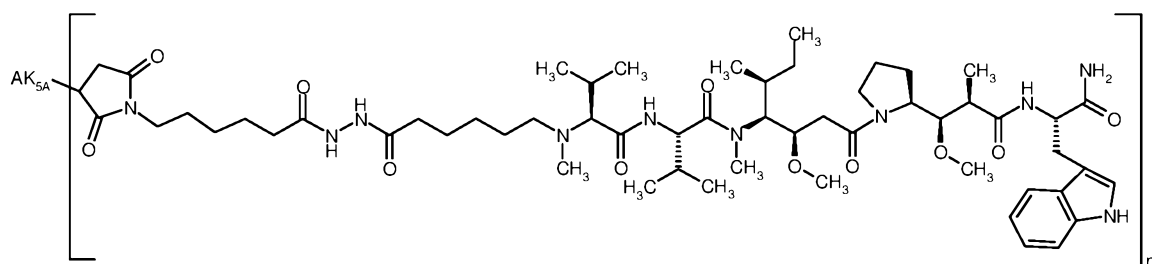
10

90 mg anti-C4.4a M31-B01 in DPBS pH 7.4 were presently used for the coupling and, after
purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 10.2 mg/ml

Drug/mAb ratio: 4.3

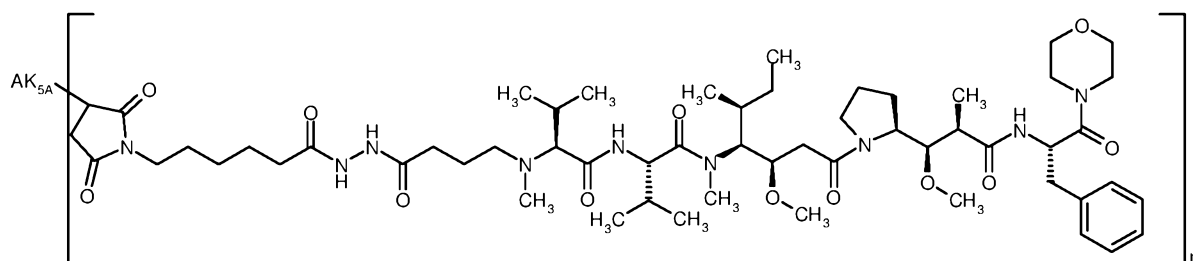
Example 196



Protein concentration: 1.37 mg/ml

5 Drug/mAb ratio: 2.6

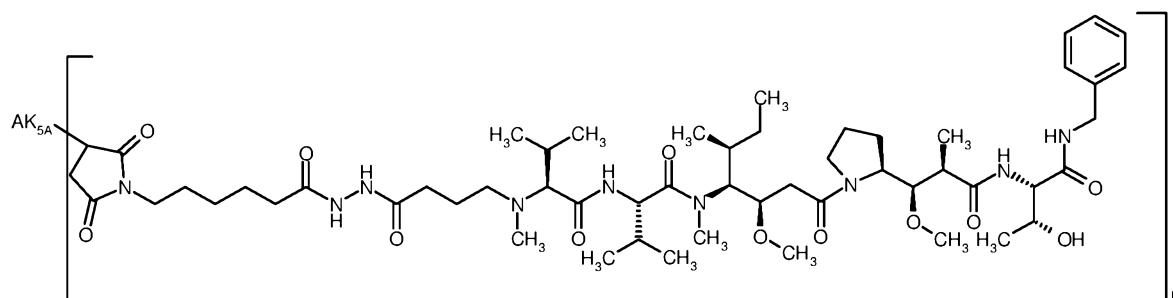
Example 197



Protein concentration: 1.14 mg/ml

10 Drug/mAb ratio: 2.0

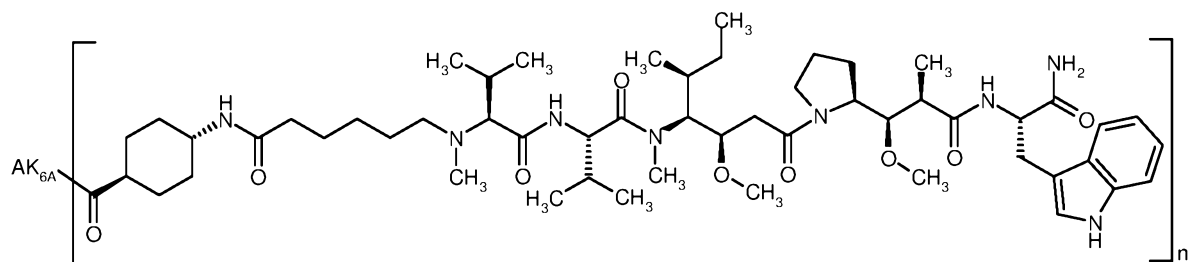
Example 198



Protein concentration: 1.07 mg/ml

Drug/mAb ratio: 3.5

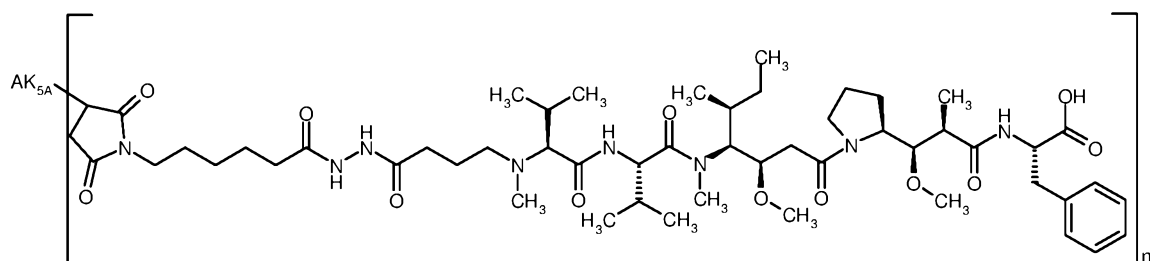
Example 199



5 Protein concentration: 1.14 mg/ml

Drug/mAb ratio: 1.9

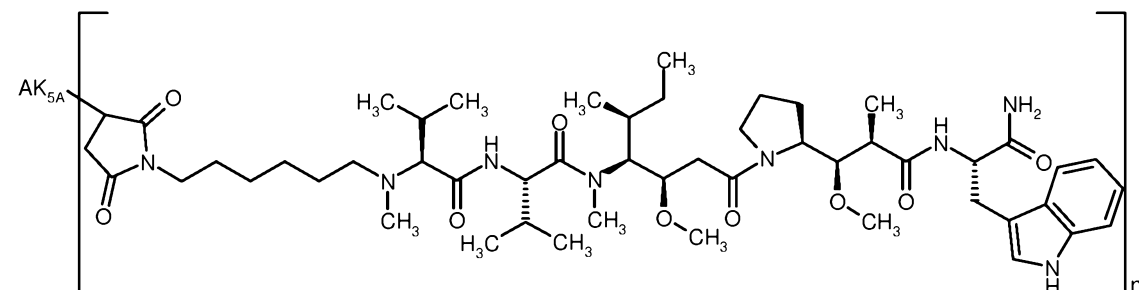
Example 200



10 Protein concentration: 1.22 mg/ml

Drug/mAb ratio: 3.3

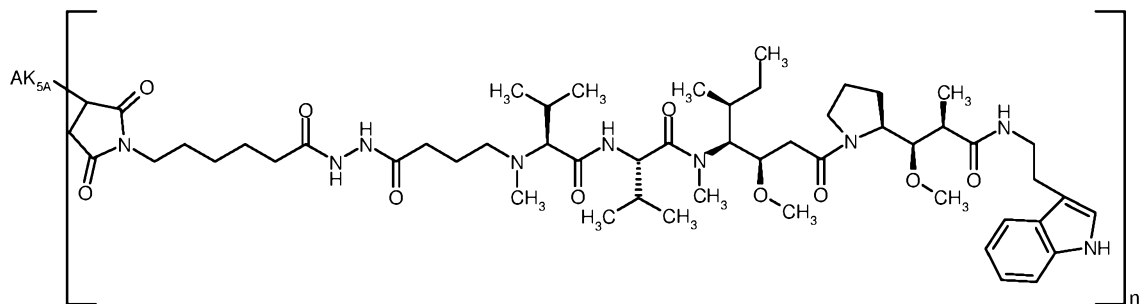
Example 201



Protein concentration: 1.3 mg/ml

Drug/mAb ratio: 3.2

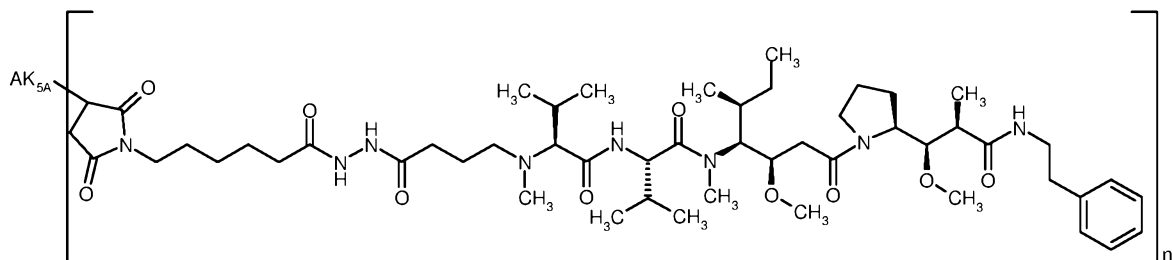
Example 202



5 Protein concentration: 1.23 mg/ml

Drug/mAb ratio: 3.3

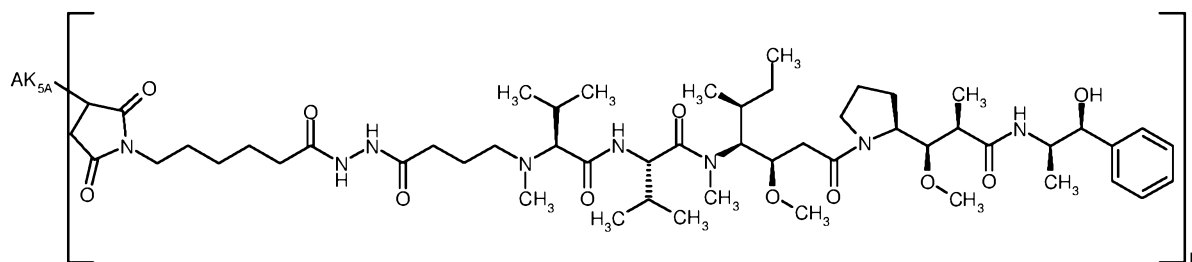
Example 203



10 Protein concentration: 1.64 mg/ml

Drug/mAb ratio: 1.8

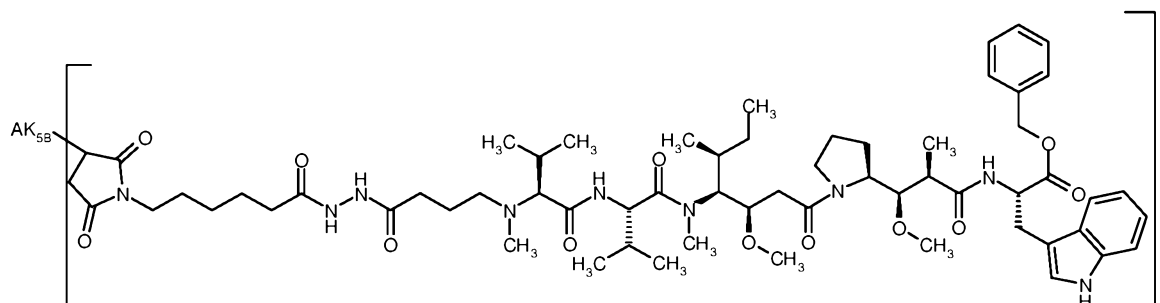
Example 204



Protein concentration: 1.07 mg/ml

5 Drug/mAb ratio: 3.1

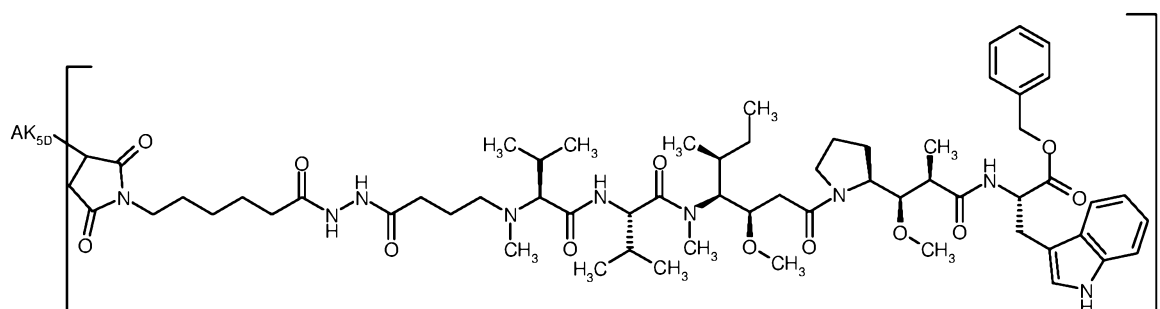
Example 205



Protein concentration: 1.14 mg/ml

10 Drug/mAb ratio: 2.3

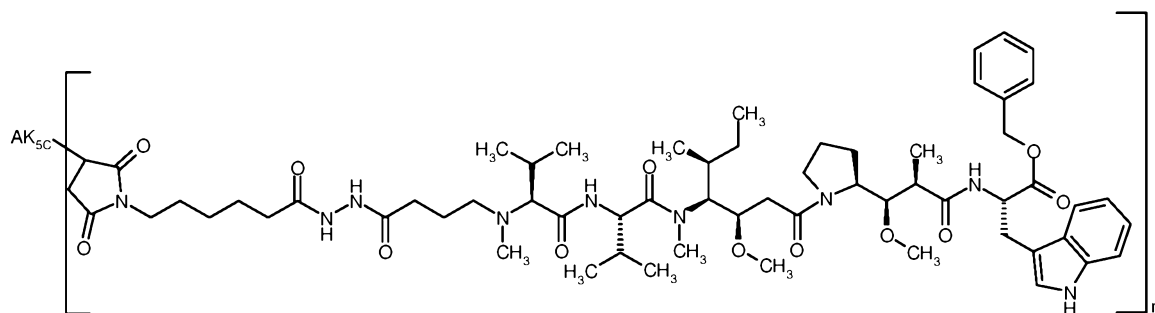
Example 206



Protein concentration: 1.23 mg/ml

Drug/mAb ratio: 3.4

Example 207

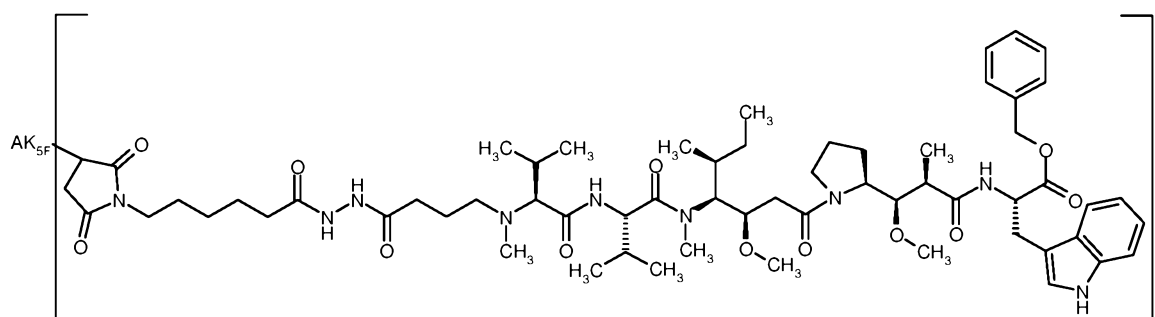


5

Protein concentration: 1.22 mg/ml

Drug/mAb ratio: 2.5

Example 208

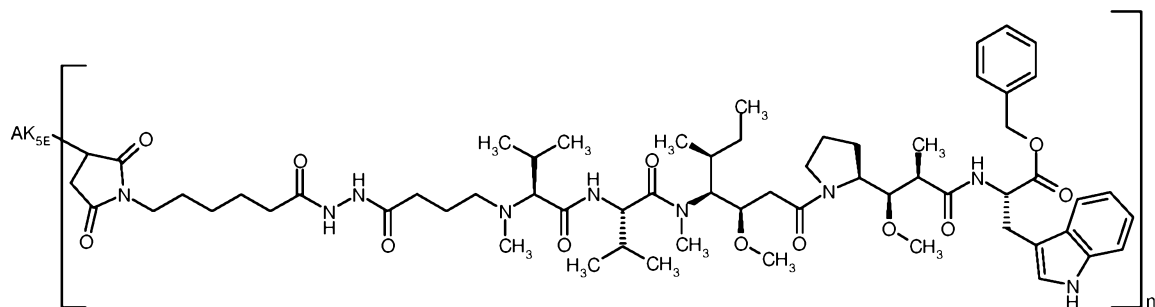


10

Protein concentration: 1.22 mg/ml

Drug/mAb ratio: 2.4

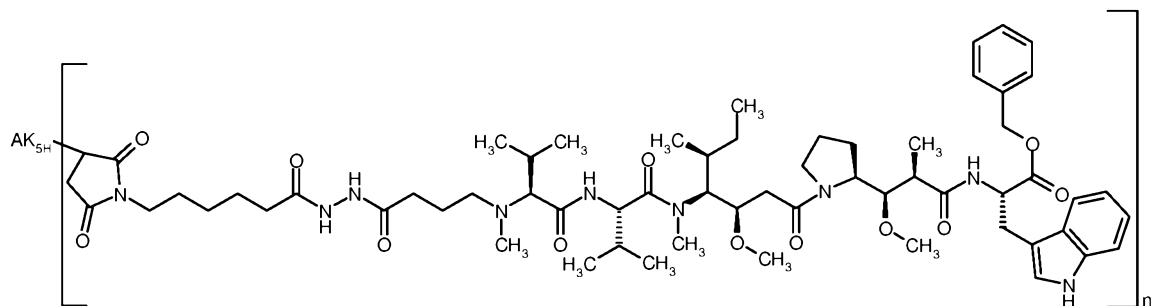
Example 209



Protein concentration: 1.32 mg/ml

- 5 Drug/mAb ratio: not detectable

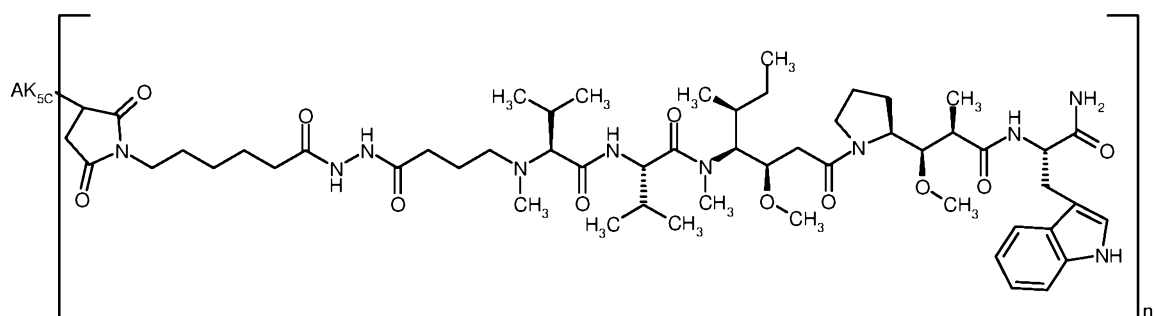
Example 210



Protein concentration: 1.44 mg/ml

- 10 Drug/mAb ratio: 2.3

Example 211

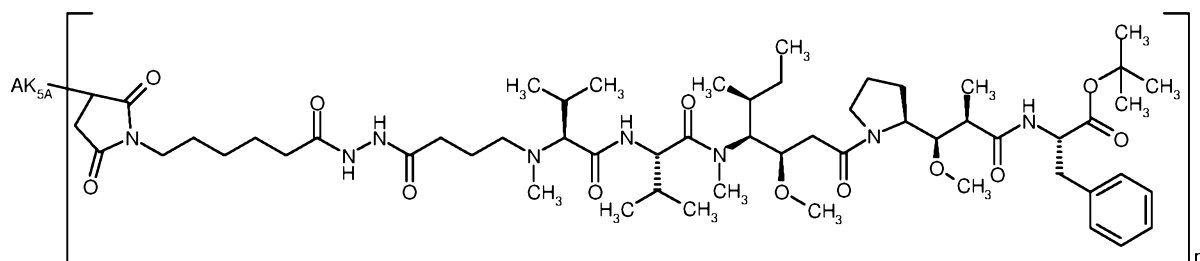


250 mg anti-C4.4a B01-10 in DPBS pH 7.4 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 12.8 mg/ml

Drug/mAb ratio: 5.2

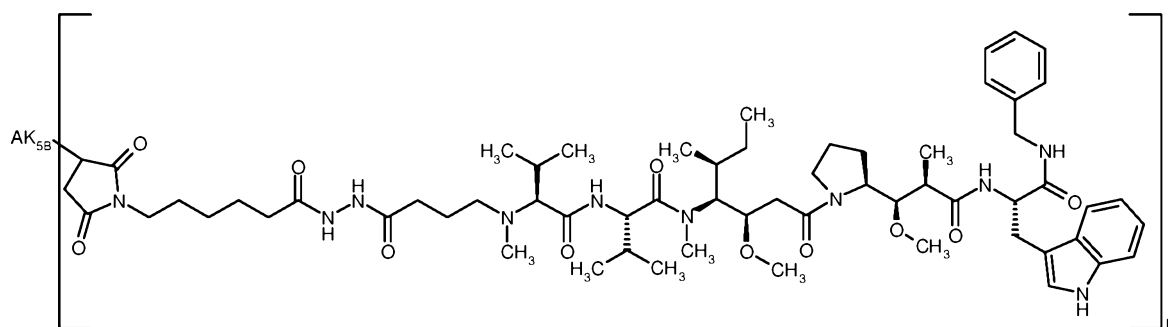
5 Example 212



Protein concentration: 0.9 mg/ml

Drug/mAb ratio: 2

10 Example 213

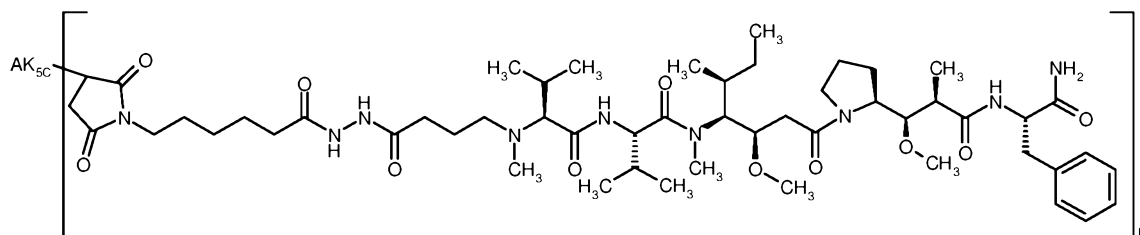


250 mg anti-C4.4a B01-3 in DPBS pH 7.4 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

15 Protein concentration: 8.0 mg/ml

Drug/mAb ratio: 4.5

Example 214

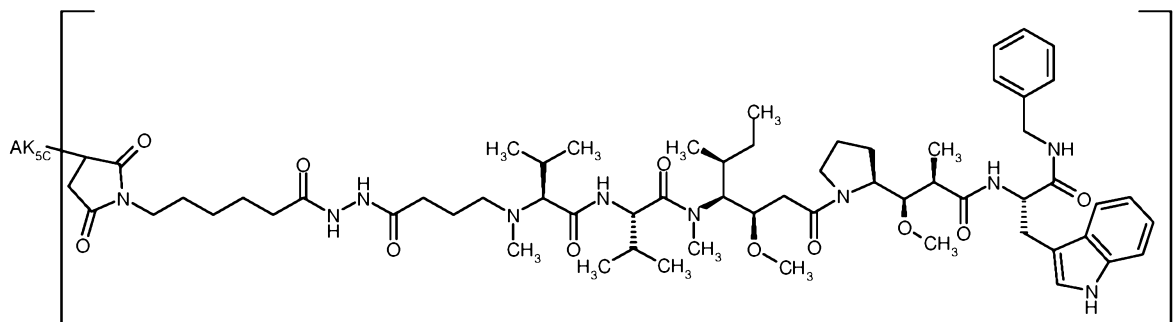


250 mg anti-C4.4a B01-10 in DPBS pH 7.4 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

5 Protein concentration: 12.3 mg/ml

Drug/mAb ratio: 5.2

Example 215

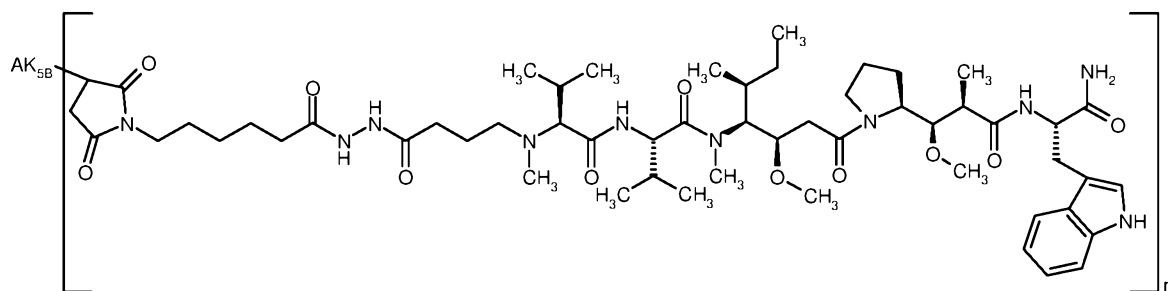


10 250 mg anti-C4.4a B01-10 in DPBS pH 7.4 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 10.2 mg/ml

Drug/mAb ratio: 4.4

Example 216

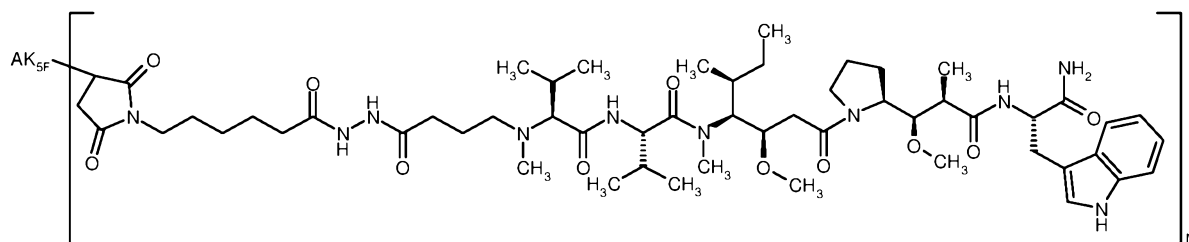


50 mg anti-C4.4a B01-3 in DPBS pH 7.4 were presently used for the coupling and, after
 5 purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 11.5 mg/ml

Drug/mAb ratio: 5.2

Example 217



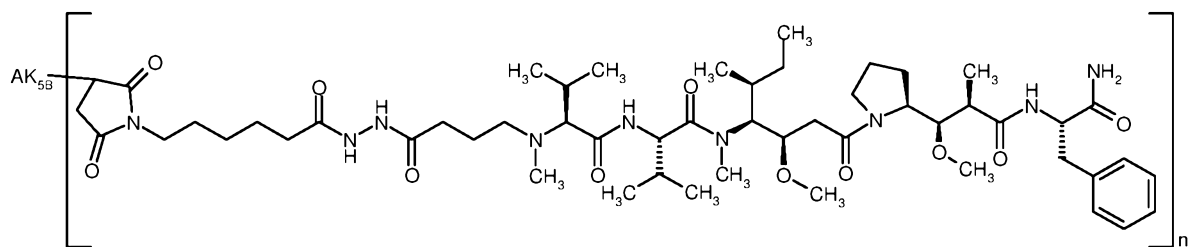
10

250 mg anti-C4.4a D02-6 in DPBS pH 7.4 were presently used for the coupling and, after
 purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 13 mg/ml

Drug/mAb ratio: 5.2

Example 218

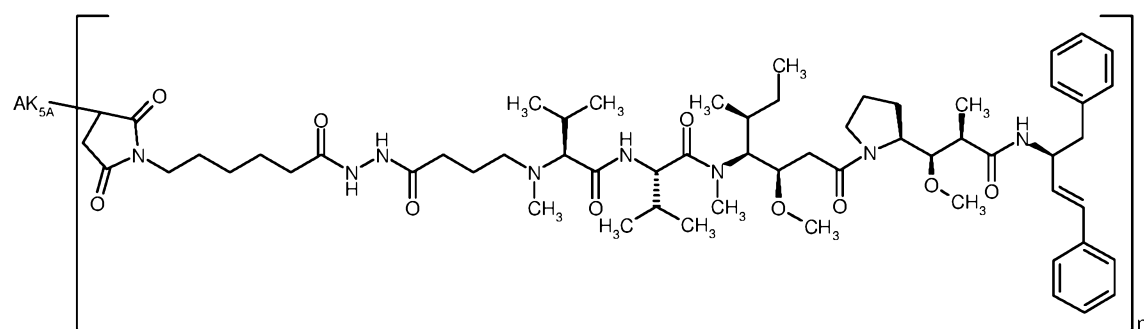


250 mg anti-C4.4a B01-3 in DPBS pH 7.4 were presently used for the coupling and, after
 5 purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 10.3 mg/ml

Drug/mAb ratio: 4.9

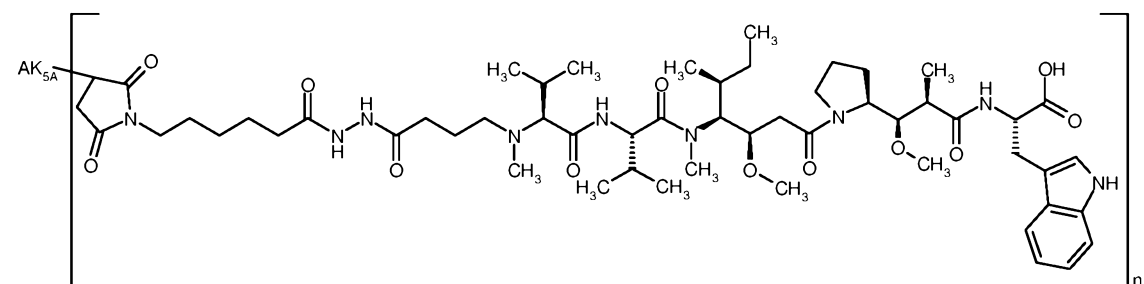
Example 219



10 Protein concentration: 0.88 mg/ml

Drug/mAb ratio: 3.2

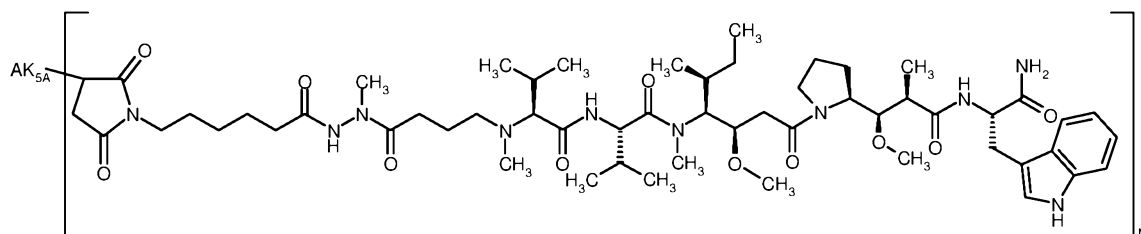
Example 220



Protein concentration: 1.18 mg/ml

Drug/mAb ratio: 3.4

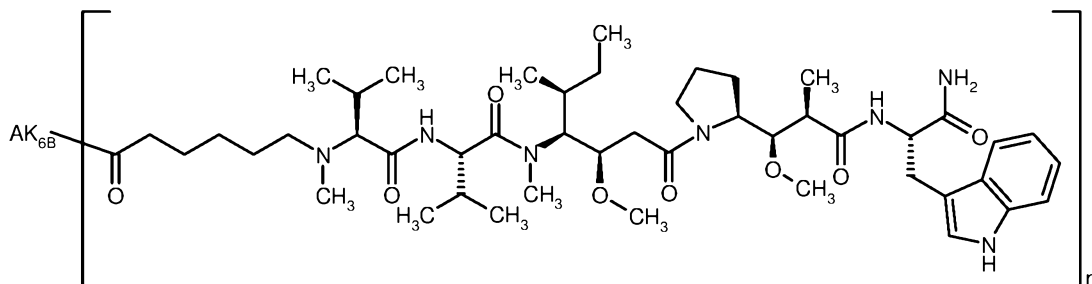
Example 221



5 Protein concentration: 1.23 mg/ml

Drug/mAb ratio: 3.0

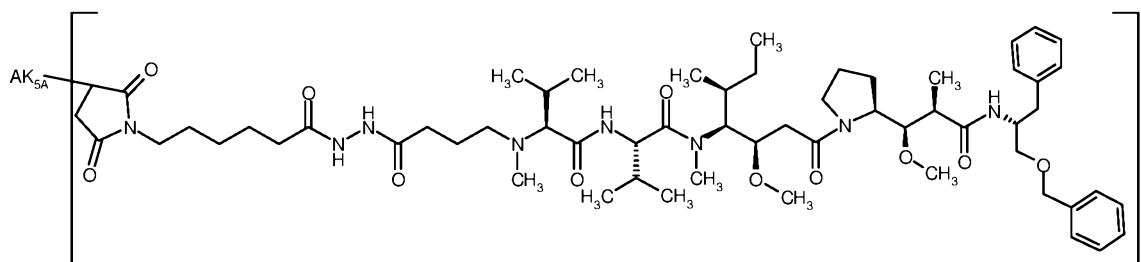
Example 222



Protein concentration: 1.3 mg/ml

10 Drug/mAb ratio: 3.3

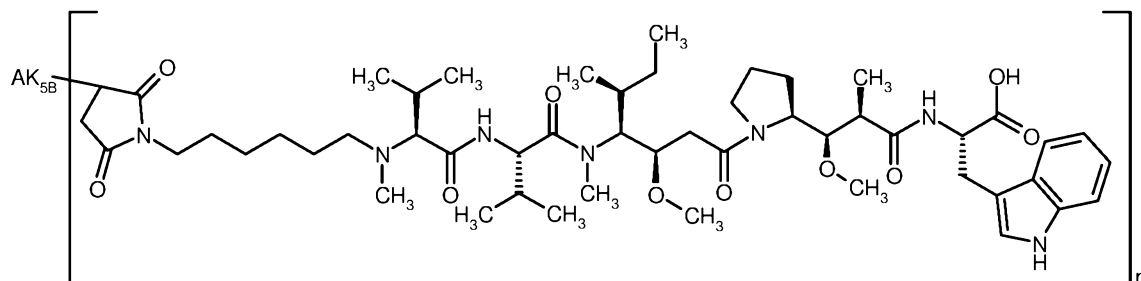
Example 223



Protein concentration: 1.11 mg/ml

Drug/mAb ratio: not detectable

Example 224

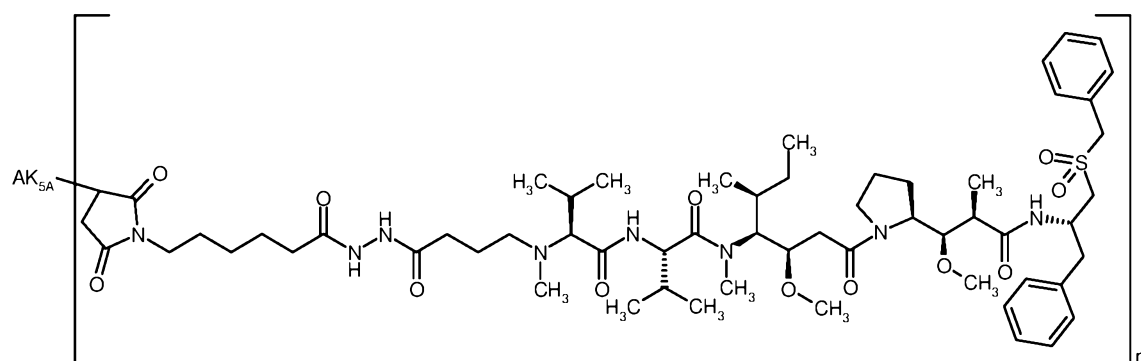


5

Protein concentration: 1.25 mg/ml

Drug/mAb ratio: 2.4

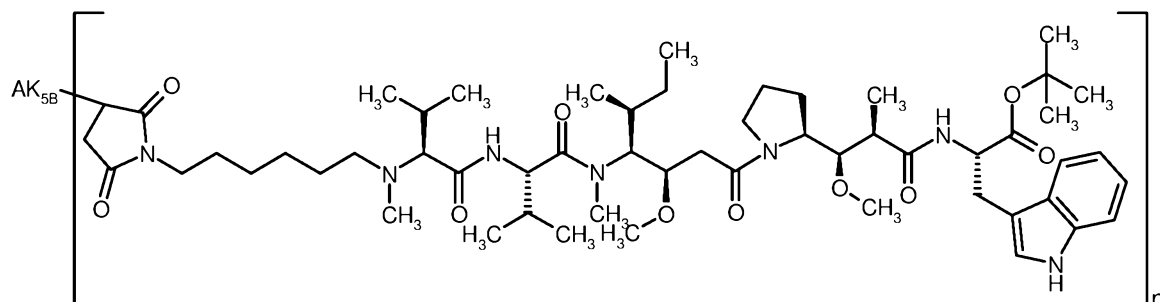
Example 225



10 Protein concentration: 0.88 mg/ml

Drug/mAb ratio: 5.0

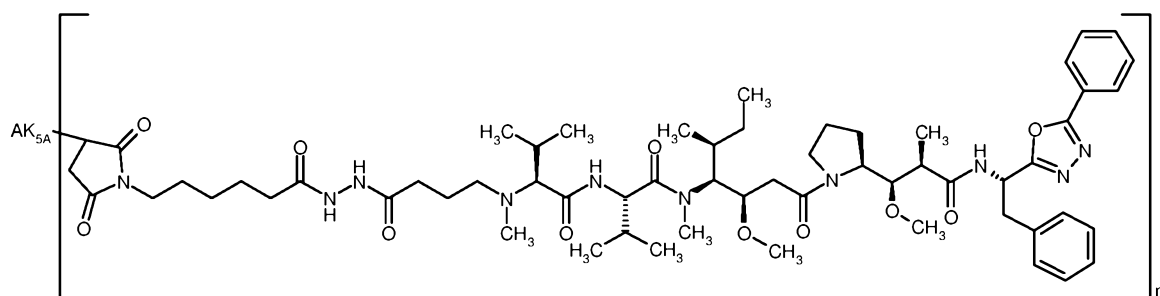
Example 226



Protein concentration: 1.23 mg/ml

5 Drug/mAb ratio: 3.3

Example 227

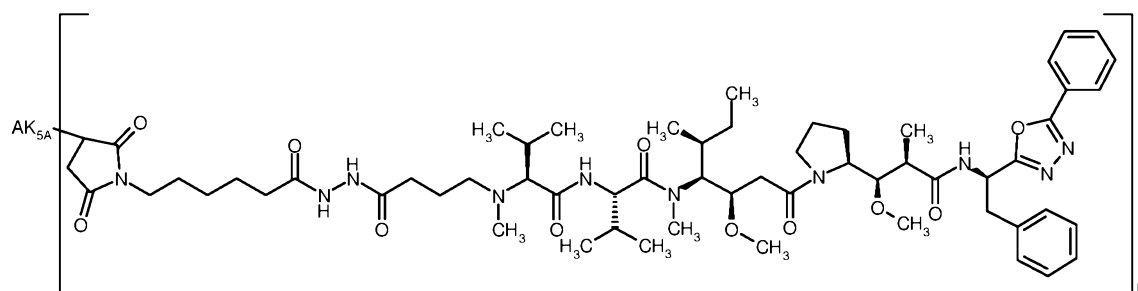


10

Protein concentration: 0.93 mg/ml

Drug/mAb ratio: 1.8

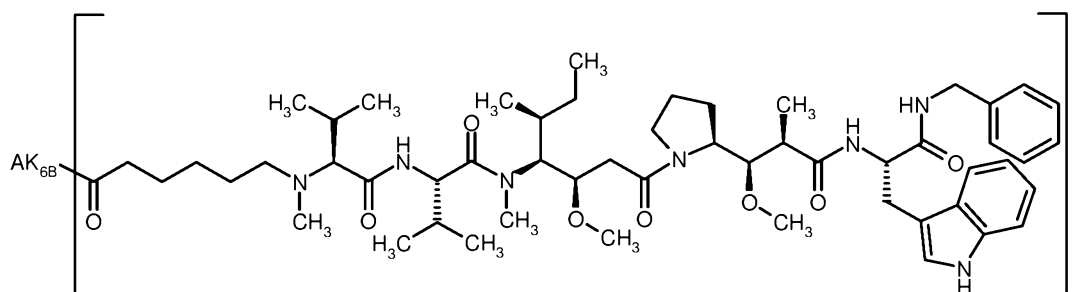
Example 228



Protein concentration: 0.85 mg/ml

Drug/mAb ratio: 5.3

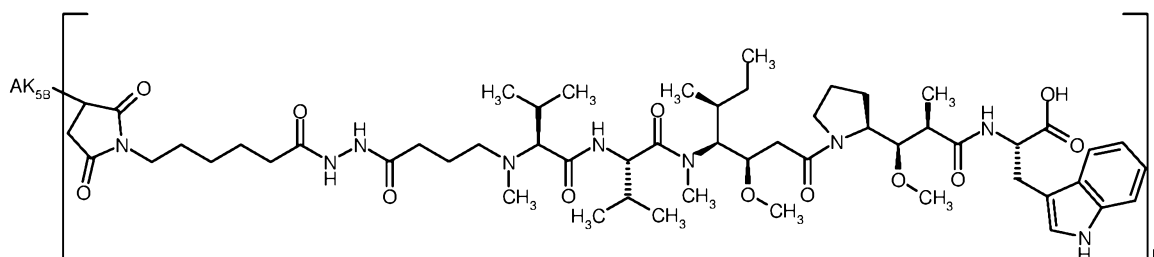
5 Example 229



Protein concentration: 1.51 mg/ml

Drug/mAb ratio: 1.4

Example 230



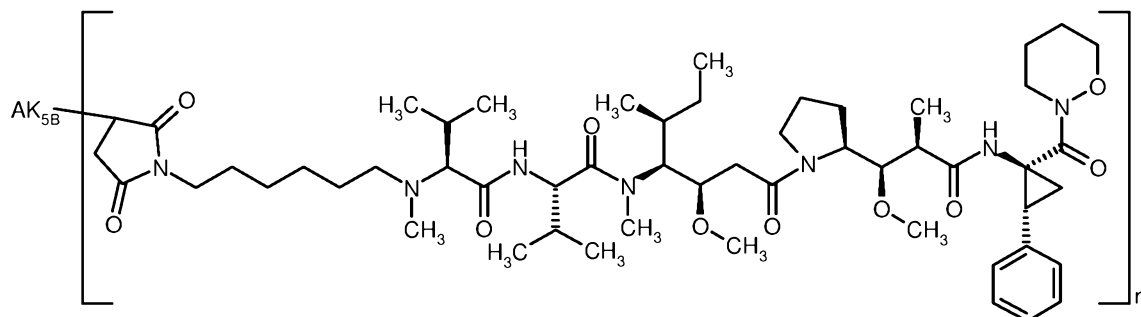
10

150 mg anti-C4.4a B01-3 in DPBS pH 7.4 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 11.0 mg/ml

Drug/mAb ratio: 4.5

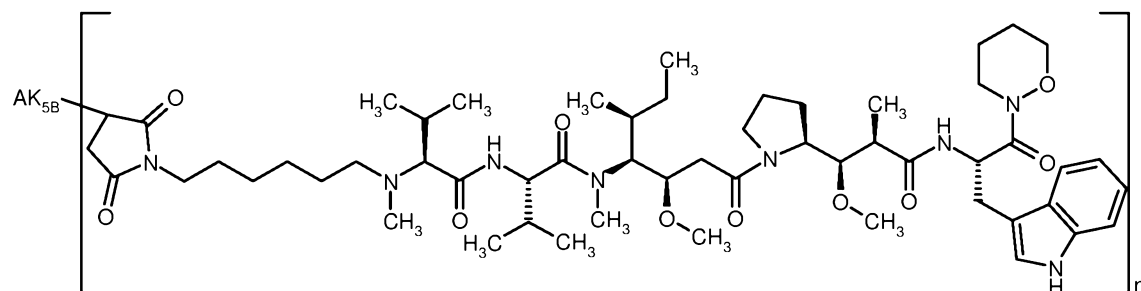
Example 231



5 Protein concentration: 1.2 mg/ml

Drug/mAb ratio: 3.3

Example 232



Protein concentration: 1.25 mg/ml

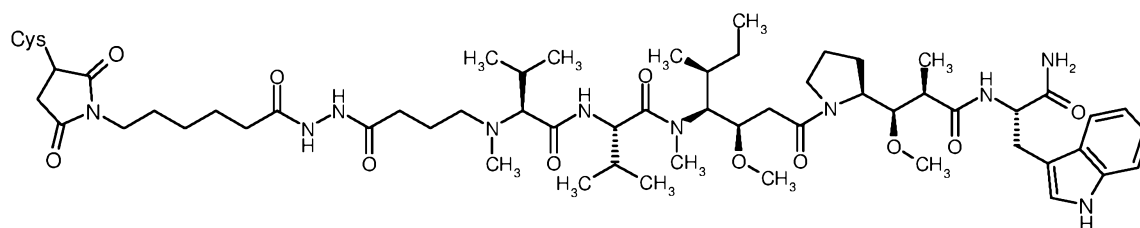
10 Drug/mAb ratio: 3.1

Example 233

N-(4-{2-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]

hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-

15 3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



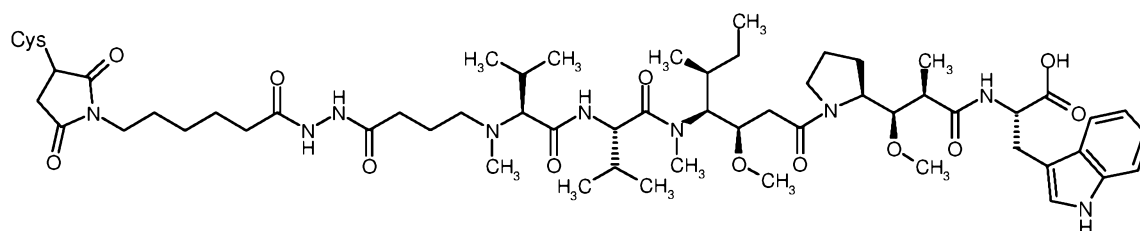
- 10 mg (10 μ mol) of Intermediate 157 weretaken up in 5.2 ml DMF and mixed with 2.28 mg (20 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 5.8 mg (48% of theory) of the title compound.

HPLC (Method 5): R_t = 1.45 min;

LC-MS (Method 1): R_t = 0.74 min; MS (ESIpos): m/z = 1184 (M+H)⁺.

Example 234

- 10 N-(4-{2-[6-(3-{{(2R)-2-amino-2-carboxyethyl}sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{{(2S)-2-[(1R,2R)-3-{{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



15

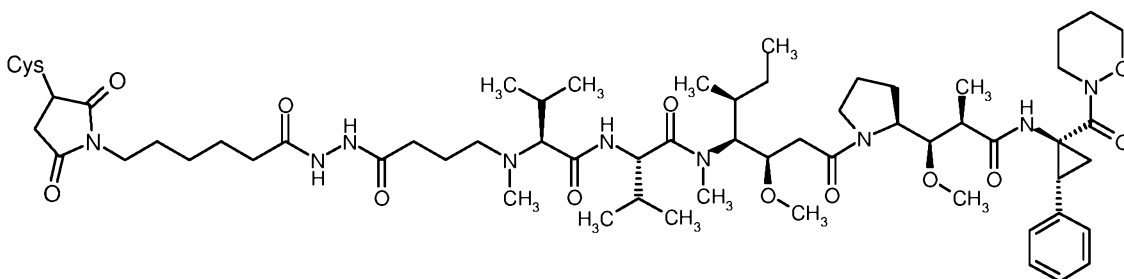
10 mg (10 μ mol) of Intermediate 113 weretaken up in 5.2 ml DMF and mixed with 2.28 mg (20 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 6 mg (54% of theory) of the title compound.

- 20 HPLC (Method 5): R_t = 1.5 min;

LC-MS (Method 1): R_t = 0.77 min; MS (ESIpos): m/z = 1185 (M+H)⁺.

Example 235

N-(4-{2-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



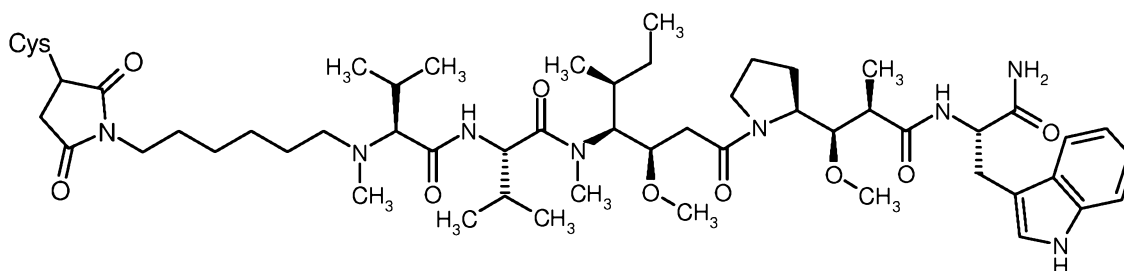
9 mg (8.3 μ mol) of Intermediate 132 were taken up in 4 ml DMF and mixed with 3 mg (24.4 μ mol) L-cysteine. The reaction mixture was stirred overnight at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 6.8 mg (68% of theory) of the title compound.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.78 min; MS (ESIpos): m/z = 1227 ($M+H$)⁺.

Example 236

N-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



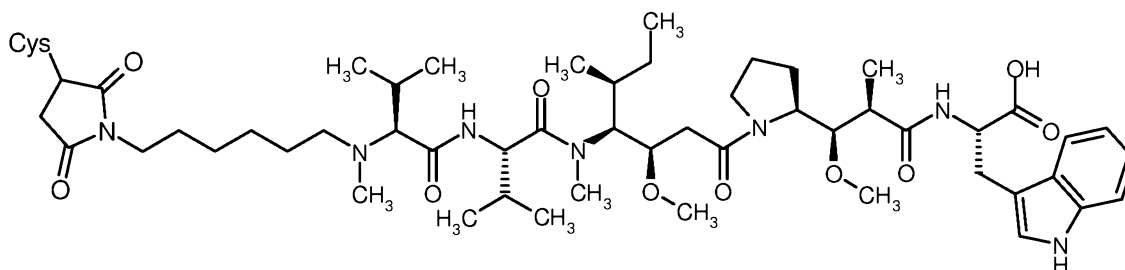
10 mg (10 μ mol) of Intermediate 106 weretaken up in 5.8 ml DMF and mixed with 2.5 mg (20 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 5.2 mg (46% of theory) of the title compound.

5 HPLC (Method 5): $R_t = 1.5$ min;

LC-MS (Method 11): $R_t = 0.71$ min; MS (ESIpos): $m/z = 1070$ (M+H)⁺.

Example 237

N-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



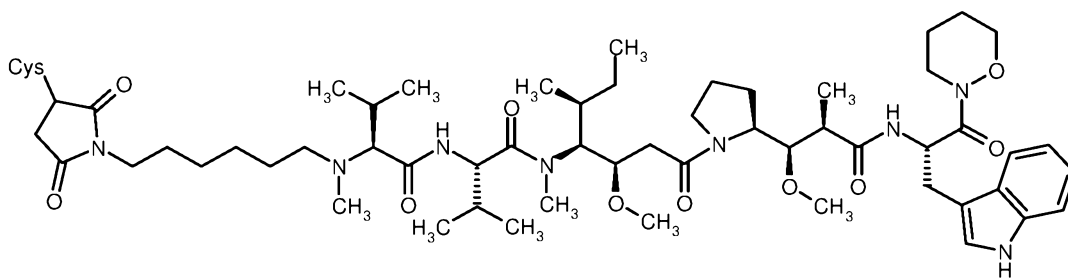
10 mg (10 μ mol) of Intermediate 124 was taken up in 4 ml DMF and mixed with 2.5 mg (20 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 7.2 mg (64% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.8$ min; MS (ESIpos): $m/z = 1071$ (M+H)⁺.

Example 238

20 N-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



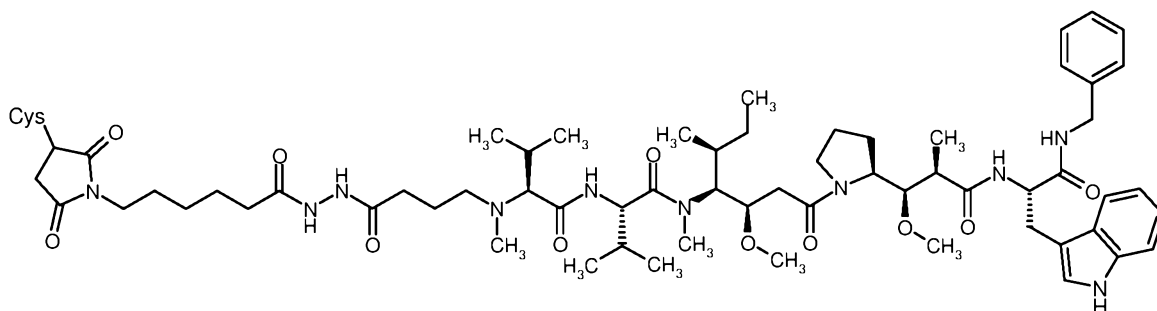
10 mg (10 μ mol) of Intermediate 125 weretaken up in 4 ml DMF and mixed with 2.4 mg (20 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 7.7 mg (69% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 2): $R_t = 1.91$ min; MS (ESIpos): $m/z = 1140$ ($M+H$)⁺.

Example 239

N-(4-{2-[6-(3-[(*(2R)*)-2-amino-2-carboxyethyl]sulfanyl)-2,5-dioxopyrrolidin-1-yl]hexanoyl}hydrazino)-4-oxobutyl)-N-methyl-L-valyl-N-[(*(3R,4S,5S)*)-1-[(*(2S)*)-2-[(*(1R,2R)*)-3-[(*(2S)*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



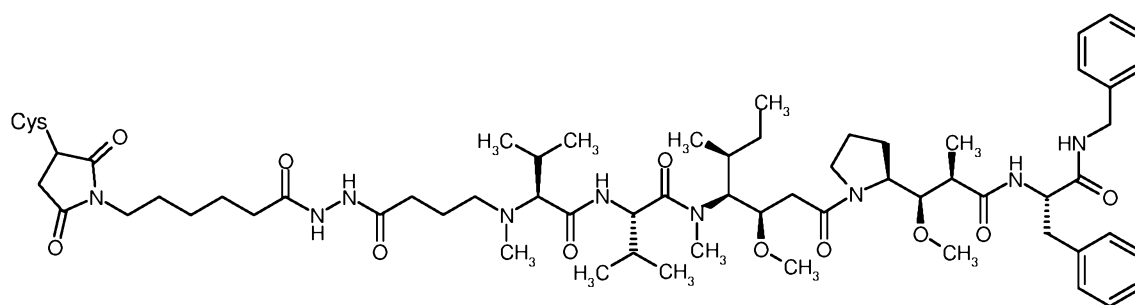
10 mg (10 μ mol) of intermediate 160 weretaken up in 3 ml DMF and mixed with 2.1 mg (20 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 8.1 mg (73% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 1274$ ($M+H$)⁺.

Example 240

- 5 N-(4-{2-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-(benzylamino)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



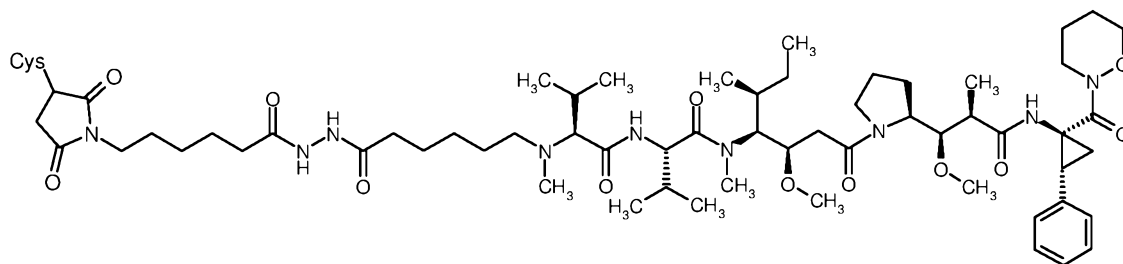
- 10 3.5 mg (3 μ mol) of Intermediate 159 weretaken up in 1 ml DMF and mixed with 0.76 mg (6 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 2.6 mg (65% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.75$ min;

LC-MS (Method 1): $R_t = 0.85$ min; MS (ESIpos): $m/z = 1235$ ($M+H$)⁺.

- 15 Example 241

N-(6-{2-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

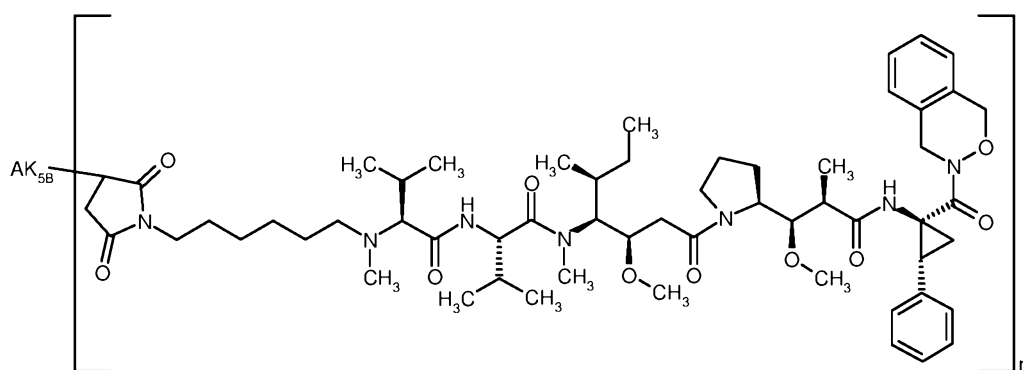


3.6 mg (3 μ mol) of Intermediate 129 were taken up in 1 ml DMF and mixed with 0.77 mg (6 μ mol) L-cysteine. The reaction mixture was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 1.55 mg (39% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.6$ min;

LC-MS (Method 1): $R_t = 0.87$ min; MS (ESIpos): $m/z = 1255$ ($M+H$) $^+$.

Example 242

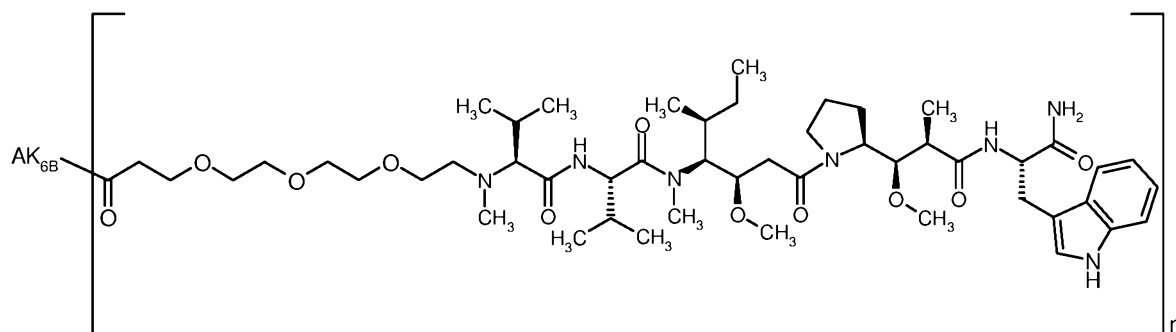


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 0.83 mg/ml

Drug/mAb ratio: 1.6

Example 243



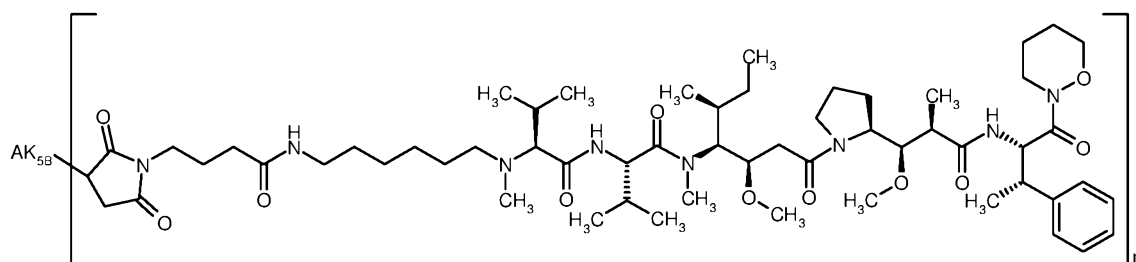
5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 5 Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.59 mg/ml

Drug/mAb ratio: 3.1

Drug/mAb ratio: 2.9

10 Example 244

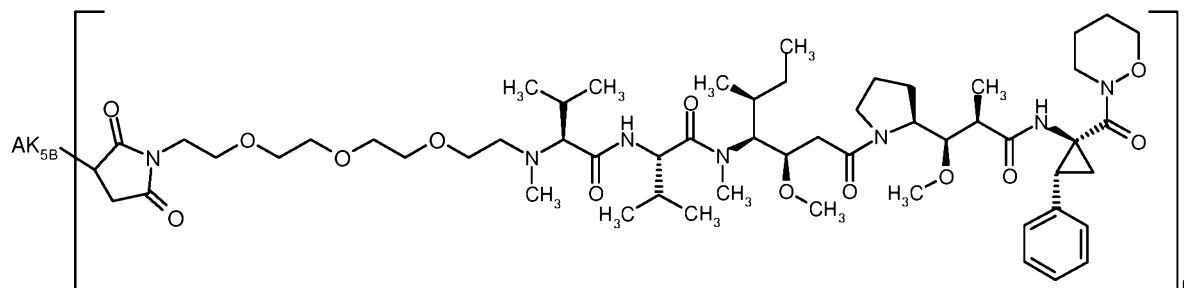


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

15 Protein concentration: 1.25 mg/ml

Drug/mAb ratio: 4.0

Example 245

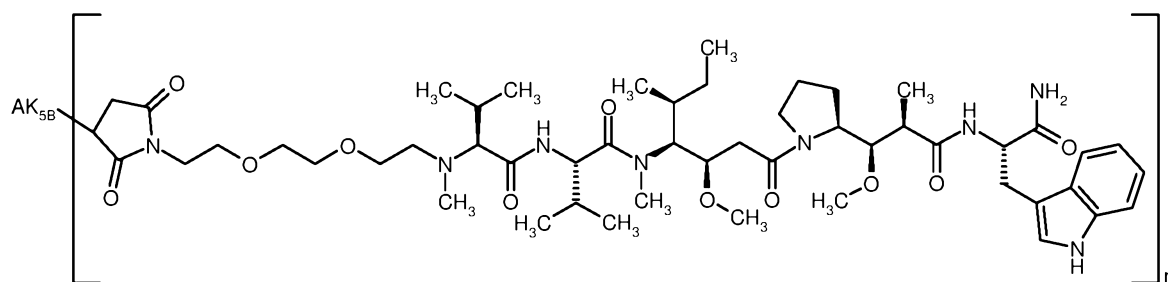


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 5 Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.27 mg/ml

Drug/mAb ratio: 3.6

Example 246



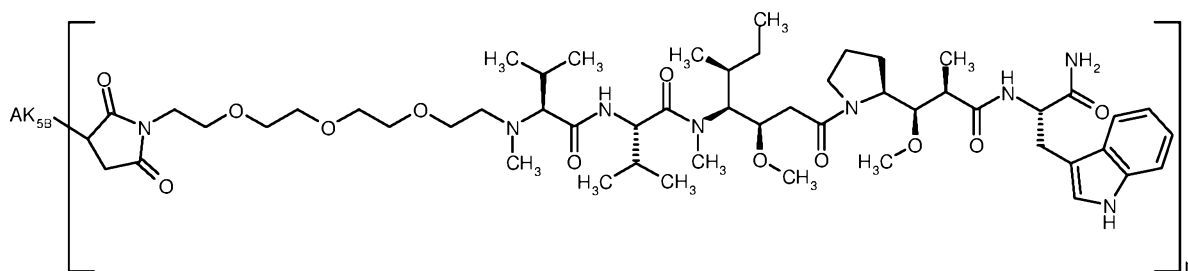
10

5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

15 Protein concentration: 1.54 mg/ml

Drug/mAb ratio: 4.7

Example 247

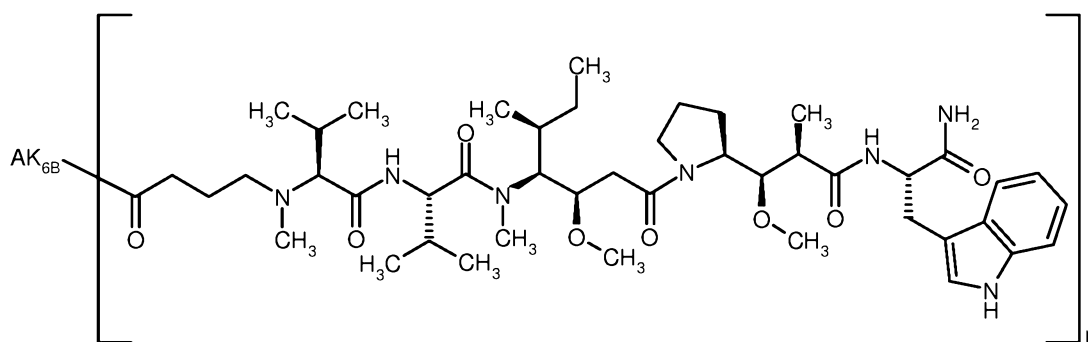


5 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.73 mg/ml

Drug/mAb ratio: 4.7

Example 248



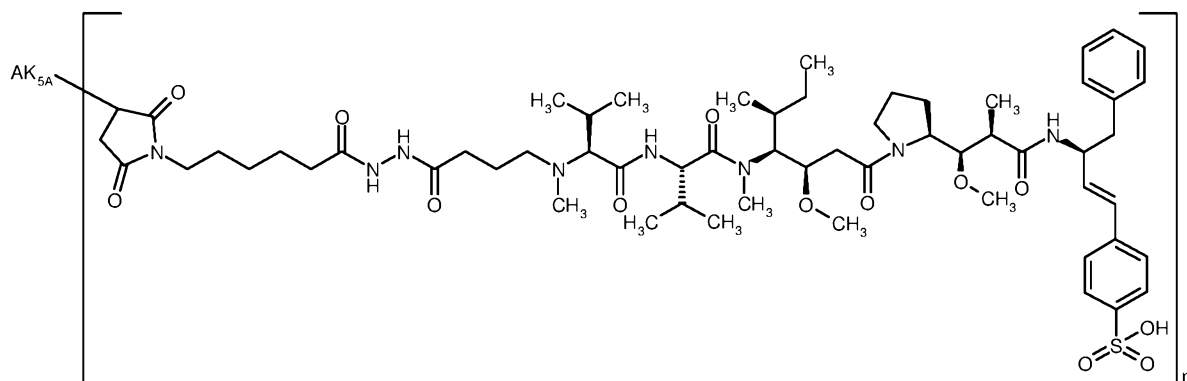
10

5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the reaction mixture was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.66 mg/ml

15 Drug/mAb ratio: 1.3

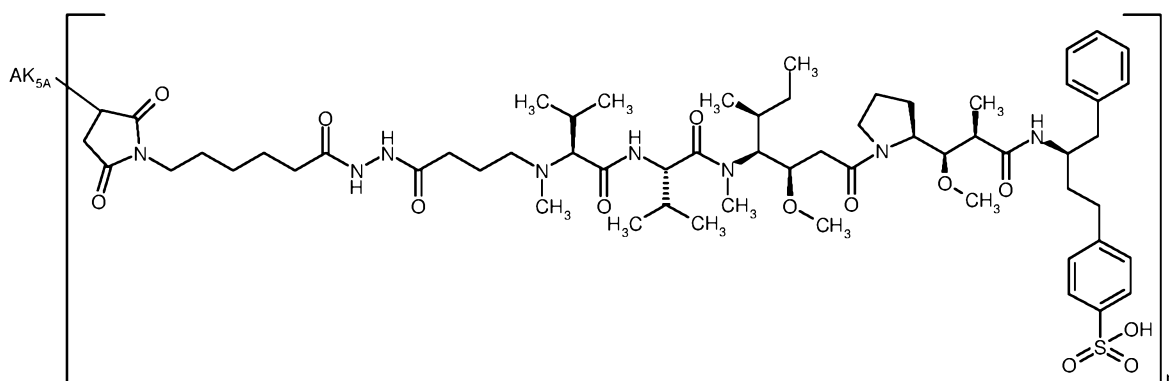
Example 249



Protein concentration: 2.11 mg/ml

5 Drug/mAb ratio: 5.5

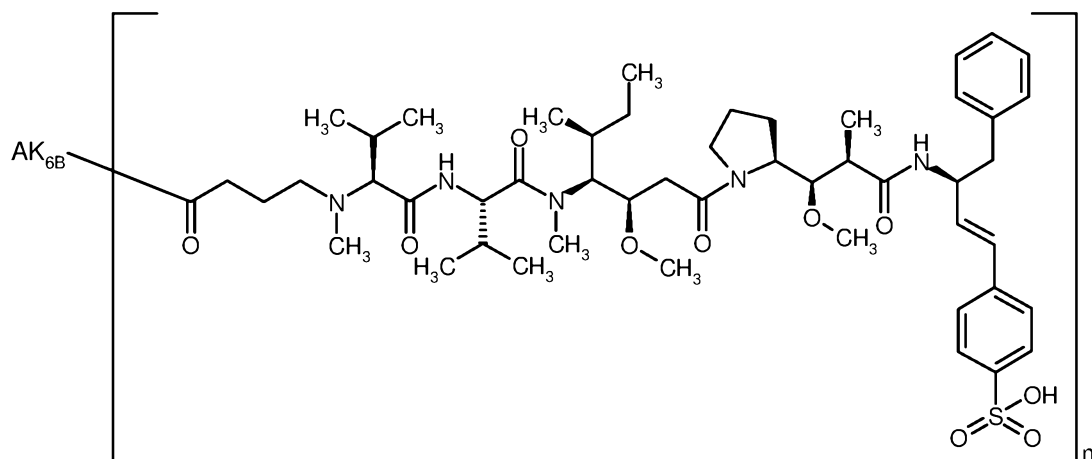
Example 250



Protein concentration: 1.53 mg/ml

10 Drug/mAb ratio: 3.4

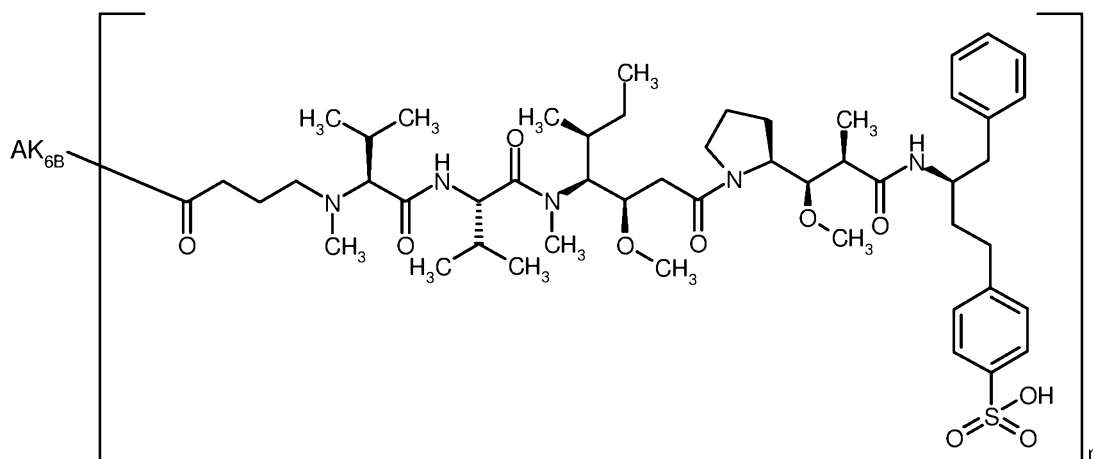
Example 251



Protein concentration: 1.5 mg/ml

Drug/mAb ratio: 0.2

5 Example 252

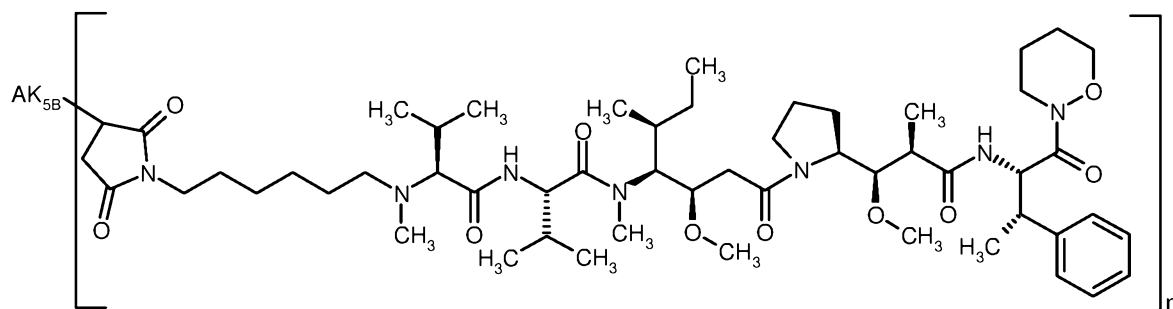


Protein concentration: 1.32 mg/ml

Drug/mAb ratio: 0.1

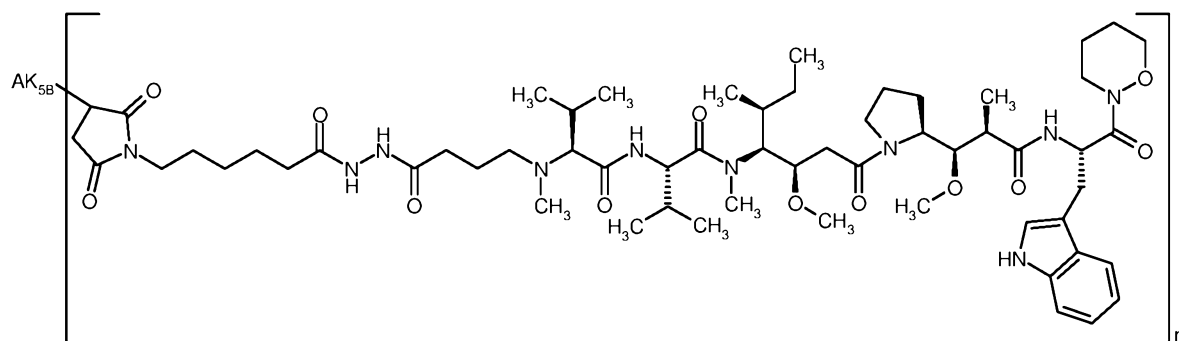
CN(C)CCCCC(=O)N(C)[C@H](C)C(=O)N[C@@H](C)C(=O)N(C)[C@H](C)[C@H](OC)CC(=O)N1CC[C@H]1[C@H](OC)C(=O)N[C@@H](C2=CN3C(=O)N(C4CCOCC4)C3=CC=C5C2=CN=C5)C2

Example 254



Drug/mAb ratio: 1.8

Example 255

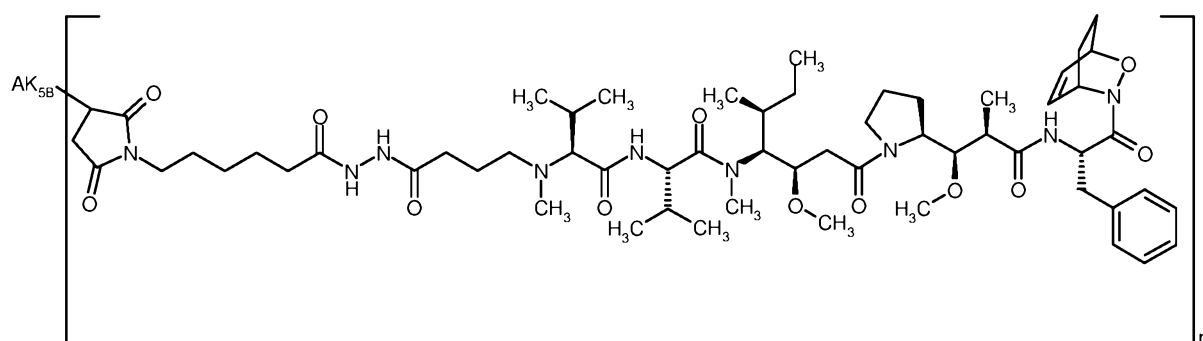


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.52 mg/ml

Drug/mAb ratio: 4.2

Example 256



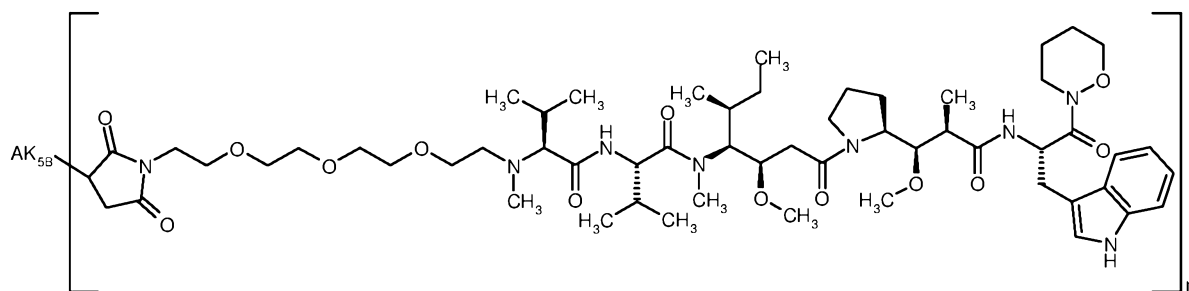
10

5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.1 mg/ml

Drug/mAb ratio: 3.3

Example 257

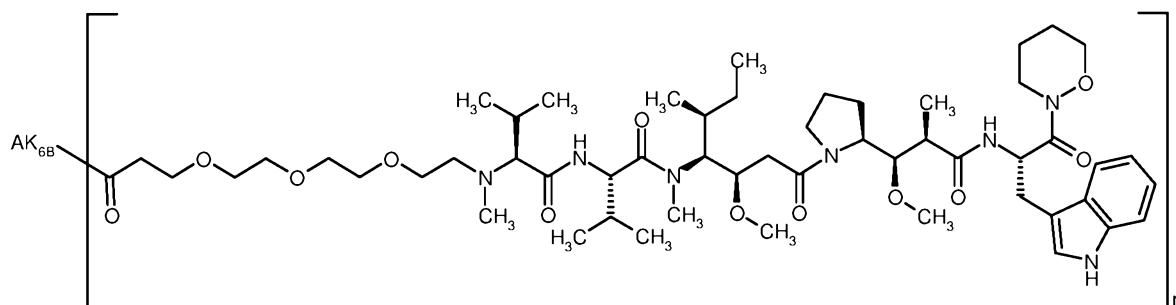


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.43 mg/ml

Drug/mAb ratio: 4.8

Example 258



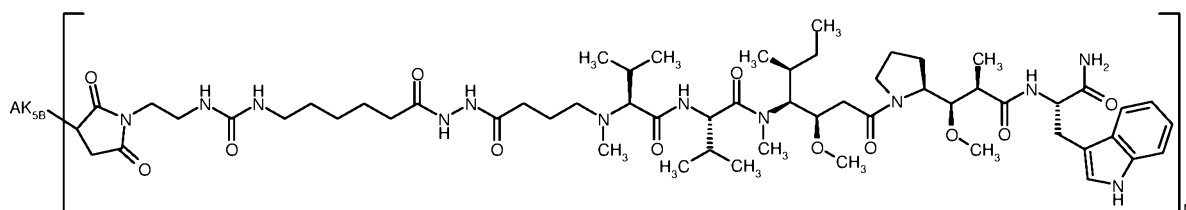
10

5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 Sephadex column, the batch was concentrated by ultracentrifugationm diluted again with PBS
 and reconcentrated.

Protein concentration: 1.36 mg/ml

15 Drug/mAb ratio: 4.6

Example 259

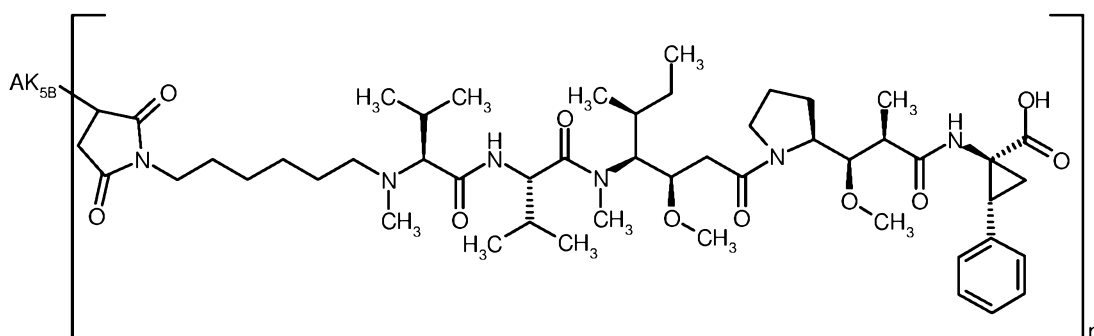


- 5 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.33 mg/ml

Drug/mAb ratio: 4.0

Example 260

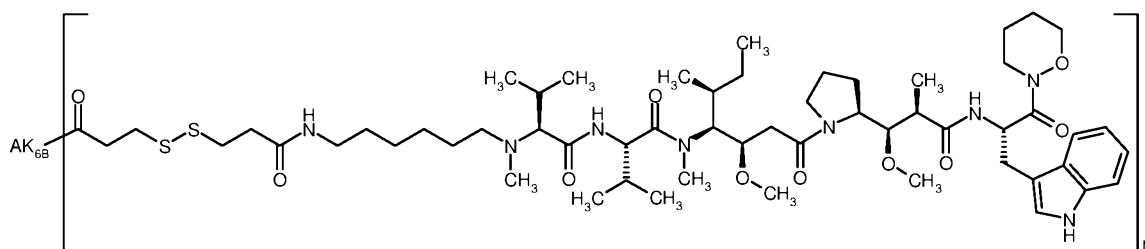


- 10 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.33 mg/ml

Drug/mAb ratio: 4.6

Example 261

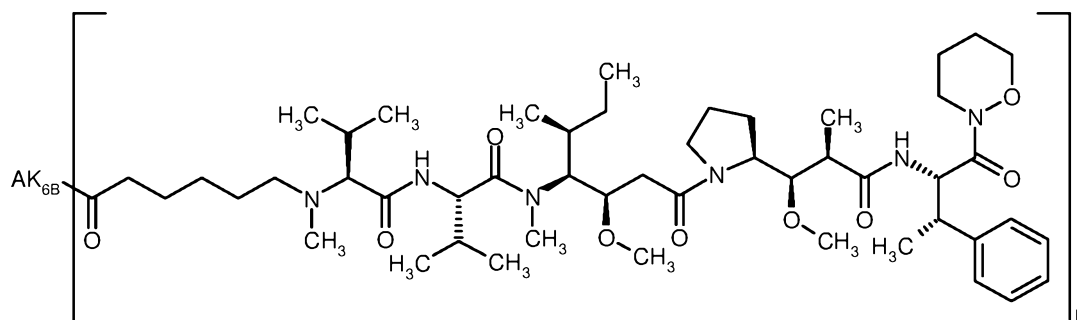


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.47 mg/ml

Drug/mAb ratio: 1.6

Example 262

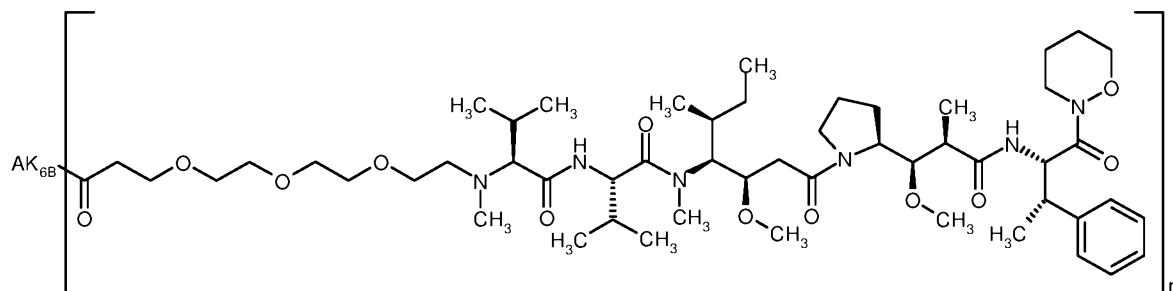


10 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.49 mg/ml

Drug/mAb ratio: 4.5

Example 263

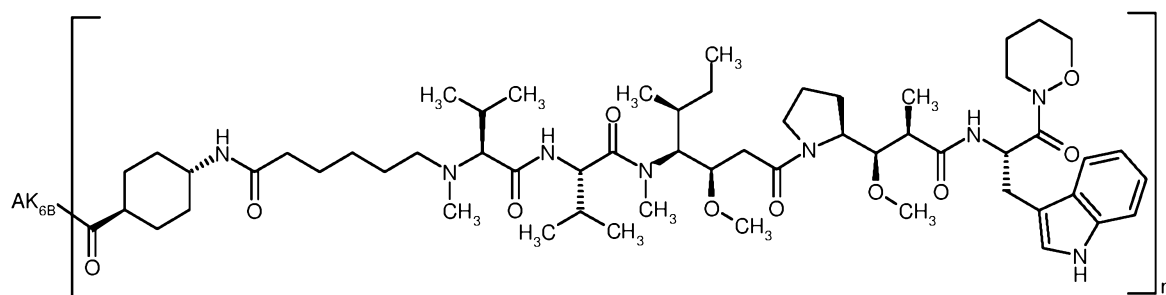


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.29 mg/ml

Drug/mAb ratio: 3.3

Example 264



10

5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

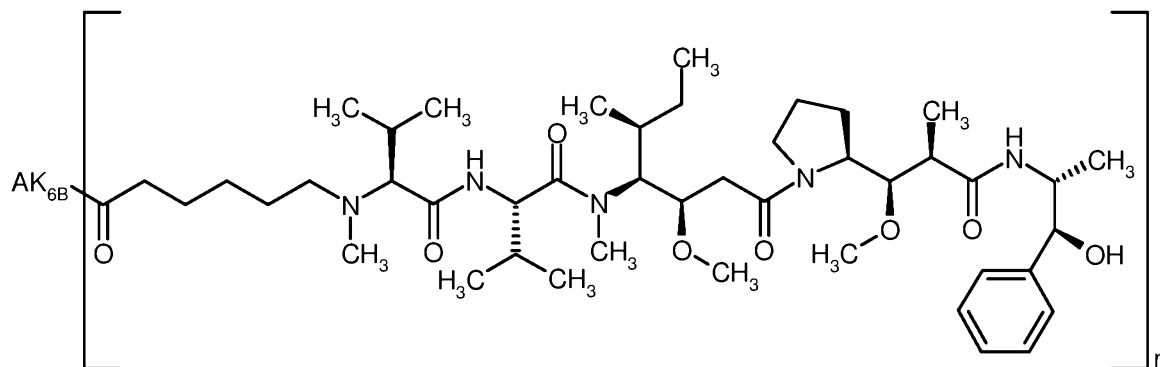
Protein concentration: 1.74 mg/ml

Drug/mAb ratio: 3.5

C[C@H](O)[C@@H](Cc1ccccc1)C(=O)N[C@@H](C)[C@H](OC(=O)C[C@H](OC)C[C@H](OC(=O)N[C@@H](C)[C@H](C)C(=O)N[C@@H](C)[C@H](C)C(=O)OCC)C(=O)OCCCN(C)C[C@H](C)[C@@H](C(=O)NC[C@H](C)C(=O)N[C@@H](C)[C@H](COCC(=O)N1CCCC1[C@H](OC)[C@@H](C)C(=O)NC[C@@H]2c3ccccc3n(c2)c4ccccc44)C(=O)Oc5ccc(cc5)C(=O)O[K+]

Drug/mAb ratio: 0.2

Example 267

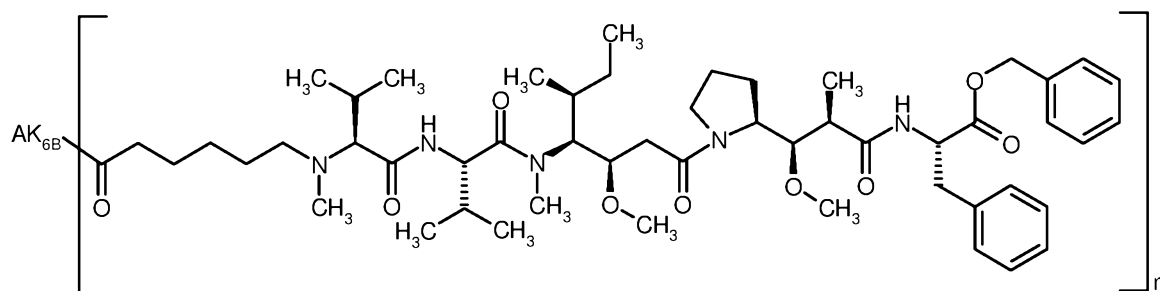


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.41 mg/ml

Drug/mAb ratio: 7.6

Example 268



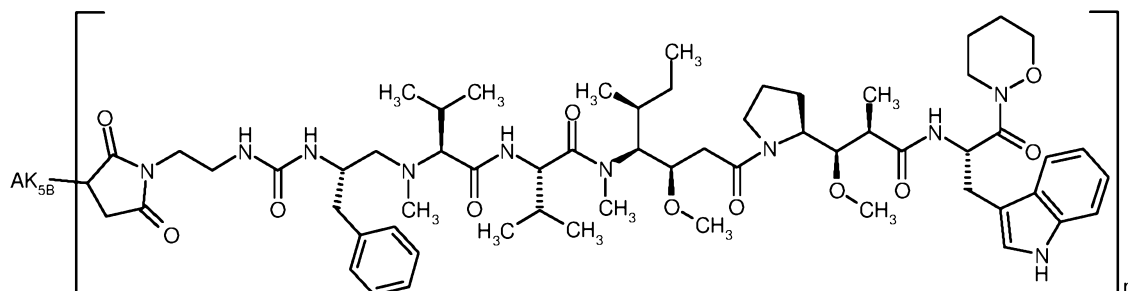
10

5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 2.0 mg/ml

Drug/mAb ratio: 1.6

Example 269

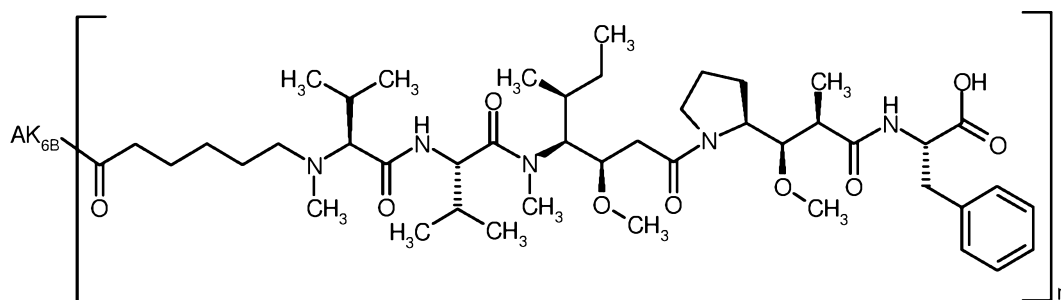


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

5 Protein concentration: 1.67 mg/ml

Drug/mAb ratio: 2.8

Example 270

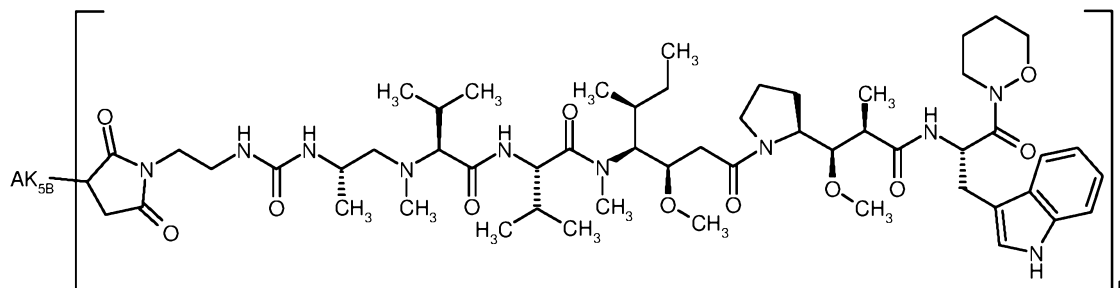


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.91 mg/ml

Drug/mAb ratio: 5.3

Example 271

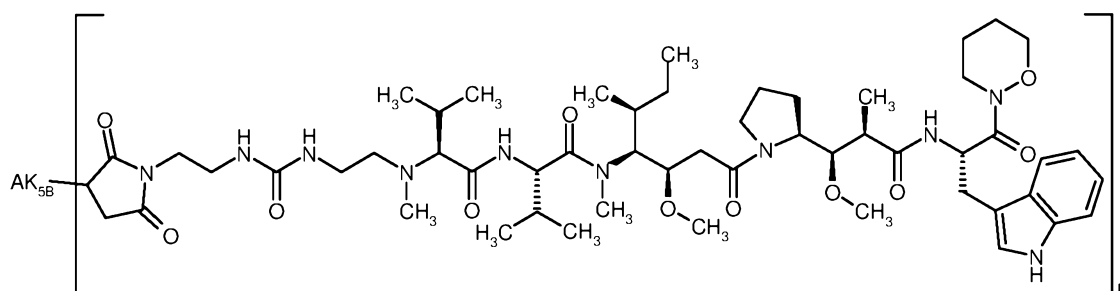


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

5 Protein concentration: 1.82 mg/ml

Drug/mAb ratio: 4.6

Example 272

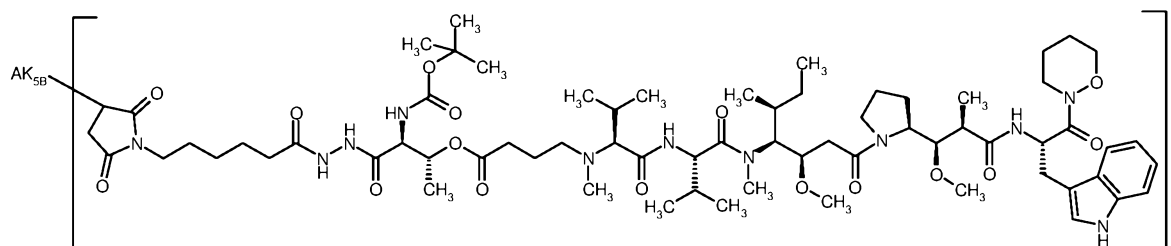


10 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.9 mg/ml

Drug/mAb ratio: 4.2

Example 273

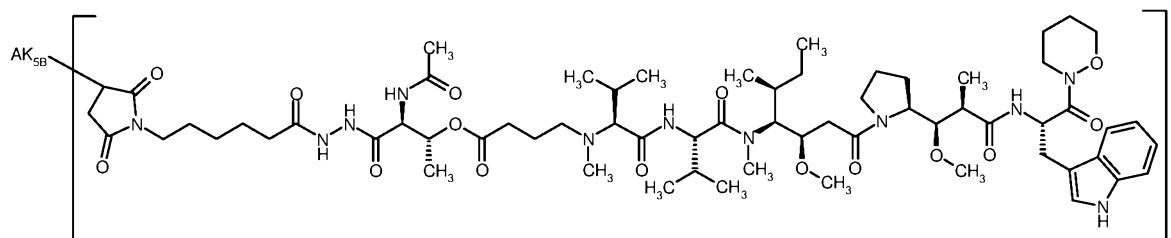


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.89 mg/ml

Drug/mAb-Ratio: 2.7

Example 274



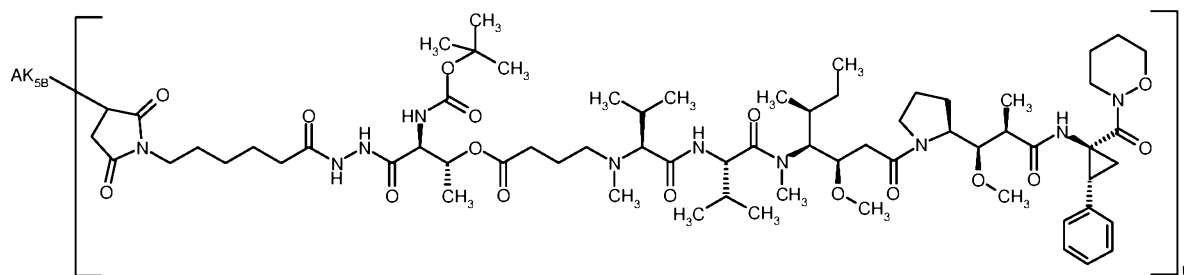
10

5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.73 mg/ml

Drug/mAb-Ratio: 2.3

Example 275

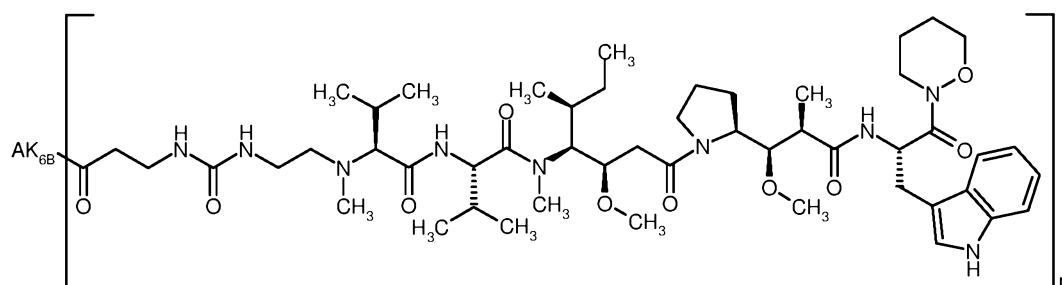


- 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.71 mg/ml

Drug/mAb-Ratio: 3.3

Example 276



- 10 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a
 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

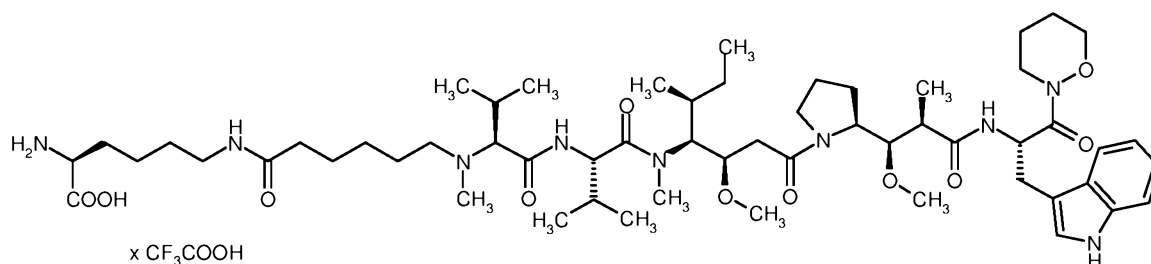
Protein concentration: 1.47 mg/ml

Drug/mAb ratio: 3.9

Example 277

- 15 N-(6-[(5S)-5-amino-5-carboxypentyl]amino)-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-
 {(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]amino}-1-

methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



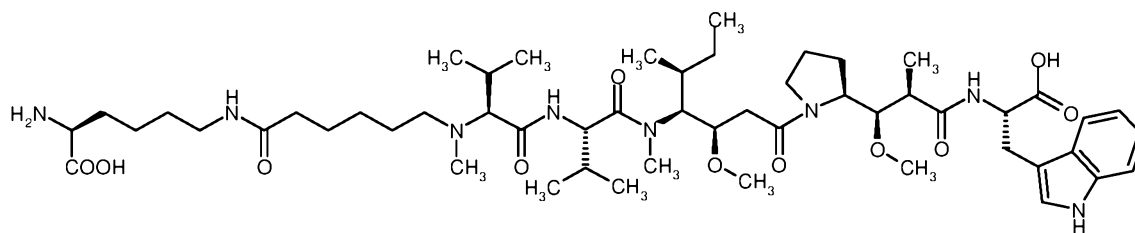
- 5 15.5 mg (15 μ mol) of Intermediate 210 were taken up in 5 ml DMF and mixed with 4.4 mg (18 μ mol) N²-(tert.-butoxycarbonyl)-L-lysine as well as 7.7 μ L (44 μ mol) *N,N*-diisopropylethylamine. The reaction mixture was stirred overnight at RT and then concentrated *in vacuo*. Next, the residue was purified by means of preparative HPLC. The yield was 14 mg (81% of theory) of the protected intermediate of the title compound that was subsequently taken up in 1
- 10 ml dichloromethane and deprotected with 1 ml trifluoroacetic acid. The batch was concentrated and, following lyophilization of the residue from acetonitrile/water (1:1), 15 mg (97% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.79 min; MS (ESIpos): m/z = 1083 (M+H)⁺.

15 Example 278

N-(6-{[(5*S*)-5-amino-5-carboxypentyl]amino}-6-oxohexyl)-N-methyl-L-valyl-N-[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



40 mg (40 μ mol) of Intermediate 227 weretaken up in 5 ml DMF and mixed with 11.5 mg (40 μ mol) N²-[(benzyloxy)carbonyl]-L-lysineas well as 13 μ l (80 μ mol) *N,N*-diisopropylethylamine. The reaction mixture was stirred overnight at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 32.5 mg (70% of theory) of the protected
 5 intermediate of the title compound.

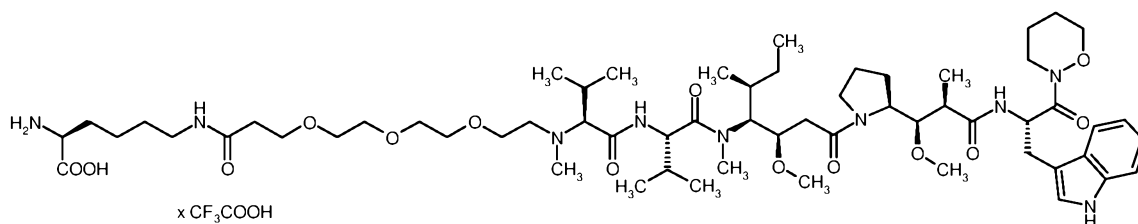
These 32.5 mg of the intermediate were dissolved in 10 ml methanol and, after adding 2 mg 10% palladium on activated carbon, hydrogenated for 30 min at RT under standard hydrogen pressure. The catalyst was then filtered off and the solvent removed *in vacuo*. Following lyophilization of the residue from dioxane/water 1:1, 26 mg (99% of theory) of the title compound were obtained.

10 HPLC (Method 12): R_t = 1.7 min;

LC-MS (Method 1): R_t = 0.76 min; MS (ESIpos): m/z = 1014 (M+H)⁺.

Example 279

N-[(18S)-18-amino-18-carboxy-12-oxo-3,6,9-trioxa-13-azaoctadec-1-yl]-N-methyl-L-valyl-N-
 [(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-
 15 2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate

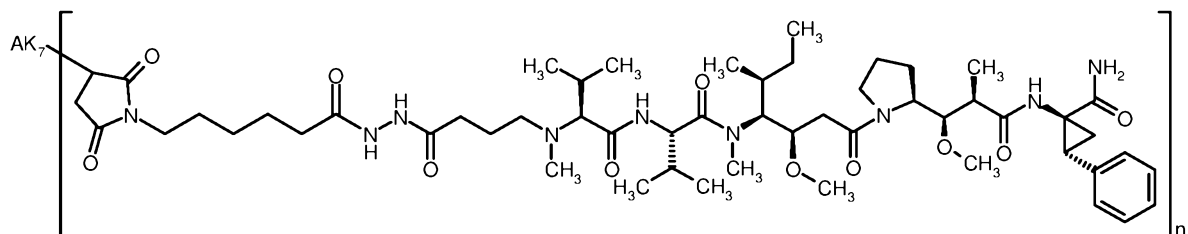


3.5 mg (3 μ mol) of Intermediate 202 weretaken up in 2 ml DMF and mixed with 0.8 mg (3 μ mol) N²-(tert. butoxycarbonyl)-L-lysine as well as 1.6 μ l (10 μ mol) *N,N*-diisopropylethylamine. The reaction mixture was stirred overnight at RT and then concentrated *in vacuo*. Next, the residue was taken up in acetonitrile/water (1:1), adjusted to pH 2 with trifluoroacetic acid, and purified by means of preparative HPLC. The yield was 1 mg (25% of theory) of the protected intermediate of the title compound that was subsequently taken up in 500 μ ldichloromethane and deprotected with 500 μ ltrifluoroacetic acid. The batch was concentrated and, following lyophilization of the residue from acetonitrile/water (1:1), 1 mg (89% of theory) of the title
 20
 25 compound was obtained.

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.82$ min; MS (ESIpos): $m/z = 1173$ (M+H)⁺.

Example 280

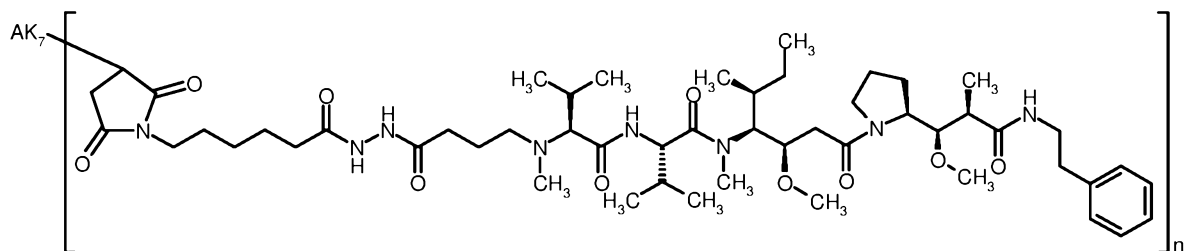


5

Protein concentration: 0.9 mg/ml

Drug/mAb ratio: 2.8

Example 281

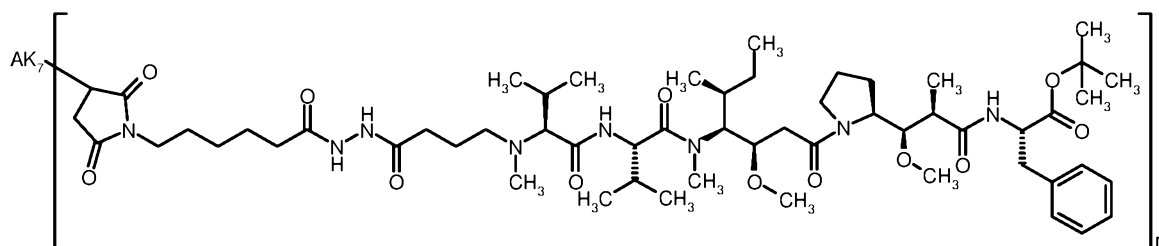


10

Protein concentration: 1.08 mg/ml

Drug/mAb ratio: 1.1

Example 282

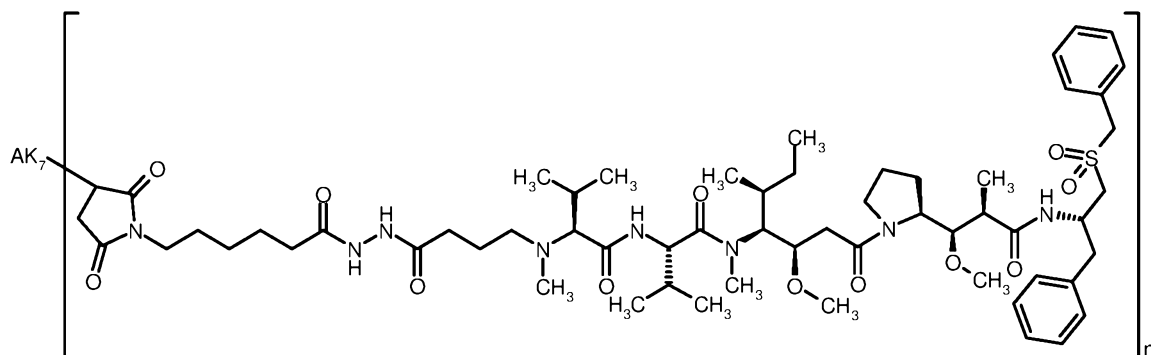


15

Protein concentration: 0.98 mg/ml

Drug/mAb ratio:2.4

Example 283

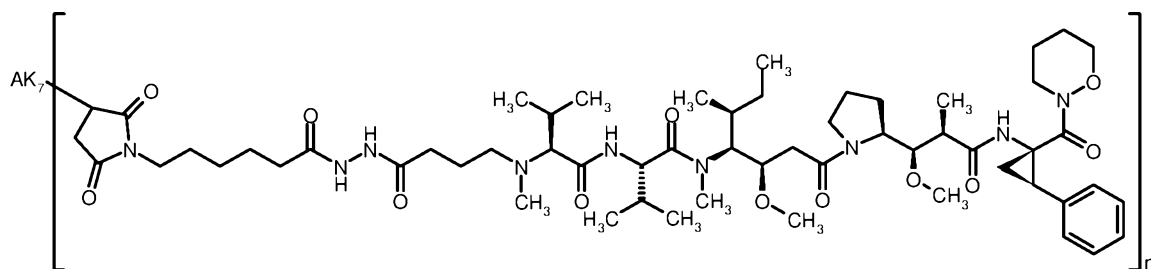


5

Protein concentration: 1.23 mg/ml

Drug/mAb ratio:4.6

Example 284



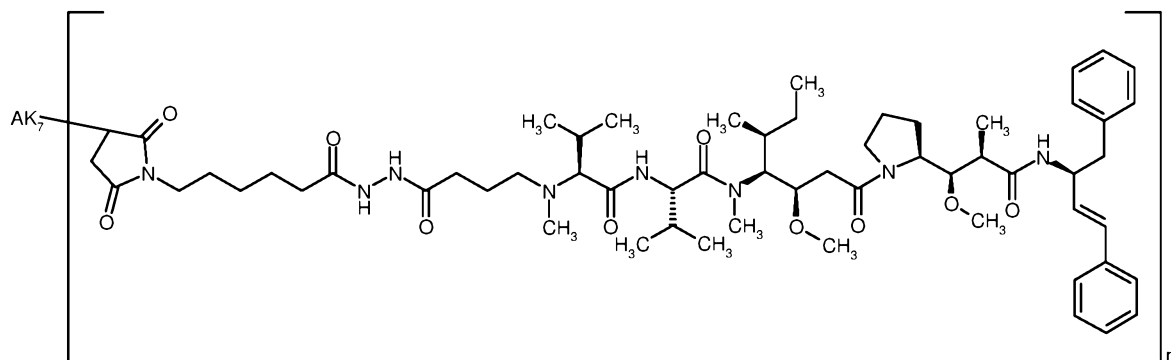
10

100 mg anti-CA9 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation. The solution was then diluted again, reconcentrated, and the process was repeated one more time.

Protein concentration: 9.2 mg/ml

15 Drug/mAb ratio:3.2

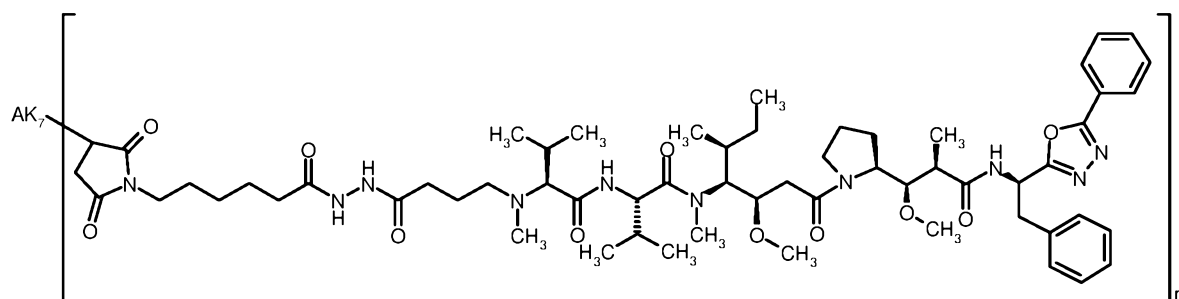
Example 285



Protein concentration: 1.21 mg/ml

5 Drug/mAb ratio: not detectable

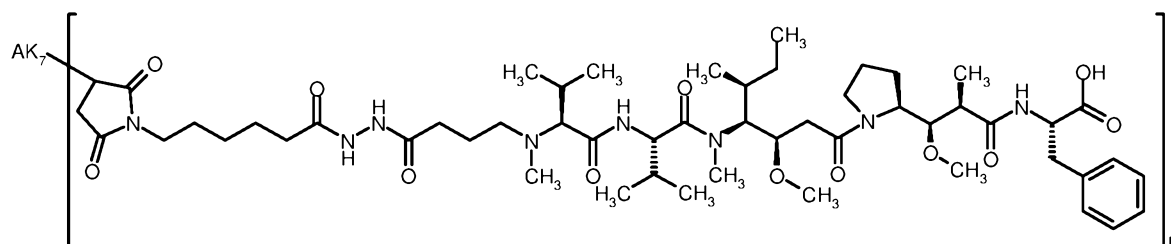
Example 286



Protein concentration: 1.26 mg/ml

10 Drug/mAb ratio: 4.2

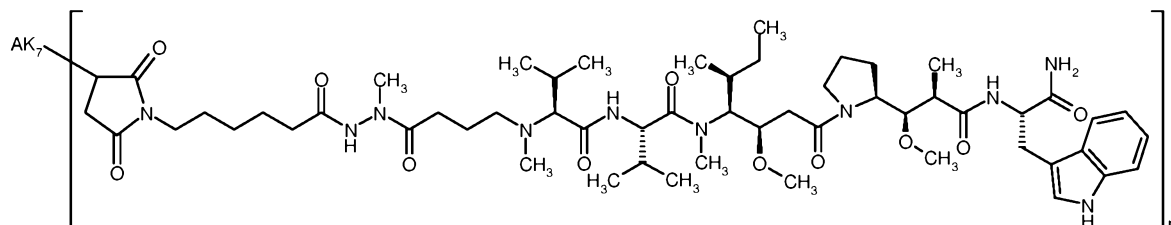
Example 287



Protein concentration: 1.01 mg/ml

Drug/mAb ratio:3.0

Example 288

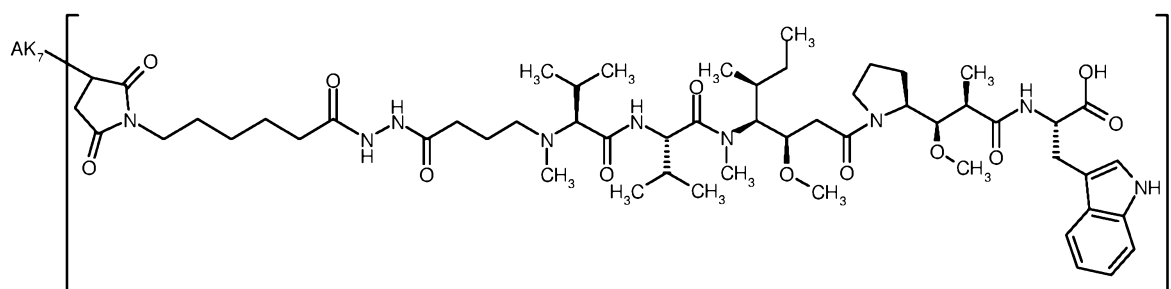


5

Protein concentration: 1.28 mg/ml

Drug/mAb ratio: 2.3

Example 289

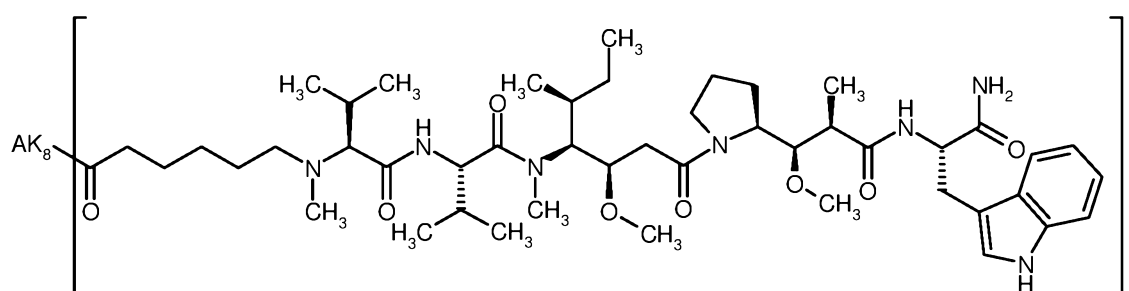


10

Protein concentration: 1.12 mg/ml

Drug/mAb ratio:2.6

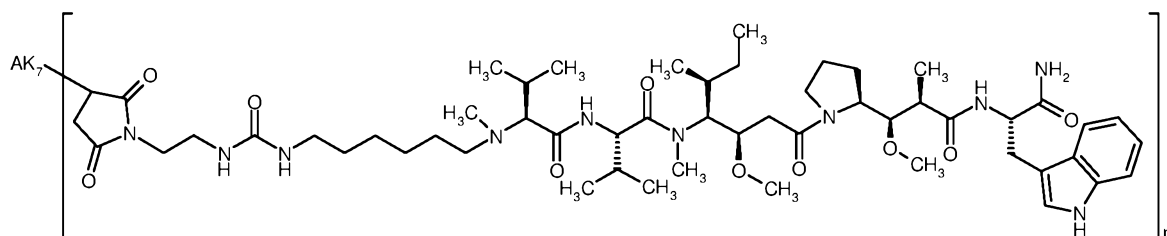
Example 290



Protein concentration: 1.4 mg/ml

Drug/mAb ratio:3.3

Example 291

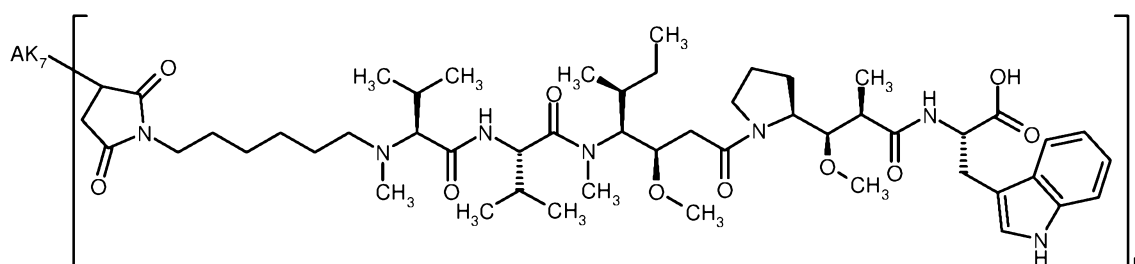


5

Protein concentration: 1.3 mg/ml

Drug/mAb ratio:2.5

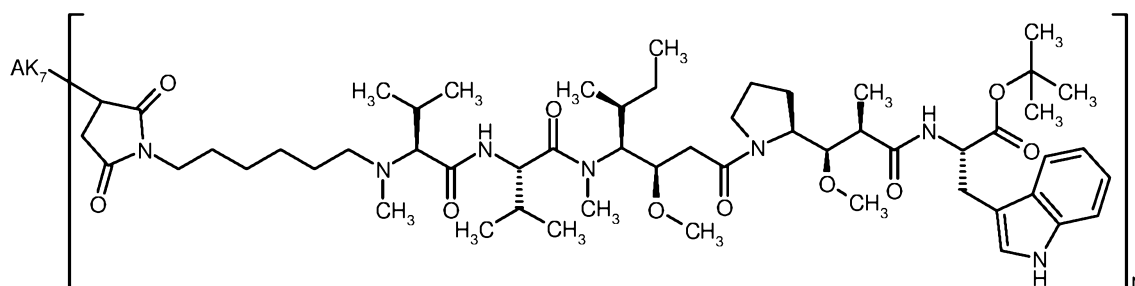
Example 292



10 Protein concentration: 1.27 mg/ml

Drug/mAb ratio: 2.6

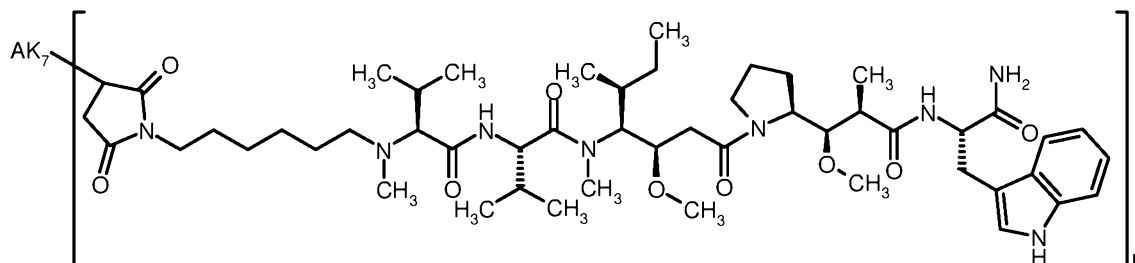
Example 293



Protein concentration: 1.55 mg/ml

Drug/mAb ratio: not detectable

Example 294

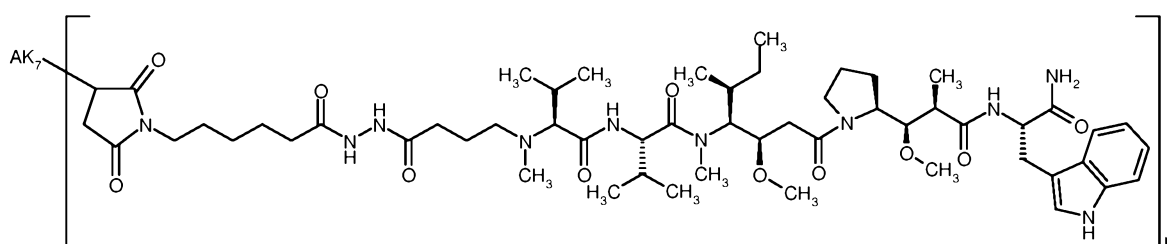


- 5 100 mg anti-CA9 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 11.8 mg/ml

Drug/mAb ratio: 4.4

Example 295



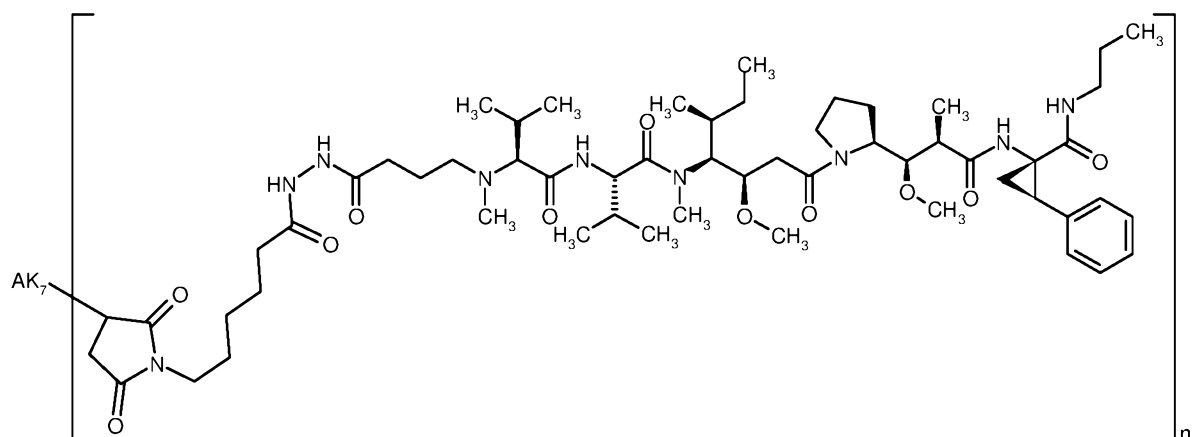
10

100 mg anti-CA9 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 12.29 mg/ml

- 15 Drug/mAb ratio: 3.8

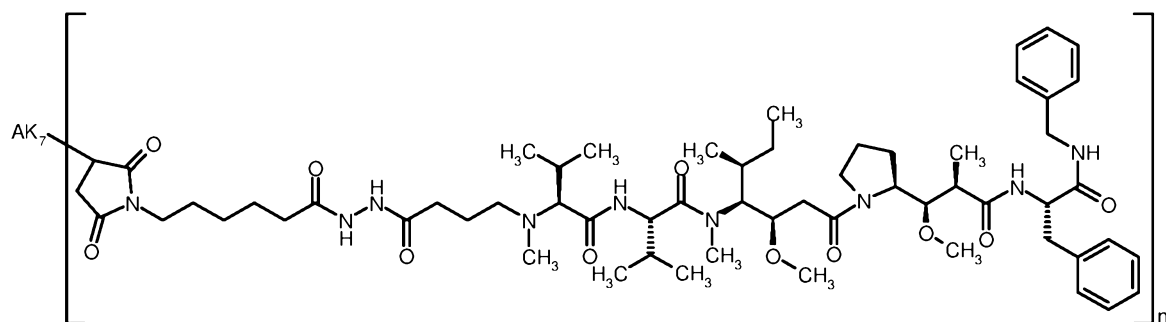
Example 296



Protein concentration: 1.1 mg/ml

5 Drug/mAb ratio: 1.6

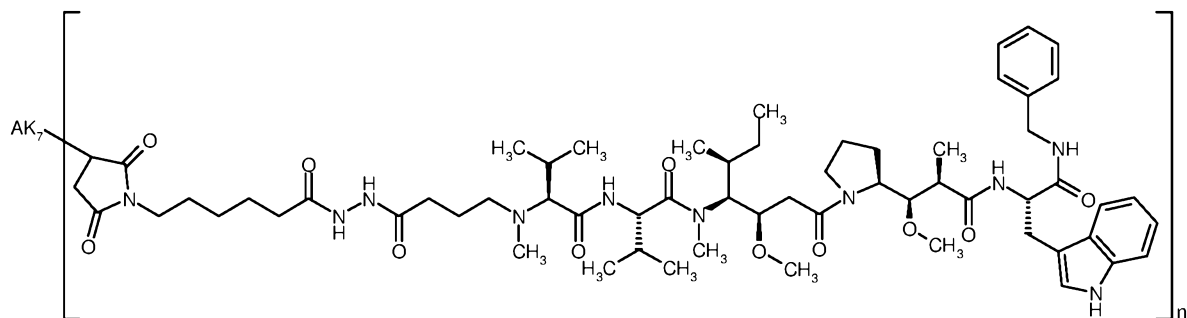
Example 297



Protein concentration: 1.00 mg/ml

10 Drug/mAb ratio: 1.9

Example 298

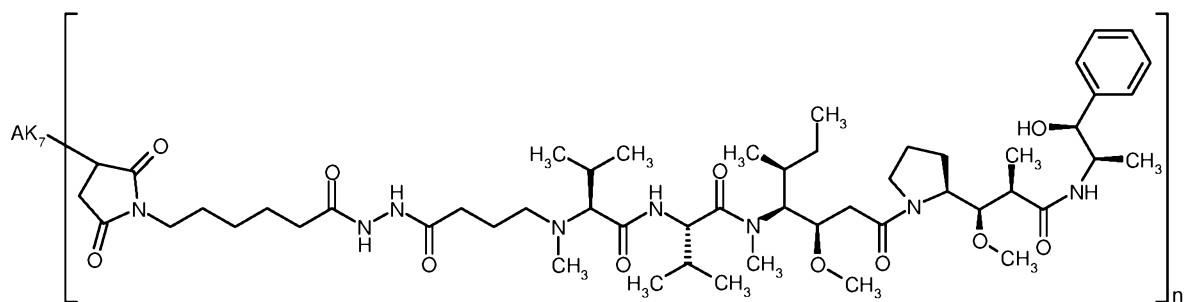


100 mg anti-CA9 were presently used for the coupling and, after purification on a Sephadex
 5 column, the batch was concentrated by ultracentrifugation.

Protein concentration: 11.5 mg/ml

Drug/mAb ratio: 4.9

Example 299

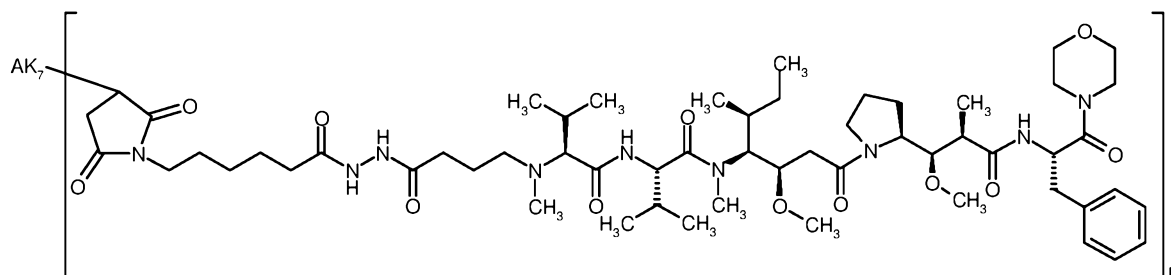


10

Protein concentration: 0.98 mg/ml

Drug/mAb ratio: 2.5

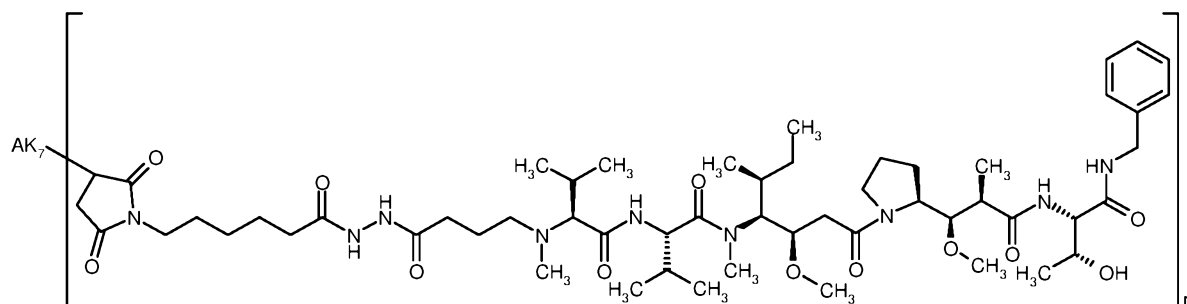
Example 300



Protein concentration: 0.99 mg/ml

5 Drug/mAb ratio: 2.0

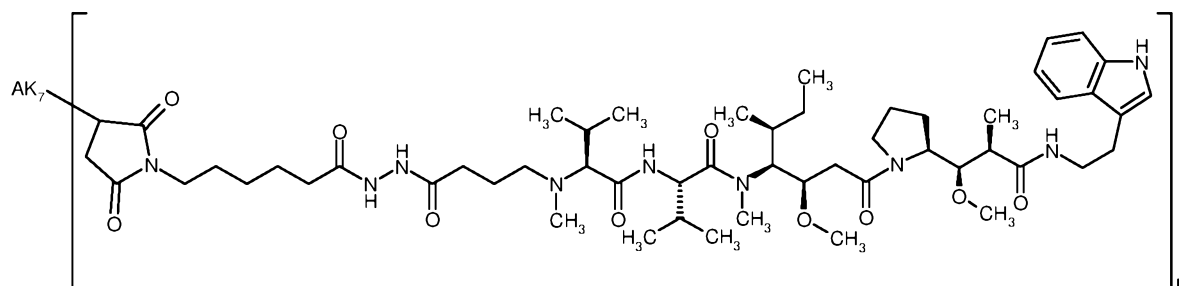
Example 301



Protein concentration: 0.87 mg/ml

10 Drug/mAb ratio: 2.1

Example 302



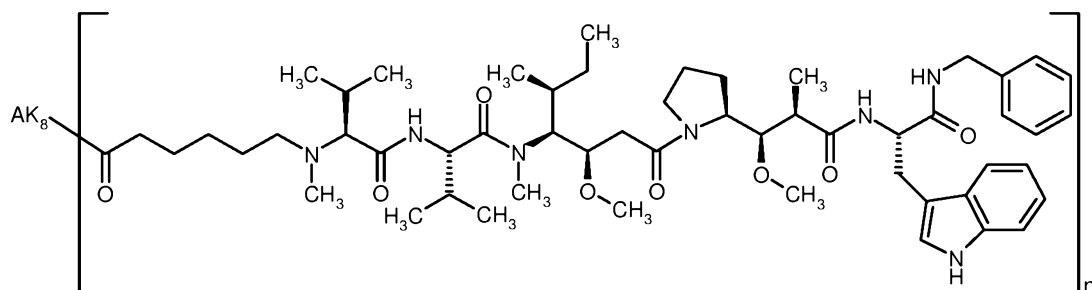
100 mg anti-CA9 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

15

Protein concentration: 12.2 mg/ml

Drug/mAb ratio: 4.6

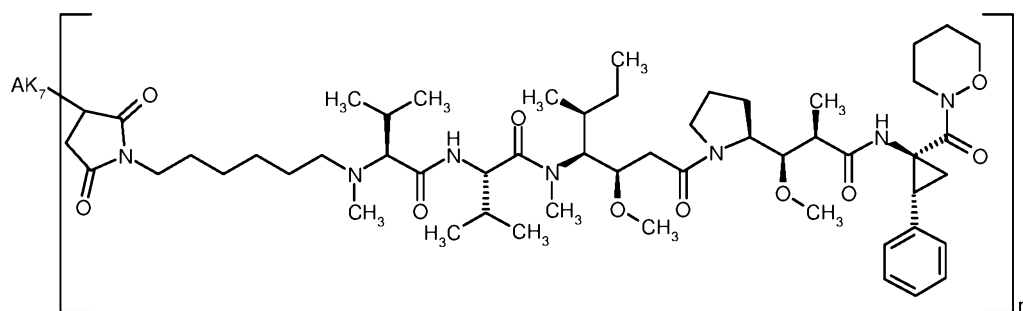
Example 303



5 Protein concentration: 1.58 mg/ml

Drug/mAb ratio: 1.9

Example 304

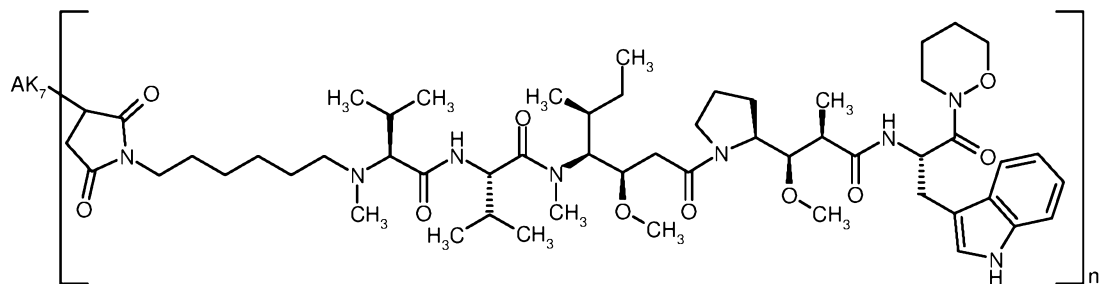


70 mg anti-CA9 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 11.5 mg/ml

Drug/mAb ratio: 3.9

Example 305

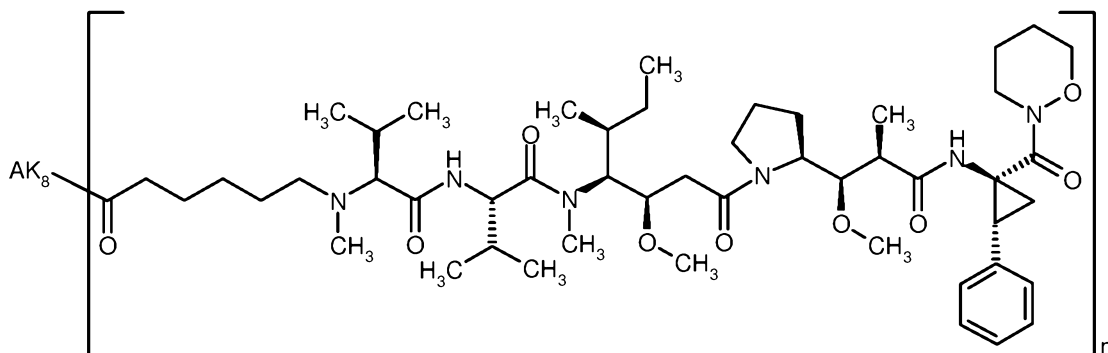


60 mg anti-CA9 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

5 Protein concentration: 11.6 mg/ml

Drug/mAb ratio: 3.9

Example 306



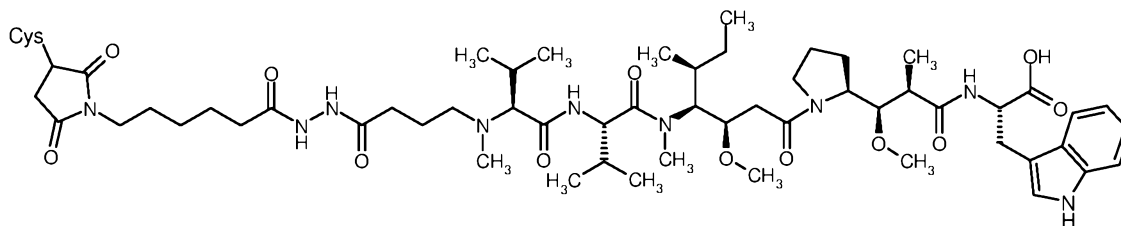
60 mg anti-CA9 were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation.

Protein concentration: 10 mg/ml

Example 307

N-(4-{2-[6-(3-[(2R)-2-amino-2-carboxyethyl]sulfanyl)-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-

{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



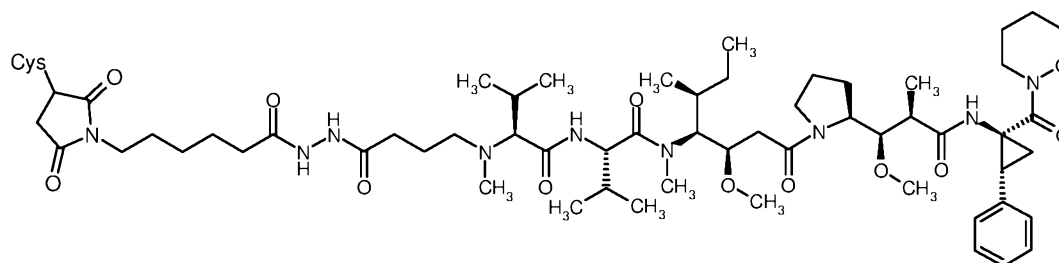
- 5 10 mg (10 μmol) of Intermediate 113 were taken up in 5.2 ml DMF and mixed with 2.28 mg (20 μmol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 6 mg (54% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.5$ min;

- 10 LC-MS (Method 1): $R_t = 0.77$ min; MS (ESIpos): $m/z = 1185$ (M+H)⁺.

Example 308

- N-(4-{2-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(1S,2R)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



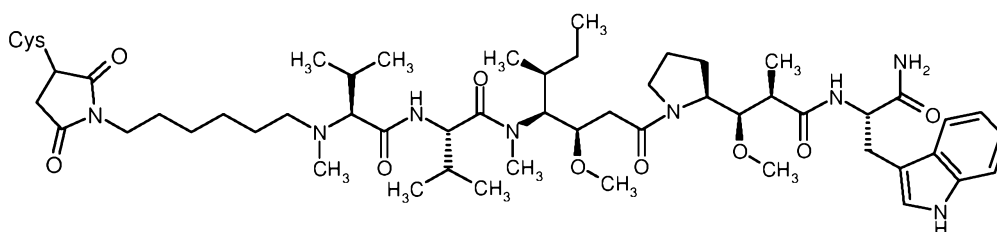
- 9 mg (8.3 μmol) of Intermediate 132 were taken up in 4 ml DMF and mixed with 3 mg (24.4 μmol) L-cysteine. The batch was stirred overnight at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 6.8 mg (68% of theory) of the title compound.
- 20

HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.78$ min; MS (ESIpos): $m/z = 1227$ (M+H)⁺.

Example 309

N-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-amino-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



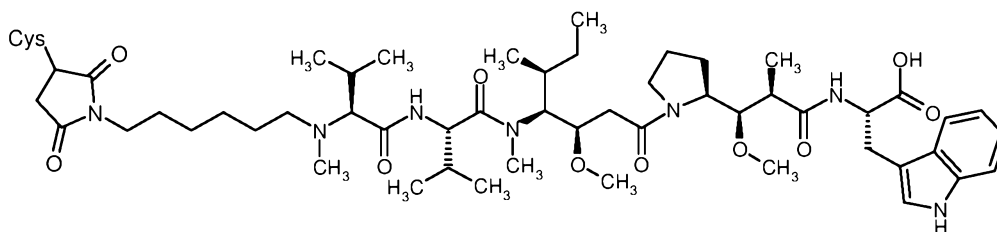
10 mg (10 μ mol) of Intermediate 106 were taken up in 5.8 ml DMF and mixed with 2.5 mg (20 μ mol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 5.2 mg (46% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.5$ min;

LC-MS (Method 11): $R_t = 0.71$ min; MS (ESIpos): $m/z = 1070$ (M+H)⁺.

15 Example 310

N-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



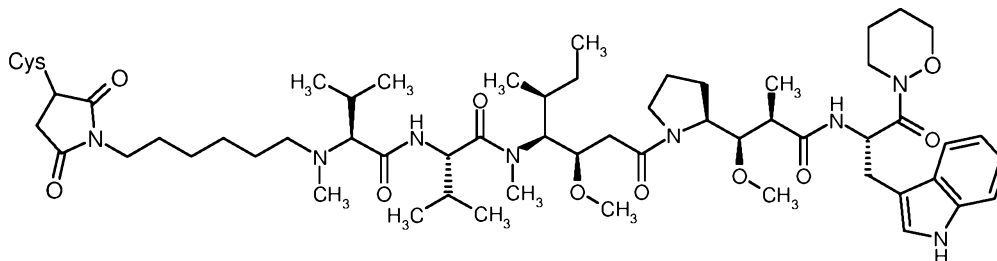
10 mg (10 μ mol) of Intermediate 124 weretaken up in 4 ml DMF and mixed with 2.5 mg (20 μ mol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 7.2 mg (64% of theory) of the title compound.

5 HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 1): R_t = 0.8 min; MS (ESIpos): m/z = 1071 (M+H)⁺.

Example 311

N-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



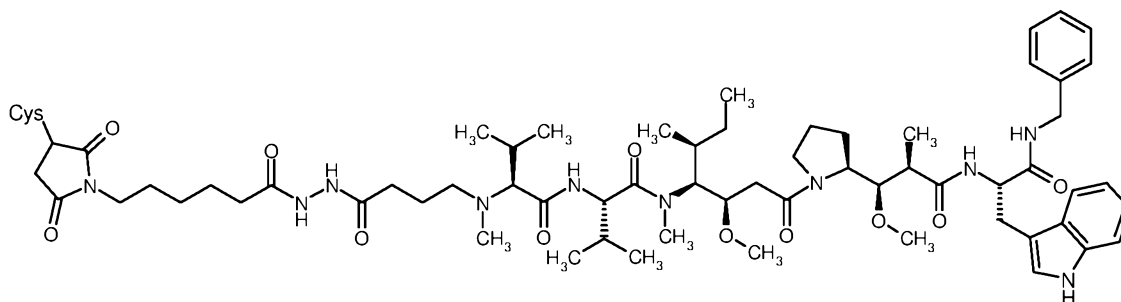
10 mg (10 μ mol) of Intermediate 125 weretaken up in 4 ml DMF and mixed with 2.4 mg (20 μ mol) L-cysteine. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 7.7 mg (69% of theory) of the title compound.

HPLC (Method 5): R_t = 1.7 min;

LC-MS (Method 2): R_t = 1.91 min; MS (ESIpos): m/z = 1140 (M+H)⁺.

Example 312

20 N-(4-{2-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexanoyl]hydrazino}-4-oxobutyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-(benzylamino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide

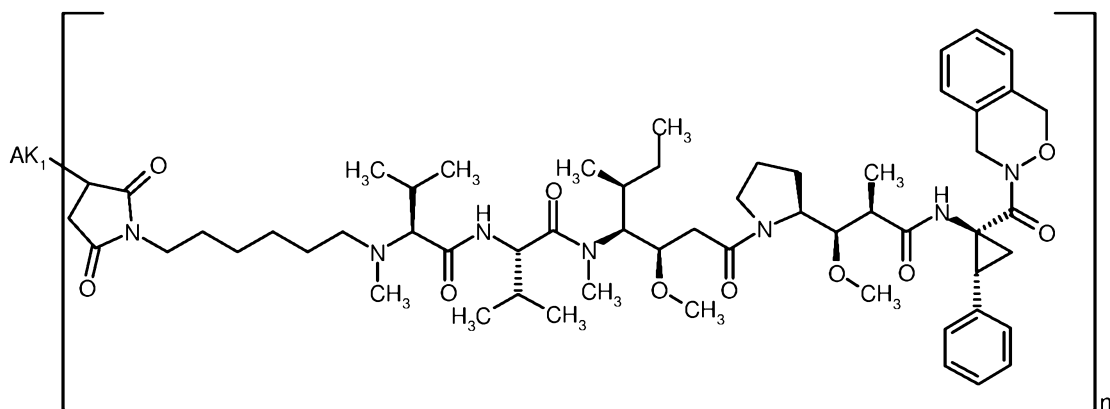


10 mg (10 μ mol) of Intermediate 160 was taken up in 3 ml DMF and mixed with 2.1 mg (20 μ mol) L-cystein. The batch was stirred for 2 hours at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 8.1 mg (73% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.86$ min; MS (ESIpos): $m/z = 1274$ ($M+H$)⁺.

Example 313

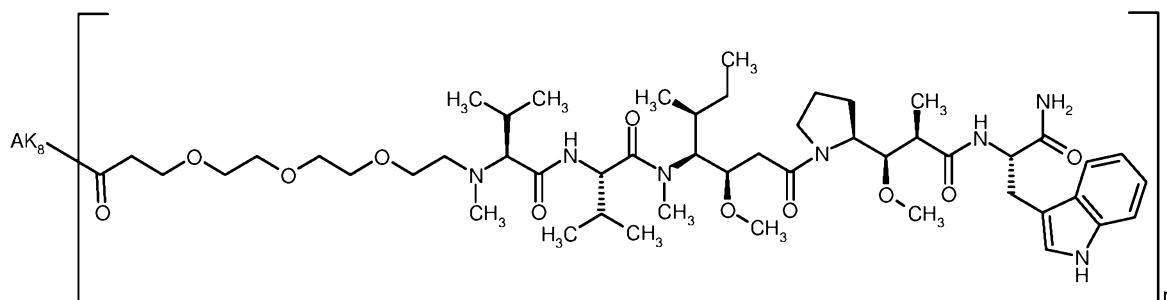


10 5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.23 mg/ml

Drug/mAb ratio: ~1-1.5

Example 314

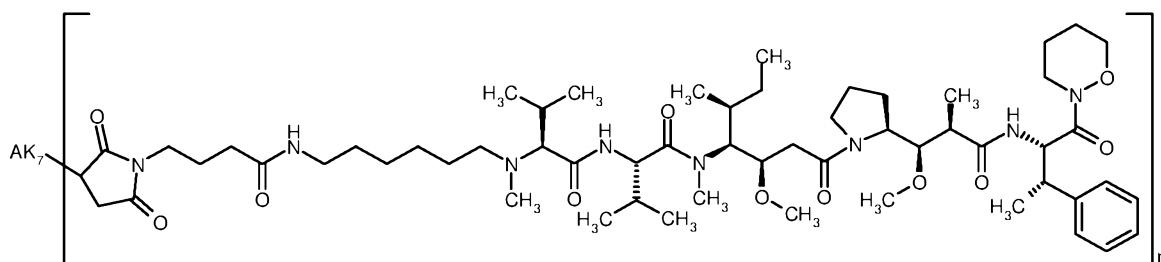


5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex
 5 column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.98 mg/ml

Drug/mAb ratio: 2.8

Example 315



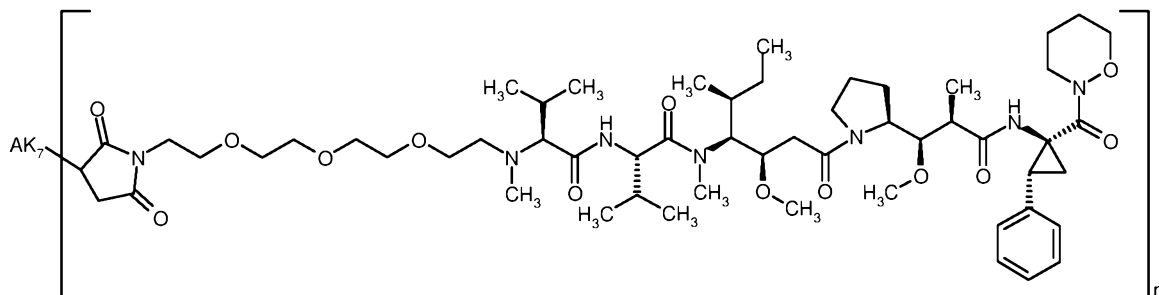
10

5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex
 column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.0 mg/ml

Drug/mAb ratio: 3

Example 316

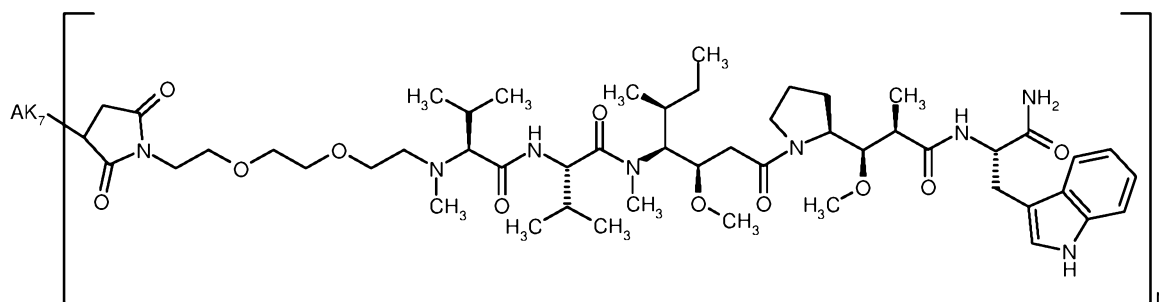


5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex
5 column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.59 mg/ml

Drug/mAb ratio: 3.1

Example 317



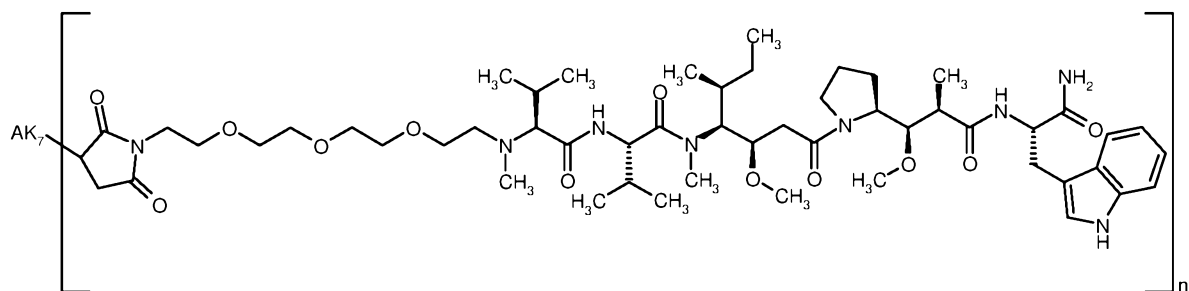
10

5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.75 mg/ml

Drug/mAb ratio: 3.3

Example 318

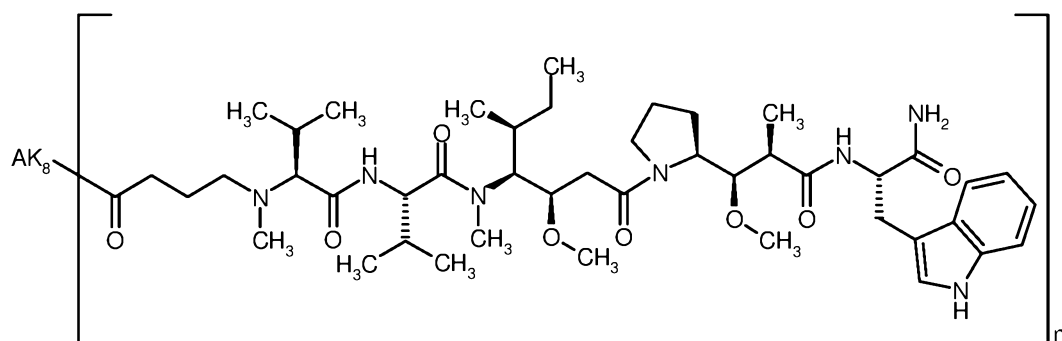


5 5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.54 mg/ml

Drug/mAb ratio: 3.5

Example 319

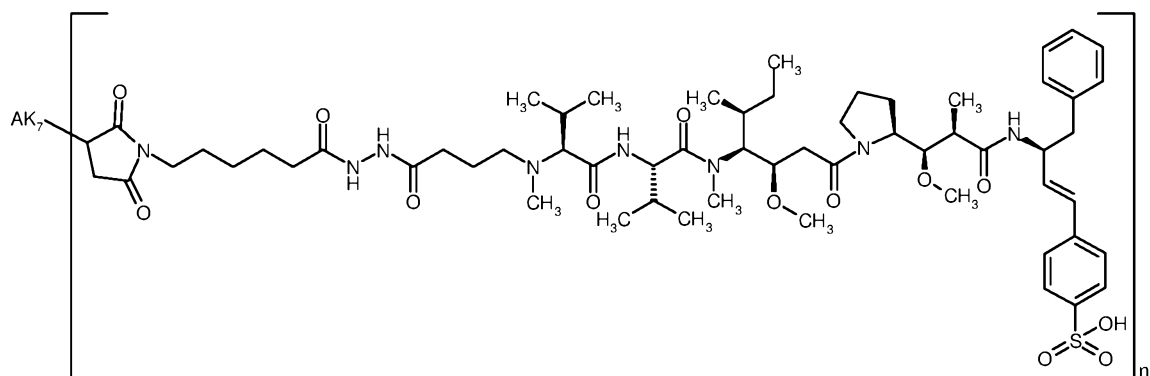


10 5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 2 mg/ml

Drug/mAb ratio: 1.1

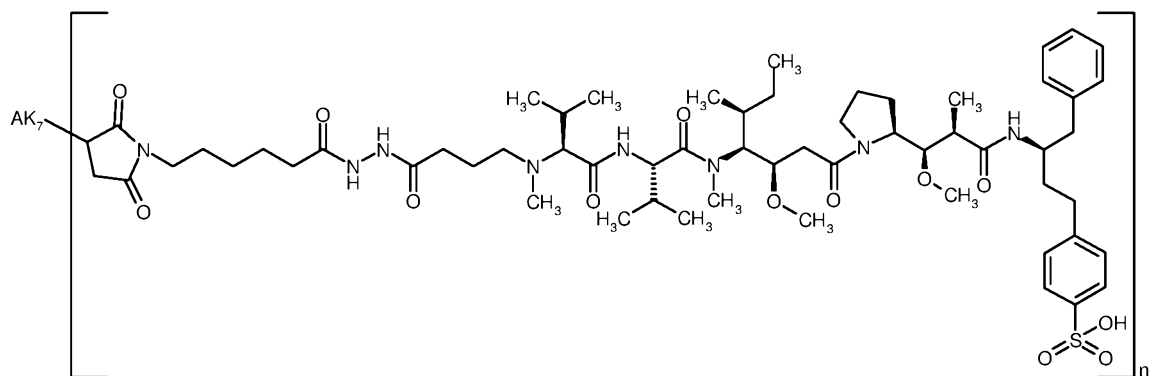
Example 320



Protein concentration: 1.66 mg/ml

Drug/mAb ratio: 4.9

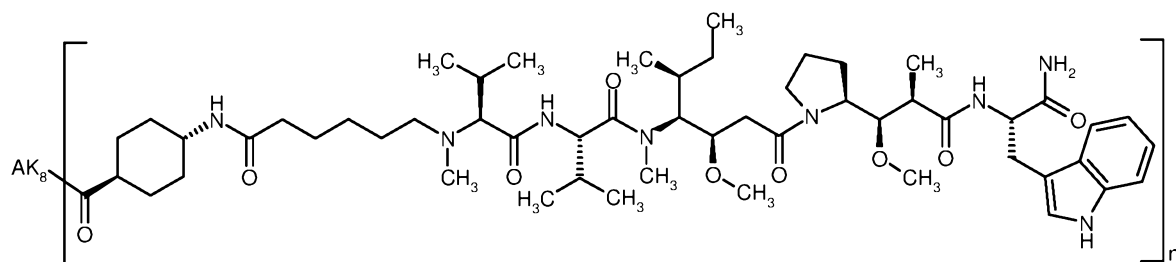
5 Example 321



Protein concentration: 1.7 mg/ml

Drug/mAb ratio: 3.0

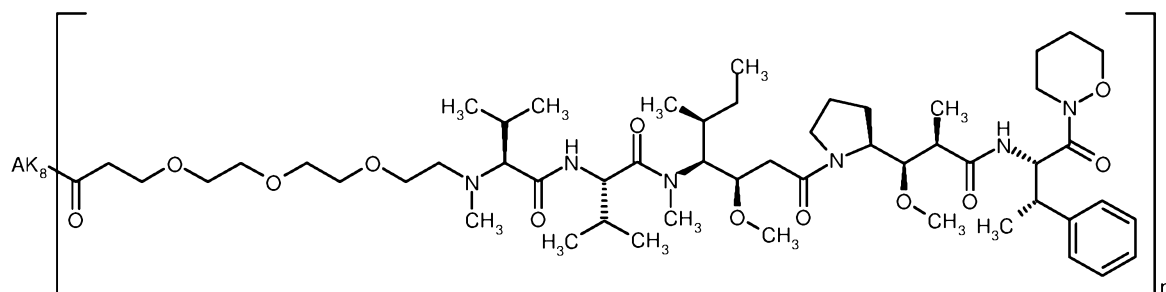
Example 322



Protein concentration: 1.08 mg/ml

5 Drug/mAb ratio: 1.9

Example 323

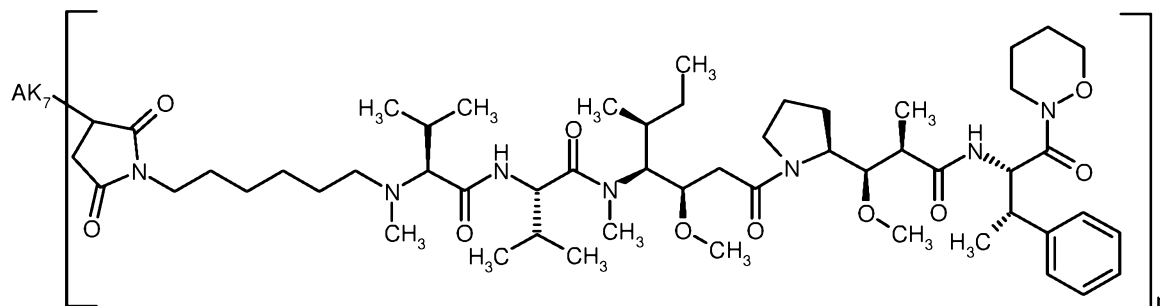


5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex
 10 column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.57 mg/ml

Drug/mAb ratio: 2.9

Example 324

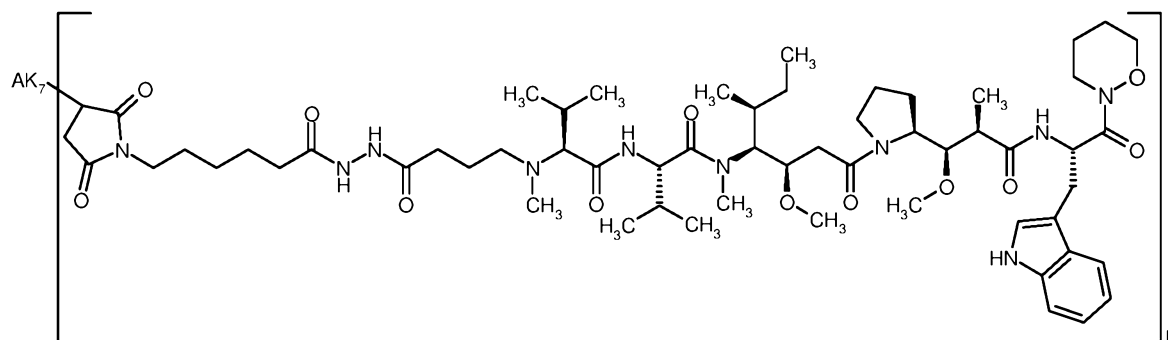


5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex
 5 column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.7 mg/ml

Drug/mAb ratio: 1.4

Example 325



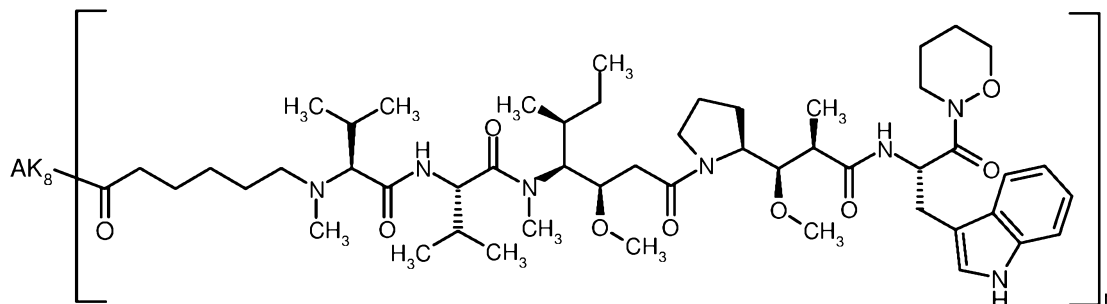
10

5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex
 column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.53 mg/ml

Drug/mAb ratio: 3.6

Example 326

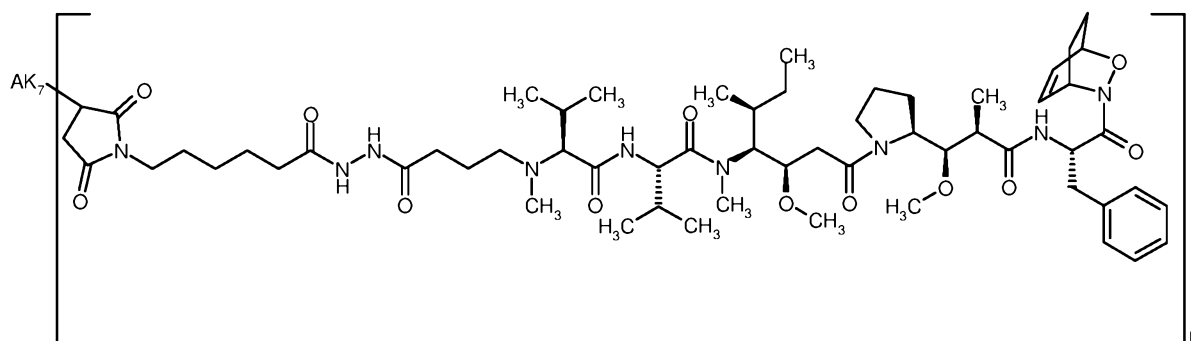


5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

5 Protein concentration: 1.77 mg/ml

Drug/mAb ratio: 6.1

Example 327

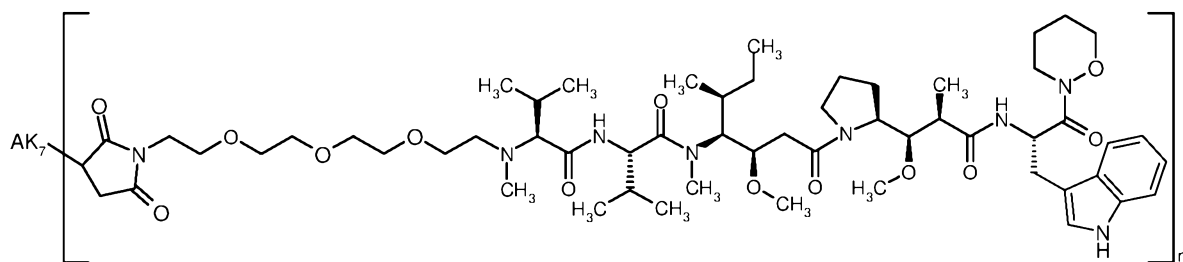


10 5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.14 mg/ml

Drug/mAb ratio: 2.5

Example 328

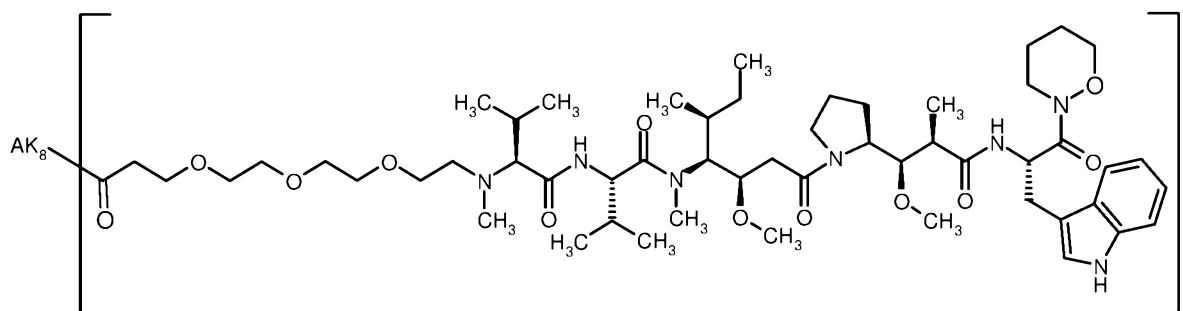


5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex
5 column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.57 mg/ml

Drug/mAb ratio: 3.8

Example 329



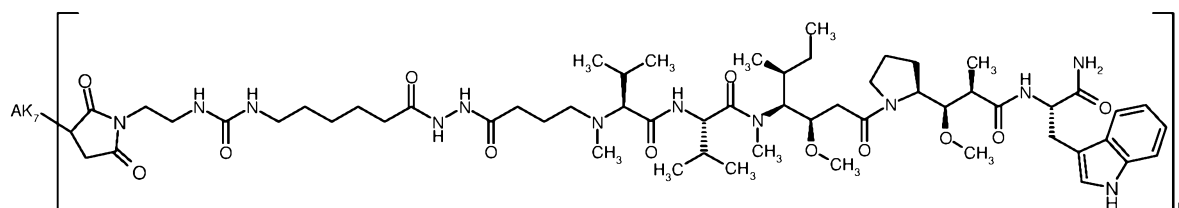
10

5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex
column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.72 mg/ml

Drug/mAb ratio: 3.9

Example 330

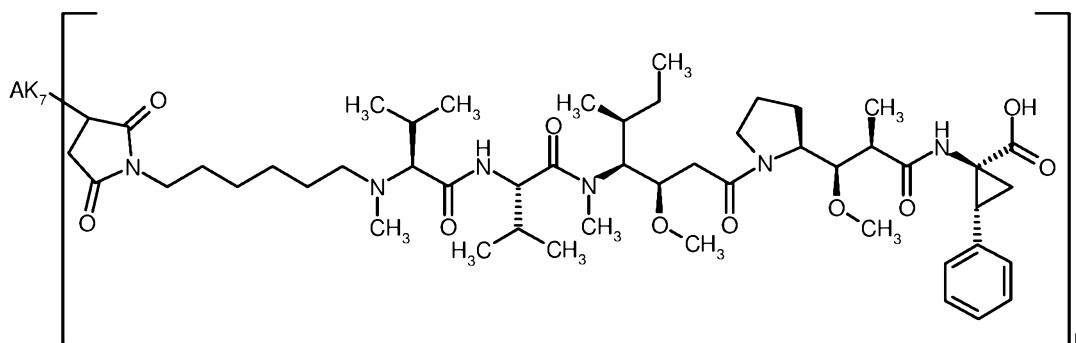


- 5 5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.56 mg/ml

Drug/mAb ratio: 2.9

Example 331

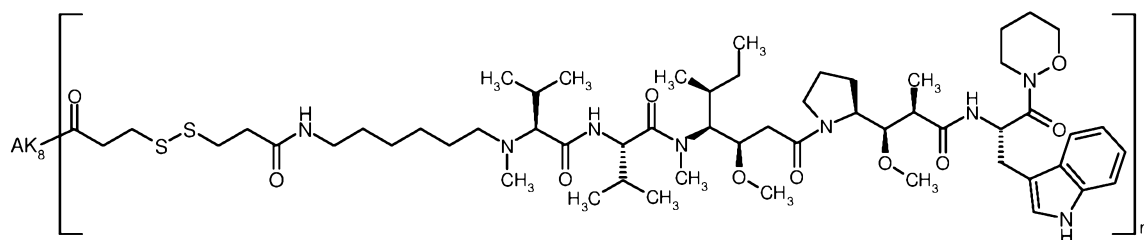


- 10 5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.81 mg/ml

Drug/mAb ratio: 3.5

Example 332

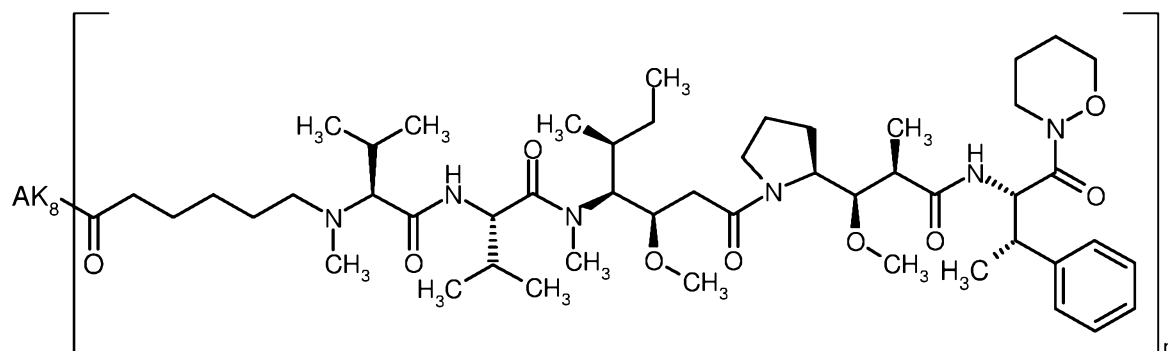


5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

5 Protein concentration: 1.54 mg/ml

Drug/mAb ratio: 1.3

Example 333



10 5mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

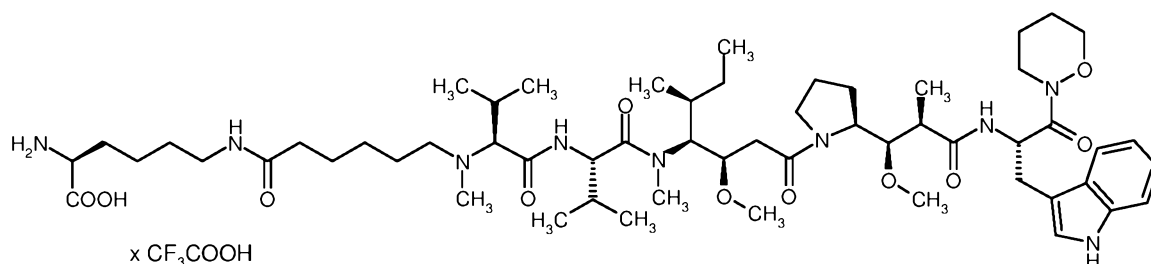
Protein concentration: 1.72 mg/ml

Drug/mAb ratio: 4.0

Example 334

15 N-(6-[(5S)-5-amino-5-carboxypentyl]amino)-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-

methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



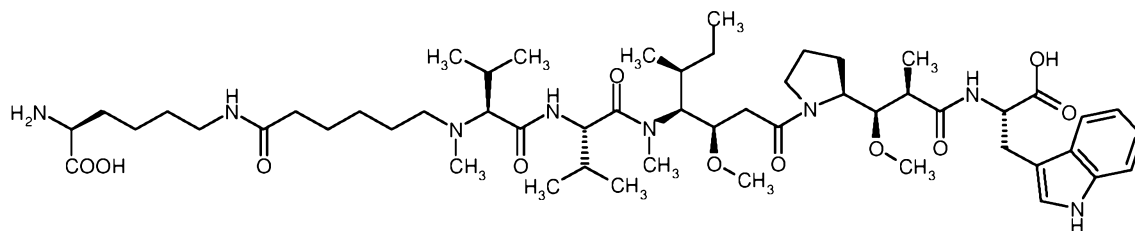
- 5 15.5 mg (15 μ mol) of Intermediate 210 weretaken up in 5 ml DMF and mixed with 4.4 mg (18 μ mol) N²-(tert.-butoxycarbonyl)-L-lysine as well as 7.7 μ l (44 μ mol) *N,N*-diisopropylethylamine. The reaction mixture was stirred overnight at RT and then concentrated *in vacuo*. Next, the residue was purified by means of preparative HPLC. 14 mg (81% of theory) of the protected intermediate of the title compound were obtained, which were then taken up in 1
- 10 mldichloromethane and deprotected with 1 mltrifluoroascetic acid.The reaction mixture was concentrated and, after lyophilization of the residue from acetonitrile/water (1:1), 15 mg (97% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.79 min; MS (ESIpos): m/z = 1083 (M+H)⁺.

15 Example 335

N-(6-{[(5*S*)-5-amino-5-carboxypentyl]amino}-6-oxohexyl)-N-methyl-L-valyl-N-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-(1*H*-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



20

40 mg (40 μ mol) of Intermediate 227 weretaken up in 5 ml DMF and mixed with 11.5 mg (40 μ mol) N²-[(benzyloxy)carbonyl]-L-lysine as well as 13 μ l (80 μ mol) *N,N*-diisopropylethylamine.

The reaction mixture was stirred overnight at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 32.5 mg (70% of theory) of the protected intermediate of the title compound.

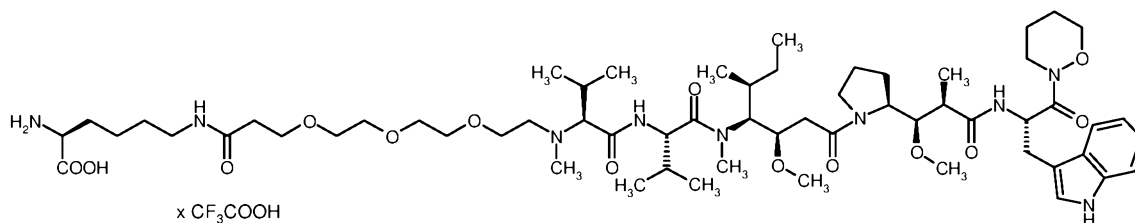
32.5 mg of this intermediate was dissolved in 10 ml methanol and, after adding 2 mg 10% palladium on activated carbon, hydrogenated for 30 min at RT under standard hydrogen pressure. The catalyst was then filtered off and the solvent removed *in vacuo*. After lyophilization of the residue from dioxane/water (1:1), the yield was 26 mg (99% of theory) of the title compound.

HPLC (Method 12): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.76$ min; MS (ESIpos): $m/z = 1014$ (M+H)⁺.

10 Example 336

N-[(18S)-18-amino-18-carboxy-12-oxo-3,6,9-trioxa-13-azaoctadec-1-yl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



15

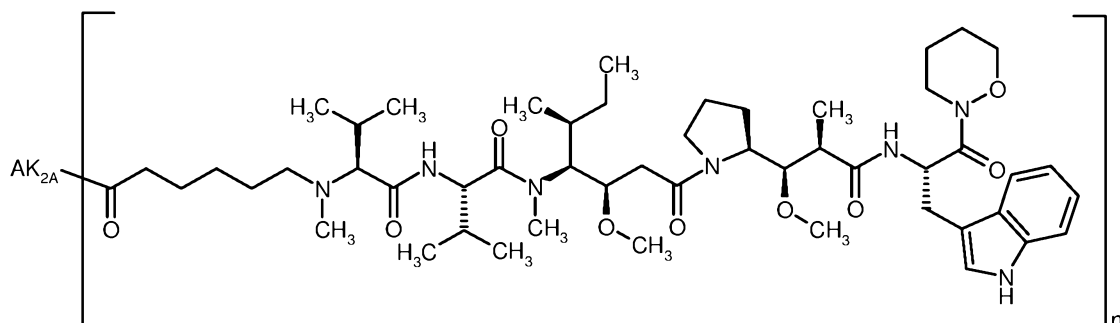
3.5 mg (3 μ mol) of Intermediate 202 were taken up in 2 ml DMF and mixed with 0.8 mg (3 μ mol) N²-(tert.-butoxycarbonyl)-L-lysine as well as 1.6 μ l (10 μ mol) *N,N*-diisopropylethylamine. The reaction mixture was stirred overnight at RT and then concentrated *in vacuo*. Next, the residue was taken up in acetonitrile/water (1:1), adjusted to pH 2 with trifluoroacetic acid and then purified by means of preparative HPLC. 1 mg (25% of theory) of the protected intermediate of the title compound was obtained, which was subsequently taken up in 500 μ l dichloromethane and deprotected with 500 μ l trifluoroacetic acid. The reaction mixture was concentrated and, after lyophilization of the residue from acetonitrile/water (1:1), the yield was 1 mg (89% of theory) of the title compound.

25

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.82$ min; MS (ESIpos): $m/z = 1173$ ($M+H$)⁺.

Example 337

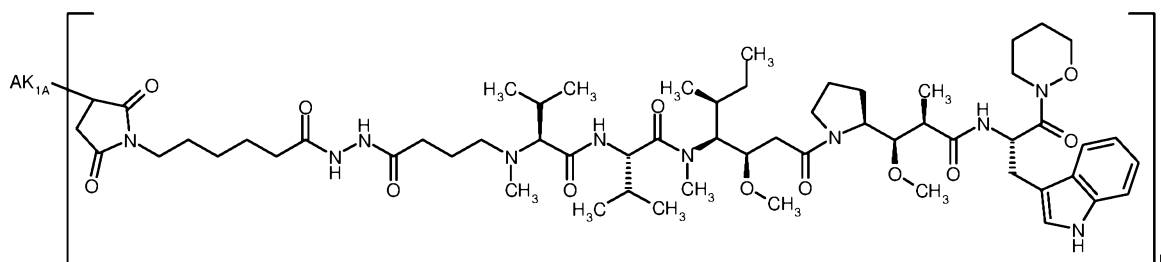


5 mg cetuximab in PBS were presently used for the coupling and, following purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.57 mg/ml

Drug/mAb ratio: 4.6

Example 338



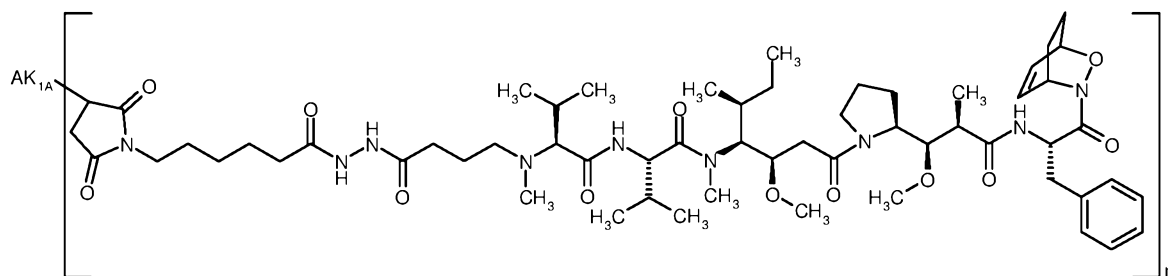
10

5 mg cetuximab were presently used for the coupling and, following purification on a Sephadex
 column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.48 mg/ml

Drug/mAb ratio: 3.4

Example 339

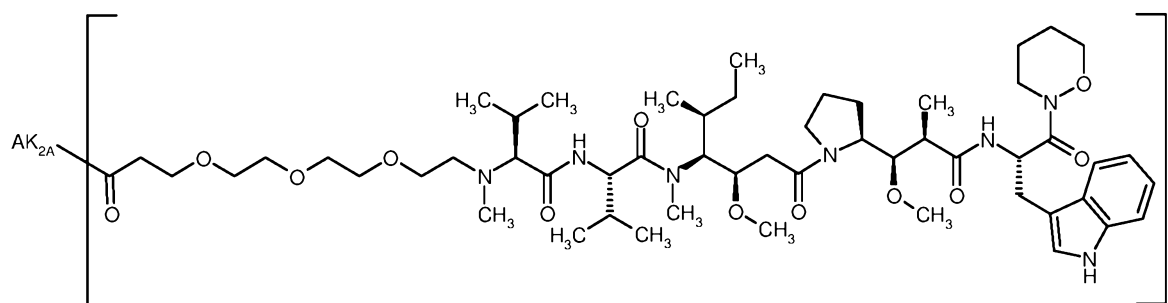


5 mg cetuximab in PBS were presently used for the coupling and, following purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.21 mg/ml

Drug/mAb ratio: 2.4

Example 340



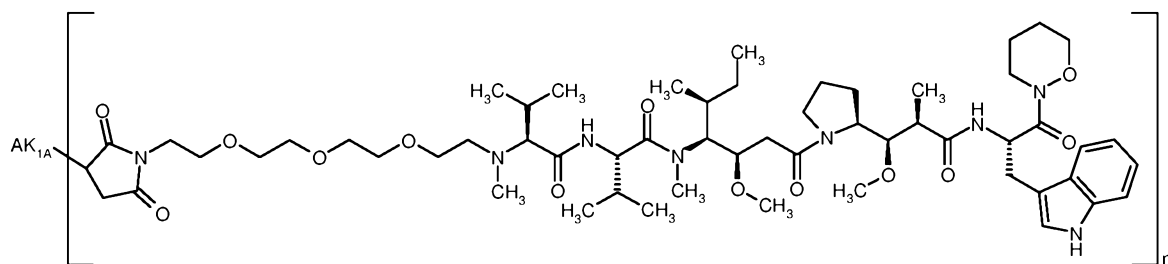
10

5 mg cetuximab in PBS were presently used for the coupling and, following purification on a
 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.75 mg/ml

Drug/mAb ratio: 3.4

Example 341

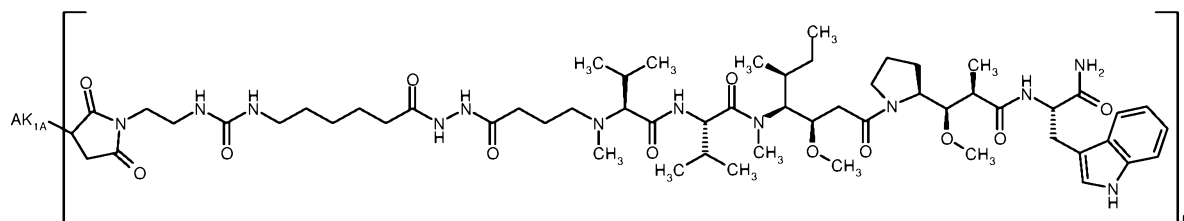


5 mg cetuximab in PBS were presently used for the coupling and, following purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.69 mg/ml

Drug/mAb ratio: 2.9

Example 342



10

5 mg cetuximab were presently used for the coupling and, following purification on a Sephadex
 column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.27 mg/ml

Drug/mAb ratio: 2.9

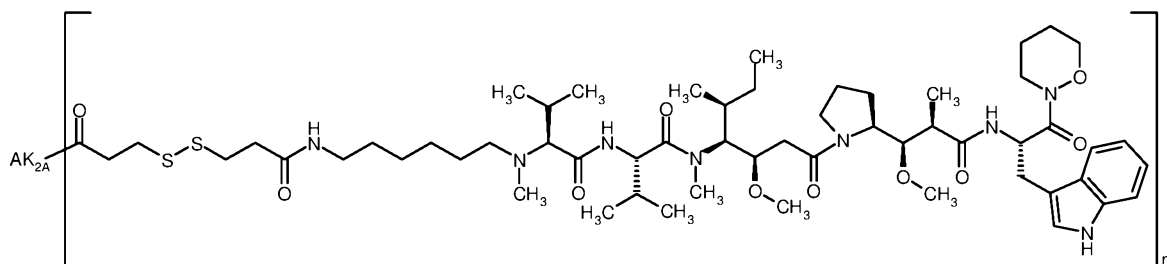
CN(C)C(=O)[C@H](NC(=O)[C@@H](OC)[C@H](COCC(=O)N[C@@H]([C@H](OC)CN1CCCC1)CCC(=O)N[C@H](C)C[C@H](OC)C)C[C@H](C)C

AK_{1c}

[illegible]

Drug/mAb ratio: 3.1

Example 347

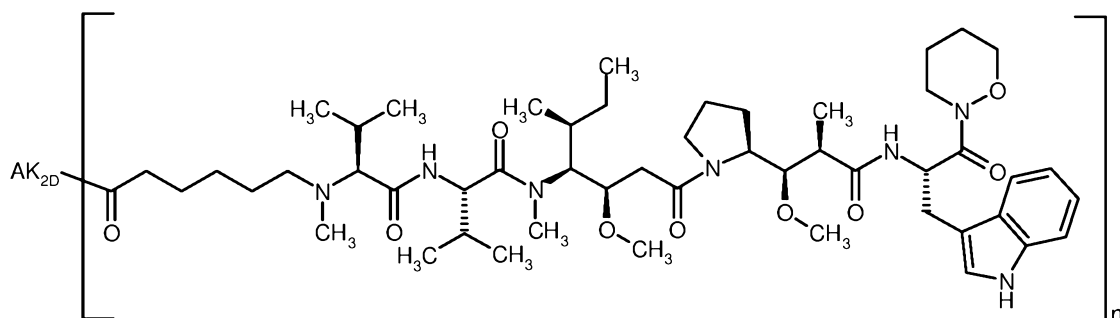


5 mg cetuximab in PBS were presently used for the coupling and, following purification on a
5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.73 mg/ml

Drug/mAb ratio: 1.2

Example 348

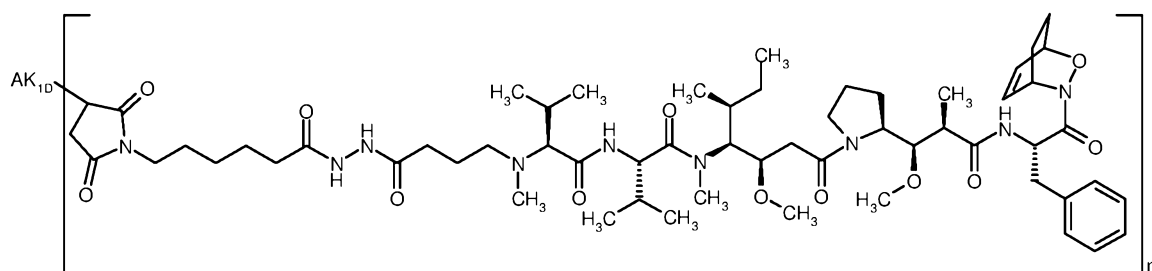


2 mg anti-PDL1 in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.04 mg/ml

Drug/mAb ratio: 4.8

Example 349

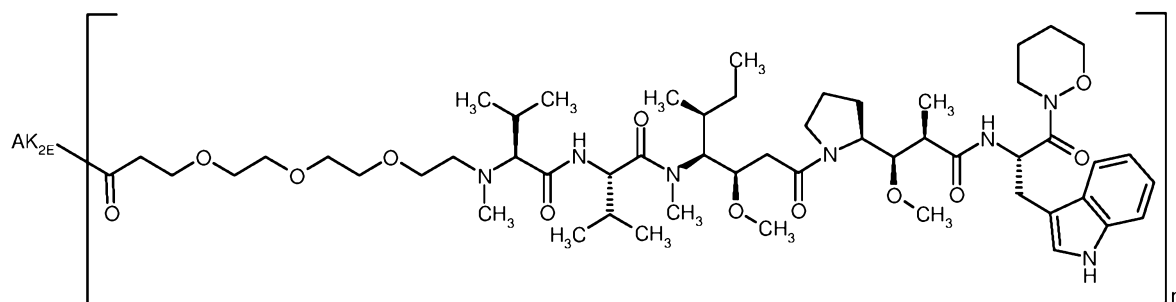


3 mg anti-PDL1 in PBS were presently used for the coupling and, following purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.7 mg/ml

Drug/mAb ratio: 2.3

Example 350



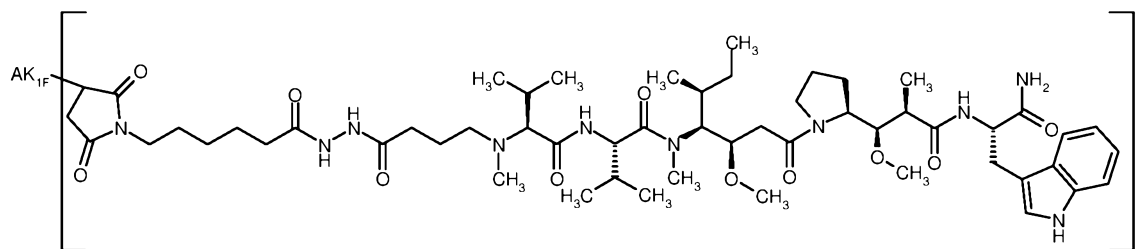
10

2 mg anti-ICOSLGIN PBS were presently used for the coupling and, following purification on a
 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.77 mg/ml

Drug/mAb ratio: 3.7

Example 351

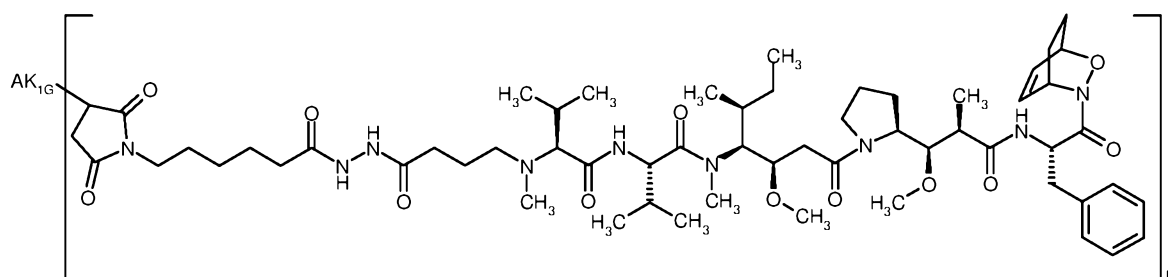


4 mg anti-FGFR3in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentratedby ultracentrifugation and diluted again with PBS.

5 Protein concentration: 1.41 mg/ml

Drug/mAb ratio: 1.8

Example 352

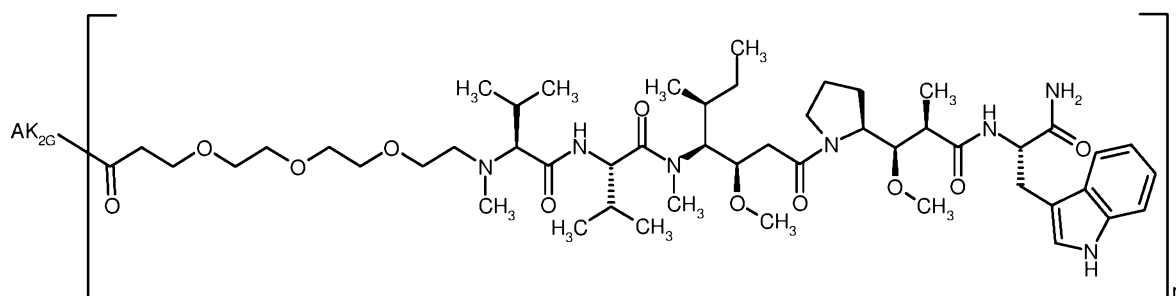


10 3 mg herceptin in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentratedby ultracentrifugation and diluted again.

Protein concentration: 1.49 mg/ml

Drug/mAb ratio: 2.3

Example 353

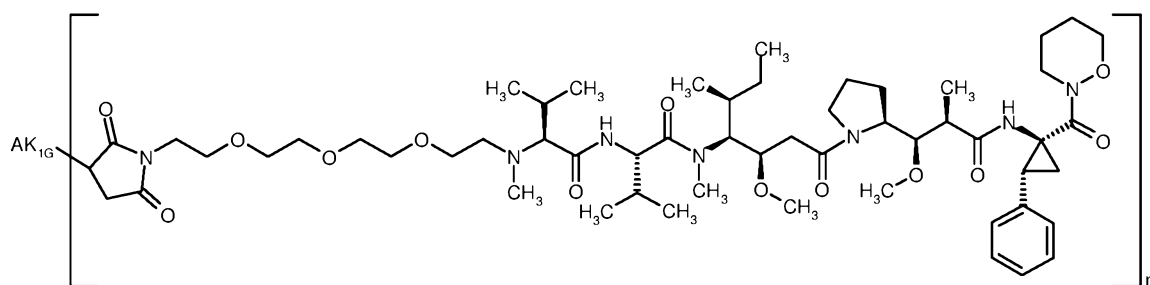


5 mg herceptin in PBS were presently used for the coupling and, following purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.62 mg/ml

Drug/mAb ratio: 5.0

Example 354



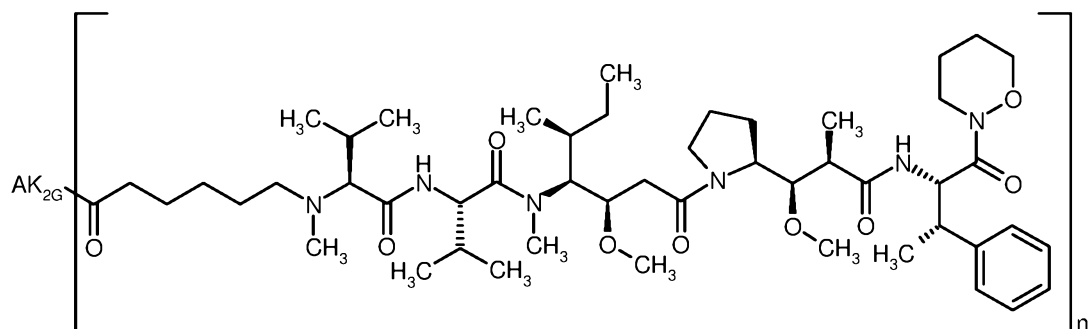
10

5 mg herceptin in PBS were presently used for the coupling and, following purification on a
 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.63 mg/ml

Drug/mAb ratio: 2.4

Example 355

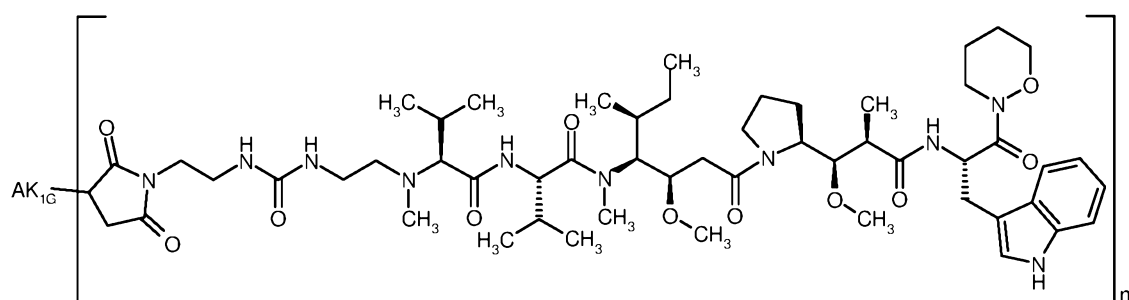


5 mg herceptin in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

5 Protein concentration: 1.65 mg/ml

Drug/mAb ratio: 3.9

Example 356

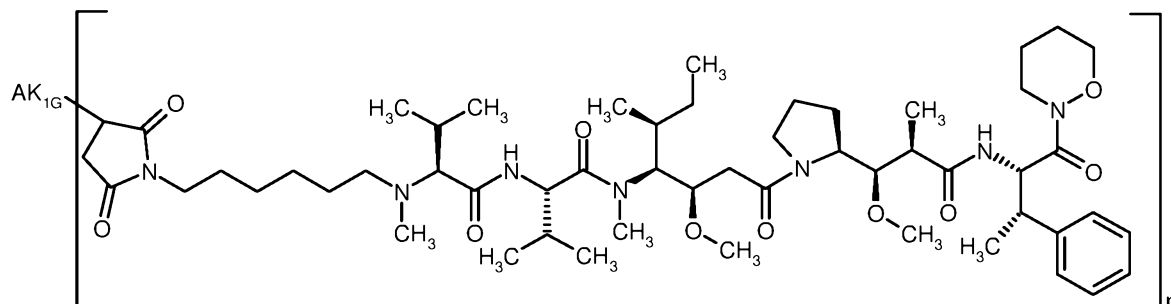


10 5 mg herceptin in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.78 mg/ml

Drug/mAb ratio: 3.1

Example 357

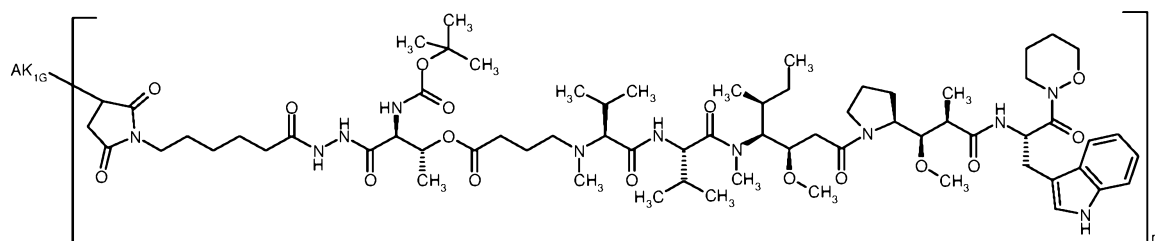


5 5 mg herceptin in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.72 mg/ml

Drug/mAb ratio: 3.2

Example 358



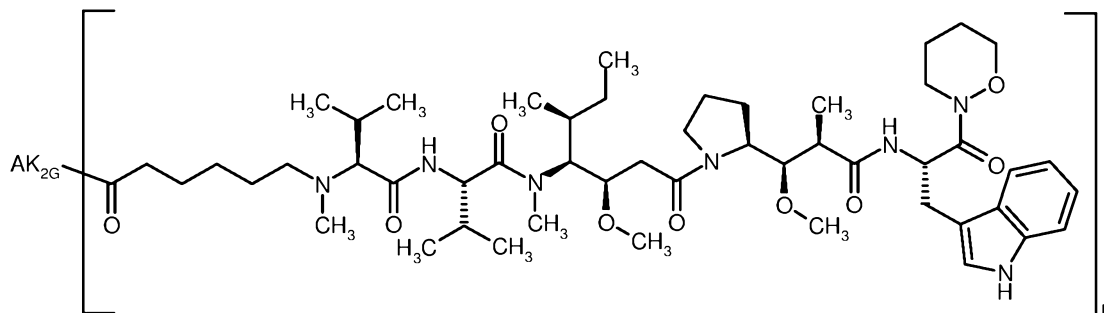
10

5mg herceptin in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Proteinkonzentration: 1.95 mg/ml

Drug/mAb-Ratio: ~3.7

Example 359



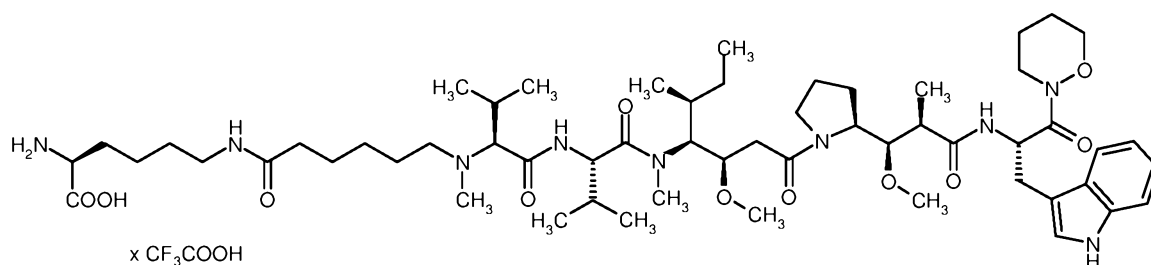
5 mg herceptin in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

5 Protein concentration: 1.79 mg/ml

Drug/mAb ratio: 9.1

Example 360

N-(6-{[(5S)-5-amino-5-carboxypentyl]amino}-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-
 {(2S)-2-[(1R,2R)-3-[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-
 10 methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-
 methyl-L-valinamide trifluoroacetate



15.5 mg (15 μ mol) of Intermediate 210 were taken up in 5 ml DMF and mixed with 4.4 mg (18 μ mol) N^2 -(tert.-butoxycarbonyl)-L-lysine as well as 7.7 μ l (44 μ mol) *N,N*-diisopropylethylamine. The reaction mixture was stirred overnight at RT, then concentrated *in vacuo*. The residue was purified by means of preparative HPLC. 14 mg (81% of theory) of the protected intermediate of the title compound were obtained, which were then taken up in 1 ml dichloromethane and deprotected with 1 ml trifluoroacetic acid. The batch was concentrated and, after lyophilization of

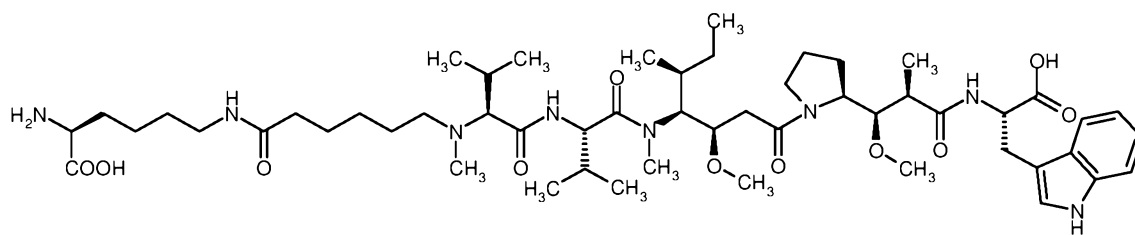
the residue from acetonitrile/water (1:1), the yield was 15 mg (97% of theory) of the title compound.

HPLC (Method 12): $R_t = 1.8$ min;

LC-MS (Method 1): $R_t = 0.79$ min; MS (ESIpos): $m/z = 1083$ ($M+H$)⁺.

5 Example 361

N-(6-([(5S)-5-amino-5-carboxypentyl]amino)-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



10

40 mg (40 μ mol) of Intermediate 227 weretaken up in 5 ml DMF and mixed with 11.5 mg (40 μ mol) N^2 -[(benzyloxy)carbonyl]-L-lysine as well as 13 μ l (80 μ mol) N,N -diisopropylethylamine. The reaction mixture was stirred overnight at RT, then concentrated *in vacuo* and next purified by means of preparative HPLC. The yield was 32.5 mg (70% of theory) of the protected intermediate of the title compound.

15

32.5 mg of this intermediate weretaken up in 10 ml methanol and, after adding 2 mg 10% palladium on activated carbon, hydrogenated at RT under hydrogen normal pressure. The catalyst was then filtered off and the solvent removed *in vacuo*. After lyophilization of the residue from dioxane/water (1:1), the yield was 26 mg (99% of theory) of the title compound.

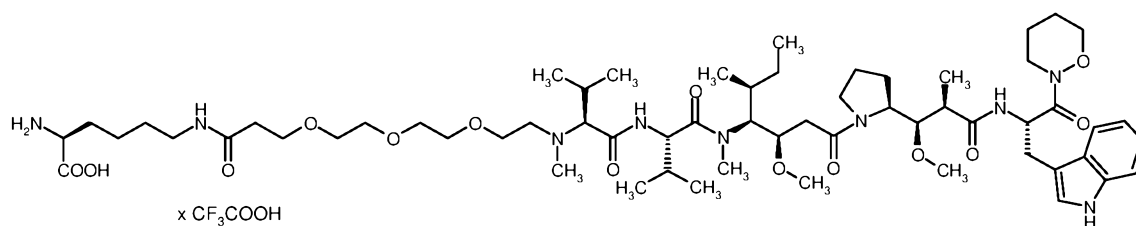
20 HPLC (Method 12): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.76$ min; MS (ESIpos): $m/z = 1014$ ($M+H$)⁺.

Example 362

N-[(18S)-18-amino-18-carboxy-12-oxo-3,6,9-trioxa-13-azaotadec-1-yl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-

2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate

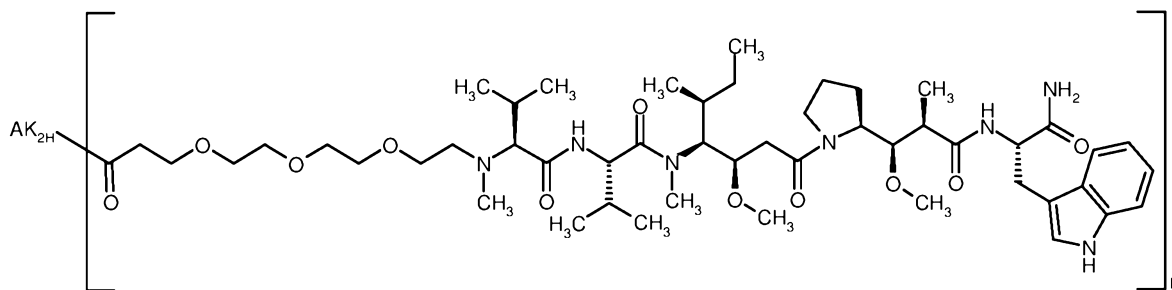


- 5 3.5 mg (3 μ mol) of Intermediate 202 were taken up in 2 ml DMF and mixed with 0.8 mg (3 μ mol) N^2 -(tert.-butoxycarbonyl)-L-lysine as well as 1.6 μ l (10 μ mol) N,N -diisopropylethylamine. The reaction mixture was stirred overnight at RT, then concentrated *in vacuo*. The residue was taken up in acetonitrile/water (1:1), adjusted to pH 2 with trifluoroacetic acid, then purified by means of preparative HPLC. The yield was 1 mg (25% of theory) of the protected intermediate of
- 10 the title compound that was subsequently taken up in 500 μ l dichloromethane and deprotected with 500 μ l trifluoroacetic acid. The batch was concentrated and, after lyophilization of the residue from acetonitrile/water (1:1), the yield was 1 mg (89% of theory) of the title compound.

HPLC (Method 12): $R_t = 1.9$ min;

LC-MS (Method 1): $R_t = 0.82$ min; MS (ESIpos): $m/z = 1173$ ($M+H$) $^+$.

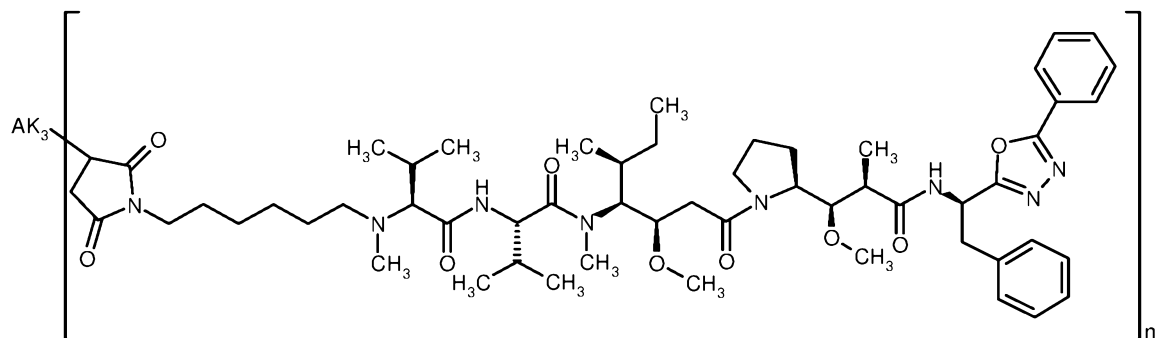
15 Example 363



2.2 mg anti-TYRP1 in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

20 Protein concentration: 1.14 mg/ml

Example 366

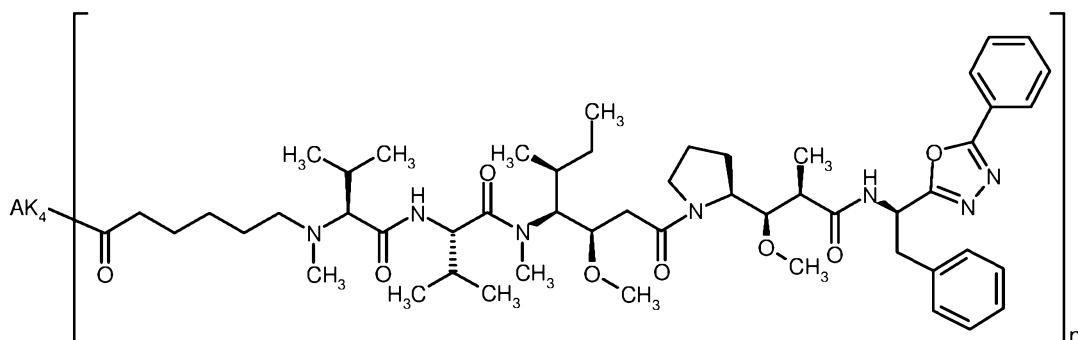


5 mg MF-Ta in PBS were presently used for the coupling and, following purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 0.81 mg/ml

Drug/mAb ratio: 2.5

Example 367

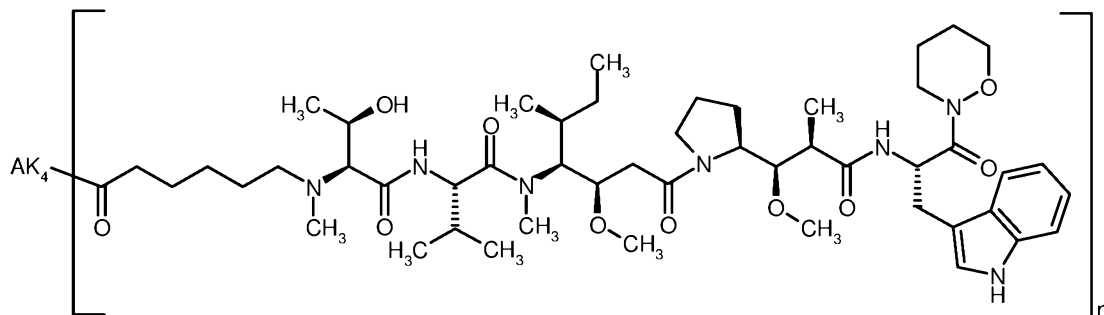


10 5 mg MF-Ta in PBS were presently used for the coupling and, following purification on a
 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.06 mg/ml

Drug/mAb ratio: 1.8

Example 368

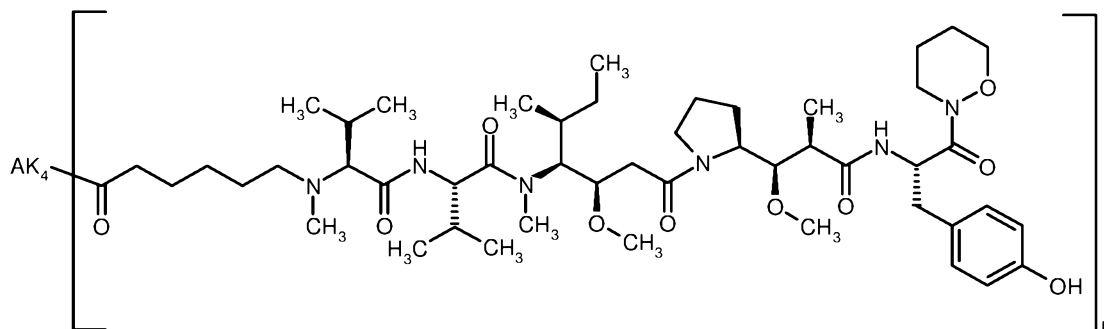


5 mg MF-Ta in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

5 Protein concentration: 1.36 mg/ml

Drug/mAb ratio: 7.2

Example 369

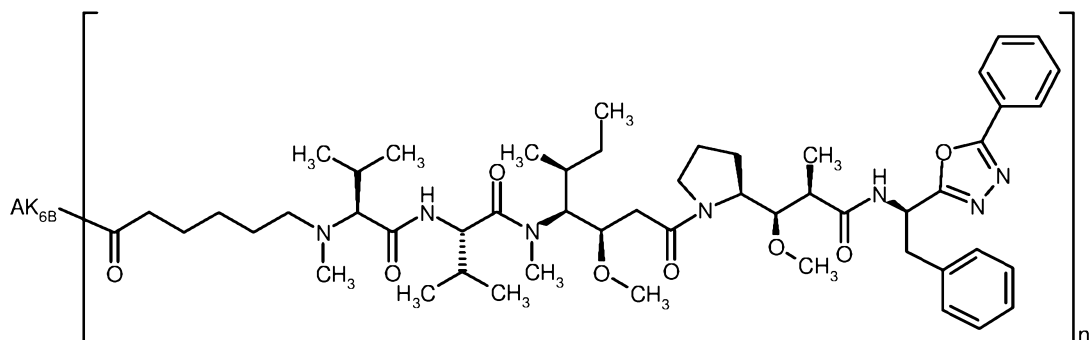


10 5 mg MF-Ta in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.57 mg/ml

Drug/mAb ratio: 2.9

Example 370

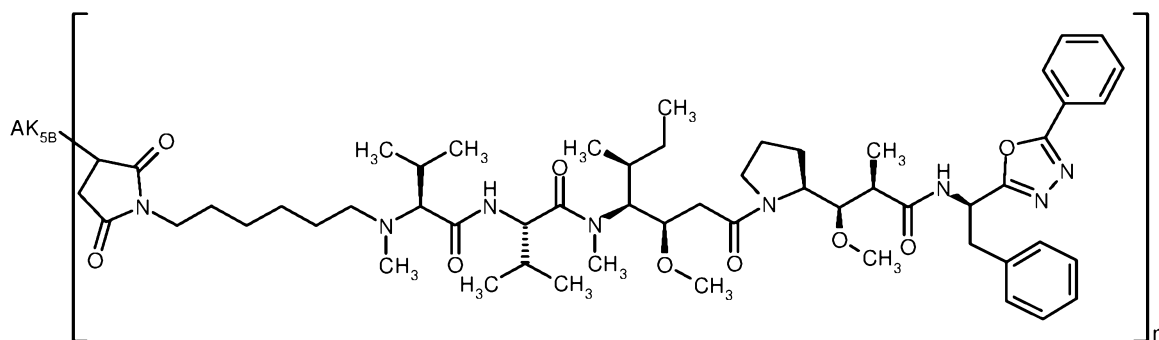


5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

5 Protein concentration: 0.89 mg/ml

Drug/mAb ratio: 1.8

Example 371

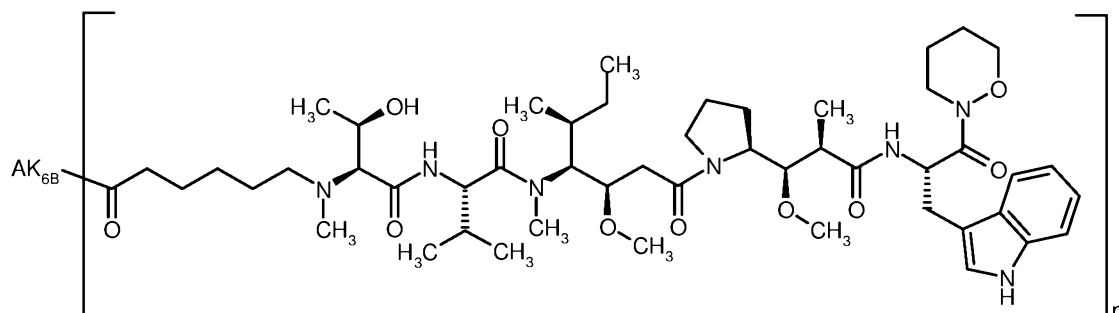


10 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 0.57 mg/ml

Drug/mAb ratio: 1.5

Example 372

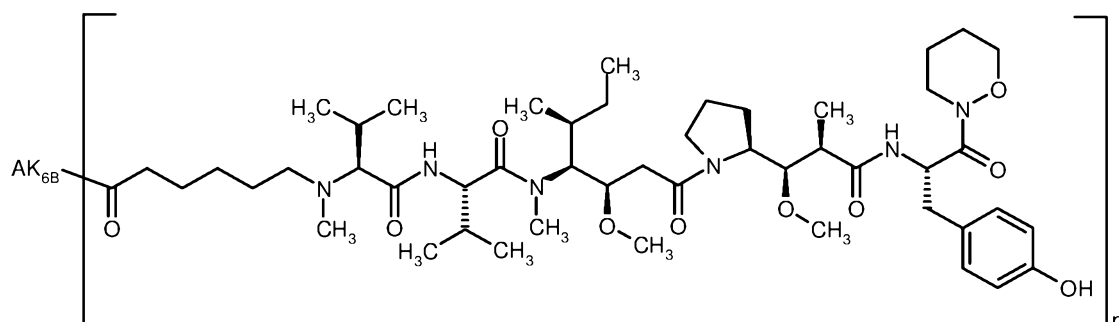


5 mg anti-C4.4a B01-3 were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

5 Protein concentration: 1.39 mg/ml

Drug/mAb ratio: 7.1

Example 373

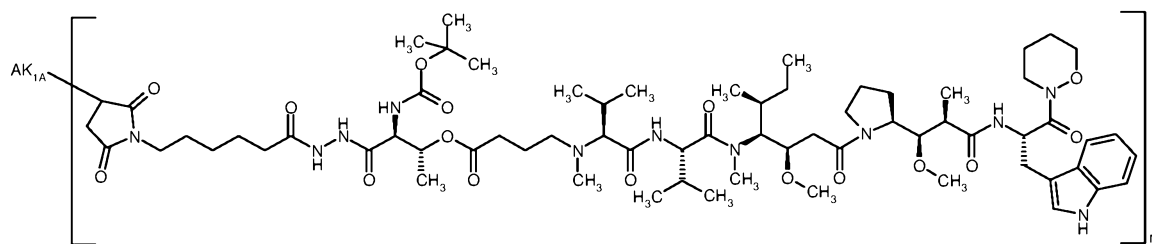


5 mg anti-C4.4a B01-3 were presently used for the coupling and, following purification on a
10 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.54 mg/ml

Drug/mAb ratio: 2.4

Example 374

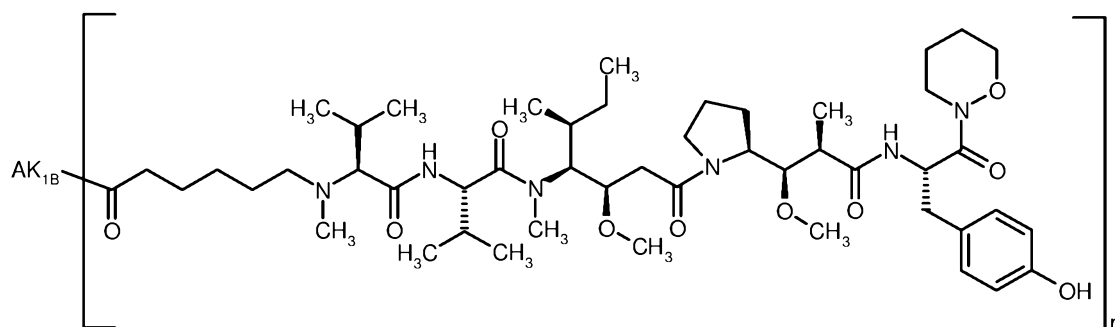


- 5 mg cetuximab in PBS were presently used for the coupling and, following purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.55 mg/ml

Drug/mAb-Ratio: 1.8

Example 375



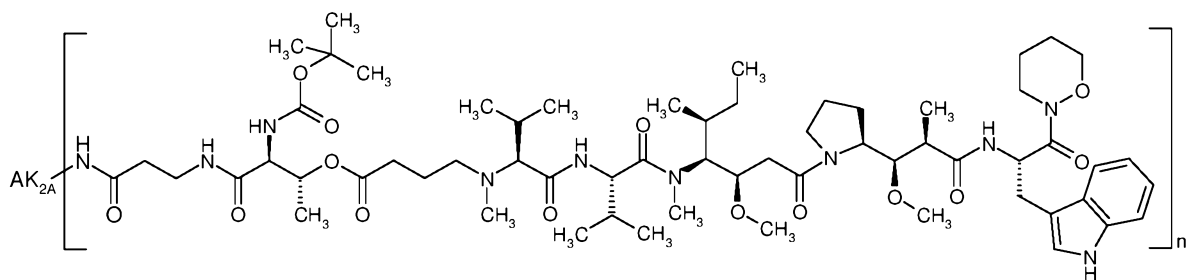
10

5 mg cetuximab were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.36 mg/ml

Drug/mAb ratio: 1.9

Example 376

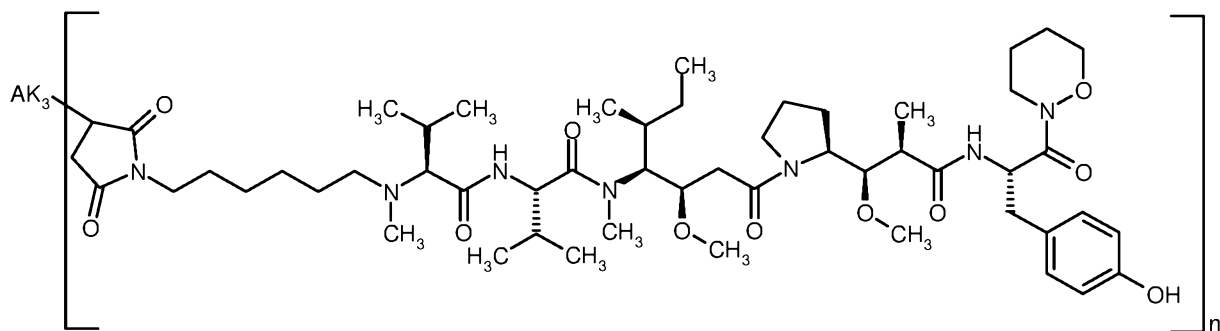


5 mg cetuximab in PBS were presently used for the coupling and, following purification on a
5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.73 mg/ml

Drug/mAb-Ratio: 3.7

Example 377



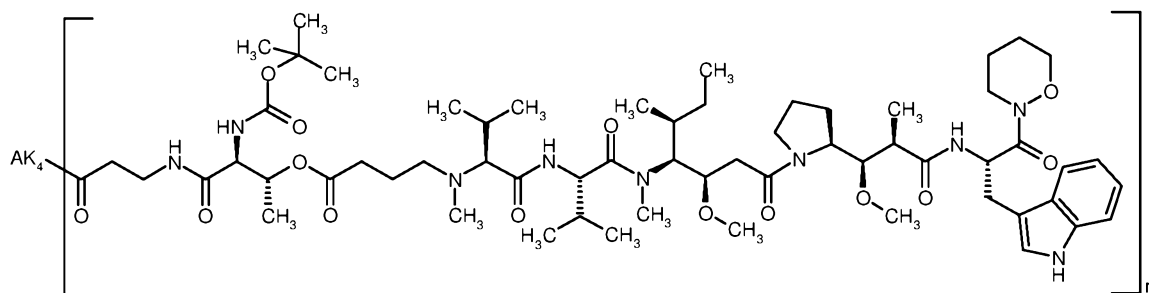
10

5 mgMF-Ta in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.44 mg/ml

15 Drug/mAb ratio: 2.5

Example 378

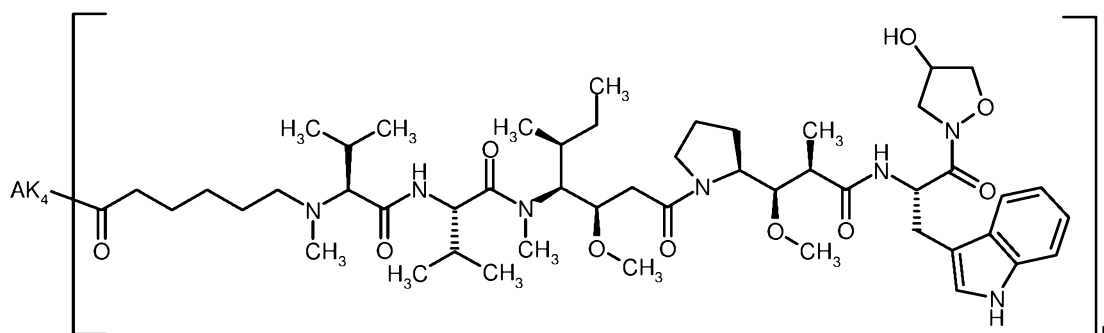


5 mgMF-Ta in PBS were presently used for the coupling and, following purification on a
 5 Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.74 mg/ml

Drug/mAb-Ratio: 3.6

Example 379 (Diastereomer 1)

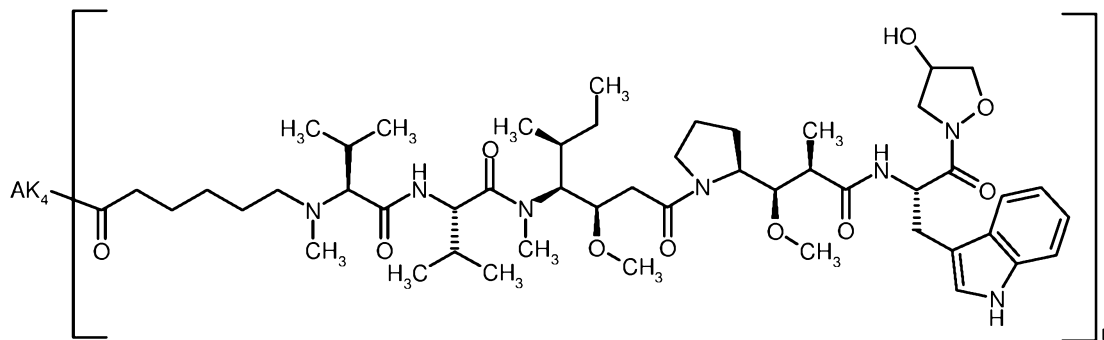


10 Intermediate 247a and 5 mgMF-Ta in PBS were presently used for the coupling and, following
 purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted
 again with PBS.

Protein concentration: 1.57 mg/ml

Drug/mAb ratio: 4.2

Example 380 (Diastereomer 2)



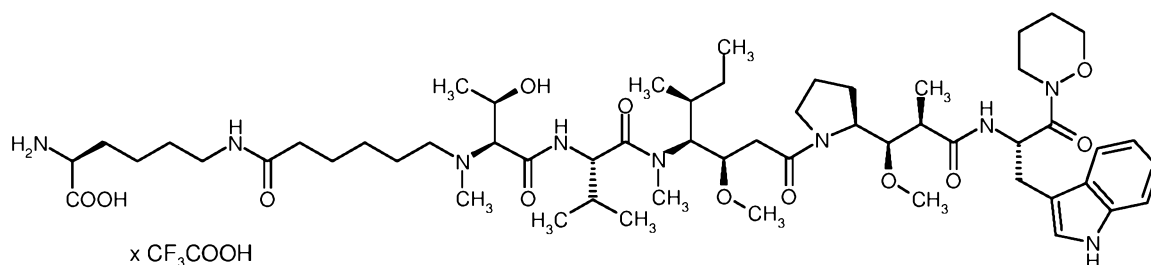
Intermediate 247a and 5 mg MF-Ta in PBS were presently used for the coupling and, following purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.42 mg/ml

Drug/mAb ratio: 4.0

Example 381

N-(6-([(5S)-5-amino-5-carboxypentyl]amino)-6-oxohexyl)-N-methyl-L-threonyl-N-[(3R,4S,5S)-1-
 {(2S)-2-[(1R,2R)-3-([(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino)-1-
 methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-
 L-valinamide trifluoroacetate



8.6 mg (8 μ mol) of Intermediate 240 were taken up in 5 ml DMF and mixed with 4.0 mg (16 μ mol) N²-(tert.-butoxycarbonyl)-L-lysine as well as 2 μ l (16 μ mol) N,N-diisopropylethylamine. The reaction mixture was stirred for 4 hours at RT, then mixed again with the same quantities of N²-(tert.-butoxycarbonyl)-L-lysine and N,N-diisopropylethylamine and

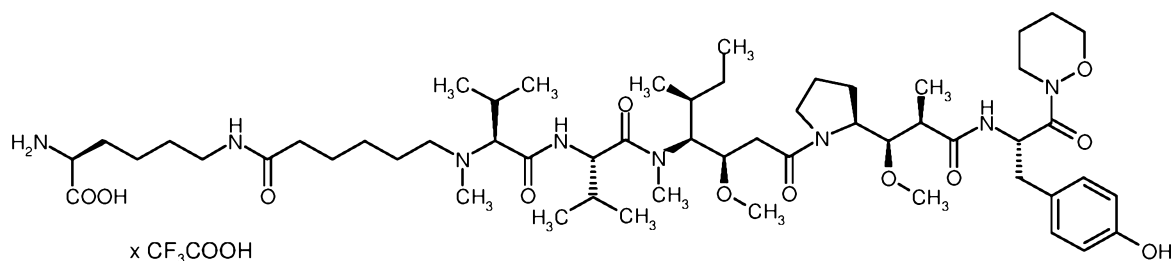
stirred overnight at RT. The batch was then concentrated *in vacuo*. Next, the residue was purified by means of preparative HPLC. The yield was 7 mg (72% of theory) of the protected intermediate of the title compound that was then taken up in 1 ml of dichloromethane and deprotected with 0.5 ml trifluoroacetic acid. The batch was concentrated and the residue purified by means of preparative HPLC. After drying under high vacuum, the yield was 3.3 mg (47% of theory) of the title compound.

HPLC (Method 5): $R_t = 1.5$ min;

LC-MS (Method 1): $R_t = 0.8$ min; MS (ESIpos): $m/z = 1084$ (M+H)⁺.

Example 382

- 10 N-(6-[(5S)-5-amino-5-carboxypentyl]amino)-6-oxohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-[(2S)-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate



15

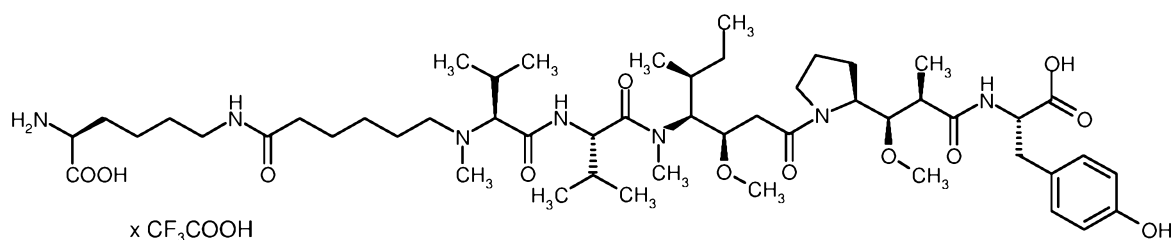
- 8 mg (8 μ mol) of Intermediate 242 were taken up in 3 ml DMF and mixed with 2.9 mg (12 μ mol) N²-(tert.-butoxycarbonyl)-L-lysine as well as 2.7 μ l (16 μ mol) *N,N*-diisopropylethylamine. The reaction mixture was stirred overnight at RT, then mixed again with the same quantities of N²-(tert.-butoxycarbonyl)-L-lysine and *N,N*-diisopropylethylamine, then stirred for another 4 hours at RT. The batch was then concentrated *in vacuo*. Next, the residue was purified by means of preparative HPLC. Following lyophilization from acetonitrile/water, the yield was 6.5 mg (72% of theory) of the protected intermediate of the title compound that was then taken up in 5 ml dichloromethane and deprotected with 0.75 ml trifluoroacetic acid. The batch was concentrated and, following lyophilization of the residue from dioxane/water, the yield was 5 mg (76% of theory) of the title compound.
- 20
- 25

HPLC (Method 12): $R_t = 1.7$ min;

LC-MS (Method 1): $R_t = 0.69$ min; MS (ESIpos): $m/z = 1059$ (M+H)⁺.

Example 383

N-(6-[[[(5S)-5-amino-5-carboxypentyl]amino]-6-oxohexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-
{(2S)-2-[(1R,2R)-3-[(1S)-1-carboxy-2-(4-hydroxyphenyl)ethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide
5 trifluoroacetate



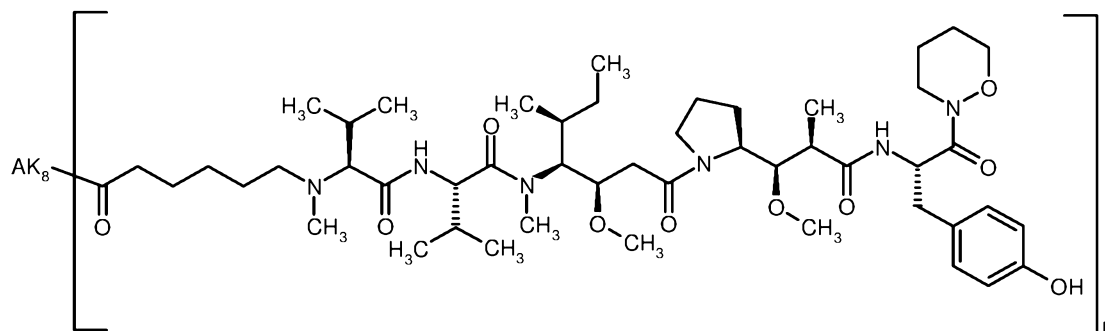
38 mg (41 μ mol) of Intermediate 248 were first converted to the N-hydroxysuccinimide ester. 72
10 mg of the obtained raw product were taken up in 5 ml DMF and mixed with 24 mg (100 μ mol)
N²-(tert.-butoxycarbonyl)-L-lysine as well as 23 μ l N,N-diisopropylethylamine. The reaction
mixture was stirred overnight at RT, then mixed again with 16 mg N²-(tert.-butoxycarbonyl)-L-
lysine and 12 μ l N,N-diisopropylethylamine and finally treated for another 2 hours in an ultrasonic
15 bath. The batch was then concentrated *in vacuo* and the residue purified by means of preparative
HPLC. After lyophilization from acetonitrile/water, the yield was 20 mg (50% of theory) of the
protected intermediate of the title compound.

15 mg (12 μ mol) of this intermediate were then taken up in 3 ml dichloromethane and mixed
with 1 ml trifluoroacetic acid. After stirring for 40 min at RT, further 1.5 ml trifluoroacetic acid
were added, and the batch was treated for 1 h in an ultrasonic bath. The batch was then
20 concentrated and, after lyophilization of the residue from dioxane/water, the yield was 13 mg
(90% of theory) of the title compound.

HPLC (Method 12): $R_t = 1.5$ min;

LC-MS (Method 1): $R_t = 0.68$ min; MS (ESIpos): $m/z = 990$ (M+H)⁺.

Example 384

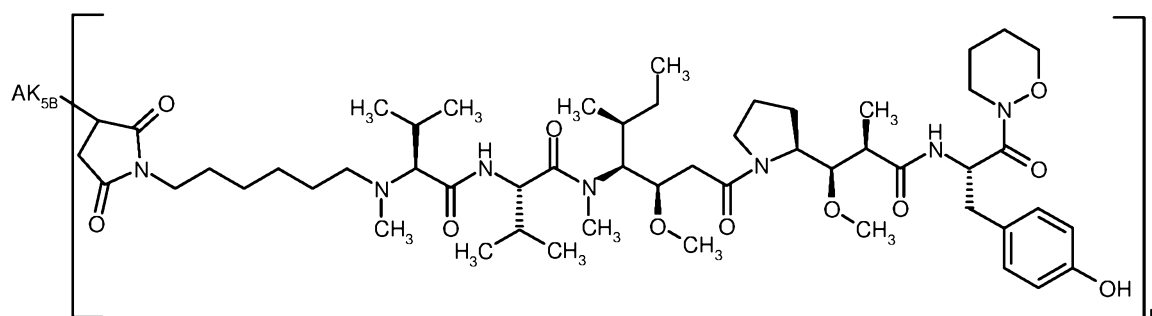


5 mg anti-CA9 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

5 Protein concentration: 1.4 mg/ml

Drug/mAb ratio: 3.0

Example 385

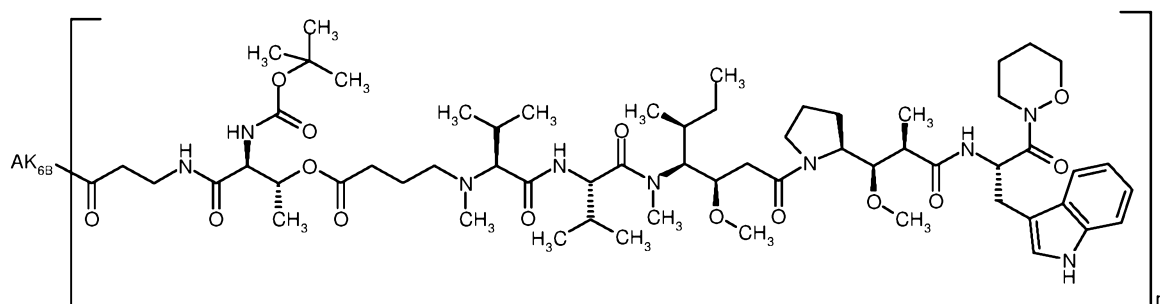


10 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Protein concentration: 1.48 mg/ml

Drug/mAb ratio: 2.4

Example 386

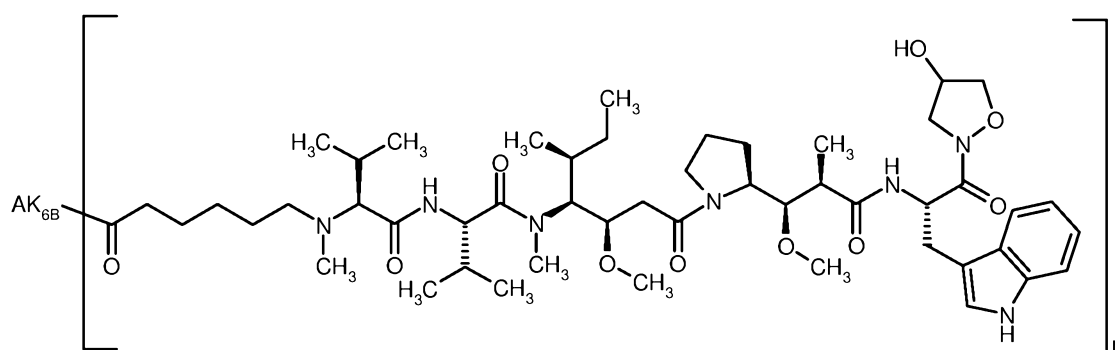


- 5 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again.

Proteinkonzentration: 1.43mg/ml

Drug/mAb-Ratio: 3.6

Example 387 Diastereomer 1

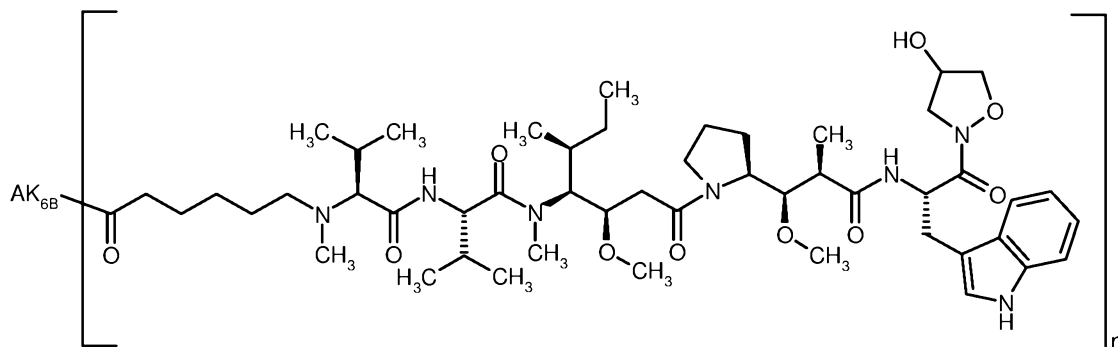


- 10 Intermediate 247a and 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and diluted again with PBS.

Protein concentration: 1.45 mg/ml

Drug/mAb ratio: 3.8

Example 388 Diastereomer 2



Intermediate 247a and 5 mg anti-C4.4a B01-3 in PBS were presently used for the coupling and, after purification on a Sephadex column, the batch was concentrated by ultracentrifugation and
 5 diluted again with PBS.

Protein concentration: 1.42mg/ml

Drug/mAb ratio: 4.0

C. Evaluation of biological efficacy

The biological activity of the compound according to the invention can be demonstrated by *in*
 10 *vitro* and *in vivo* tests, such as those with which those skilled in the art are familiar.

The biological effect of the compounds according to the invention was revealed in the assays described below:

C-1.1 In vitro cell proliferation test

Human EGFR-expressing tumors cells are used to test the efficacy of anti-EGFR ADCs. The
 15 cells may be, for example, NCI-H292 or A431 with a high expression. Cells with a low EGFR expression, such as HT29 or cells with practically no EGFR expression such as NCI-H520 are used as the controls for the EGFR-dependent cytotoxicity.

Description of the experiment

Day 1: The cells are plated out in the medium in 100 μ L/well in a 96-well plate (Perkin Elmer,
 20 white, catalog 6005680). Cells for determination of the time zero are plated out in a parallel plate. All plates are incubated overnight at 37°C.

Day 2: A three-fold dilution series of the test substances in medium is prepared and 100 µL of the three-fold dilutions is pipetted into each well in the plates. The plates are incubated for 96 hours at 37°C in an incubator. The time zero plate is measured: 100 µL/well CTG solution (Promega Cell Titer Glo solution (catalog nos. G755B and G756B)) are pipetted into the
 5 corresponding wells and incubated for 2 min on a shaker for +10 minutes in the dark. Next the luminescence is measured using a VICTOR V instrument (Perkin Elmer).

Day 6: Measurement of all other batches: 100 µL/well CTG solution (Promega Cell Titer Glo solution (catalog nos. G755B and G756B)) is pipetted into the corresponding wells and incubated for 2 min on a shaker for +10 minutes in the dark. Then the luminescence is measured using a
 10 VICTOR V instrument (Perkin Elmer).

The luminescence is used as a marker for the number of viable cells.

The measured value of the time zero plate is equated with zero, and the measured value of the cells incubated only in medium without active ingredient is equated with 100%. The result is a sigmoidal dose-effect curve from which the IC₅₀ can be determined (GraphPad Prism software).

15 A431: 2500 cells/well, medium: DMEM Hams, Biochrom, #FG4815 + 10% FCS

NCI-H292: 2500 cells/well, medium: RPMI1640; Biochrom, #FG1215 + 10% FCS

HT29L 2500 cells/well, medium: DMEM Hams; Biochrom, #FG4815 + 10% FCS

Substances that inhibit cell proliferation at $<1 \times 10^{-7}$ M are classified as effective.

Substances that inhibit cell proliferation at $<1 \times 10^{-9}$ M are classified as especially effective.

20 Table 3 below lists the IC₅₀ values¹⁾ of representative exemplary embodiments from this assay.

Table 3

Example	IC ₅₀ (nM) A431	IC ₅₀ (nM) NCI-H292	IC ₅₀ (nM) HT29
1	0.03	<0.03	<0.03
2	1.50		1.47
3	0.28		0.12
4	0.19		0.17
5	<0.03	<0.03	<0.03

6	2.79	0.79	6.22
7	0.91	0.06	10.40
10	0.08	<0.03	2.46
11	2.91	0.36	46.70
12	0.39	<0.03	0.27
13	1.59	0.49	2.94
38	0.60	0.24	1.06

¹⁾ The efficacy data given are based on the ADC batches described in concrete terms here and may deviate in other batches having a different drug/antibody ratio.

C-1.2 Protocol of proliferation assay with short substance incubation (pulse assay)

The protocol is performed as described above but the substance is removed by suction after 4 hours of incubation with the test substances and is replaced by fresh medium. The analysis is performed as described above after a total of 96 hours.

Table 4 below lists the IC₅₀ values^{1,2)} of representative exemplary embodiments from this assay.

Table 4

Example	Antibody	A431	NCI H292
7	Cetuximab	0.99	0.01
8	Nimotuzumab	>200	34.4
9	Panitumumab	90.5	26.4
10	Cetuximab	0.0526	0.0884
14	Cetuximab	0.258	0.248
15	Cetuximab	13.4	3.5
16	Cetuximab	0.943	0.689
17	Cetuximab	0.285	0.133
18	Cetuximab	17.4	3.63
19	Cetuximab		0.89
20	Cetuximab	31.9	42.3

Example	Antibody	A431	NCI H292
21	Nimotuzumab	54.6	120
22	Panitumumab	44.3	51.8
23	Cetuximab	88.1	124
24	Nimotuzumab	111	>200
25	Panitumumab	53	77
26	Cetuximab	10.6	3.02
27	Nimotuzumab	11.4	20.4
28	Panitumumab	5.78	8.6
29	Cetuximab	0.37	0.03
30	Panitumumab	23.2	3.34
31	Nimotuzumab	13.71	4.24
32	Cetuximab	50.8	20.4
33	Nimotuzumab	49	42
34	Panitumumab	51.1	32.2
35	Cetuximab	0.716	0.125
36	Cetuximab	0.357	0.0589
37	Cetuximab	0.52	0.17
38	Cetuximab		7.21
39	Cetuximab	1.49	0.03
40	Nimotuzumab	36	2.38
41	Panitumumab	110	6.91
42	Cetuximab	2.84	0.0949
43	Cetuximab	2.44	0.587
44	Cetuximab	0.456	0.571
52	Cetuximab	>200	0.961
53	Cetuximab	0.253	0.133
54	Cetuximab	166	1.83

Example	Antibody	A431	NCI H292
55	Cetuximab	1.47	0.11
337	Cetuximab	0.175	0.0746
338	Cetuximab	1.79	0.152
339	Cetuximab	2.57	0.194
340	Cetuximab	0.225	0.101
341	Cetuximab	2.25	0.232
342	Cetuximab	24.5	0.267
343	Panitumumab	>200	20.3

¹⁾ The efficacy data given are based on the ADC batches described in concrete terms here and may deviate in other batches having a different drug/antibody ratio.

²⁾ This shows the averages of two experiments (A431) or three (NCI-H292) experiments.

C-1.3 Determination of the influence on tubulin polymerization

- 5 Cancer cells are degenerate cells which often lead to development of a tumor due to an increased cell division. Microtubules form the spindle fibers of the spindle apparatus and are an essential component of the cell cycle. Regulated synthesis and degradation of microtubules permits an accurate classification of chromosomes on daughter cells and constitutes a continuous dynamic process. A disturbance in the dynamics leads to a faulty cell division and ultimately leads to cell
- 10 death. However, the increased cell division of cancer cells also makes them especially sensitive to spindle fiber toxins which are a fixed component of chemotherapy. Spindle fiber toxins such as paclitaxel or epothilone lead to a greatly increased polymerization rate of the microtubules, whereas the vinca alkaloids or monomethyl auristatin E (MMAE) lead to a greatly reduced polymerization rate of the microtubules. Those cases involve a sensitive disturbance in the
- 15 essential dynamics of the cell cycle. The compounds tested within the scope of the present invention result in a reduced polymerization rate of the microtubules.

The "Fluorescence-Based Microtubule Polymerization Assay Kit" from Cytoskeleton (Denver, Colorado, USA, order no. BK011) was used to investigate tubulin polymerization. In this assay, GTP is added to unpolymerized tubulin, so that polymerization can take place spontaneously.

- 20 The assay is based on binding of the fluorophore 4',6'-diamidino-2-phenylindole (DAPI) to tubulin. Free and bound DAPI can be differentiated on the basis of their different emission spectra. Tubulin polymerization can be tracked according to the increase in fluorescence of

bound DAPI fluorophores, because DAPI has a much higher affinity for polymerized tubulin in comparison with unpolymerized tubulin.

To perform this assay, the test substances dissolved in DMSO were diluted from their initial concentration of 10 mM to 1 μ M in water. In addition to the buffer controls, polymerization-increasing paclitaxel was also included as an assay control, and on the other hand, polymerization-inhibiting vinblastine was also included. For the measurement 96-well plates with a half bottom area were used, tracking the kinetics of tubulin polymerization for one hour at 37°C in a fluorimeter. The excitation wavelength was 355 nm, and the emission was tracked at 460 nm. For the range of the linear increase within the first 10 minutes, the change in fluorescence per minute ($\Delta F/\text{min}$) which represents the polymerization rate of the microtubules was calculated. The potency of the test substances was quantified on the basis of the respective reduction in polymerization rate.

The value of the inhibition of MMAF at a concentration of 1 μ M is set at 100%.

Table 5 shows the data for inhibition of tubulin polymerization of representative exemplary embodiments.

Table5

Toxophore/ Example	Concentration (μM)	Percentage inhibition of tubulin polymerization
MMAF	1	100
MMAF	10	34
MMAF	100	0
360	1	45
360	10	1
361	1	80
361	10	14
362	1	60
362	10	0
233	1	88
233	10	25
234	1	109

Toxophore/ Example	Concentration (μ M)	Percentage inhibition of tubulin polymerization
234	10	27
235	1	120
236	1	117
236	10	64
237	1	107
237	10	25
238	1	121
238	10	35
239	1	111
239	10	45
240	1	110
381	1	102
381	10	31
382	1	88
382	10	21
383	1	90
383	10	17

The toxophore MMAF and the exemplary embodiments inhibit tubulin polymerization in a concentration-dependent. Tubulin polymerization is completely inhibited at 100 μ M MMAF. Example 115 inhibits the tubulin polymerization rate at 1 μ M to 45% of the value measured at 1 μ M MMAF.

C-1.4 Efficacy test *in vivo*

The efficacy of the conjugates according to the invention was tested *in vivo*, e.g., by means of xenograft models. Those skilled in the art are familiar with state-of-the-art methods for testing the efficacy of a conjugate according to the invention (see, for example, WO 2005081711; Polson et al., *Cancer Res.* March 15, 2009, **69**(6):2358-64). For example, a tumor cell line that expresses the target molecule of the binder would be implanted in rodents (e.g., mice). Then a

conjugate according to the invention or a control antibody or an isotonic saline solution would be administered to the implant animals. The substance would be administered either one or more times. After an incubation time of several days, the tumor size would be determined in comparison with animals treated with conjugate and with the control group.

5 **C-1.4a** Growth inhibition/regression of experimental tumors in the mouse

Human tumor cells that express the antigen for ADC are injected subcutaneously into the flanks of immunosuppressed mice for inoculation, for example, nude mice or SCID mice. Of the cell culture, 1-10 million cells are isolated, centrifuged and resuspended with Medium or Medium/Matrigel. The cell suspension is then injected subcutaneously into the mice.

- 10 A tumor will then grow within a few days. The treatment begins after establishment of the tumor but at a tumor size of 20 mm². To investigate the effect of larger tumors, the treatment may be started only when the tumor size reaches 50-100 mm².

Treatment with ADCs is performed via the intravenous route into the caudal vein of the mouse. The ADC is administered in a volume of 5 mL/kg.

- 15 The treatment regime depends on the pharmacokinetics of the antibody. The standard treatment consists of treatment three times every fourth day. However, the treatment may also be continued further or a second cycle with three days of treatment may also follow at a later point in time.

- 20 Eight animals are used per treatment group as the standard. This number may be increased if especially great fluctuations in tumor growth or according to treatment are to be expected. In addition to the groups receiving the active substances, one group as the control group is treated only with the buffer according to the same scheme.

In the case of this experiment, the area of the tumor is measured regularly using a caliper in two dimensions (length/width). The area of the tumor is determined by length × width.

- 25 At the end of the experiment, the tumors are excised and weighed. The comparison of the average tumor weights of the treatment group with the control group is reported as T/C.

C-1.4b Efficacy in the BxPC3 pancreatic carcinoma tumor model

Two million BxPC3 cells are injected subcutaneously into the flanks of female NMRI nude mice for inoculation.

In a tumor cell of 40 mm² on day 15, the treatment is initiated with an intravenous dose of 10 mg/kg (days 15, 19, 22). Following the treatment, the tumor growth is tracked until day 77. The animals in the control group had to be sacrificed on day 50 for veterinary reasons because of the large tumors.

- 5 A naked anti-EGFR antibody shows a delayed tumor growth at approx. 14 days.

Animals treated with the anti-EGFR ADCs (Example 7 and Example 10) did not exhibit any further tumor growth until the end of the experiment on day 77.

C-1.4c Efficacy in the NCI-H292 NSCLC tumor model

- 10 Five million NCI-H292 cells were injected subcutaneously into the flanks of female NMRI nude mice for inoculation.

At a tumor size of 100 mm² on day 15, the treatment was initiated with an intravenous dose of 3 mg/kg (days 15, 19, 23). The tumor growth was tracked until day 27 after the treatment. At the end of the experiment, the tumors were excised and weighed. The experiment was terminated on day 27 because the animals in the control group had to be euthanized due to the large tumors.

- 15 The animals treated with the naked anti-EGFR antibody exhibited inhibited tumor growth. The animals treated with the anti-EGFR ADCs show regression of the tumor. After an incubation time of several days, the tumor size was determined in comparison of conjugate-treated animals with the control group (T/C). The animals treated with the conjugate had tumors of a smaller size.
- 20 The T/C ratio for the ADCs is between 0.05 (Example 10) and 0.1 (Example 7), whereas that with the naked anti-EGFR antibody is 0.3.

C-2.1 In vitro cell proliferation tests

- 25 The cytotoxic effect of the conjugates according to the invention was tested in an *in vitro* cell proliferation test by incubating a mammal cell that expresses the target molecule of the binder either endogenously or recombinantly with the conjugate according to the invention. After an incubation time of several hours to several days, cell proliferation was determined on the basis of the cell count in comparison with controls to which no conjugated was added. The unconjugated toxophore alone may be added as additional controls and/or cells that do not express the target molecule of the binder may be used. The cell count was determined by methods with which those
- 30 skilled in the art are familiar, for example, by counting or by using a test kit which allows a determination of the cell count based on a measurement of ATP (e.g., ATPlite™, Perkin Elmer).

The IC₅₀ value of the conjugates according to the invention was determined in this way. The selectivity of the conjugate could be determined by comparing the IC₅₀ value of the conjugate in measurements on cells carrying the target molecule of the binder and cells not carrying that molecule.

5 **C-2.2** Determination of the antiproliferative effect of anti-mesothelin ADC on the human colon carcinoma cell line HT29

A defined cell count of the human colon carcinoma cell line HT29wt (2500 c/well, wild type) was sown in a 96-well MTP in whole medium (10% FCS RPMI) and incubated overnight at 37°C, 5% carbon dioxide. In parallel with that transfected HT29 cells with stable mesothelin
10 expression were plated out in a 96-well MTP in whole medium and incubated overnight (2500 c/well, 37°C, 5% carbon dioxide).

After 18 hours, the inoculation medium was replaced by fresh medium with 10% FCS. The treatment was initiated with the addition of the compounds according to the invention. The transfected cells and the HT29wt cells were treated identically here.

15 Of the substances to be tested, dose-effect curves were determined in a concentration range from 10⁻⁵ M to 10⁻¹⁴ M (1:10 dilution series).

Incubation times of 48-96 hours were selected.

Proliferation was determined with the help of the MTT assay (ATCC, Manassas, Virginia, USA, catalog no. 30-1010K). After the selected time had elapsed, the HT29wt cells were incubated for
20 4 hours with MTT before lysis of the cells was performed overnight by adding detergent.

The dye thus formed was detected at 570 nm.

The 100% value was defined as the proliferation not with test substance but otherwise identically treated cells. The data obtained from this test represents triple determinations and at least two independent experiments were conducted.

25 Table 6 below lists the IC₅₀ values¹⁾ of representative exemplary embodiments of this assay:

Table 6

Example	HT29 meso ⁺ IC ₅₀ (nM)	HT29 wt IC ₅₀ (nM)	Example	HT29 meso ⁺ IC ₅₀ (nM)	HT29 wt IC ₅₀ (nM)

Example	HT29 meso⁺ IC₅₀(nM)	HT29 wt IC₅₀(nM)	Example	HT29 meso⁺ IC₅₀(nM)	HT29 wt IC₅₀(nM)
56	0.17	5.3	101	3	500
57	0.4	162	102	7.2	294
58	5	22	103	10	32
59	1	13	104	1.8	0.9
60	0.06	8	105	0.6	1.4
60	0.16	5.4	106	0.8	15
61	0.019	0.27	107	3	2
62	20	>300	108	0.74	9.2
63	0.03	1	109	0.09	1.1
64	0.11	1.4	110	0.002	0.28
65	0.14	3.3	111	0.7	162
66	1.5	8.6	112	0.018	4
67	0.015	11	113	0.4	2.5
68	2	9	114	0.6	4.1
69	0.07	20	115	0.06	10
70	0.02	2	129	5.3	57
71	0.6	11	130	0.13	672
72	0.05	8	131	0.09	769
73	25	221	132	3.3	>1000
74	9	>50	133	2.1	219
75	0.2	0.29	134	0.03	199
76	0.35	3	135	0.25	317
77	0.8	3.6	136	1.6	3000
78	1.1	6	137	0.04	10
79	0.5	5	138	0.3	>1000
80	4.4	14	139	70	>1000
81	0.02	1.4	140	6.8	>1000
82	24	>1000	141	43	>1000

Example	HT29 meso ⁺ IC ₅₀ (nM)	HT29 wt IC ₅₀ (nM)	Example	HT29 meso ⁺ IC ₅₀ (nM)	HT29 wt IC ₅₀ (nM)
83	0.3	31	142	0.27	554
84	6	14	143	0.3	54
85	0.2	2	144	2.7	74
86	0.45	>500	145	7.4	135
87	0.9	3	146	13	>1000
88	0.01	0.2	147	5.6	18
89	0.06	3.1	148	425	>1000
90	2.6	60	149	3.1	33
91	0.35	1.1	150	<0,1	0,15
92	38	167	151	>1000	>1000
93	52	89	152	4.1	>1000
94	0.6	>500	153	30	>1000
95	0.013	4.9	154	2.2	>1000
96	25	>500	155	0.5	>500
97	0.2	0.2	156	4.6	>500
98	1.7	6.3	157	0.09	839
99	0.27	1.6	158	0.18	602
100	2	44	159	177	>500
366	1.3	16	367	0.06	10
368	3.1	>500	369	0.02	14
377	1.6	>500	378	0.04	>500
379	7	>500	380	15	>500

¹⁾Efficacy data listed here is based on the exemplary embodiments described in the present experimental section with the drug/mAb ratios indicated. The values may deviate with different drug/mAb ratios.

C-2.3 Pharmacokinetics in the HT29 tumor model with mesothelin-transfected HT29 cells and non-transfected cells

After i.v. administration of 16 mg/kg from Example **60**, the plasma and tumor concentrations of Example **60** were measured along with potentially occurring metabolites such as those of Example **119**, for example. The area under the curve (AUC) of the compound from Example **119** in the plasma of animals with mesothelin-transfected tumors was approx. 0.50 mg·h/L; in the tumor, the exposure of the compound from Example **119** was approx. 400 times higher (AUC = 203 mg·h/L). In the animals with non-transfected tumors, the exposure in the plasma in Example **119** was identical to the exposure in the plasma of animals with transfected tumors. However, the AUC in the tumor of the non-transfected animals was approx. eight times lower than in the transfected animals. This is indicative of a definite targeting effect in the tumor in the presence of the antigen.

Analysis for quantitation of the compound from Example **119**

The measurement of the compound from Example **119** in the plasma and the tumor was performed after precipitation of the proteins with methanol by high pressure liquid chromatography (HPLC) coupled to a tandem mass spectrometer (MS).

For workup of 100 µL plasma, it was mixed with 400 µL methanol and 10 µL internal standard (ISTD, 50 ng/mL methanol) and agitated for 10 seconds. After centrifuging for 5 minutes at 16,000 g, 250 µL supernatant was transferred to an autosampler vial, topped off with 250 µL ammonium acetate buffer (AAC, 10 mM, pH 6.8) and agitated again.

In workup of a tumor, it was mixed with a four-fold amount of methanol. The sample was pulverized for 6 minutes at 30 beats per minute in the TissueLyser II (Quiagen) and then centrifuged for 5 minutes at 16,000 g; 50 µL of the supernatant was transferred to an autosampler vial and topped off with 50 µL ammonium acetate buffer (10 mM, pH 6.8) and 5 µL ISTD. After agitating again, the tumor sample was ready for measurement.

Finally, the measurement of both matrix samples was performed on the HPLC coupled atmospheric pressure ionization/tandem mass spectrometer by means of turbo ion spray interface (TISP) on an API 4000 instrument from the company SCIEX. The following m/z transitions were measured:

Example 119 (quantifier)	614.652 → 570.9
Example 119 (qualifier 1)	614.652 → 555.0
Example 119 (qualifier 2)	614.652 → 500.4
Internal standard (ISTD)	726.665 → 694.5

HPLC/LC MSMS (TISP) analysis was performed under the following gradient conditions on an HP1100 pump (Agilent) using the Gemini column (5 μ L C18 110 A, 50 $\times$ 3 mm, Phenomenex); flow rate 0.4 mL/min; gradient: 0.0 min to 1.0 min 10% acetonitrile/90% AAC, 1.0 min to 3.0 min 10% acetonitrile/90% AAC $\rightarrow$ 50% acetonitrile/50% AAC, 3.0 min to 5.5 min 50% acetonitrile/50% AAC, 5.5 min to 5.6 min 50% acetonitrile/50% AAC $\rightarrow$ 10% acetonitrile/90% AAC, 5.6 min to 6.0 min 10% acetonitrile/90% AAC.

For the calibration, plasma samples with concentrations of 0.5-2000 μ g/L were mixed. The limit of quantitation (LOQ) was 2 μ g/L. The linear range was from 2 to 1000 μ g/L.

For calibration of the tumor samples, the supernatants of untreated tumors with concentrations of 0.5 to 200 μ g/L were mixed. The limit of quantitation was 5 μ g/L. The linear range was from 5 to 200 μ g/L.

Quality controls for validity testing contained 5 and 50 μ g/L, with an additional 500 μ g/L in the plasma. The concentrations determined on these samples deviated by as much as 20% from the ideal value (data not included).

15 **C-2.4** Efficacy test *in vivo*

The efficacy of the conjugates according to the invention was tested *in vivo* by using xenograft models, for example. Those skilled in the art are familiar with the state-of-the-art methods for testing the efficacy of a conjugate according to the invention (see, for example, WO 2005081711; Polson *et al.*, *Cancer Res.* March 15, 2009, **69**(6):2358-64). For example, a tumor cell line that expresses the target molecule of the binder would be implanted in rodents (e.g., mice). Then a conjugate according to the invention or a control antibody or an isotonic saline solution would be administered to the implant animals. The substance would be administered one or more times. After an incubation time of several days, the tumor size would be determined in comparison with animals treated with the conjugate and with the control group.

25 The size of the tumors was smaller in the animals treated with the conjugate.

C-2.4a Testing anti-mesothelin ADCs in experimental tumors in the mouse

Human mesothelin-expressing tumor cells are injected subcutaneously into the flanks of immunosuppressed mice, for example, nude or SCID mice; 1-10 million cells are isolated from the cell culture, centrifuged and resuspended in 100 μ L Medium or 1:1 Medium/Matrigel. The cell suspension is then injected subcutaneously into the mice.

30

A tumor will then grow within a few days. The treatment begins after establishment of the tumor but at a tumor size of 20 mm². To investigate the effect of larger tumors, the treatment may be started only when the tumor size reaches 50-100 mm².

Treatment with ADCs is performed via the intravenous route into the caudal vein of the mouse.

- 5 The ADC is dissolved in PBS and administered in a volume of 5 mL/kg.

The treatment regime depends on the pharmacokinetics of the antibody. The standard treatment consists of treatment three times every fourth day. However, the treatment may also be continued further or a second cycle with three days of treatment may also follow at a later point in time.

- 10 Eight animals are used per treatment group as the standard. This number may be increased if especially great fluctuations in tumor growth or according to treatment are to be expected. In addition to the groups receiving the active substances, one group as the control group is treated only with the buffer according to the same scheme.

In this experiment, the area of the tumor is measured regularly in two dimensions (length/width) using a caliper. The area of the tumor is determined by length × width.

- 15 At the end of the experiment, the tumors are excised and weighed. The comparison of the average tumor weights of the treatment group (T) with the control group (C) is reported as T/C.

C-2.4b Efficacy in the HT29 colon carcinoma tumor model with mesothelin-transfected HT29 cells

- 20 One million HT29 cells (stable transfection with mesothelin) were injected subcutaneously into the flanks of NMRI nude mice for inoculation. At a tumor size of 20-30 mm² on day 6, intravenous treatment begins (days 6, 10, 14). Following the treatment, the tumor growth was tracked until day 48.

C-2.4c Efficacy in the Ovar3 ovarian carcinoma tumor model

- 25 Seven million Ovar3 cells were injected subcutaneously into the flanks of NMRI nude mice for inoculation.

At a tumor size of 25-30 mm² on day 31, the intravenous treatment was initiated in the dosage range of 5-30 mg/kg (days 31, 35, 39). Following the treatment, the tumor growth was tracked until day 94. At the end of the experiment, the tumors were excised and weighed.

C-3.1 Analysis of the cytotoxic effect of the ADCs directed against C4.4a

The cytotoxic effect of the anti-C4.4a ADCs was analyzed on various cell lines:

- A549 (CCL-185, ATCC) transfected with the sequence for the complete C4.4a receptor
- A549, mock transfected
- 5 – A549 wild type (DSMZ, lot 11)
- NCI-H292, endogenous C4.4a-expressing lung cancer cell line CRL-1848, ATCC)
- SCC-4 endogenous C4.4a-expressing squamous epithelial cell carcinoma cell line (CRL-1624, ATCC)
- SCC-9 endogenous C4.4a-expressing squamous epithelial cell carcinoma cell line
10 (CRL-1629, ATCC)
- HCT-116 endogenous C4.4a-expressing colon carcinoma cell line (CCL-247, ATCC)
- HCT-116/VM46, HCT-116 transfected with VM46
- A431NS (CRL-2592, ATCC)

The cells were cultured according to the standard method as stipulated in the American Tissue
15 Type Collection (ATCC) for the respective cell lines. For this procedure, the cells were isolated with a solution of trypsin (0.05%) and EDTA (0.02%) in PBS (Biochrom AG #L2143), pelleted, resuspended in culture medium, counted and sown in a 96-hole culture plate with a white bottom (Costar #3610) (2500 cells in 100 μ L/well) and then incubated at 37°C and 5% carbon dioxide in an incubator. After 24 hours, the antibody-drug conjugates were added to
20 the cells in 100 μ L culture medium in concentrations of 10^{-7} M to 10^{-11} M (double values) and incubated at 37°C and 5% carbon dioxide in an incubator. After 72 hours, the cell viability was determined by the Cell Titer Glow Luminescent Cell Viability Assay (Promega #G7573 and #G7571). To do so, 100 μ L of substrate was added per cell batch, then the plates were covered with aluminum foil, agitated for 2 minutes with the plate agitator at 180 rpm, left to stand for
25 8 minutes on the laboratory bench and then measured using the Victor X2 instrument (Perkin Elmer). The substrate detects the ATP content in the viable cells, thus generating a luminescence signal, the height of which is directly proportional to the vitality of the cells. The IC₅₀ is calculated from the measured data using the Graph Pad Prism laboratory software.

Table 7 below shows the IC₅₀ value¹⁾ of representative exemplary embodiments of this assay:

Table 7

Example	IC₅₀(nM) A549: C4.4a	IC₅₀ (nM) A549 Mock	Example	IC₅₀(nM) A549: C4.4a	IC₅₀ (nM) A549 Mock	Example	IC₅₀(nM) A549: C4.4a	IC₅₀ (nM) A549 Mock
163	0.081	11.15	203	0.4	2	252	>100	>100
164	0.7	50	204	0.1	14	253	0.15	kH
165	0.47	4.75	205	0.062	6.33	254	0.29	>100
166	0.6	100	206	0.044	6.93	255	0.04	>100
167	0.4 0.2 0.1	20 26 17	207	0.058	4.01	256	0.035	100
168	0.53	4.50	208	0.062	7.74	257	0.036	>100
169	0.39	32	209	0.066	9.11	258	0.018	>100
170	0.01	0.15	210	0.061	6.78	259	0.062	>100
171	0.43	10	211	0.076	100	260	0.06	>100
172	0.01	25	212	0.02	0.02	261	0.1	80
173	4	>100	213	0.044	44	262	0.1	kH
174	0.58	6.36	214	0.04	45	263	0.3	kH
175	0.7	14.9	215	0.046	26	264	0.1	kH
176	0.1	65.5	216	0.074	>100	265	0.2	30
177	0.030	9.53	217	0.053	>100	266	3	kH
178	3.8	21	218	0.037	60	267	0.03	50
179	0.62	4.19	219	0.3	1	268	0.05	20
180	0.4	>100	220	0.04	>100	269	>100	kH
181	1.2	66.1	221	0.1	>100	270	0.03	>100
182	0.46	4.20	222	0.04	>100	271	1	>100
183	4.5	12.7	223	0.44	6.8	272	0.2	kH
184	5	16	224	0.09	50			
185	0.4	0.7	225	0.1	0.4			
186	0.3	23	226	0.04	0,52	373	0.03	7
187	5.4	53	227	0.03	0,04	385	0.04	>100
188	0.052	11.27	228	0.03	0,04	386	0.02	>100
189	0.65	6.70	229	0.08	26	387	0.04	>100
190	0.062	>100	230	0.02	>100	388	0.04	>100

Example	IC ₅₀ (nM) A549: C4.4a	IC ₅₀ (nM) A549 Mock	Example	IC ₅₀ (nM) A549: C4.4a	IC ₅₀ (nM) A549 Mock	Example	IC ₅₀ (nM) A549: C4.4a	IC ₅₀ (nM) A549 Mock
191	0.02	2.5	231	0.17	0,27			
192	0.1	71	212	0.06	7			
193	0.32	9	242	3.0	>100			
194	0.035	6.19	243	0.045	>100			
195	0.037	~30	244	0.06	>100			
196			245	0.27	>100			
197	0.3	20	246	0.13	>100			
198	0.08	>100	247	0,14	>100			
199	0.1	kH	248	0.17	>100			
200	0.03	50	249	0,28	>100			
201	0.04	1.5	250	1.1	>100			
202	0.6	50	251	1.3	>100			

¹⁾ Efficacy data listed here is based on the exemplary embodiments described in the present experimental section with the drug/mAb ratios indicated. The values may deviate with different drug/mAb ratios.

C-3.3 In vitro tests for determining cell permeability

- 5 The cell permeability of a substance can be investigated by means of *in vitro* testing in a flux assay using Caco2 cells (M. D. Troutman and D. R. Thakker, *Pharm. Res.* **20**(8):1210-1224 (2003)). To do so, the cells are cultured on 24-hole filter plates for 15-16 days. To determine the permeation, the respective test substance in a HEPES buffer was applied to the cells either apically (A) or basally (B) and incubated for 2 hours. After 0 h and 2 h, samples were taken from
- 10 the *cis* and *trans* compartments. The samples were separated by HPLC (Agilent 1200, Böblingen, Germany) using reverse-phase columns. The HPLC system was linked to a triple quadrupole mass spectrometer API 4000 (Applied Biosystems Applera, Darmstadt, Germany) via a turbo ion spray interface. Permeability was evaluated on the basis of a P_{app} value which was calculated by means of the formula published by Schwab *et al.* (D. Schwab *et al.*, *J. Med. Chem.* **46**, 1716-
- 15 1725 (2003)). A substance was classified as having active transport when the ratio of P_{app} (B - A) to P_{app} (A - B) was >2 or <0.5.

The permeability of B to A (P_{app} (B - A)) is of crucial importance for toxophores but are released intracellularly: the lower this permeability, the longer is the dwell time of the substance in the

cell after intracellular release and thus also the time available for interaction with the biochemical target (here tubulin).

The following table shows permeability data of representative exemplary embodiments of this assay:

5 **Table 8**

Exemplary embodiment	P_{app} (B-A) (nm/s)
233	2
234	1.6
235	2.5
236	5
237	1
239	7
277	2
278	1

In comparison with this monomethylauristatin E (MMAE) and monomethylauristatin F (MMAF) have a P_{app} (B-A) value of 73 nm/s in this test.

C-3.4 In vitro tests for determining the substrate properties for P-glycoprotein (P-gp)

- 10 Many tumor cells express transporter proteins for drugs, which is often associated with the development of a resistance to cytostatics. Substances that are not substrates of such transporter proteins such as P-glycoprotein (P-gp) or BCRP, for example, might thus have an improved profile of effect.

- 15 The substrate properties of a substance for P-gp (ABCB1) are determined by means of flux assay using LLC-PK1 cells that overexpress P-gp (L-MDR1 cells) (A. H. Schinkel *et al.*, *J. Clin. Invest.* **96**, 1698-1705 (1995)). To do so, LLC-PK1 cells or L-MDR1 cells were cultured on 96-hole filter plates for 3-4 days. To determine the permeation, the respective test substance was applied to the cells and incubated for 2 hours, either alone or in the presence of an inhibitor (e.g., ivermectin or verapamil) in a HEPES buffer either apically (A) or basally (B). After 0 h and 2 h,
20 samples were taken from the *cis* and *trans* compartments. The samples were separated by HPLC

using reverse-phase columns. The HPLC system was coupled to a triple quadrupole mass spectrometer API 3000 (Applied Biosystems Applera, Darmstadt, Germany) by way of a turbo ion spray interface. The permeability was evaluated on the basis of a P_{app} value which was calculated by using the formula published by Schwab *et al.* (D. Schwab *et al.*, *J. Med. Chem.* **46**, 1716-1725 (2003)). A substance was classified as being a P-gp substrate if the efflux ratio P_{app} (B - A) to P_{app} (A - B) was >2 .

The efflux ratios in L-MDR1 and LLC-PK1 cells or the efflux ratio in the presence or absence of an inhibitor can be compared as additional criteria for evaluating the P-gp substrate properties. The respective substance is considered to be a P-gp substrate if these values differ by more than a factor of 2.

Table 9

Exemplary embodiment	P_{app} (B-A) (nm/s)
233	3
234	3.6
235	2.1
236	3.6
237	4
239	2
277	6
278	4

Again in this assay the permeability of the examples of B to A cited here is low, i.e., the dwell time of the toxophores in the cells is long.

C-3.5 Efficacy test *in vivo*

The efficacy of the conjugates according to the invention was tested *in vivo*, for example, by means of xenograft models. Those skilled in the art are familiar with the state-of-the-art methods for testing the efficacy of a conjugate according to the invention (see, for example, WO 2005081711; Polson *et al.*, *Cancer Res.* March 15, 2009, **69**(6):2358-64). For example, a tumor cell line that expresses the target molecule of the binder would be implanted in rodents (e.g., mice). Then a conjugate according to the invention or a control antibody or an isotonic saline

solution would be administered to the implant animals. The substance would be administered either one or more times. The tumor growth was determined using a caliper twice a week. After an incubation time of several days, the tumor size would be determined in comparison with animals treated with conjugate and with the control group.

5 **C-3.5a** Testing of ADCs in experimental tumors in the mouse

Human tumor cells that express the antigen for ADC are injected subcutaneously into the flanks of immunosuppressed mice for inoculation, for example, nude mice or SCID mice. Of the cell culture, 1-10 million cells are isolated, centrifuged and resuspended with 100 μ L medium or 50% medium/50% Matrigel. The cell suspension is then injected subcutaneously into the mice.

- 10 A tumor will then grow within a few days. The treatment begins after establishment of the tumor but at a tumor size of 25 mm².

Treatment with ADCs is performed via the intravenous route into the caudal vein of the mouse. The ADC is dissolved in PBS and administered in a volume of 10 mL/kg.

- 15 The treatment regime depends on the pharmacokinetics of the antibody. The standard treatment consists of treatment three times every fourth day. However, the treatment may also be continued further or a second cycle with three days of treatment may also follow at a later point in time.

- 20 Eight animals are used per treatment group as the standard. This number may be increased if especially great fluctuations in tumor growth or according to treatment are to be expected. In addition to the groups receiving the active substances, one group as the control group is treated only with the buffer according to the same scheme.

In the case of this experiment, the area of the tumor is measured regularly using a caliper in two dimensions (length/width). The area of the tumor is determined by length $\times$ width.

- 25 At the end of the experiment, the tumors are excised and weighed. The quotient of the average tumor weights of the treatment group (T) and the control group (C) is given as T/C. If the control group and treatment group are ended at different points in time, the T/C value is calculated on the basis of the tumor areas of the last joint measurement of all treatment groups and control groups.

One million SCC-4 cells are injected subcutaneously into the flanks of female NMRI nude mice for inoculation.

- 30 The intravenous treatment with the ADCs is started when the average tumor size has reached 30-35 mm². When the control groups have reached the maximum allowed size, the test is

terminated and the tumors are excised and weighed. All the C-4.4a targeting ADCs tested were found to have inhibited tumor growth in a dose dependent ratio. In a dose of 30 mg/kg all the tested ADCs achieved a T/C ratio of <0.1 (Example 216, Example 211, Example 215, Example 213). A significant antitumor effect was achieved in comparison with the controls for all the tested ADCs down to a dose of 15 mg/kg.

One million NCI-H292 cells are injected subcutaneously into the flanks of female NMRI nude mice for inoculation.

The intravenous treatment with the ADCs was initiated at an average tumor size of 30-35 mm². The control groups and treatment groups were terminated whenever the maximum allowed tumor size was reached. Therefore, differences in the subsequent growth of tumors after the end of treatment can contribute toward a further characterization of the ADCs. The tumor areas at the last joint measurement time were therefore used to determine the antitumor effect in comparison with the controls (T/C). In the NCI-H292 mouse model used in this experiment, it is demonstrated that all the tested ADCs were able to reduce tumor growth in a dose dependent manner in comparison with the control. For Example 216, a significant antitumor effect was achieved down to a dose of 1.9 mg/kg; for Example 211, this was achieved down to a dose of 3.75 mg/kg. The minimal T/C values achieved in this model include a T/C of 0.16 at 30 mg/kg for Example 216; a T/C of 0.17 at 30 mg/kg for Example 211; a T/C of 0.16 at 30 mg/kg for Example 215, and a T/C of 0.17 at 15 mg/kg for Example 213.

C-4. Pharmacokinetics in the A549 tumor model with C4.4a-transfected and non-transfected A549 cells

After i.v. administration of 7-30 mg/kg of various ADCs, the plasma and tumor concentrations of ADC and any potential metabolites that might occur were measured and the pharmacokinetic parameters such as the clearance (CL), area under the curve (AUC) and half-life (t_{1/2}) were calculated.

Analysis for quantitation of the metabolites occurring potentially

After precipitation of the proteins with methanol, the compounds in the plasma and tumor were measured by high pressure liquid chromatography (HPLC) coupled to a tandem mass spectrometer (MS).

For workup of 100 µL plasma, it was mixed with 400 µL methanol and 10 µL internal standard (ISTD, 50 ng/mL in methanol) and shaken for 10 seconds. After centrifuging for 5 minutes at

16,000 g, 250 µL supernatant was transferred to an autosampler vial, topped off with 250 µL ammonium acetate buffer (AAC, 10 mM, pH 6.8) and shaken again.

In workup of a tumor, it was mixed with a four-fold amount of methanol. The sample was pulverized for 6 minutes at 30 beats per minute in the TissueLyser II (Quiagen) and then centrifuged for 5 minutes at 16,000 g; 50 µL of the supernatant was transferred to an autosampler vial and topped off with 50 µL ammonium acetate buffer (10 mM, pH 6.8) and 5 µL ISTD. The tumor sample was ready for measurement.

Finally, the two matrix samples were measured on the atmospheric pressure ionization/tandem mass spectrometer by means of turbo ion spray interface (TISP) on an API4000 instrument from the company SCIEX with the mass spectrometer linked to an HPLC.

HPLC/LC-MSMS (TISP) analysis was performed on an HP1100 pump (Agilent) with a Gemini column (5 µm C18 110 A, 50 × 3 mm, Phenomenex).

For calibration, plasma samples with concentrations of 0.5-2000 µg/L were mixed. The limit of quantitation (LOQ) was 2 µg/L. The linear range extended from 2 to 1000 µg/L.

For calibration of the tumor samples, the supernatant of untreated tumors with concentrations of 0.5-200 µg/L was mixed. The limit of quantitation was 5 µg/L. The linear range extended from 5 to 200 µg/L.

Quality controls for validity testing contained 5 and 50 µg/L plus 500 µg/L in the plasma. The concentrations determined on these samples deviated as much as 20% from the ideal value (data not included).

C-4.1 In vitro cell proliferation tests

The cytotoxic effect of the conjugates according to the invention was tested in an *in vitro* cell proliferation test by incubating a mammalian cell that expresses the target molecule of the binder either endogenously or recombinantly with the conjugate according to the invention. After an incubation time of several hours to several days, cell proliferation was determined on the basis of the cell count in comparison with controls to which no conjugate was added. The unconjugated toxophore alone may be added as additional controls and/or cells that do not express the target molecule of the binder may be used. The cell count was determined by methods with which those skilled in the art are familiar, for example, by counting or by using a test kit which allows a determination of the cell count based on a measurement of ATP (e.g., ATPlite™, Perkin Elmer). The IC₅₀ value of the conjugates according to the invention was determined in this way. The

selectivity of the conjugate was determined by comparing the IC₅₀ value of the conjugate in measurements on cells carrying the target molecule of the binder and cells not carrying that molecule.

C-4.2 *In vitro* cytotoxicity on the cell lines HT29, DLD-1 and SNU-5

- 5 For testing the CA9 selective, cytotoxic effect on tumor cells that are endogenously CAIX-positive or CAIX-negative, the human colon carcinoma cell lines HT29 (CA9-positive) and DLD-1 (CA9-negative) as well as the gastric carcinoma cell line SNU-5 (CA9-positive) were used. A defined cell count of the cell line HT29 (5000 c/well) was sown in a 96-well MTP for luminescent in whole medium (DMEM / HAM's F12, 10% FCS heat inactivated) and incubated
- 10 overnight at 37°C, 5% CO₂. A similar procedure was followed with the SNU-5 cell line, but the medium here was ISCOVE's + 10% FCS (heat inactivated). The antigen-negative cell line DLD-1 was plated out in parallel in a 96-well MTP for luminescence in whole medium (RPMI 1640, 10% FCS, heat inactivated) and incubated overnight (5000 c/well, 37°C, 5% CO₂).

- After 24 hours, the substances to be tested were concentrated three times in RPMI / 5% FCS and
- 15 prepared. The treatment began with the addition of the substances to be tested and/or the ADC to the cells. The HT29, SNU-5 and DLD-1 cells were treated identically.

Of the substances to be tested, dose-effect curves were determined in a concentration range of 3×10^{-7} M to 10^{-12} M.

Incubation times of 2 to 96 hours were selected.

- 20 Detection of proliferation was performed with the help of the Cell Titer Glo Luminescent Cell Viability Assay (PROMEGA catalog no. #G7571). After the selected incubation time had elapsed, the Cell Titer Glo reagent was incubated with the cells for 20 minutes and then the measurement of the luminescence was performed with the luminescence reader VICTOR Light (Perkin Elmer).
- 25 The proliferation not with test substance but with otherwise identically treated cells was defined as the 100% value. The data obtained from this test represents triple determinations and at least two independent experiments were performed.

The following table lists the IC₅₀ values of representative exemplary embodiments from this assay:

Table 10

Example	EC ₅₀ (nM) HT29	EC ₅₀ (nM) DLD-1	EC ₅₀ (nM) SNU-5
280	2.9	58	n.d.
281	1.7	23	n.d.
282	<0.01	<0.01	n.d.
283	0.3	20	n.d.
284	0.7	39	3
284	1.6	5.8	2.2
285	0.5	11.1	n.d.
286	0.03	6.6	n.d.
287	>30	>68	n.d.
288	39	72	n.d.
289	49	>300	n.d.
290	115	7938	84
291	27	107	n.d.
292	223	74	n.d.
293	0.1	0.6	n.d.
294	13.6	5.5	21.4
295	>100	>100	63
296	0.4	16.4	n.d.
297	<0.01	<0.01	n.d.
298	2.5	31	7.8
299	4.1	32.3	n.d.
300	5	41	n.d.

Example	EC ₅₀ (nM) HT29	EC ₅₀ (nM) DLD-1	EC ₅₀ (nM) SNU-5
301	>30	>300	n.d.
302	>30	>300	n.d.
303	2.2	18.3	3.3
304	0.05	8	0.6
305	0.9	23	2.2
306	66	>100	33

C-4.3 Determining the antiproliferative effect of anti-CAIX ADC on the human pancreatic carcinoma cell line MIAPaCa 2 and colon carcinoma cell line HT29

A defined cell count of the human pancreatic carcinoma cell line MIAPaCa 2 (2500 c/well, wild type) was sown in a 96-well MTP in whole medium (DMEM, 10% FCS, 2.5% equine serum) and incubated overnight at 37°C, 5% CO₂. Transfected MIAPaCa 2 cells (MIAPaCaMSL) with stable CAIX expression were plated out in a 96-well MTP in whole medium and incubated overnight (2500 c/well, 37°C, 5% CO₂).

To test the cytotoxic effect on cells that endogenously express CAIX, the colon carcinoma cell line HT29 was used. The cells (2500 c/well, wild type) were also sown in a 96-well MTP and incubated overnight in whole medium (RPMI, 10% FCS).

After 18 hours, the inoculation medium was replaced by fresh medium with serum. Treatment was initiated by adding the substances to be tested and/or the ADC. The transfected cells and MIAPaCa2 cells as well as the HT29 cells were treated identically.

Of the substances to be tested, dose-effect curves in a concentration range of 10⁻⁵M to 10⁻¹⁴ M (1:10 dilution series) were determined.

Incubation times of 48-96 hours were selected.

Proliferation was detected with the help of the MTT assay (ATCC, Manassas, Virginia, USA, catalog no. 30-1010K). After the end of the selected incubation time, the MTT reagent was incubated for 4 hours with the cells before lysis of the cells was performed overnight by adding the detergent.

The dye that was formed was detected at 570 nm.

Proliferation not with test substance but otherwise identically treated cells was defined as the 100% value. Data obtained from this test represents triple determinations and at least two independent experiments were conducted in each case.

- 5 The following table shows the IC₅₀ values of representative exemplary embodiments from this assay:

Table 11

Example	MIAPaCa 2/CA9+IC₅₀(nM)	MIAPaCa 2 wt IC₅₀(nM)	Example	MIAPaCa 2/CA9+IC₅₀(nM)	MIAPaCa 2 wtIC₅₀(nM)
280	0.4	8	296	0.6	0.9
281	1.2	4.1	297	0.02	0.4
282	0.05	0.5	298	1.1	9
283	0.4	2.1	299	0.8	3.3
284	0.9	5	300	0.3	1.1
285	0.6	1.8	301	22	34
286	0.8	2.7	302	3.9	39
287	69	>1000	303	0.009	0.015
288	12	42	304	0.019	0.03
289	78	>1000	305	2.6	3.7
290	1.9	357	306	21	25

Example	MIAPaCa 2/CA9+IC ₅₀ (nM)	MIAPaCa 2 wt IC ₅₀ (nM)	Example	MIAPaCa 2/CA9+IC ₅₀ (nM)	MIAPaCa 2 wtIC ₅₀ (nM)
29 1	22	n.d.	31 3	3.8	44
29 2	35	1686	31 4	150	>1000
29 3	<0.1	<0.1	31 5	3.2	23
29 4	1	7	32 0	19	78
29 5	25	1400	32 1	220	1142
32 2	172	>1000	32 5	137	>1000
32 3	238	>500	32 6	2	>500
32 4	11	15	32 7	19	26

C-4.4 Efficacy test *in vivo*

The efficacy of the conjugates according to the invention was tested *in vivo*, for example, by means of xenograft models. Those skilled in the art are familiar with the state-of-the-art methods for testing the efficacy of a conjugate according to the invention (see, for example, 5 WO 2005081711; Polson *et al.*, *Cancer Res.* March 15, 2009, **69**(6):2358-64). For example, a tumor cell line that expresses the target molecule of the binder would be implanted in rodents (e.g., mice). Then a conjugate according to the invention or a control antibody or an isotonic saline solution would be administered to the implant animals. The substance would be 10 administered either one or more times. After an incubation time of several days, the tumor size would be determined in comparison with animals treated with conjugate and with the control group.

C-4.4a Testing the efficacy of anti-CA9 ADCs in experimental tumors in the mouse

Human CA9-expressing tumor cells are injected s.c. into the flanks of immunosuppressed mice, for example, nude or SCID mice; 1-10 million cells are isolated from the cell culture, centrifuged and resuspended with 100 μ L Medium or Medium/Matrigel. The cell suspension is then injected subcutaneously into the mice.

- 5 A tumor will then grow within a few days. The treatment begins after establishment of the tumor but at a tumor size of 20 mm². To investigate the effect of larger tumors, the treatment may be started only when the tumor size reaches 50-100 mm².

Treatment with ADCs is performed via the intravenous route into the caudal vein of the mouse. The ADC is dissolved in PBS and administered in a volume of 5 mL/kg.

- 10 The treatment regime depends on the pharmacokinetics of the antibody. The standard treatment consists of treatment three times every fourth day. However, the treatment may also be continued further or a second cycle with three days of treatment may also follow at a later point in time.

- Eight animals are used per treatment group as the standard. This number may be increased if especially great fluctuations in tumor growth or according to treatment are to be expected. In addition to the groups receiving the active substances, one group as the control group is treated only with the buffer according to the same scheme.
- 15

In the case of this experiment, the area of the tumor is measured regularly using a caliper in two dimensions (length/width).

- At the end of the experiment, the tumors are excised and weighed. The quotient of the average tumor weights of the treatment group (T) and the control group (C) is given as T/C. If the control group and treatment group are terminated on different days, the tumor area at the last measurement point at which all the groups were still in the experiment was used to calculate the T/C.
- 20

C-4.4b Efficacy in SNU-5 gastric carcinoma tumor model

- 25 Three million SNU-5 cells were injected subcutaneously into the flanks of nodSCID mice for inoculation.

- At a tumor size of 30-40 mm² on day 15, the treatment is initiated by intravenous injection of doses in the range between 5 and 30 mg/kg (days 15, 19, 23). Following the treatment the tumor growth of all groups was tracked. The control groups were ended when the tumors had reached the maximum allowed tumor size. Either all the groups were terminated together at this point in time, the tumors were excised and weighed and the T/C value was formed based on the tumor
- 30

weight, or the treatment groups were observed with regard to further growth of the tumors. In the latter case, the T/C value was calculated based on the tumor area at the last joint measurement time. The animals treated with the anti-CA9 ADCs showed inhibited tumor growth.

C-4.4c HT29 model

- 5 Female athymic nude mice carrying the nu/nu gene were used for the human xenograft study of mice. These inbred mice (NMRI background) were obtained from Taconic, Denmark with a body weight between 18 and 21 g. Human HT29 colon carcinoma cells were cultured in the recommended medium with 10% fetal calf serum (FCS) in accordance with the ATCC protocol. The cells were harvested for transplantation in the subconfluent state (80% confluence). On day
- 10 0, tumors were initiated by subcutaneous (s.c.) injection of 1×10^6 HT29 cells in 50% Matrigel/50% culture medium (without FCS) in the mice. The transplantation volume was 100 μ L, the transplantation site was the left flank. Tumor growth was determined by measuring the area of the tumor (calculation: longest diameter $\times$ length of the perpendicular to that diameter), measured by caliper. According to the statement of object, the tumors were used up to
- 15 a predefined size of 20-30 or 40-50 mm². At this point in time, the animals were randomized and assigned to the individual test groups – control groups and treatment groups. The treatment with ADCs was administered every fourth day for a total of three times (Q4D $\times$ 3). The form of the treatment was an intravenous (i.v.) injection into the caudal vein. The treatment of each animal was based on the individual body weight and the treatment volumes were 5-10 mL/kg body
- 20 weight. The area of the tumor and the animal's weight were determined twice a week, monitoring the body weight as a measure of the treatment-associated toxicity. Animals were euthanized when signs of toxicity developed or when the tumor reached the size of 150 mm² or when tumors became necrotic. At the time of termination of a group, the animals were euthanized, the tumors were excised and the respective tumor wet weight was determined. The response to treatment
- 25 was calculated as the ratio of treatment to controls based on the tumor area or the final tumor weight where appropriate.

D. Exemplary embodiments for pharmaceutical compositions

The compounds according to the invention can be converted to pharmaceutical preparations by the following procedure:

30 **i.v. solution:**

The compound according to the invention is dissolved in a concentration below the saturation solubility in a physiologically safe solvent (e.g., isotonic saline solution, D-PBS or a formulation

with glycine and sodium chloride in citrate puffer with the addition of polysorbate 80). The solution is sterile-filtered and bottled in sterile and pyrogen-free injection containers.

i.v. solution

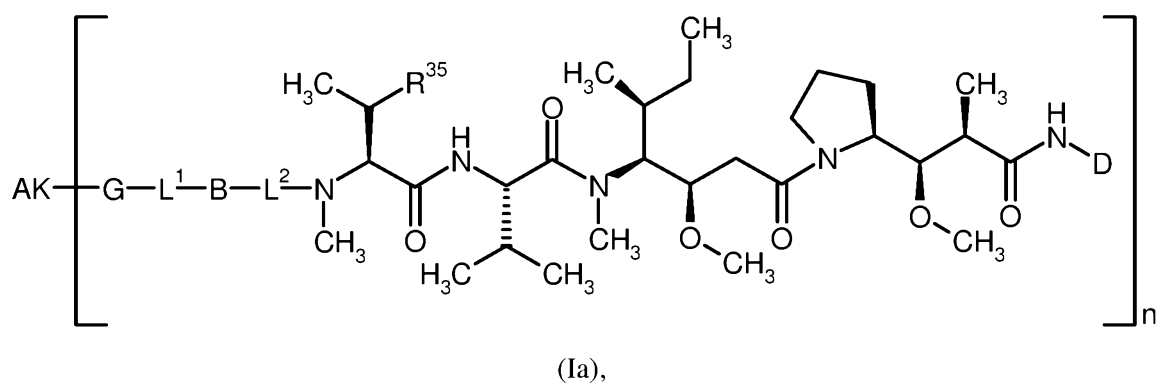
The compounds according to the invention can be converted to the dosage forms indicated. This may be done in a known way by "mixing with" or "dissolving in" inert nontoxic pharmaceutical suitable excipients (e.g., buffer substances, stabilizers, solubilizers, preservatives). The following may be included for example: amino acids (glycine, histidine, methionine, arginine, lysine, leucine, isoleucine, threonine, glutamic acid, phenylalanine and others), sugars and related substances (glucose, saccharose, mannitol, trehalose, sucrose, mannose, lactose, sorbitol), glycerol, sodium, potassium, ammonium and calcium salts (e.g., sodium chloride, potassium chloride or disodium hydrogen phosphate and many more) acetate/acetic acid buffer systems, phosphate buffer systems, citric acid and citrate buffer systems trometamol (TRIS and TRIS salts), polysorbates (e.g., polysorbate 80 and polysorbate 20), poloxamers (e.g., poloxamer 188 and poloxamer 171), macrogols (PEG- derivatives, e.g., 3350), Triton X-100, EDTA salts, glutathione, albumins (e.g., human), urea, benzyl alcohol, phenol, chlorocresol, metacresol, benzalkoniumchloride and many others.

Lyophilisate for later conversion to an i.v., s.c. or i.m. solution:

Alternatively, the compounds according to the invention may be converted to a stable lyophilisate (possibly with the help of the excipients listed above) and reconstituted with a suitable solvent (e.g., water for injection, isotonic, saline solution) before administration and then administered.

Claims

1. Binder-drug ingredient conjugates of general formula (Ia)



in which

n stands for a number from 1 to 50,

AK stands for a binder preferably a chimeric humanized or human antibody, especially preferably an anti-EGFR antibody,

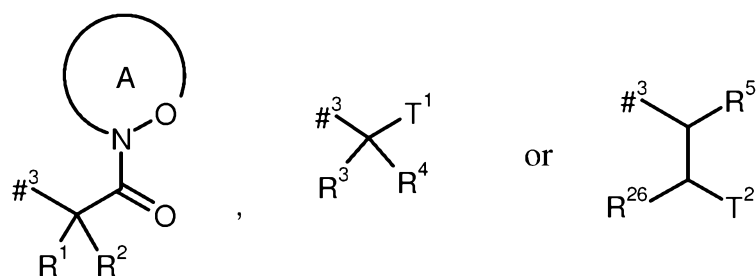
10 the group §-G-L¹-B-L²-§§ stands for a linker,

wherein

§ denotes the linkage site to the group AK and

§§ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

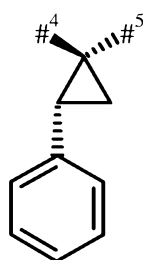
#³ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen or methyl,

R^2 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

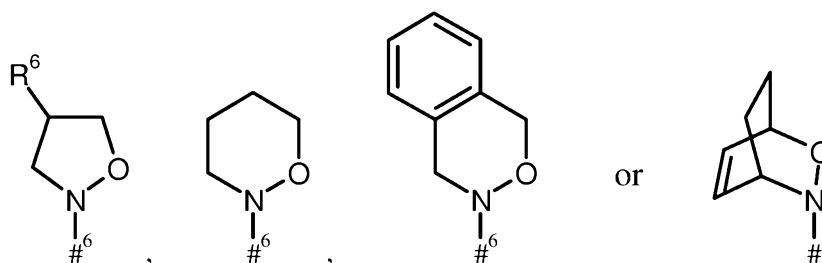


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

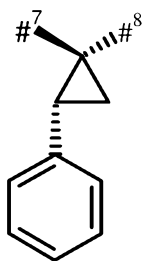
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxy-ethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

5 R^3 and R^4 together with the carbon atom to which they are attached form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

$\#^7$ denotes the linkage site with the vicinal nitrogen atom,

10 $\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

wherein

15 R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

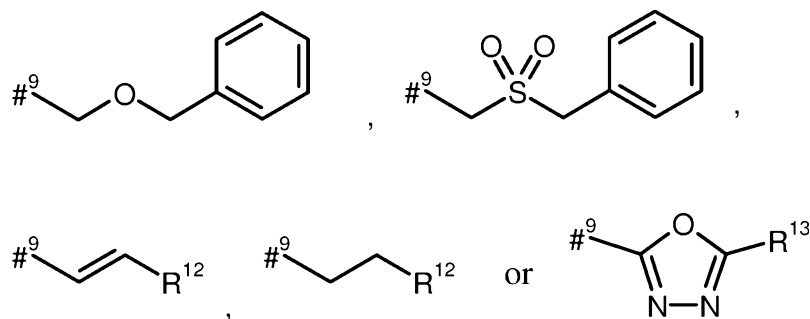
or

20 R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



5 wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(\text{R}^{26})-\text{T}^2$,

R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

10 R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

T^2 stands for phenyl, benzyl, *1H*-indol-3-yl or *1H*-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts, solvates and solvates of the salts.

15 2. Binder-drug conjugates of general formula (Ia) according to claim 1, wherein

n stands for a number from 1 to 50,

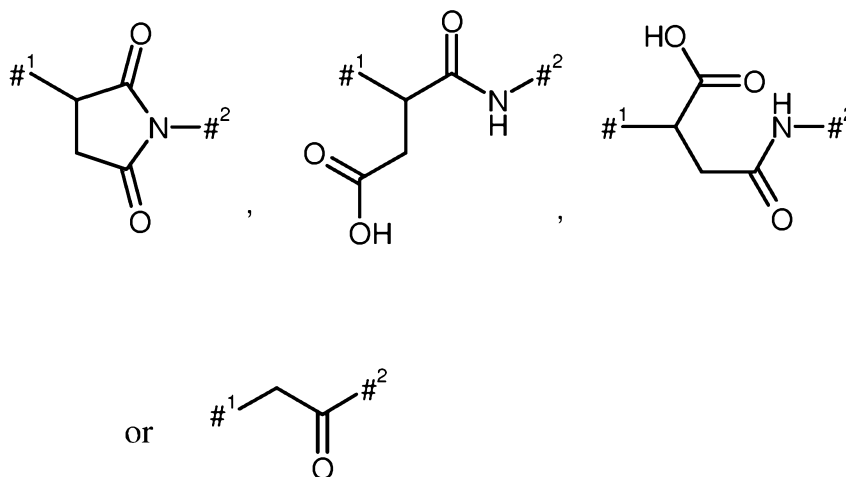
AK stands for AK_1 or AK_2

wherein

20 AK_1 stands for a binder (preferably for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody) which is bound to the group G by a sulfur atom of the binder,

AK₂ stands for a binder (preferably for a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody) which is bound to the group G by a nitrogen atom of the binder,

G for the case when AK = AK₁ stands for a group of the formula



5

wherein

#¹ denotes the linkage site to the sulfur atom of the binder,

#² denotes the linkage site to the group L¹,

or

10 for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₁-C₁₀)-alkanediyl, a group of the formula



wherein

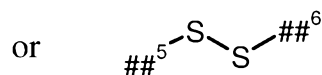
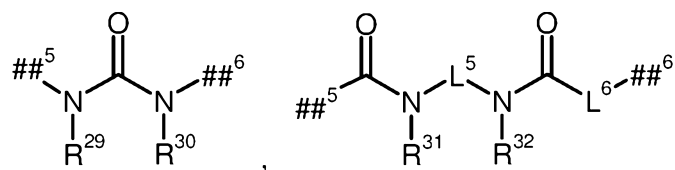
m stands for a number from 2 to 6,

15 ##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

L^{1A} stands for linear (C₂-C₁₀)-alkanediyl,

B¹ stands for a group of the formula



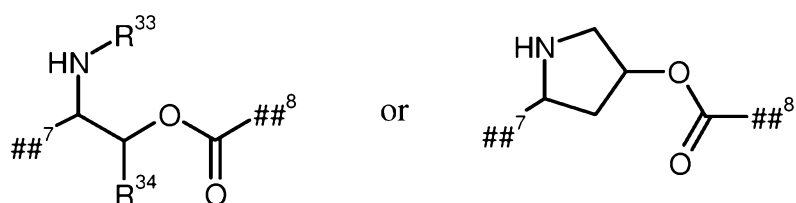
wherein

##⁵ denotes the linkage site to the group L^{1A},

5 ##⁶ denotes the linkage site to the group L^{1B},

L⁵ stands for a bond or (C₂-C₄)-alkanediyl,

L⁶ stands for a bond or a group with the formula



wherein

10 ##⁷ denotes the linkage site to the carbonyl group,

##⁸ denotes the linkage site to L^{1B},

R³³ stands for hydrogen, (C₁-C₄)-alkylcarbonyl, *tert*-butyloxy-carbonyl or benzyloxycarbonyl,

R³⁴ stands for hydrogen or methyl,

15 R²⁹ stands for hydrogen or (C₁-C₄)-alkyl,

R³⁰ stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{29} and R^{30} together with the atoms to which they are bound form a five- or six-membered heterocycle,

R^{31} stands for hydrogen or (C₁-C₄)-alkyl,

R^{32} stands for hydrogen or (C₁-C₄)-alkyl,

5 or

R^{31} and R^{32} together with the atoms to which they are bound form a five- or six-membered heterocycle,

L^{1B} stands for linear (C₂-C₁₀)-alkanediyl,

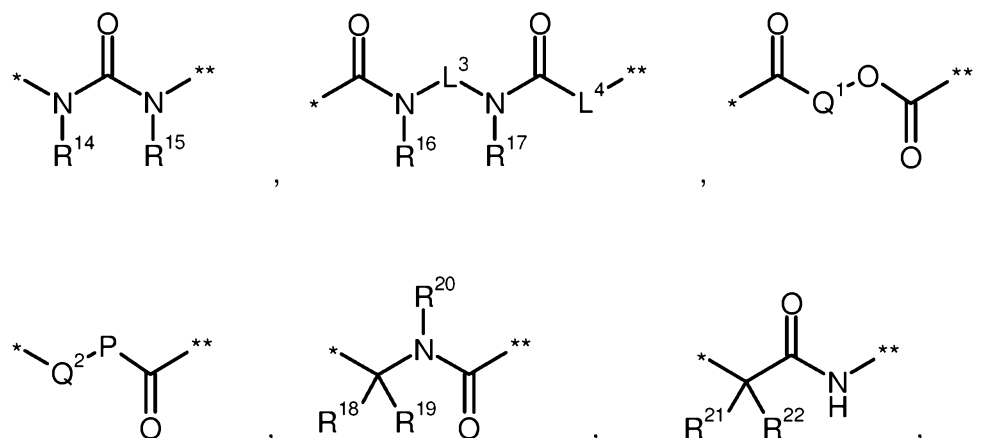
and

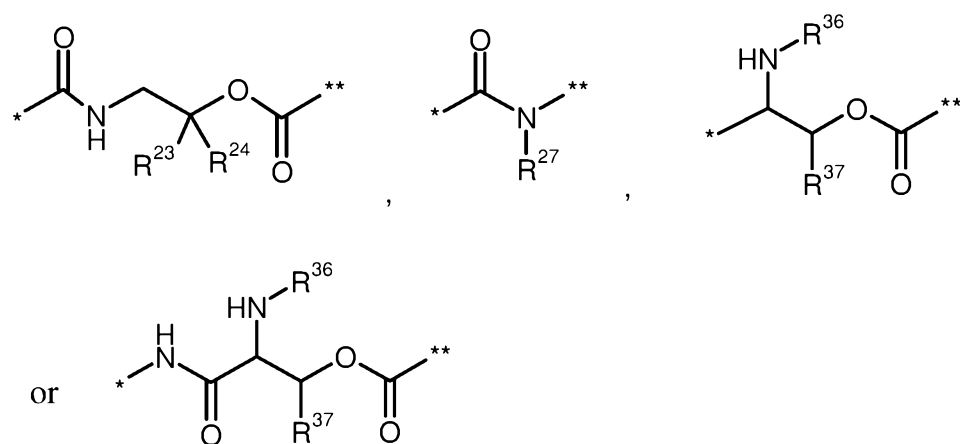
10 wherein (C₁-C₁₀)-alkanediyl may be substituted with one to four substituents selected independently from one another from the group comprising methyl, hydroxyl and benzyl

and

wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4- relation to one another, including the carbon atoms optionally situated between them, to
15 form a (C₃-C₆)-cycloalkyl ring or a phenyl ring,

B stands for a bond or a group of the formula





wherein

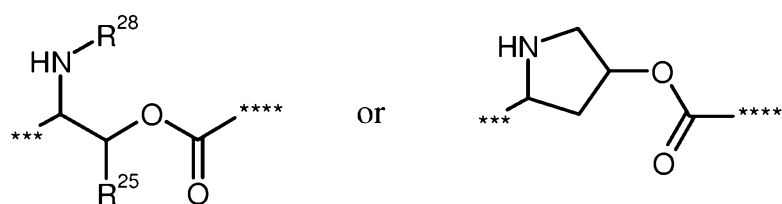
* denotes the linkage site to L¹,

** denotes the linkage site to L²,

5 P stands for O or NH,

L³ stands for a bond or (C₂-C₄)-alkanediyl,

L⁴ stands for a bond or a group of the formula



wherein

10 *** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L²,

R²⁵ stands for hydrogen or methyl,

R²⁸ stands for hydrogen, (C₁-C₄)-alkylcarbonyl, *tert*-butoxycarbonyl or benzyloxycarbonyl,

15 Q¹ stands for a four- to seven-membered heterocycle,

- Q² stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,
- R¹⁴ stands for hydrogen or (C₁-C₄)-alkyl,
- R¹⁵ stands for hydrogen or (C₁-C₄)-alkyl,
- 5 or
- R¹⁴ and R¹⁵ together with the atoms to which they are bound form a five- or six-membered heterocycle,
- R¹⁶ stands for hydrogen or (C₁-C₄)-alkyl,
- R¹⁷ stands for hydrogen or (C₁-C₄)-alkyl,
- 10 or
- R¹⁶ and R¹⁷ together with the atoms to which they are bound form a five- or six-membered heterocycle,
- R¹⁸ stands for hydrogen or (C₁-C₄)-alkyl,
- R¹⁹ stands for hydrogen or the side group of a natural α -amino acid or its homologs or isomers,
- 15 R²⁰ stands for hydrogen or (C₁-C₄)-alkyl,
- or
- R¹⁹ and R²⁰ together with the atoms to which they are bound form a pyrrolidinyl ring,
- 20 R²¹ stands for hydrogen or (C₁-C₄)-alkyl,
- R²² stands for hydrogen or (C₁-C₄)-alkyl,
- or
- R²¹ and R²² together with the atoms to which they are bound form a three- to seven-membered carbocycle,
- 25 R²³ stands for (C₁-C₄)-alkyl,

R^{24} stands for hydrogen or (C_1-C_4) -alkyl,

R^{27} stands for hydrogen or (C_1-C_4) -alkyl,

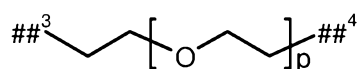
R^{36} stands for hydrogen, (C_1-C_4) -alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

5 R^{37} stands for hydrogen or methyl,

or

R^{36} and R^{37} together with the atoms to which they are bound form a pyrrolidine ring,

L^2 stands for linear (C_2-C_{10}) -alkanediyl or for a group of the formula



10 wherein

p stands for a number from 2 to 6,

##^3 denotes the linkage site to the group B,

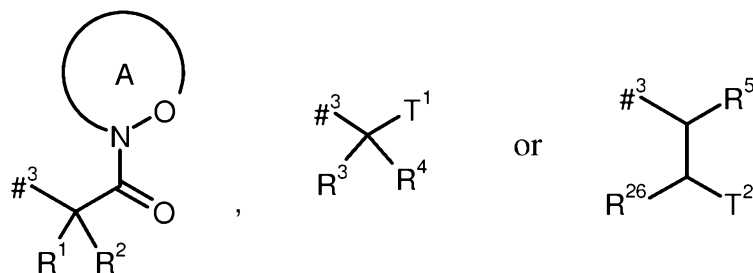
##^4 denotes the linkage site to the nitrogen atom,

15 wherein (C_2-C_{10}) -alkanediyl may be substituted with 1 to 4 substituents selected independently of one another from the group comprising methyl, hydroxyl and benzyl,

and

wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally present between them to form a (C_3-C_6) cycloalkyl ring or a phenyl ring,

20 D stands for a group of the formula



wherein

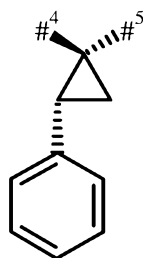
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen or methyl,

5 R² stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

or

10 R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

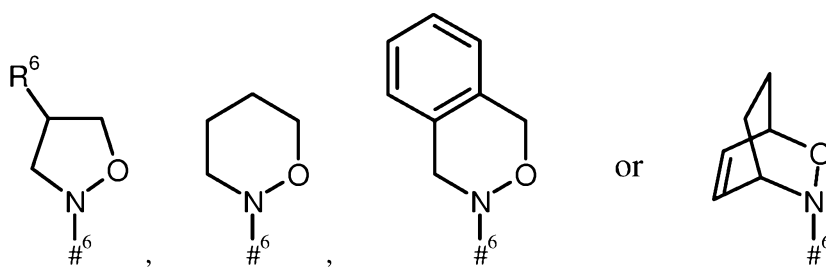


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

15 #⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O grouping contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

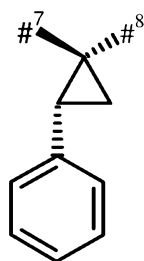
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen or methyl,

R⁴ stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

15 #⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

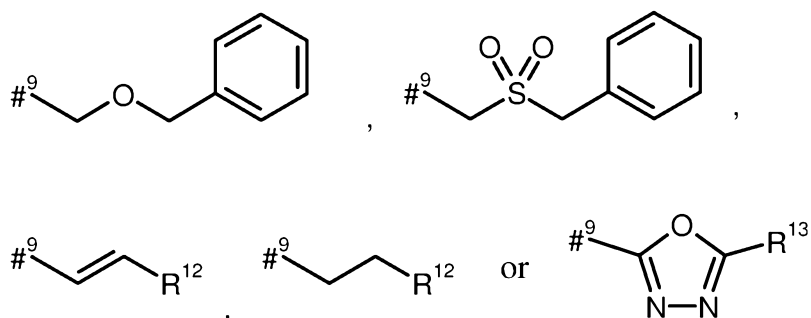
5 or

R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $\text{-CHC(R}^{26}\text{)-T}^2$,

15 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $\text{-S(O)}_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

20 T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts, solvates and solvates of the salts.

3. Binder-drug conjugates of general formula (Ia) according to claim 1 or 2, wherein

n stands for a number from 1 to 20,

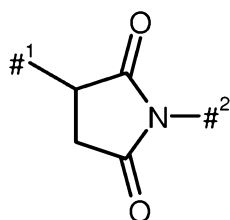
AK stands for AK₁ or AK₂

5 wherein

AK₁ stands for an antibody or an antigen-binding antibody fragment which binds to EGFR and is bound to the group G via the sulfur atom of a cysteine radical of the binder,

10 AK₂ stands for an antibody or an antigen-binding antibody fragment which binds to EGFR and is bound to the group G via the NH side group of a lysine radical of the binder,

G for the case when AK = AK₁, stands for a group of the formula



wherein

15 #¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

or

for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



20

wherein

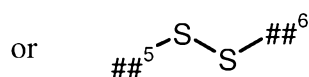
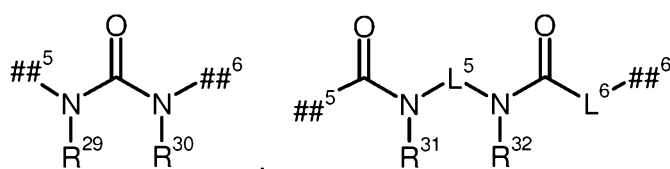
m stands for a number from 2 to 6,

$\#\#^1$ denotes the linkage site to the group G,

$\#\#^2$ denotes the linkage site to the group B,

L^{1A} stands for linear (C_2-C_6) -alkanediyl,

5 B^1 stands for a group of the formula



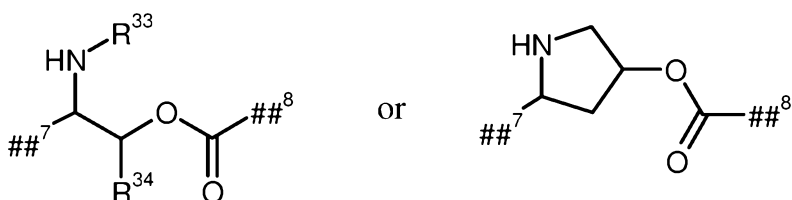
wherein

$\#\#^5$ denotes the linkage site to the group L^{1A} ,

$\#\#^6$ denotes the linkage site to the group L^{1B} ,

10 L^5 stands for a bond,

L^6 stands for a bond or a group of the formula



wherein

$\#\#^7$ denotes the linkage site to the carbonyl group,

15 $\#\#^8$ denotes the linkage site to L^{1B} ,

R^{33} for hydrogen, methylcarbonyl or *tert*-butoxycarbonyl,

R^{34} stands for hydrogen or methyl,

R^{29} stands for hydrogen,

R^{30} stands for hydrogen,

R^{31} stands for hydrogen or methyl,

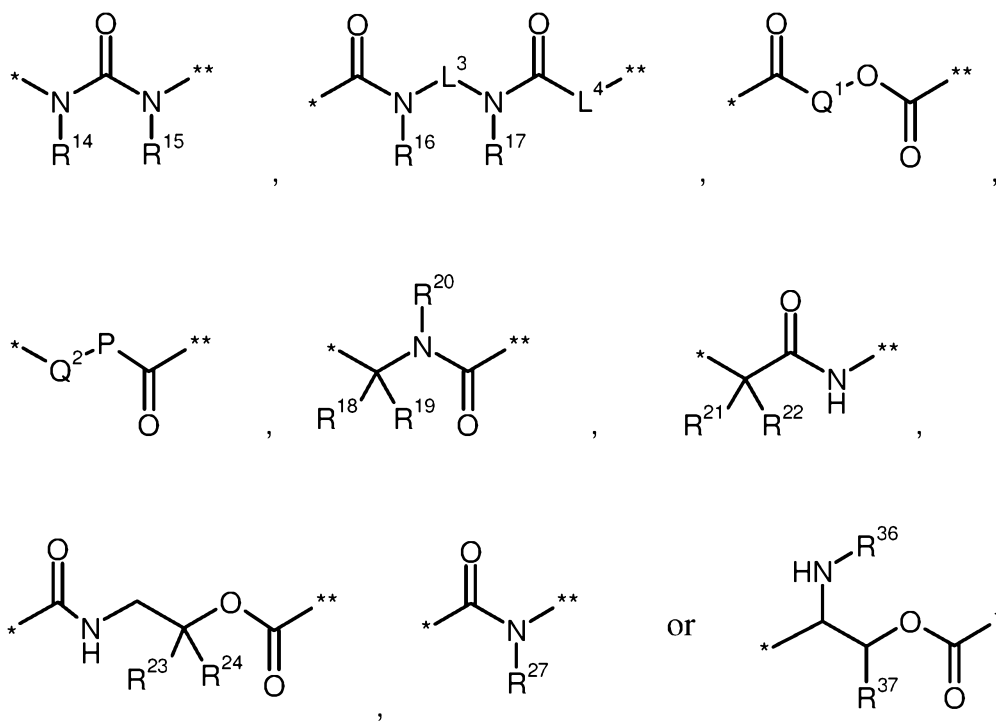
5 R^{32} stands for hydrogen or methyl,

L^{1B} stands for linear (C₂-C₆)-alkanediyl,

and

wherein (C₂-C₆)-alkanediyl may be substituted with one to two methyl substituents,

B stands for a bond or a group of the formula



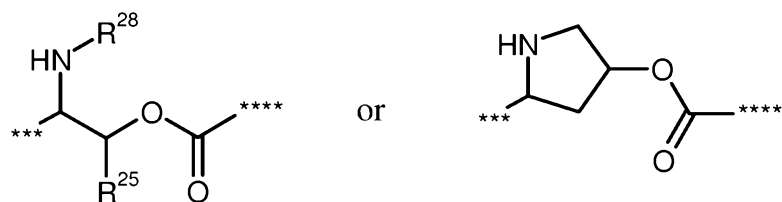
wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

L^3 stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

5 **** denotes the linkage site to L²,

R²⁵ stands for hydrogen or methyl,

R²⁸ stands for hydrogen, methylcarbonyl or *tert*-butoxycarbonyl,

Q¹ stands for a four- to seven-membered heterocycle,

R¹⁴ stands for hydrogen,

10 R¹⁵ stands for hydrogen,

R¹⁶ stands for hydrogen or methyl,

R¹⁷ stands for hydrogen or methyl,

or

R¹⁶ and R¹⁷ together with the atoms to which they are bound form a piperazinyl ring,

15 R¹⁸ stands for hydrogen,

R¹⁹ stands for hydrogen, methyl, propan-2-yl, 2-methylpropan-1-yl or 1-methylpropan-1-yl,

R²⁰ stands for hydrogen or methyl,

or

20 R¹⁹ and R²⁰ together with the atoms to which they are bound form a pyrrolidinyl ring,

R^{21} stands for hydrogen or methyl,

R^{22} stands for hydrogen or methyl,

or

5 R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

R^{23} stands for methyl,

R^{24} stands for hydrogen or methyl,

R^{27} stands for hydrogen,

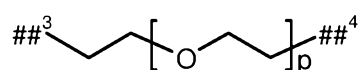
R^{36} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

10 R^{37} stands for hydrogen or methyl,

or

R^{36} and R^{37} together with the atoms to which they are bound form a pyrrolidine ring,

L^2 stands for linear (C_2 - C_6)-alkanediyl or for a group of the formula



15 wherein

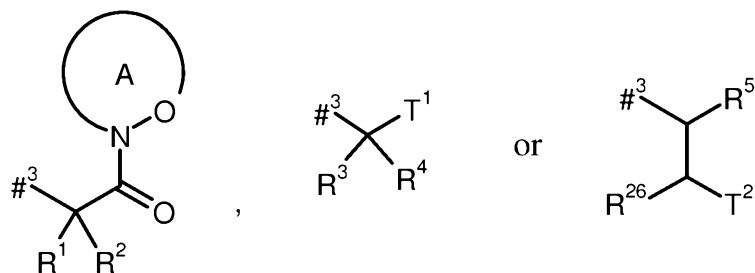
p stands for a number from 2 to 6,

$##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

wherein (C_2 - C_{10})-alkanediyl may be substituted with one or two methyl substituents.

20 D stands for a group of the formula



wherein

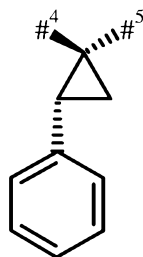
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached may form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



10

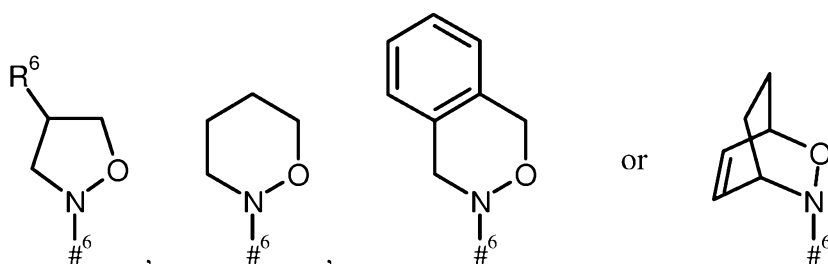
wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained therein stands for a mono- or bicyclic, optionally substituted heterocycle of the formula

15



wherein

#⁶ denotes the linkage site to the carbonyl group,

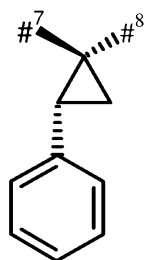
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen,

R⁴ stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or
1H-indol-3-ylmethyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached may form a
(1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen,

#⁸ denotes the linkage site to the group T¹,

15 T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-
R¹⁰ or -CH₂-O-R¹¹,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

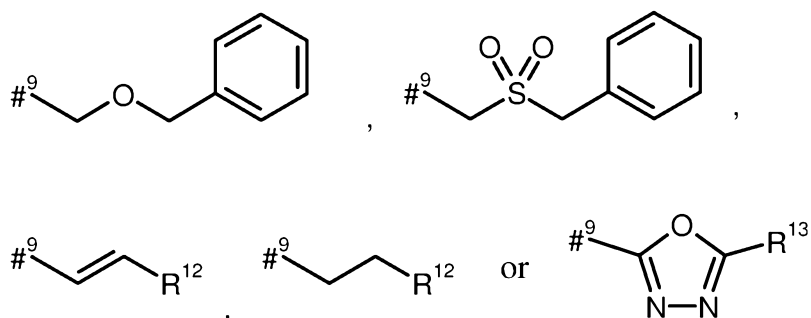
5 or

R^8 and R^9 together with the nitrogen atom which they are bound may form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl which may be substituted with methoxycarbonyl or carboxyl in the phenyl group,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(\text{R}^{26})\text{-T}^2$,

15 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

20 T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts, solvates and solvates of the salts.

4. Binder-drug conjugates of general formula (Ia) according to claims 1 to 3, wherein

n stands for a number between 1 and 10,

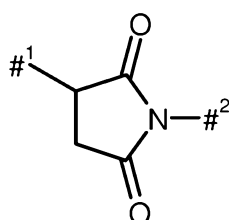
AK stands for AK₁ or AK₂

5 wherein

AK₁ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the sulfur atom of a cysteine radical of the binder,

AK₂ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

10 G for the case when AK = AK₁, stands for a group of the formula



wherein

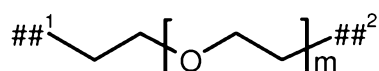
#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

15 or

for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



wherein

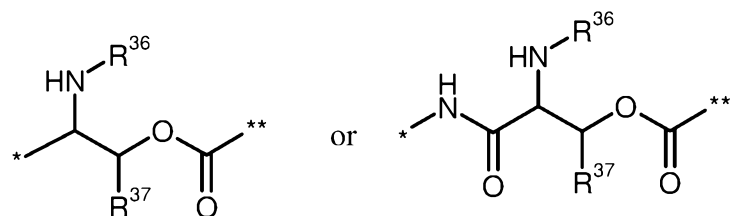
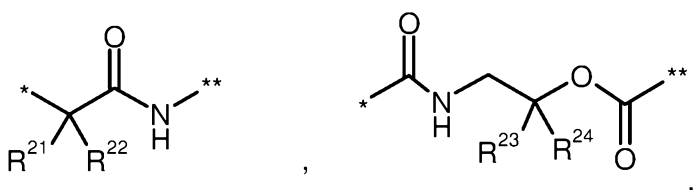
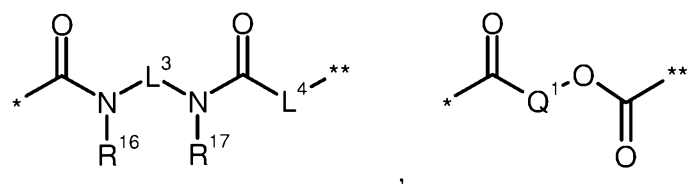
20 m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediy may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



5

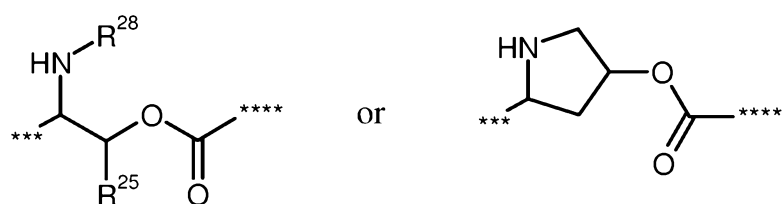
wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

L³ stands for a bond or ethane-1,2-diyl,

10 L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

R^{25} stands for methyl,

R^{28} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

5 Q^1 stands for piperidine-1,4-diyl,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

10 R^{21} stands for hydrogen or methyl,

R^{22} stands for hydrogen or methyl,

or

R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

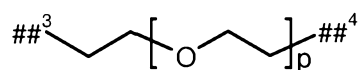
15 R^{23} stands for methyl,

R^{24} stands for hydrogen,

R^{36} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

R^{37} stands for hydrogen or methyl,

L^2 stands for linear (C_2 - C_6)-alkanediyl or for a group of the formula



20

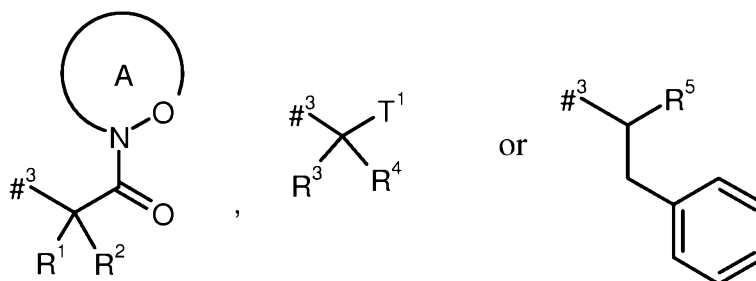
wherein

p stands for a number from 2 to 6,

$\#^3$ denotes the linkage site to the group B,

$\#^4$ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



5 wherein

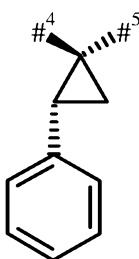
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

10 or

R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

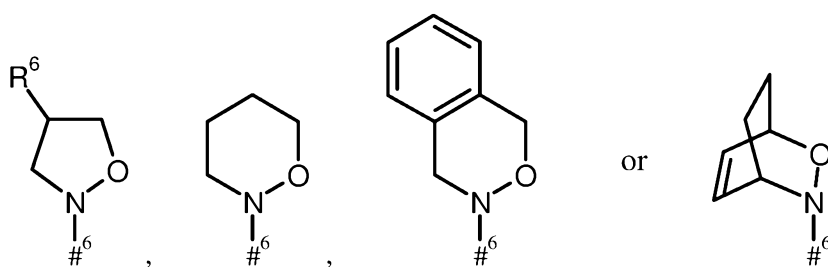


wherein

15 $\#^4$ denotes the linkage site to the vicinal nitrogen atom,

$\#^5$ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

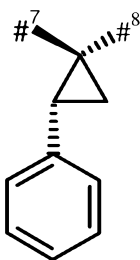
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen,

R⁴ stands for benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



10

wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹ or -CH₂-O-R¹¹,

15

wherein

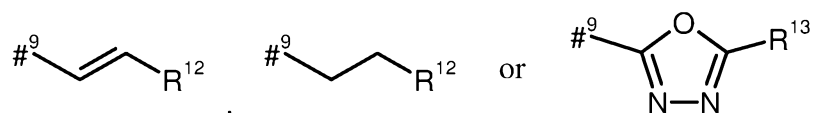
R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

5 R^5 stands for hydrogen, methyl or a group of the formula



wherein

#⁹ denotes the linkage site to -CHCH₂phenyl,

10 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula -S(O)₂OH,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts, solvates and solvates of the salts.

15

5. Binder-drug conjugates of general formula (Ia) according to one of claims 1 to 4, wherein

n stands for a number from 1 to 10,

AK stands for AK₂,

20 wherein

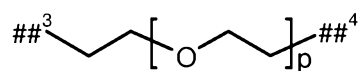
AK₂ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G stands for carbonyl,

L¹ stands for a bond,

B stands for a bond,

L² stands for linear (C₃-C₆)-alkanediyl or for a group of the formula



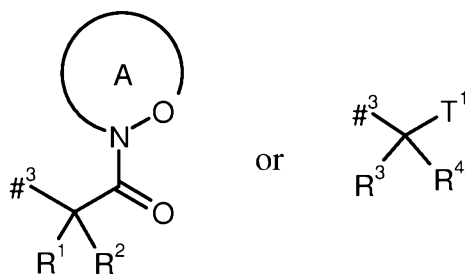
5 wherein

p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

##⁴ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



10

wherein

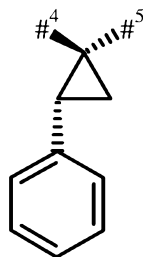
##³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

R² stands for benzyl, 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

15 or

R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

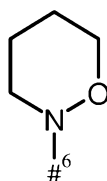


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

5 the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

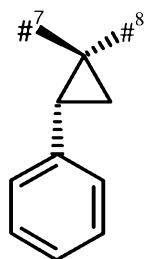
#⁶ denotes the linkage site to the carbonyl group,

10 R³ stands for hydrogen,

R⁴ stands for benzyl, 4-hydroxybenzyl or 1H-indol-3-ylmethyl,

or

R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷ or -C(=O)-NR⁸R⁹,

5 wherein

R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R⁸ stands for hydrogen,

R⁹ stands for hydrogen or benzyl,

10 R³⁵ stands for methyl,

as well as their salts, solvates and solvates of the salts.

6. Binder-drug conjugates of general formula (Ia) according to one of claims 1 to 4, wherein

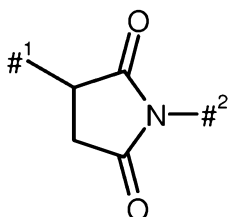
n stands for a number from 1 to 10,

15 AK stands for AK₁

wherein

AK₁ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the sulfur atom of a cysteine radical of the binder,

G stands for a group of the formula



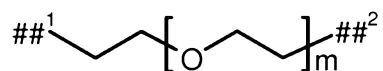
20

wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

L¹ stands for a bond, linear (C₃-C₅)-alkanediyl or a group of the formula



5 wherein

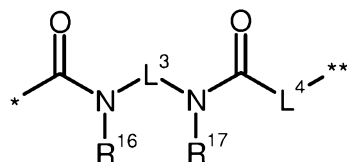
m stands for a number of 2 or 3,

\#\#¹ denotes the linkage site to the group G,

\#\#² denotes the linkage site to the group B,

wherein (C₃-C₅)-alkanediyl may be substituted with one or two methyl substituents,

10 B stands for a bond or a group of the formula



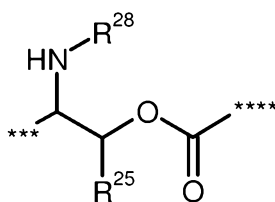
wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

15 L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

R^{25} stands for methyl,

R^{28} stands for hydrogen, methylcarbonyl or *tert*-butoxycarbonyl,

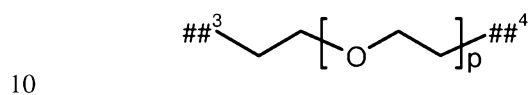
5 R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

L^2 stands for linear (C_3-C_5) -alkanediyl or for a group of the formula



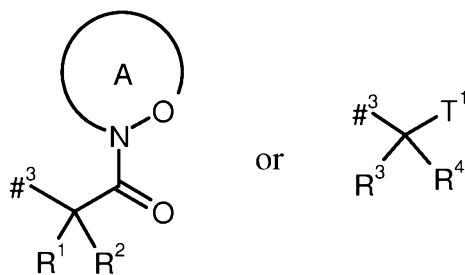
wherein

p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

##⁴ denotes the linkage site to the nitrogen atom,

15 D stands for a group of the formula



wherein

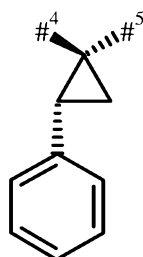
#³ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for benzyl or 1H-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



5

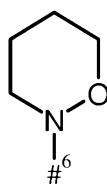
wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula

10



wherein

#⁶ denotes the linkage site to the carbonyl group,

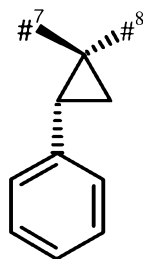
R^3 stands for hydrogen,

R^4 stands for benzyl or 1H-indol-3-ylmethyl,

15

or

R^3 and R^4 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

5 T¹ stands for a group of the formula -C(=O)-OR⁷ or -C(=O)-NR⁸R⁹,

wherein

R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

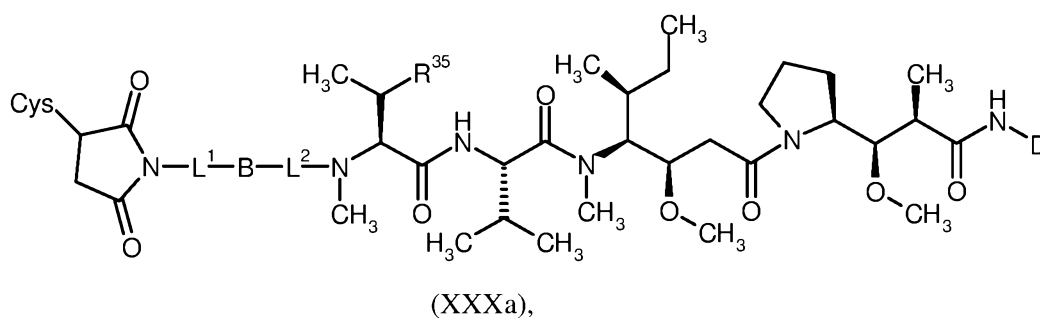
R⁸ stands for hydrogen,

10 R⁹ stands for hydrogen or benzyl,

R³⁵ stands for methyl,

as well as their salts, solvates and solvates of the salts.

7. Compounds of formula (XXXa)



15

in which

Cys stands for a cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

L^1 stands for a bond, linear (C_1 - C_{10})-alkanediyl, a group of the formula



wherein

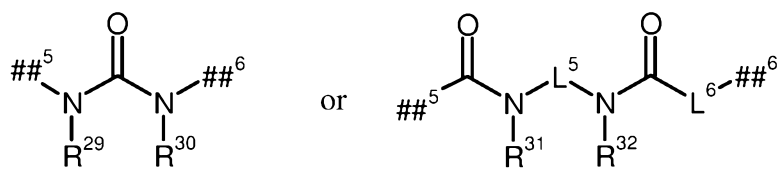
m stands for a number from 2 to 6,

5 ##^1 denotes the linkage site to the group G,

##^2 denotes the linkage site to the group B,

L^{1A} stands for linear (C_2 - C_{10})-alkanediyl,

B^1 stands for a group of the formula



10 wherein

##^5 denotes the linkage site to the group L^{1A} ,

##^6 denotes the linkage site to the group L^{1B} ,

L^5 stands for a bond or (C_2 - C_4)-alkanediyl,

L^6 stands for a bond,

15 R^{29} stands for hydrogen or (C_1 - C_4)-alkyl,

R^{30} stands for hydrogen or (C_1 - C_4)-alkyl,

or

R^{29} and R^{30} together with the atoms to which they are bound form a five- or six-membered heterocycle,

20 R^{31} stands for hydrogen or (C_1 - C_4)-alkyl,

R^{32} stands for hydrogen or (C_1-C_4) -alkyl,

or

R^{31} and R^{32} together with the atoms to which they are bound form a five- or six-membered heterocycle,

5 L^{1B} stands for linear (C_2-C_{10}) -alkanediyl,

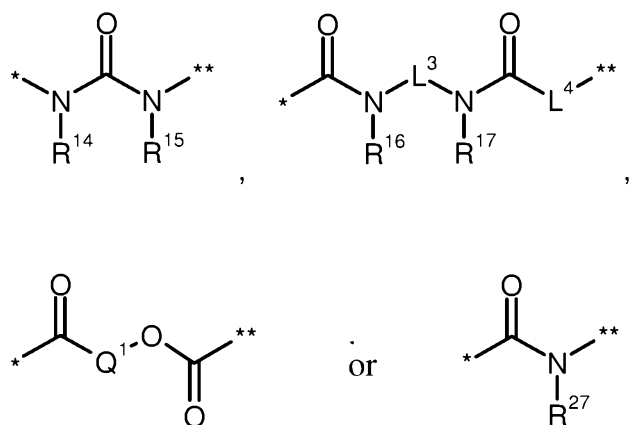
and

wherein (C_1-C_{10}) -alkanediyl may be substituted with one to four substituents selected independently from one another from the group comprising methyl, hydroxyl and benzyl

and

10 wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally situated between them, to form a (C_3-C_6) -cycloalkyl ring or a phenyl ring,

B stands for a bond or a group of the formula



15 wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

P stands for O or NH,

L^3 for a bond or (C_2-C_4) -alkanediyl,

- L^4 stands for a bond,
- Q^1 stands for a four- to seven-membered heterocycle,
- Q^2 stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,
- 5 R^{14} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{15} stands for hydrogen or (C₁-C₄)-alkyl,
- or
- R^{14} and R^{15} together with the atoms to which they are bound form a five- or six-membered heterocycle,
- 10 R^{16} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{17} stands for hydrogen or (C₁-C₄)-alkyl,
- or
- R^{16} and R^{17} together with the atoms to which they are bound form a five- or six-membered heterocycle,
- 15 R^{18} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{19} stands for hydrogen or the side group of a natural α -amino acid or its homologs or isomers,
- R^{20} stands for hydrogen or (C₁-C₄)-alkyl,
- or
- 20 R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidinyl ring,
- R^{21} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{22} stands for hydrogen or (C₁-C₄)-alkyl,
- or

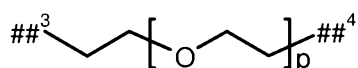
R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{23} stands for (C₁-C₄)-alkyl,

R^{24} stands for hydrogen or (C₁-C₄)-alkyl,

5 R^{27} stands for hydrogen or (C₁-C₄)-alkyl,

L^2 stands for linear (C₂-C₁₀)-alkanediyl or for a group of the formula



wherein

p stands for a number from 2 to 6,

10 $##^3$ denotes the linkage site to the group B,

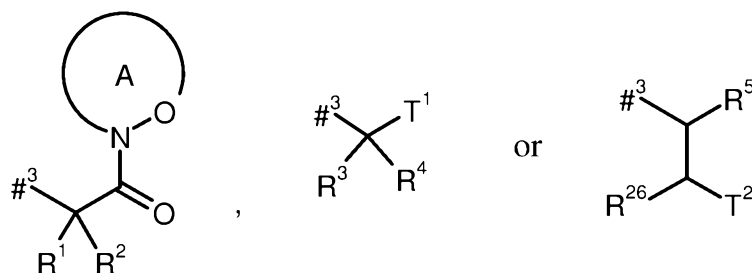
$##^4$ denotes the linkage site to the nitrogen atom,

wherein (C₂-C₁₀)-alkanediyl may be substituted with 1 to 4 substituents selected independently of one another from the group comprising methyl, hydroxyl and benzyl,

and

15 wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally present between them to form a (C₃-C₆) cycloalkyl ring or a phenyl ring,

D stands for a group of the formula



20 wherein

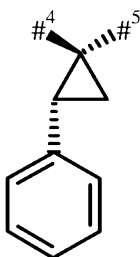
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen or methyl,

R² stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-amino-benzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

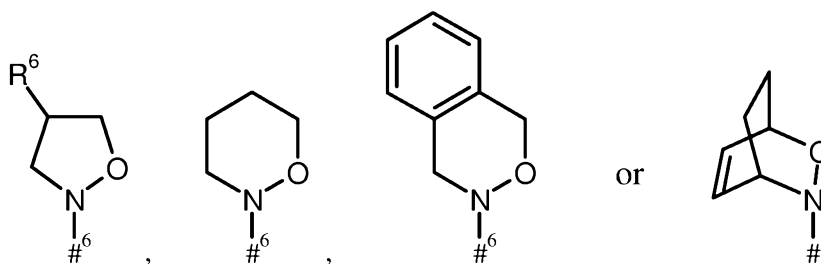


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

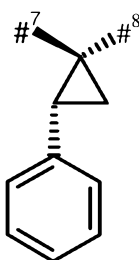
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxy-ethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

5 or

R^3 and R^4 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

10 $\#^7$ denotes the linkage site to the vicinal nitrogen atom,

$\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

wherein

15 R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

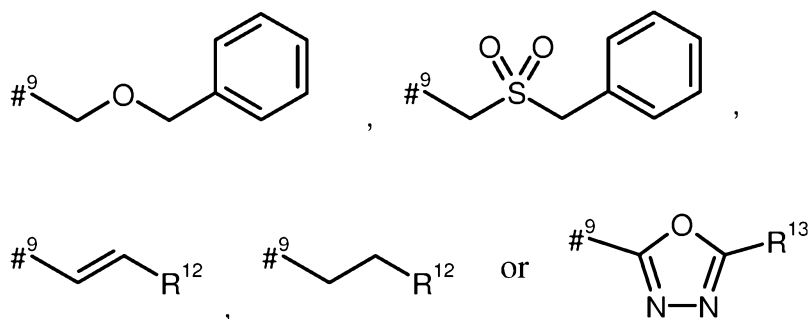
or

20 R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



5 wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(\text{R}^{26})-\text{T}^2$,

R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

10 R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts, solvates and solvates of the salts.

15 8. Compounds of formula (XXXa) according to claim 7, wherein

Cys stands for a cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

L^1 stands for a bond, linear (C_2-C_6) -alkanediyl, a group of formula



wherein

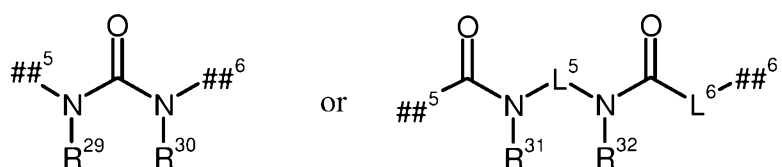
m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

5 L^{1A} stands for linear (C₂-C₆)-alkanediyl,

B¹ stands for a group of the formula



wherein

##⁵ denotes the linkage site to the group L^{1A},

10 ##⁶ denotes the linkage site to the group L^{1B},

L⁵ stands for a bond,

L⁶ stands for a bond,

R²⁹ stands for hydrogen,

R³⁰ stands for hydrogen,

15 R³¹ stands for hydrogen or methyl,

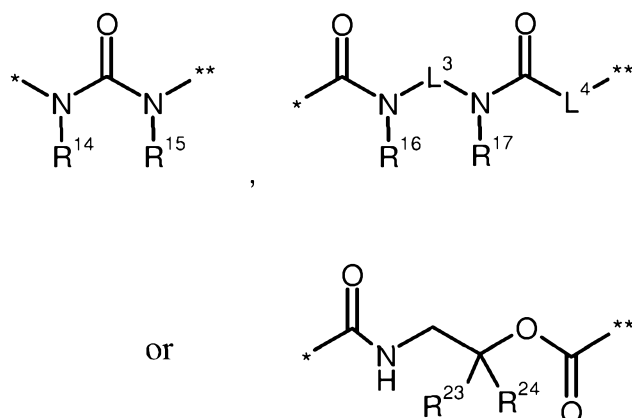
R³² stands for hydrogen or methyl,

L^{1B} stands for linear (C₂-C₆)-alkanediyl,

and

wherein (C₂-C₆)-alkanediyl may be substituted with one to two methyl substituents,

20 B stands for a bond or a group of the formula



wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

5 L^3 stands for a bond or ethane-1,2-diyl,

L^4 stands for a bond,

R^{14} stands for hydrogen,

R^{15} stands for hydrogen,

R^{16} stands for hydrogen or methyl,

10 R^{17} stands for hydrogen or methyl,

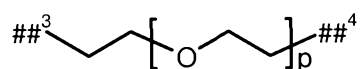
or

R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

R^{23} stands for methyl,

R^{24} stands for hydrogen or methyl,

15 L^2 stands for linear (C_2-C_6) -alkanediyl or for a group of the formula



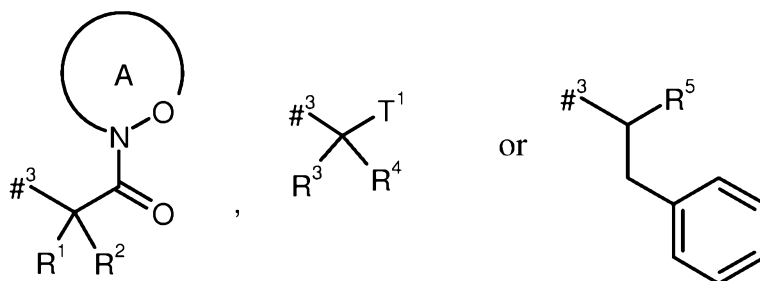
wherein

p stands for a number of 2 or 3,

$\#^3$ denotes the linkage site to the group B,

$\#^4$ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



5

wherein

$\#^3$ denotes the linkage site to the nitrogen atom,

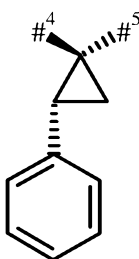
R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

10

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



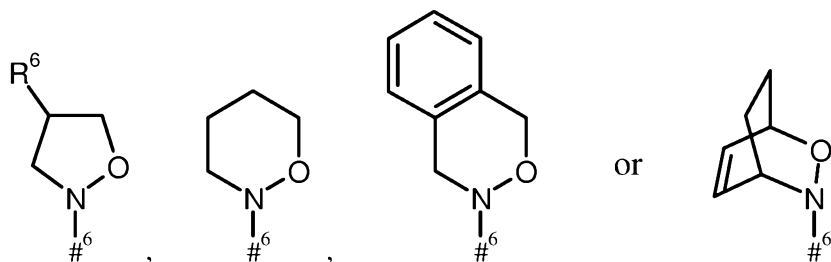
15

wherein

$\#^4$ denotes the linkage site to the vicinal nitrogen atom,

$\#^5$ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

5 $\#^6$ denotes the linkage site to the carbonyl group,

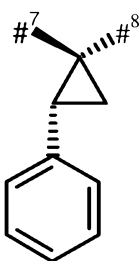
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

10 or

R^3 and R^4 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

15 $\#^7$ denotes the linkage site to the vicinal nitrogen atom,

$\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

5 R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

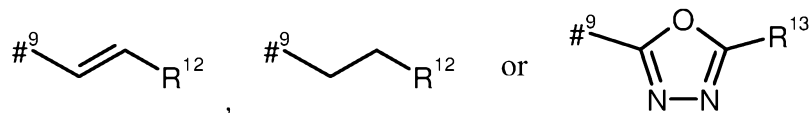
or

R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

15 $\#^9$ denotes the linkage site to $\text{---CHCH}_2\text{phenyl}$,

R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $\text{---S(O)}_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

20 R^{35} stands for methyl or hydroxyl,

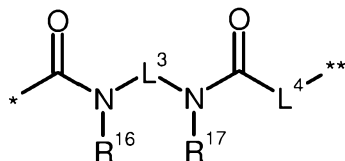
as well as their salts, solvates and solvates of the salts.

9. Compounds of formula (XXXa) according to claims 7 and 8, wherein

Cys stands for a cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

L^1 stands for a bond or linear (C₂-C₆)-alkanediyl,

B stands for a bond or a group of the formula



wherein

5 * denotes the linkage site to L^1 ,

 ** denotes the linkage site to L^2 ,

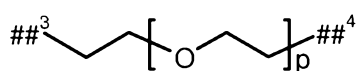
L^3 stands for a bond,

L^4 stands for a bond,

R^{16} stands for hydrogen or methyl,

10 R^{17} stands for hydrogen or methyl,

L^2 stands for linear (C₂-C₆)-alkanediyl or for a group of the formula



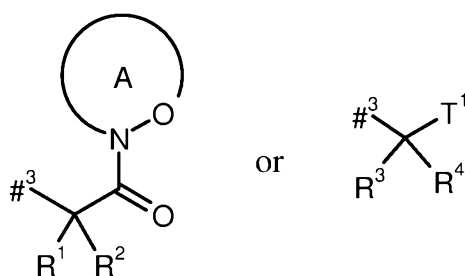
wherein

p stands for a number of 2 or 3,

15 ##³ denotes the linkage site to the group B,

 ##⁴ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

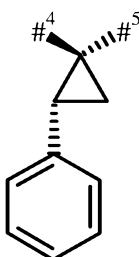
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for benzyl or 1H-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2- phenylcyclopropane-1,1-diyl group of the formula



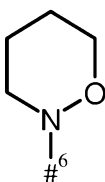
10

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



15

wherein

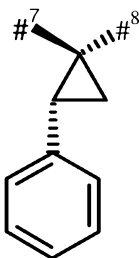
#⁶ denotes the linkage site to the carbonyl group,

R³ stands for hydrogen,

R⁴ stands for benzyl or 1H-indol-3-ylmethyl,

5 or

R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

10 #⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷ or -C(=O)-NR⁸R⁹,

wherein

R⁷ stands for hydrogen,

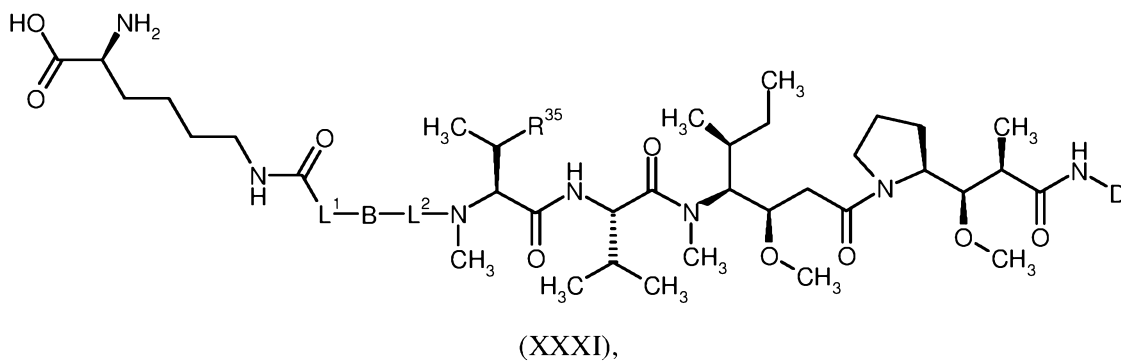
15 R⁸ stands for hydrogen,

R⁹ stands for hydrogen,

R³⁵ stands for methyl,

as well as their salts, solvates and solvates of the salts.

10. Compounds of formula (XXXI)



in which

L^1 stands for a bond, linear (C_1-C_{10}) -alkanediyl, a group of the formula



5

wherein

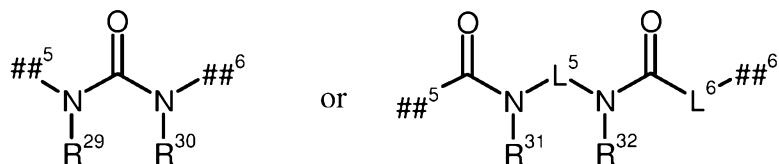
m stands for a number from 2 to 6,

$\# \#^1$ denotes the linkage site to the group G,

$\# \#^2$ denotes the linkage site to the group B,

10 L^{1A} stands for linear (C_2-C_{10}) -alkanediyl,

B^1 stands for a group of the formula



wherein

$\# \#^5$ denotes the linkage site to the group L^{1A} ,

15 $\# \#^6$ denotes the linkage site to the group L^{1B} ,

L^5 stands for a bond or (C_2-C_4) -alkanediyl,

L^6 stands for a bond,

R^{29} stands for hydrogen or (C₁-C₄)-alkyl,

R^{30} stands for hydrogen or (C₁-C₄)-alkyl,

or

5 R^{29} and R^{30} together with the atoms to which they are bound form a five- or six-membered heterocycle,

R^{31} stands for hydrogen or (C₁-C₄)-alkyl,

R^{32} stands for hydrogen or (C₁-C₄)-alkyl,

or

10 R^{31} and R^{32} together with the atoms to which they are bound form a five- or six-membered heterocycle,

L^{1B} stands for linear (C₂-C₁₀)-alkanediyl,

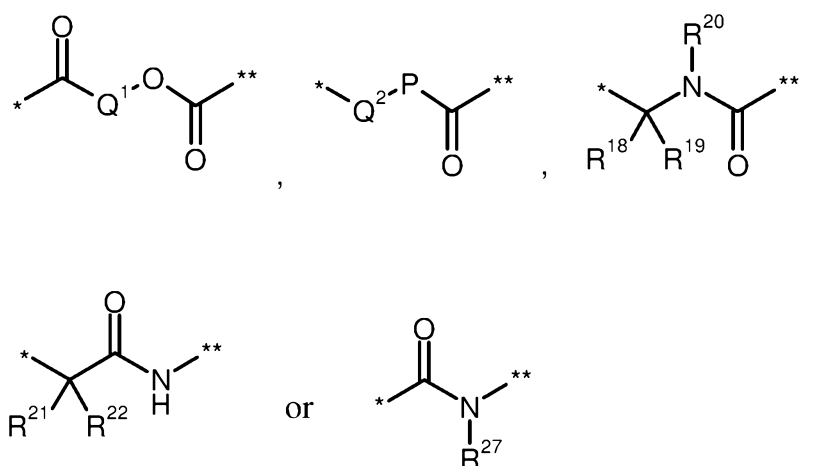
and

wherein (C₁-C₁₀)-alkanediyl may be substituted with one to four substituents selected independently from one another from the group comprising methyl, hydroxyl and benzyl

15 and

wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally situated between them, to form a (C₃-C₆)-cycloalkyl ring or a phenyl ring,

B stands for a bond or a group of the formula



wherein

* denotes the linkage site to L^1 ,

** denotes the linkage site to L^2 ,

5 P stands for O or NH,

Q^1 stands for a four- to seven-membered heterocycle,

Q^2 stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,

R^{18} stands for hydrogen or (C₁-C₄)-alkyl,

10 R^{19} stands for hydrogen or the side group of a natural α -amino acid or its homologs or isomers,

R^{20} stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidinyl ring,

15 R^{21} stands for hydrogen or (C₁-C₄)-alkyl,

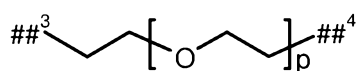
R^{22} stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{27} stands for hydrogen or (C₁-C₄)-alkyl,

L^2 stands for linear (C₂-C₁₀)-alkanediyl or for a group of the formula



5

wherein

p stands for a number from 2 to 6,

$\#^3$ denotes the linkage site to the group B,

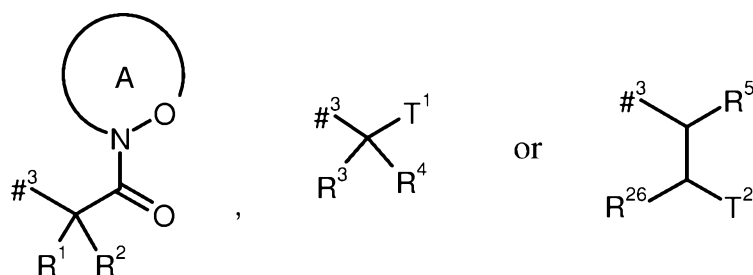
$\#^4$ denotes the linkage site to the nitrogen atom,

10 wherein (C₂-C₁₀)-alkanediyl may be substituted with 1 to 4 substituents selected independently of one another from the group comprising methyl, hydroxyl and benzyl,

and

15 wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4-relation to one another, including the carbon atoms optionally present between them to form a (C₃-C₆) cycloalkyl ring or a phenyl ring,

D stands for a group of the formula



wherein

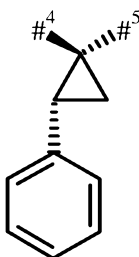
$\#^3$ denotes the linkage site to the nitrogen atom,

20 R^1 stands for hydrogen or methyl,

R^2 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-amino-benzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

5 or

R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

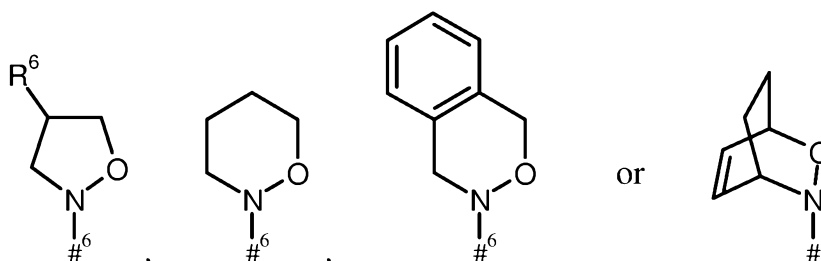


wherein

10 #⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



15 wherein

#⁶ denotes the linkage site to the carbonyl group,

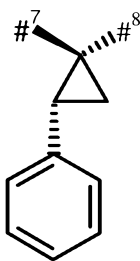
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxy-ethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

or

5 R^3 and R^4 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

$\#^7$ denotes the linkage site to the vicinal nitrogen atom,

10 $\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

wherein

15 R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

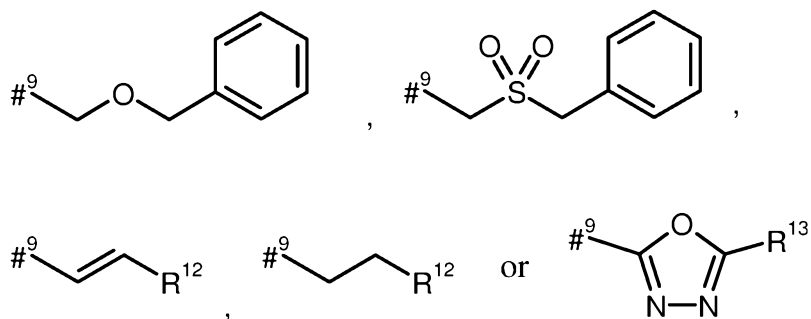
or

20 R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



5 wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(R^{26})-\text{T}^2$,

R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

10 R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

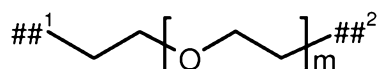
T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts, solvates and solvates of the salts.

15 11. Compounds of formula (XXXI) according to claim 10, wherein

L^1 stands for a bond, linear (C_2-C_6) -alkanediyl or a group of the formula



wherein

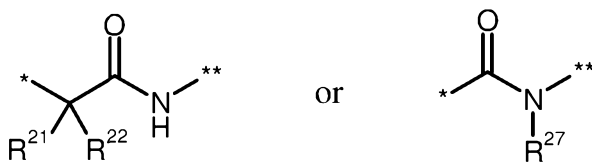
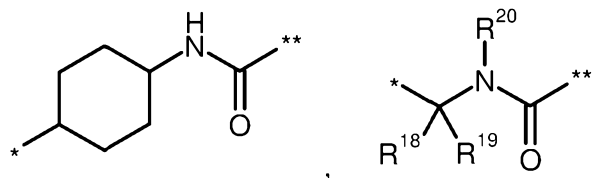
m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



5

wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

R¹⁸ stands for hydrogen,

10 R¹⁹ stands for hydrogen, methyl, propan-2-yl, 2-methylpropan-1-yl or 1-methylpropan-1-yl,

R²⁰ stands for hydrogen or (C₁-C₄)-alkyl,

or

R¹⁹ and R²⁰ together with the atoms to which they are bound form a pyrrolidinyl ring,

15 R²¹ stands for hydrogen or methyl,

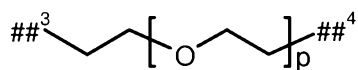
R²² stands for hydrogen or methyl,

or

R²¹ and R²² together with the atoms to which they are bound form a cyclopropyl ring,

R^{27} stands for hydrogen or methyl,

L^2 stands for linear (C_2 - C_6)-alkanediyl or for a group of the formula



wherein

5 p stands for a number of 2 or 3,

$##^3$ denotes the linkage site to the group B,

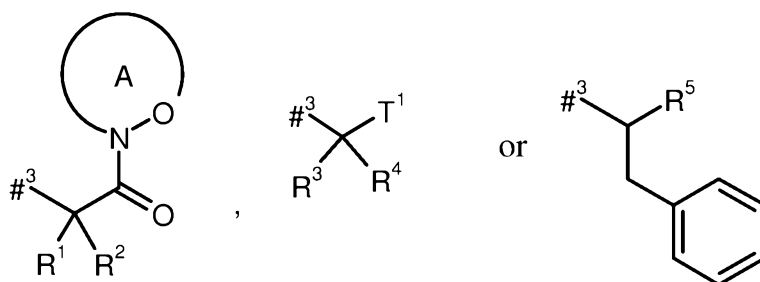
$##^4$ denotes the linkage site to the nitrogen atom,

wherein (C_2 - C_{10})-alkanediyl may be substituted with one or two methyl substituents,

and

10 wherein two carbon atoms of the alkanediyl chain may be bridged in 1,4-relation to one another, including the carbon atoms optionally present between them to form a phenyl ring,

D stands for a group of the formula



15 wherein

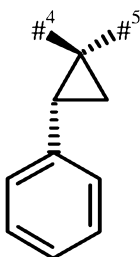
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

20 or

R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

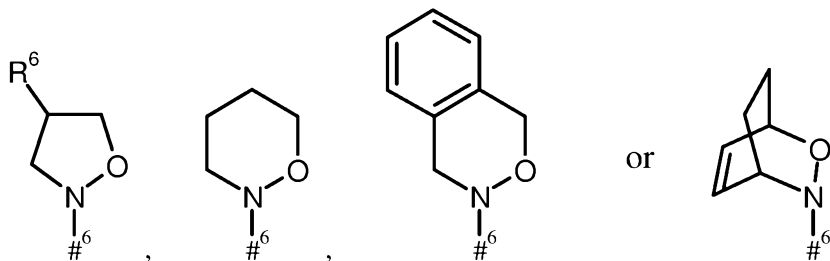


wherein

5 $\#^4$ denotes the linkage site to the vicinal nitrogen atom,

$\#^5$ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



10 wherein

$\#^6$ denotes the linkage site to the carbonyl group,

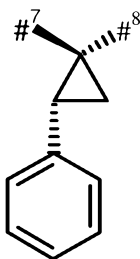
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

15 R^4 stand for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

5 T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

10 R⁸ stands for hydrogen or methyl,

R⁹ stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

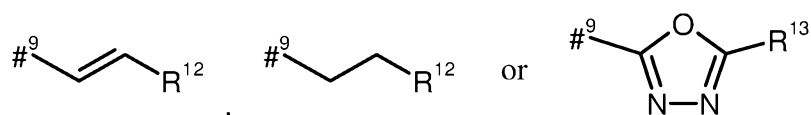
or

R⁸ and R⁹ together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

15 R¹⁰ stands for benzoyl,

R¹¹ stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R⁵ stands for hydrogen, methyl or a group of the formula



20 wherein

#⁹ denotes the linkage site to -CHCH₂phenyl,

R¹² stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula -S(O)₂OH,

R¹³ stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R³⁵ stands for methyl or hydroxyl,

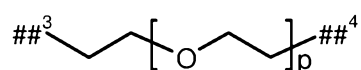
as well as their salts, solvates and solvates of the salts.

12. Compounds of formula (XXXI) according to claims 10 and 11, wherein

L^1 stands for a bond,

10 B stands for a bond,

L² stands for linear (C₂-C₆)-alkanediyl or for a group of the formula



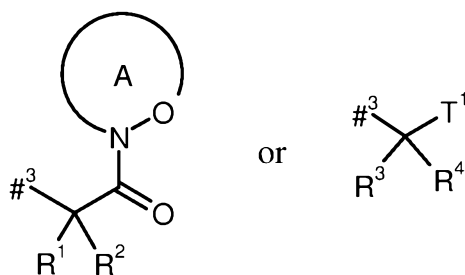
wherein

p stands for a number of 2 or 3,

15 ##³ denotes the linkage site to the group B,

##⁴ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



20 wherein

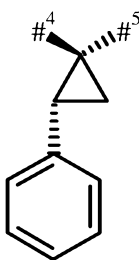
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

R² stands for benzylor 1H-indol-3-ylmethyl,

or

- 5 R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

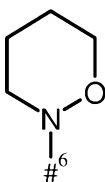


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

- 10 #⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

- 15 #⁶ denotes the linkage site to the carbonyl group,

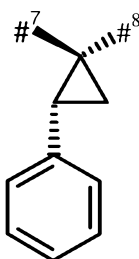
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

R³ stands for hydrogen,

R⁴ stand for benzylor 1H-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



5 wherein

$\#^7$ denotes the linkage site to the vicinal nitrogen atom,

$\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$ or $-C(=O)-NR^8R^9$,

wherein

10 R^7 stands for hydrogen,

R^8 stands for hydrogen,

R^9 stands for hydrogen,

R^{35} stands for methyl,

as well as their salts, solvates and solvates of the salts.

15 13. Compounds of formulas (XXXa) and (XXXI) selected from the group:

N-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide,

20 N-[6-(3-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}-2,5-dioxopyrrolidin-1-yl)hexyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-

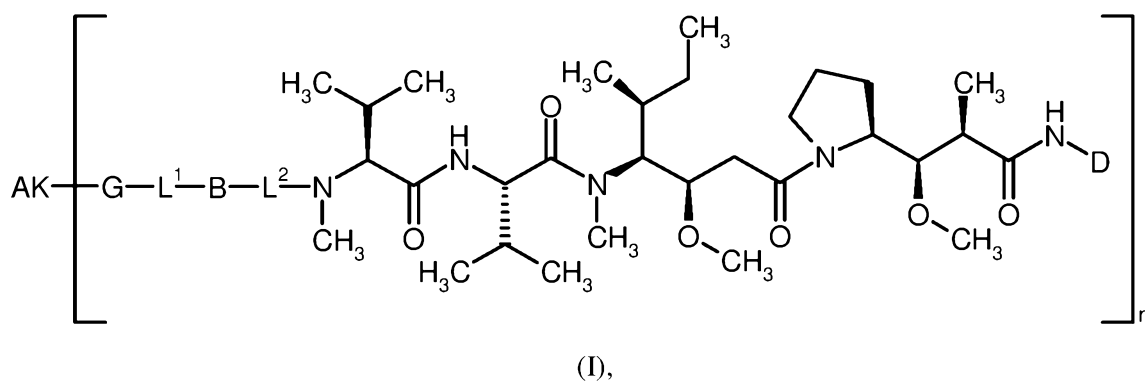
-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide,

5 N-(6-{[(5S)-5-amino-5-carboxypentyl]amino}-6-oxohexyl)-N-methyl-L-valyl-N-
-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-
-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-
-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate,

10 N-(6-{[(5S)-5-amino-5-carboxypentyl]amino}-6-oxohexyl)-N-methyl-L-valyl-N-
-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S)-1-carboxy-2-(1H-indol-3-yl)ethyl]amino}-1-
-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-
-yl]-N-methyl-L-valinamide,

as well as their salts, solvates and solvates of the salts.

14. Binder-drug conjugates of general formula (I)



15 in which

n stands for a number from 1 to 50,

AK stands for a bond,

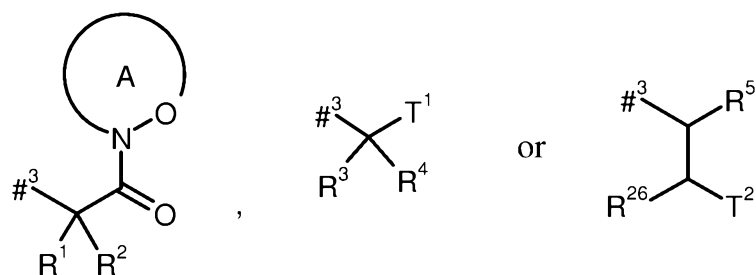
the group §-G-L¹-B-L²-§§ stands for a linker,

wherein

20 § denotes the linkage site to the group AK and

§§ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

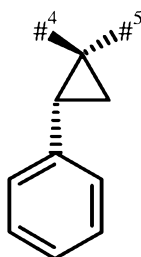
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



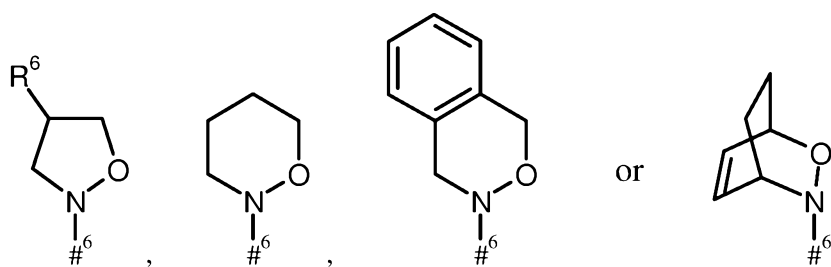
10

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

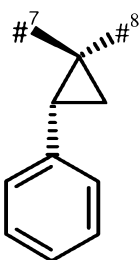
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen,

R⁴ stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-yl-methyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

15 T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

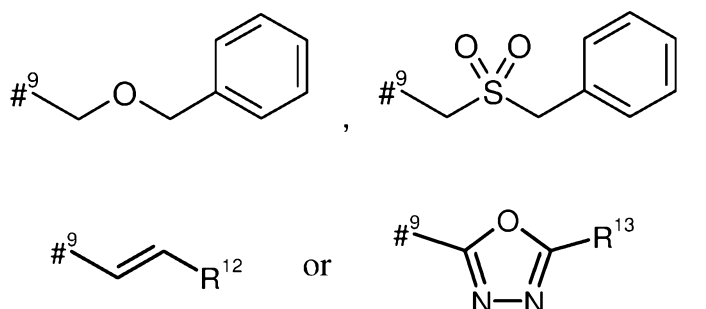
5 or

R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $\text{-CHC(R}^{26}\text{)-T}^2$,

15 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $\text{-S(O)}_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

20 T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

as well as their salts, solvates and solvates of the salts.

15. Binder-drug conjugates according to claim 14 of general formula (I),

in which

n stands for a number from 1 to 50,

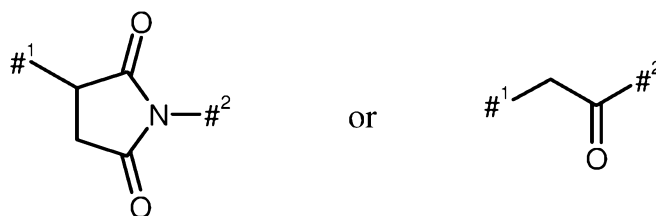
AK stands for AK₁ or AK₂

5 wherein

AK₁ stands for a binder bound to the group G via a sulfur atom of the binder,

AK₂ stands for a binder bound to the group G via a nitrogen atom of the binder,

G for the case when AK = AK₁ stands for a group of the formula



wherein

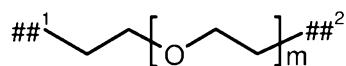
#¹ denotes the linkage site to the sulfur atom of the binder,

#² denotes the linkage site to the group L¹,

or

15 for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₁-C₁₀)-alkanediyl or stands for a group of the formula



wherein

m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

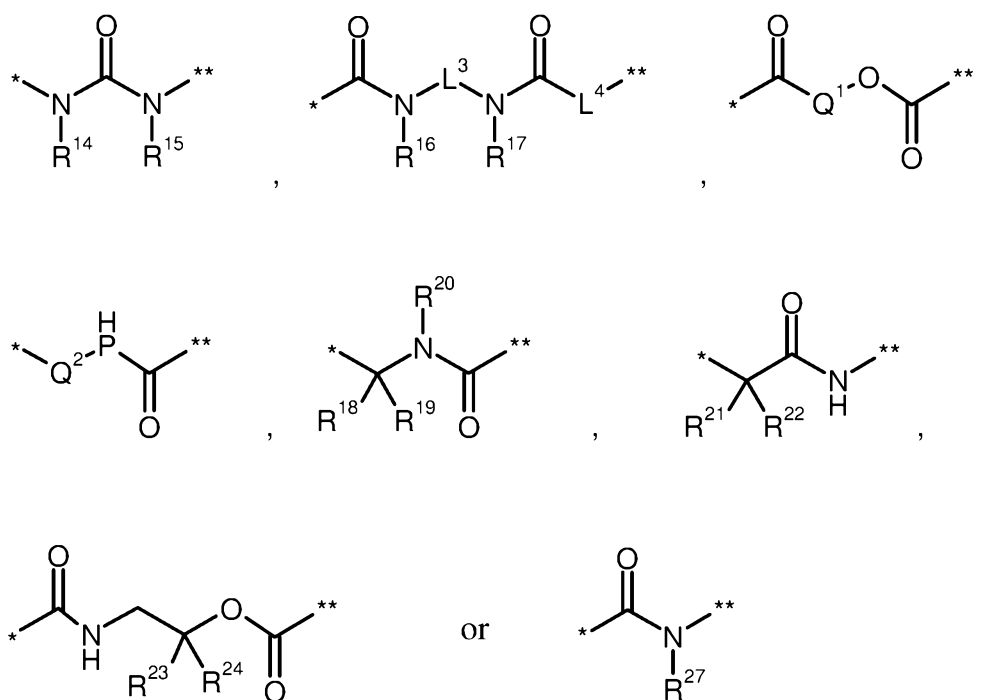
##² denotes the linkage site to the group B,

wherein (C₁-C₁₀)-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another may be bridged to form a (C₃-C₆)-cycloalkyl ring or a phenyl ring including the carbon atoms optionally situated between them,

B stands for a bond or a group of the formula



wherein

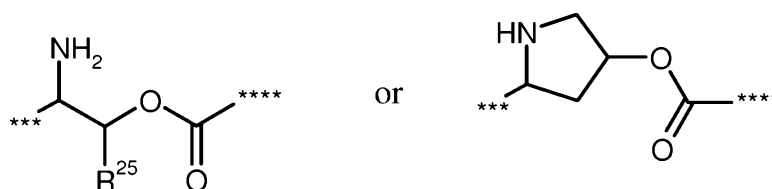
* denotes the linkage site to L¹,

** denotes the linkage site to L²,

P stands for O or NH,

L³ for a bond or (C₂-C₄)-alkanediyl,

L⁴ stands for a bond or a group of the formula



wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

5 R^{25} stands for hydrogen or methyl,

Q^1 stands for a four- to seven-membered heterocycle,

Q^2 stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,

R^{14} stands for hydrogen or (C₁-C₄)-alkyl,

10 R^{15} stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{14} and R^{15} together with the atoms to which they are bound form a five- or six-membered heterocycle,

R^{16} stands for hydrogen or (C₁-C₄)-alkyl,

15 R^{17} stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{16} and R^{17} together with the atoms to which they are bound form a five- or six-membered heterocycle,

R^{18} stands for hydrogen or (C₁-C₄)-alkyl,

20 R^{19} stands for hydrogen or the side group of a natural α -amino acid or its homologs or isomers,

R^{20} stands for hydrogen or (C₁-C₄)-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidiny ring,

R^{21} stands for hydrogen or (C₁-C₄)-alkyl,

5 R^{22} stands for hydrogen or (C₁-C₄)-alkyl,

or

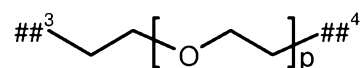
R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{23} stands for (C₁-C₄)-alkyl,

10 R^{24} stands for hydrogen or (C₁-C₄)-alkyl,

R^{27} stands for hydrogen or (C₁-C₄)-alkyl,

L^2 stands for linear (C₂-C₁₀)-alkanediyl or for a group of the formula



wherein

15 p stands for a number from 2 to 6,

$##^3$ denotes the linkage site to the group B,

$##^4$ denotes the linkage site to the nitrogen atom,

wherein (C₂-C₁₀)-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

20 wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4- relation to one another, including the carbon atoms optionally present between them to form a (C₃-C₆) cycloalkyl ring or a phenyl ring,

D has the meaning as described in claim 14,

as well as their salts, solvates and solvates of the salts.

16. Binder-drug conjugates according to claim 14 or 15 of general formula (I),

in which

n stands for a number from 1 to 50,

5 AK stands for AK₁ or AK₂

wherein

AK₁ stands for an antibody or an antigen-binding antibody fragment and is bound to the group G via a sulfur atom,

10 AK₂ stands for an antibody or an antigen-binding antibody fragment and is bound to the group G via a nitrogen atom,

n, G, L¹, B, L² and D have the meanings given in claim 14 or 15,

as well as their salts, solvates and solvates of the salts.

17. Binder-drug conjugates according to one of claims 14 to 16 of general formula (I), wherein

15 n stands for a number from 1 to 20,

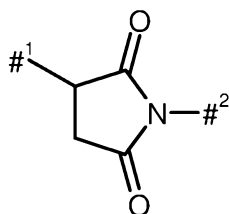
AK stands for AK₁ or AK₂

wherein

20 AK₁ stands for an antibody or an antigen-binding antibody fragment which binds to EGFR and is bound to the group G via the sulfur atom of a cysteine radical of the binder,

AK₂ stands for an antibody or an antigen-binding antibody fragment which binds to EGFR and is bound to the group G via the NH side group of a lysine radical of the binder,

G for the case when AK = AK₁ stands for a group of the formula



wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

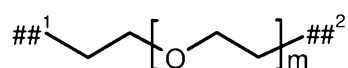
#² denotes the linkage site to the group L¹,

5

or

for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₂-C₆)-alkanediyl or stands for a group of the formula



wherein

10

m stands for a number from 2 to 6,

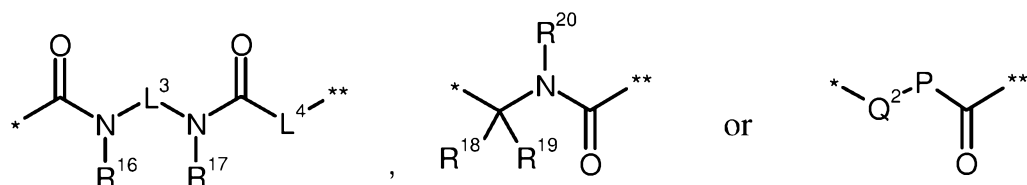
##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

15

B stands for a bond or a group of the formula



wherein

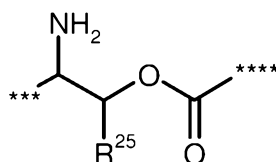
* denotes the linkage site to L¹,

** denotes the linkage site to L^2 ,

P stands for O or NH,

L^3 stands for a bond or ethane-1,2-diyl,

L^4 stands for a bond or a group of the formula



5

wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

R^{25} stands for methyl,

10 Q^2 stands for cyclopentyl or cyclohexyl,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

or

15 R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

R^{18} stands for hydrogen,

R^{19} stands for hydrogen, methyl, propan-2-yl, 2-methylpropan-1-yl or 1-methylpropan-1-yl,

R^{20} stands for hydrogen or methyl,

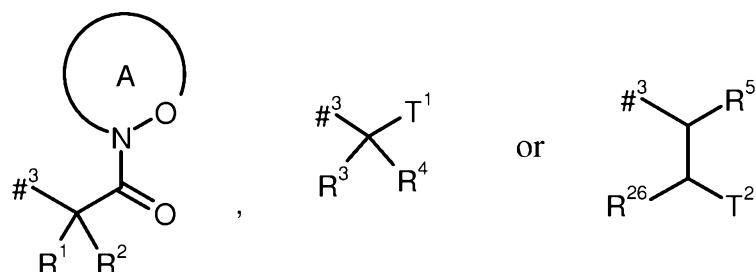
20 or

R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidinyl ring,

L² stands for linear (C₂-C₆)-alkanediyl,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

D stands for a group of the formula



wherein

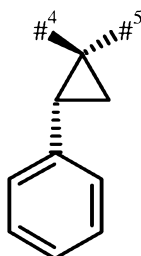
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

R² stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

10 or

R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

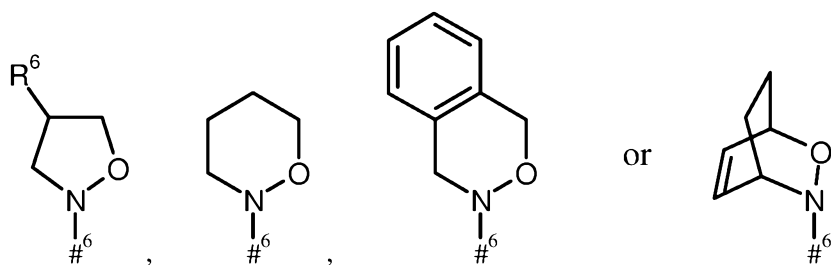


wherein

15 #⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

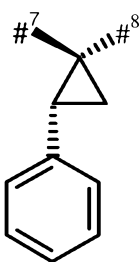
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen,

R⁴ stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



10

wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

15 T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

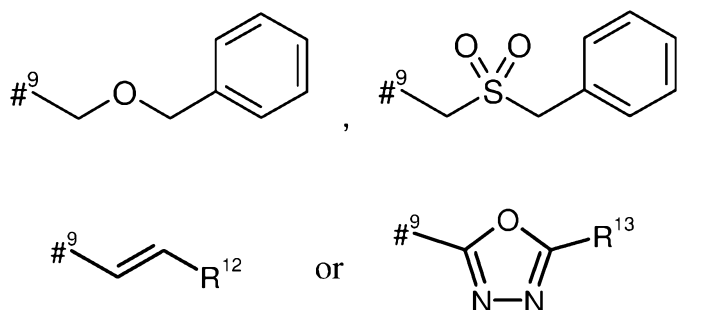
or

R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(R^{26})-\text{T}^2$,

R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

as well as their salts, solvates and solvates of the salts.

18. Binder-drug conjugates according to one of claims 14 to 17 of general formula (I), wherein

n stands for a number from 1 to 10,

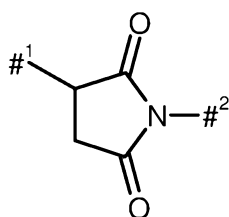
AK stands for AK₁ or AK₂

wherein

5 AK₁ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the sulfur atom of a cysteine radical of the binder,

AK₂ stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G for the case when AK = AK₁ stands for a group of the formula



10 wherein

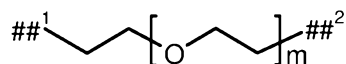
#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

or

for the case when AK = AK₂, G stands for carbonyl,

15 L¹ stands for a bond, linear (C₂-C₆)-alkanediyl or stands for a group of the formula



wherein

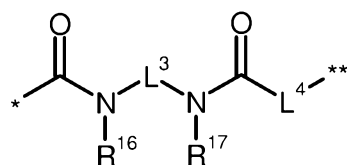
m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

20 ##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



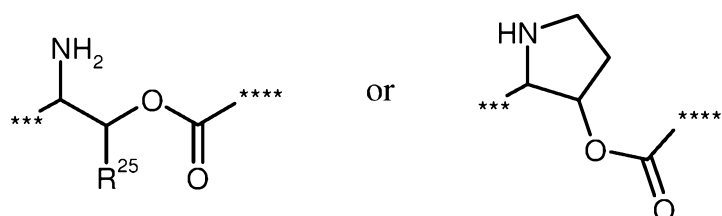
5 wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



10

wherein

*** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L²,

R²⁵ stands for methyl,

15 R¹⁶ stands for hydrogen or methyl,

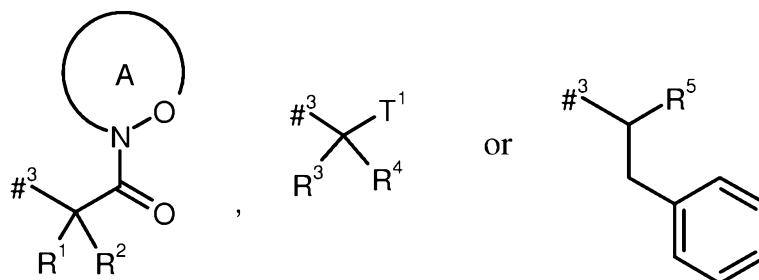
R¹⁷ stands for hydrogen or methyl,

or

R¹⁶ and R¹⁷ together with the atoms to which they are bound form a piperazinyl ring,

L² stands for linear (C₂-C₆)-alkanediyl,

D stands for a group of the formula



wherein

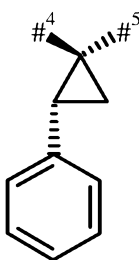
5 #³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

R² stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

10 R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

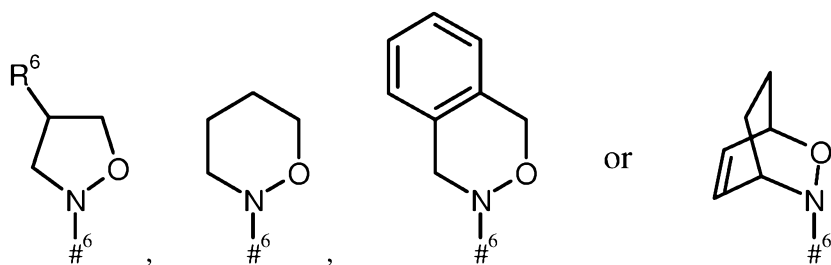


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

15 the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

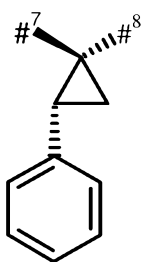
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen,

R⁴ stands for benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



10

wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T,

15 T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹ or -CH₂-O-R¹¹,

wherein

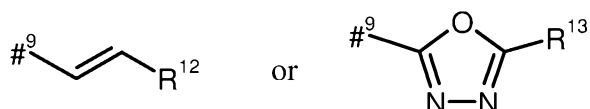
R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

5 R^5 stands for hydrogen or a group of the formula



wherein

$\#^9$ denotes the linkage site to $\text{---CHC}(R^{26})\text{phenyl}$,

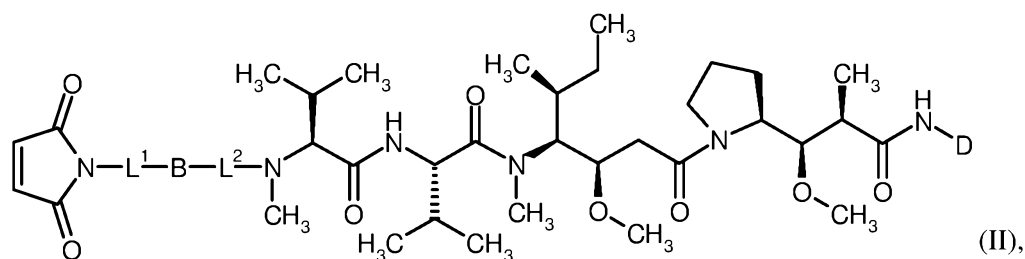
10 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $\text{---S(O)}_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

as well as their salts, solvates and solvates of the salts.

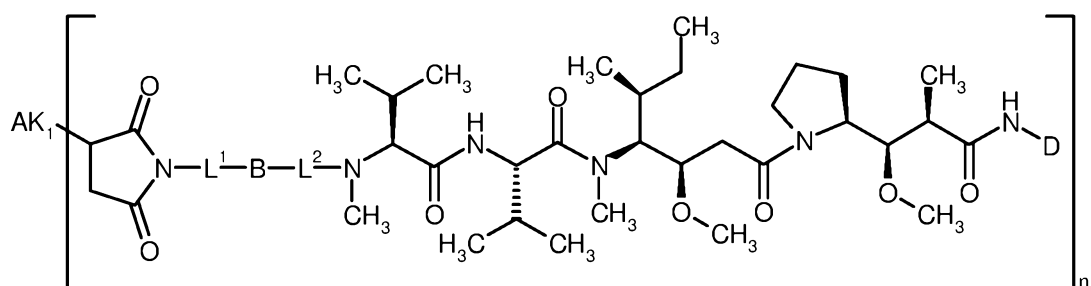
19. Method for production of inventive compounds of formula (I) as defined in one of claims
15 14 to 18, characterized in that a solution of the binder in a buffer

[A] is mixed with a suitable reducing agent such as, for example, dithiothreitol or *tris*-(2-carboxyethyl)phosphine hydrochloride and then is reacted with a compound of formula (II)



20 in which D, L^1 , B and L^2 have the meanings given in claims 14 through 18,

to form a compound of formula (I-A)

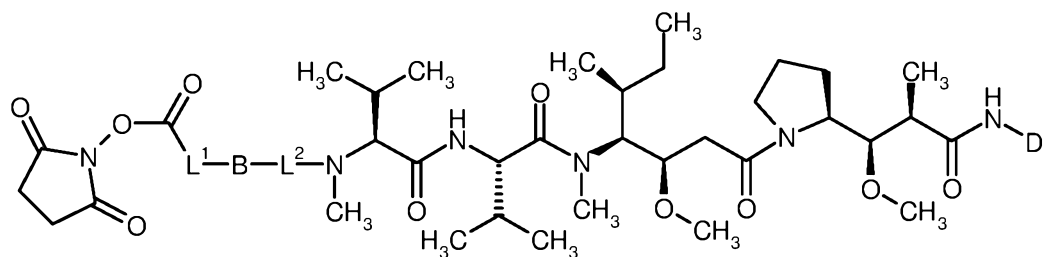


(I-A),

in which n , AK_1 , D , L^1 , B and L^2 each have the meanings given in claims 14 through 18,

or

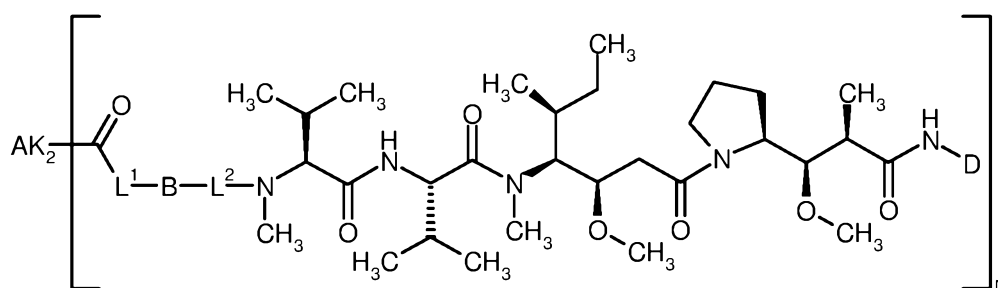
5 [B] reacting it with a compound of formula (III)



(III),

in which D , L^1 , B and L^2 each have the meanings given in claims 14 through 18,

to form a compound of formula (I-B)

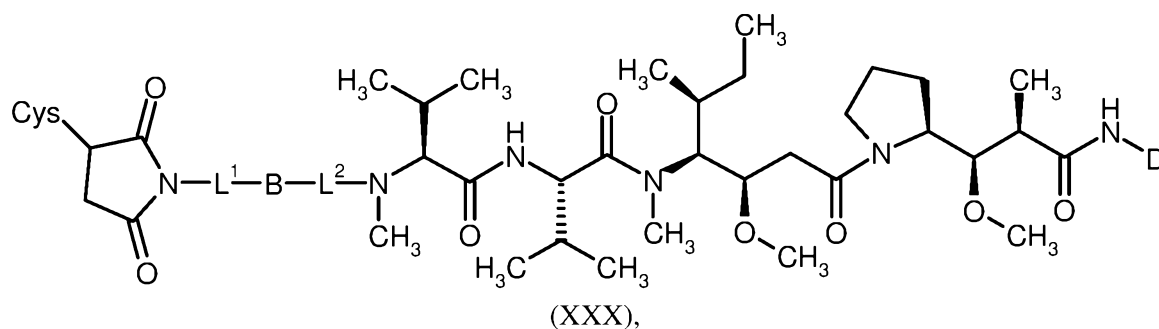


(I-B),

in which n , AK_2 , D , L^1 , B and L^2 each have the meanings given in claims 14 through 18,

20. Compounds produced by the method according to claim 19 or 51, wherein AK_1 and AK_2 stand for cetuximab, pantitumumab, nimetuzumab as well as their salts, solvates and solvates of the salts.

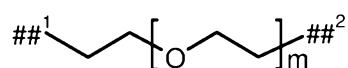
21. Compounds of formula (XXX)



in which

5 Cys stands for a cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

L^1 stands for a bond, linear (C_1-C_{10}) -alkanediyl or stands for a group of the formula



wherein

10 m stands for a number from 2 to 6,

$##^1$ denotes the linkage site to the group G,

$##^2$ denotes the linkage site to the group B,

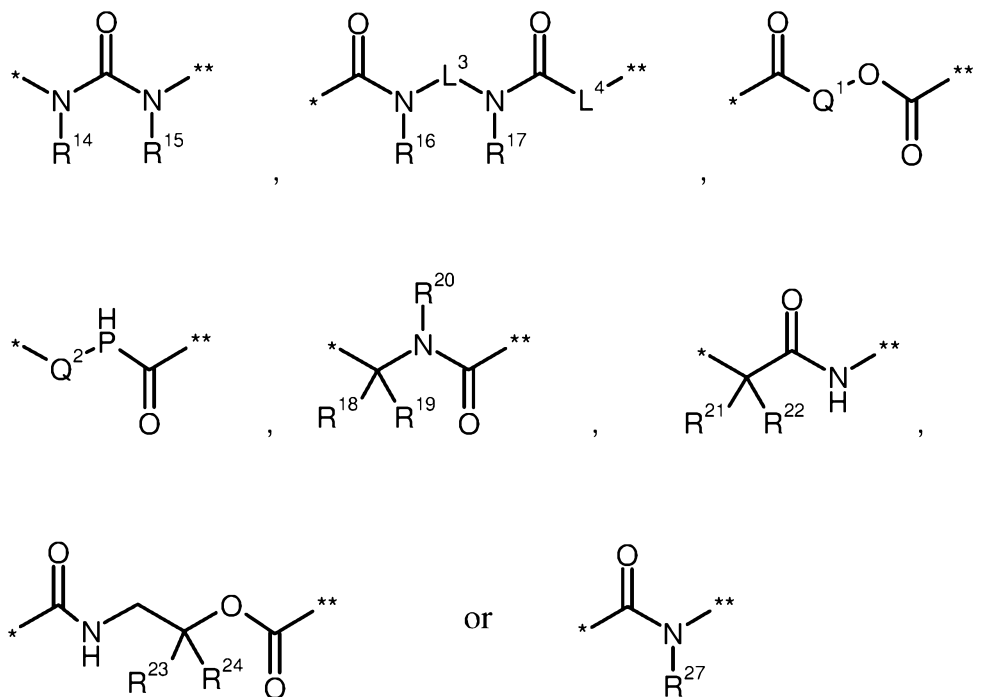
wherein (C_1-C_{10}) -alkanediyl may be substituted with 1 to 4 methyl substituents,

and

15 wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another may be bridged to form a (C_3-C_6) -cycloalkyl ring or a phenyl ring including the carbon atoms optionally situated between them,

B stands for a bond or a group of the formula

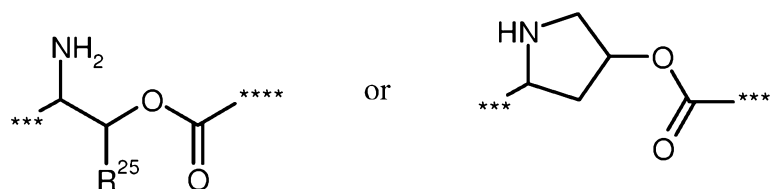
- 845 -



wherein

* denotes the linkage site to L^1 ,** denotes the linkage site to L^2 ,

5 P stands for O or NH,

 L^3 for a bond or (C₂-C₄)-alkanediyl, L^4 stands for a bond or a group of the formula

wherein

10 *** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 , R^{25} stands for hydrogen or methyl,

- 5 Q^1 stands for a three- to seven-membered carbocycle or a four- to seven-membered azaheterocycle,
- Q^2 stands for a three- to seven-membered carbocycle or a four- to seven-membered azaheterocycle,
- R^{14} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{15} stands for hydrogen or (C₁-C₄)-alkyl,
- or
- R^{14} and R^{15} together with the atoms to which they are bound form a five- or six-membered heterocycle,
- 10 R^{16} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{17} stands for hydrogen or (C₁-C₄)-alkyl,
- or
- R^{16} and R^{17} together with the atoms to which they are bound form a five- or six-membered heterocycle,
- 15 R^{18} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{19} stands for hydrogen or the side group of a natural α -amino acid or its homologs or isomers,
- R^{20} stands for hydrogen or (C₁-C₄)-alkyl,
- or
- 20 R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidinyl ring,
- R^{21} stands for hydrogen or (C₁-C₄)-alkyl,
- R^{22} stands for hydrogen or (C₁-C₄)-alkyl,
- or

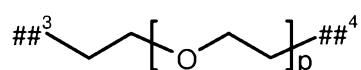
R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{23} stands for (C₁-C₄)-alkyl,

R^{24} stands for hydrogen or (C₁-C₄)-alkyl,

5 R^{27} stands for hydrogen or (C₁-C₄)-alkyl,

L^2 stands for linear (C₂-C₁₀)-alkanediyl or for a group of the formula



wherein

p stands for a number from 2 to 6,

10 $##^3$ denotes the linkage site to the group B,

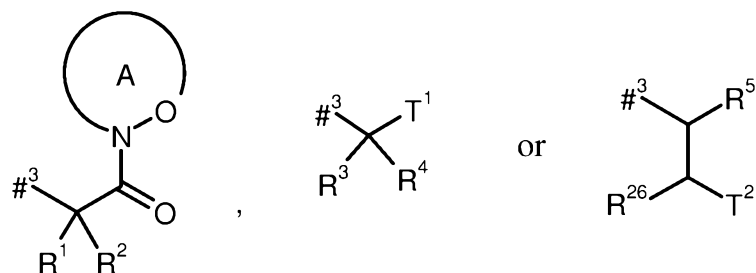
$##^4$ denotes the linkage site to the nitrogen atom,

wherein (C₂-C₁₀)-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

15 wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4- relation to one another, including the carbon atoms optionally present between them to form a (C₃-C₆) cycloalkyl ring or a phenyl ring.

D stands for a group of the formula



wherein

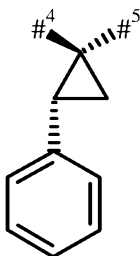
20 $#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

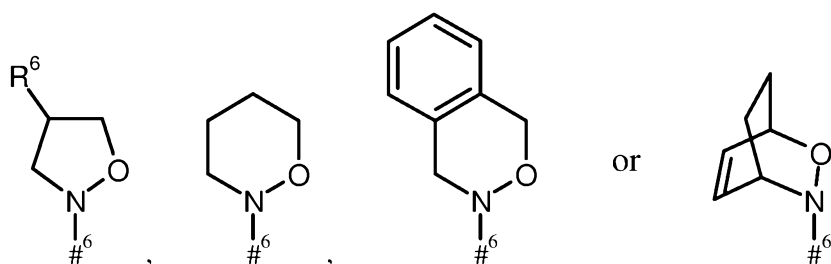


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

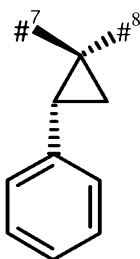
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



5 wherein

$\#^7$ denotes the linkage site to the vicinal nitrogen atom,

$\#^8$ denotes the linkage site to the group T^1 ,

T^1 stands for a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

10 wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

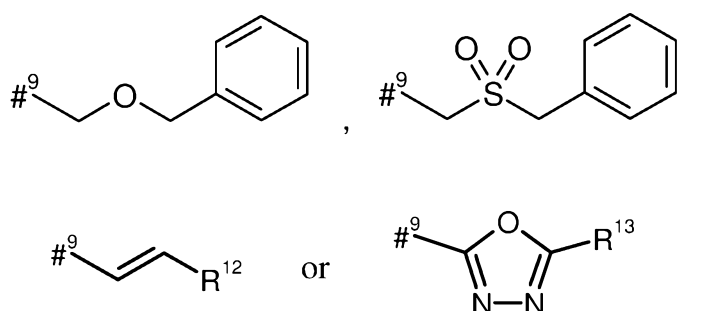
15 or

R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

20 R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

#⁹ denotes the linkage site to $-\text{CHC}(\text{R}^{26})-\text{T}^2$,

5 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

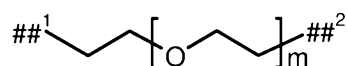
T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

10 as well as their salts, solvates and solvates of the salts.

22. Compounds of formula (XXX) according to claim 21, wherein

Cys stands for a cysteine radical which is bound to a carbon atom of the succinimide via the sulfur atom of the side chain,

L^1 stands for a bond, linear $(\text{C}_2\text{-C}_6)$ -alkanediyl or stands for a group of the formula



15

wherein

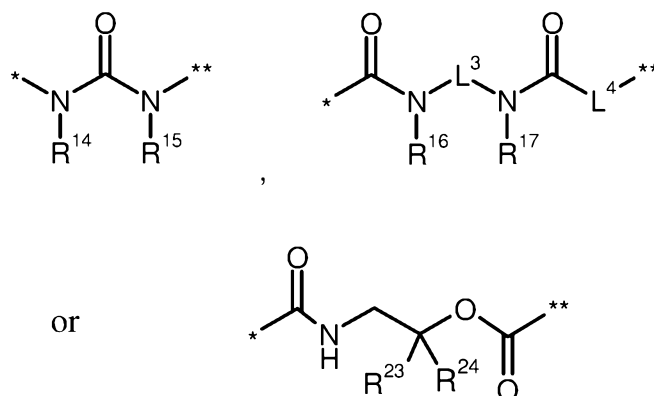
m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



5 wherein

* denotes the linkage site to L¹,

** denotes the linkage site to L²,

L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond,

10 R¹⁴ stands for hydrogen,

R¹⁵ stands for hydrogen,

R¹⁶ stands for hydrogen or methyl,

R¹⁷ stands for hydrogen or methyl,

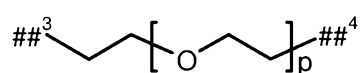
or

15 R¹⁶ and R¹⁷ together with the atoms to which they are bound form a piperazinyl ring,

R²³ stands for methyl,

R²⁴ stands for hydrogen or methyl,

L² stands for linear (C₂-C₆)-alkanediyl or for a group of the formula



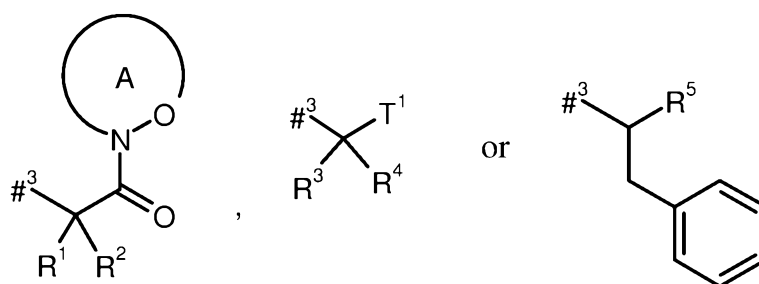
wherein

p stands for a number of 2 or 3,

##³ denotes the linkage site to the group B,

5 ##⁴ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

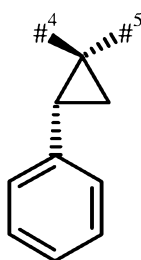
#³ denotes the linkage site to the nitrogen atom,

10 R¹ stands for hydrogen,

R² stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



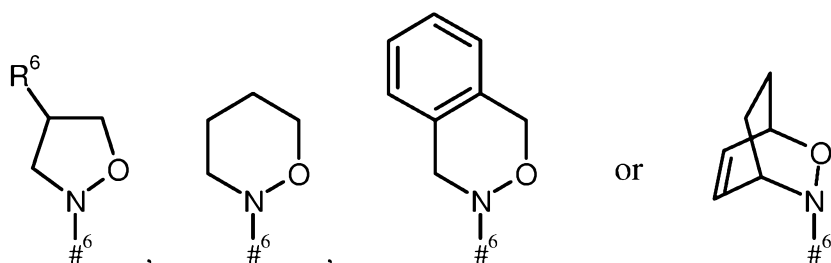
15

wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

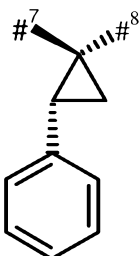
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

R³ stands for hydrogen,

10 R⁴ stands for benzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



15 wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, benzyl or adamantylmethyl,

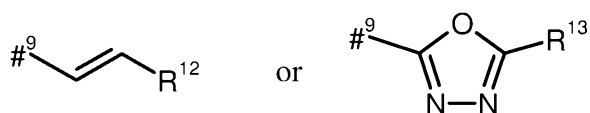
R⁸ stands for hydrogen or methyl,

R⁹ stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

R¹⁰ stands for benzoyl,

R¹¹ stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R⁵ stands for hydrogen or a group of the formula



wherein

#⁹ denotes the linkage site to -CHC(R²⁶)phenyl,

R¹² stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula -S(O)₂OH,

R¹³ stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

as well as their salts, solvates and solvates of the salts.

23. Binder-drug conjugate according to any one of the claims 1-6, 14-18 or 49-50, wherein the binder binds to a cancer target molecule.

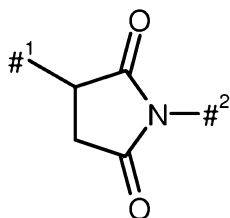
24. Binder-drug conjugate according to any one of the claims 1-6, 14-18 or 23 or 49-50, wherein the binder binds to an extracellular target molecule.

25. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23 or 24 or 49-50, wherein the binder binds to an extracellular cancer target molecule.
26. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23, 24 or 25 or 49-50, wherein the target molecule, extracellular target molecule, cancer target molecule or extracellular cancer target molecule is a protein.
27. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23, 24, 25 or 26 or 49-50, wherein the binder, after binding to the extracellular target molecule, is internalized by the cells expressing the target molecule.
28. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23, 24, 25, 26 or 27 or 49-50, wherein the binder is a binding protein.
29. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23, 24, 25, 26, 27 or 28 or 49-50, wherein the binder is an antibody or an antigen-binding antibody fragment thereof or an antibody mimetic.
30. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23, 24, 25, 26, 27, 28 or 29 or 49-50, wherein the antibody is a monoclonal antibody.
31. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23, 24, 25, 26, 27, 28, 29 or 30 or 49-50, wherein the antibody is a human, humanized or chimeric antibody.
32. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23, 24, 25, 26, 27, 28, 29, 30 or 31 or 49-50, wherein the antibody is an intact or modified intact antibody.
33. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23, 24, 25, 26, 27, 28, 29, 30, 31 or 32 or 49-50, wherein the antibody is an antibody of the IgG class.
34. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 or 33 or 49-50, wherein the binder binds to an extracellular cancer target molecule selected from the group consisting of EGF receptor (NP_005219.2), mesothelin (Q13421-3), C4.4a (NP_055215.2), carbonic dehydratase IX (CA IX; NP_001207.2), Her2, glypican-3, TYRP1, fibroblast growth factor receptor 3, single-pass type I membrane protein ICOSLG, and programmed cell death 1 ligand 1.
35. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34 or 49-50, wherein the binder binds specifically to an extracellular cancer target molecule selected from the group consisting of EGF receptor

(NP_005219.2), mesothelin (Q13421-3), C4.4a (NP_055215.2), carbonic dehydratase IX (CA IX; NP_001207.2), glypican-3, TYRPI, fibroblast growth factor receptor 3, single-pass type I membrane protein ICOSLG, and programmed cell death 1 ligand 1.

36. Binder-drug conjugate according to any one of the claims 1-6, 14-18, 23, 24, 25, 26, 27,
5 28, 29, 30, 31, 32, 33, 34 or 35 or 49-50, wherein the antibody binds to EGFR.
37. Binder-drug conjugate according to any one of the claims 1-6, 14-18 or 23-36 or 49-50,
wherein the antibody binds specifically to EGFR.
38. Binder-drug conjugate according to any one of the claims 1-6, 14-18 or 23-37 or 49-50,
wherein the anti-EGFR antibody is selected from the group consisting of cetuximab,
10 panitumumab, nimotuzumab, zalutumumab, necitumumab, matuzumab, RG-716,
GT-MAB 5.2-GEX, ISU-101, ABT-806, SYM-004, MR1-1, SC-100, MDX-447, and
DXL-1218.
39. Binder-drug conjugate according to any one of the claims 1-6, 14-18 or 23-38 or 49-50,
wherein the anti-EGFR antibody is selected from the group consisting of antibodies
15 comprising the six CDR sequences of one of the following antibodies: cetuximab,
panitumumab, nimotuzumab, zalutumumab, necitumumab, matuzumab, RG-716,
GT-MAB 5.2-GEX, ISU-101, ABT-806, SYM-004, MR1-1, SC-100, MDX-447, and
DXL-1218.
40. Binder-drug conjugate according to any one of the claims 1-6, 14-18 or 23-39, wherein
20 the anti-EGFR antibody is selected from the group consisting of antibodies comprising
the amino acid sequence of the variable light chain and the variable heavy chain of one
of the following antibodies: cetuximab, panitumumab, nimotuzumab, zalutumumab,
necitumumab, matuzumab, RG-716, GT-MAB 5.2-GEX, ISU-101, ABT-806, SYM-004,
MR1-1, SC-100, MDX-447, and DXL-1218.
- 25 41. Binder-drug conjugate according to any one of the claims 1-6, 14-18 or 23-40 or 49-50,
wherein the anti-EGFR antibody is selected from the group consisting of cetuximab,
panitumumab, nimotuzumab, zalutumumab, necitumumab and matuzumab.
42. Binder-drug conjugate or a compound according to any one of claims 1-18 or 23-41 or
49-50, for treatment and/or prevention of diseases.

43. Binder-drug conjugate or a compound according to any one of claims 1-18 or 23-41 or 49-50, for use in a method for treatment and/or prevention of hyperproliferative and/or angiogenic diseases.
44. Binder-drug conjugate or a compound according to any one of claims 1-18 or 23-41 or 49-50, for producing a drug for treatment and/or prevention of hyperproliferative and/or angiogenic diseases.
45. Pharmaceutical drug containing a binder-drug conjugate or a compound according to any one of claims 1-18 or 23-41 or 49-50, in combination with an inert nontoxic pharmaceutically suitable excipient.
46. Pharmaceutical drug containing a binder-drug conjugate or a compound according to any one of claims 1-18 or 23-41 or 49-50, in combination with one or more anti-hyperproliferative, cytostatic or cytotoxic substances.
47. Pharmaceutical drug according to claims 44 to 46 for treatment and/or for prevention of hyperproliferative and/or angiogenic diseases.
48. Method for treatment and/or prevention of hyperproliferative and/or angiogenic diseases in humans and animals using an effective amount of at least one binder-drug conjugate or a compound according to any one of claims 1-18 or 23-41 or 49-50, or a pharmaceutical drug as defined in one of claims 44 to 46.
49. Binder-drug conjugates of general formula (Ia) according to claim 1, wherein
- n stands for a number from 1 to 20,
- AK stands for AK₁ or AK₂
- wherein
- AK₁ stands for a binder bound to the group G via a sulfur atom of the binder,
- AK₂ stands for a binder bound to the group G via a nitrogen atom of the binder,
- G for the case when AK = AK₁ stands for a group of the formula



wherein

#¹ denotes the linkage site to the cysteine radical of the binder,

#² denotes the linkage site to the group L¹,

5 or

for the case when AK = AK₂, G stands for carbonyl,

L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



wherein

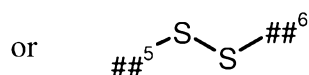
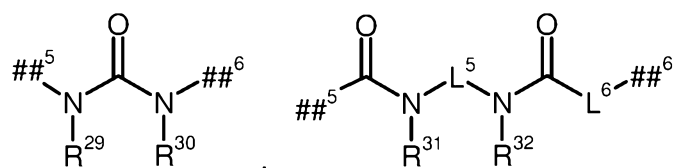
10 m stands for a number from 2 to 6,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

L^{1A} stands for linear (C₂-C₆)-alkanediyl,

B1 stands for a group of the formula



15

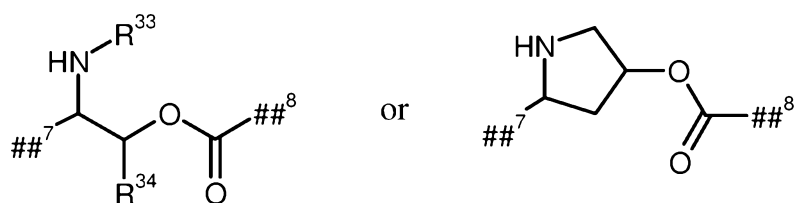
wherein

$\#\#^5$ denotes the linkage site to the group L^{1A} ,

$\#\#^6$ denotes the linkage site to the group L^{1B} ,

L^5 stands for a bond,

5 L^6 stands for a bond or a group with the formula



wherein

$\#\#^7$ denotes the linkage site to the carbonyl group,

$\#\#^8$ denotes the linkage site to L^{1B} ,

10 R^{33} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

R^{34} stands for hydrogen or methyl,

R^{29} stands for hydrogen,

R^{30} stands for hydrogen,

R^{31} stands for hydrogen or methyl,

15 R^{32} stands for hydrogen or methyl,

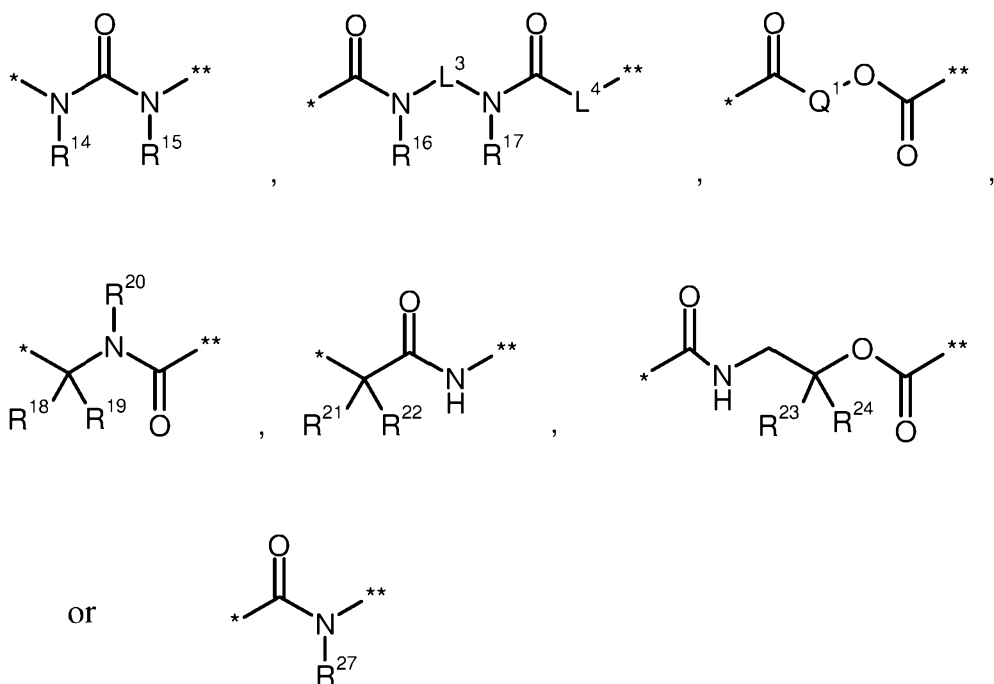
L^{1B} stands for linear (C_2-C_{10}) -alkanediyl,

and

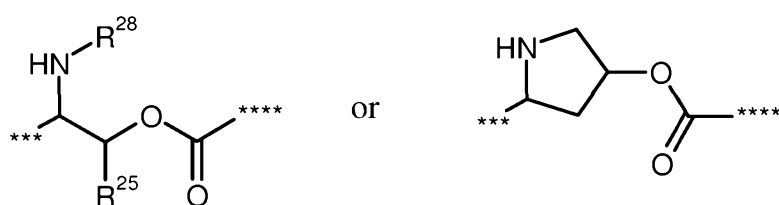
wherein (C_2-C_6) -alkanediyl may be substituted with one to two methyl substituents,

B stands for a bond or a group of the formula

- 860 -



wherein

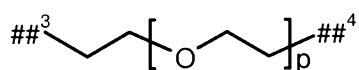
* denotes the linkage site to L^1 ,** denotes the linkage site to L^2 ,5 L^3 stands for a bond or ethane-1,2-diyl, L^4 stands for a bond or a group of the formula

wherein

*** denotes the linkage site to the carbonyl group,

10 **** denotes the linkage site to L^2 , R^{25} stands for hydrogen or methyl, R^{28} stands for hydrogen, methylcarbonyl or *tert*-butoxycarbonyl,

- Q^1 stands for a four- to seven-membered heterocycle,
 R^{14} stands for hydrogen,
 R^{15} stands for hydrogen,
 R^{16} stands for hydrogen or methyl,
 5 R^{17} stands for hydrogen or methyl,
 or
 R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,
 R^{18} stands for hydrogen,
 R^{19} stands for hydrogen, methyl, propan-2-yl, 2-methylpropan-1-yl or
 10 1-methylpropan-1-yl,
 R^{20} stands for hydrogen or methyl,
 or
 R^{19} and R^{20} together with the atoms to which they are bound form a pyrrolidinyl ring,
 R^{21} stands for hydrogen or methyl,
 15 R^{22} stands for hydrogen or methyl,
 or
 R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,
 R^{23} stands for methyl,
 R^{24} stands for hydrogen or methyl,
 20 R^{27} stands for hydrogen,
 L^2 stands for linear (C_2-C_6) -alkanediyl or for a group of the formula



wherein

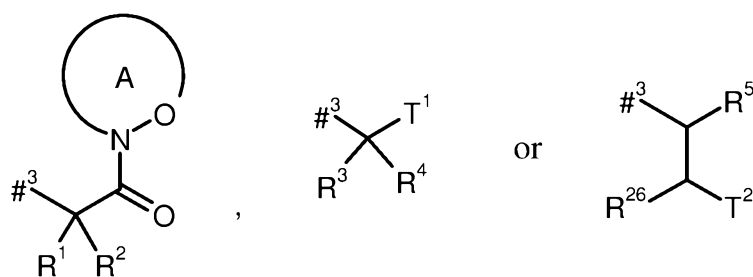
p stands for a number from 2 to 6,

$\#^3$ denotes the linkage site to the group B,

$\#^4$ denotes the linkage site to the nitrogen atom,

5 wherein (C₂-C₁₀)-alkanediyl may be substituted with one or two methyl substituents,

D stands for a group of the formula



wherein

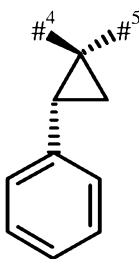
$\#^3$ denotes the linkage site to the nitrogen atom,

10 R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

15 R^1 and R^2 together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

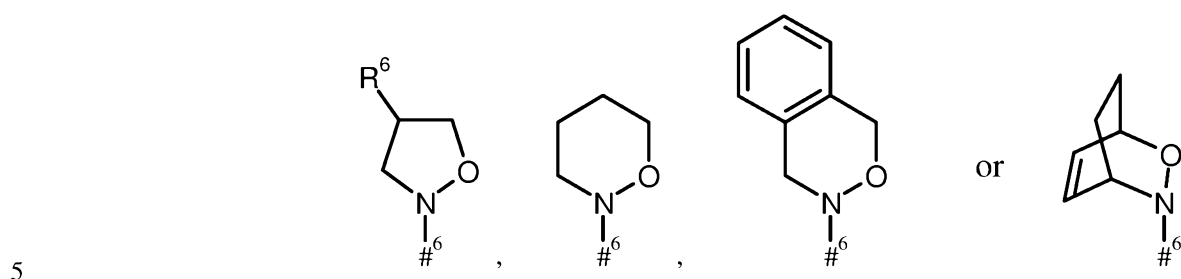


wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

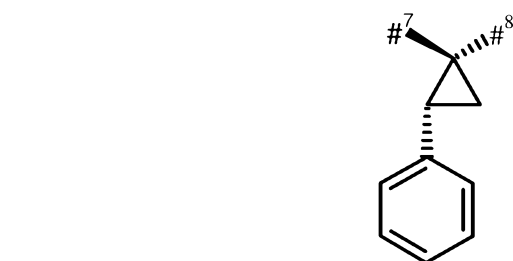
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

R³ stands for hydrogen,

10 R⁴ stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R⁸ stands for hydrogen or methyl,

R⁹ stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

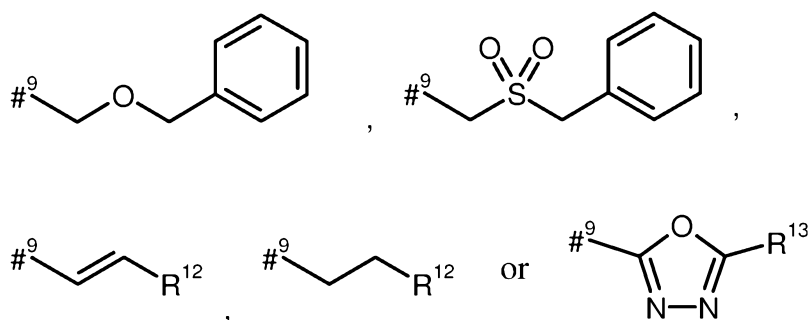
or

R⁸ and R⁹ together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R¹⁰ stands for benzoyl,

R¹¹ stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R⁵ stands for hydrogen, methyl or a group of the formula



wherein

#⁹ denotes the linkage site to -CHC(R²⁶)-T²,

R¹² stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula -S(O)₂OH,

R¹³ stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

T² stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

R³⁵ stands for methyl or hydroxyl,

as well as their salts, solvates and solvates of the salts.

5 50. Binder-drug conjugates of general formula (Ia) according to claim 1, wherein

n stands for a number from 1 to 10,

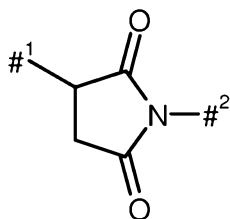
AK stands for AK₁ or AK₂

wherein

AK₁ stands for a binder bound to the group G via a sulfur atom of the binder,

10 AK_2 stands for a binder bound to the group G via a nitrogen atom of the binder,

G for the case when $AK = AK_1$ stands for a group of the formula



wherein

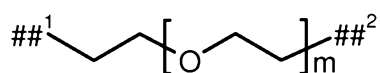
#¹ denotes the linkage site to the cysteine radical of the binder,

15 $\#^2$ denotes the linkage site to the group L¹,

or

for the case when $AK = AK_2$, G stands for carbonyl,

L¹ stands for a bond, linear (C₂-C₆)-alkanediyl, a group of the formula



wherein

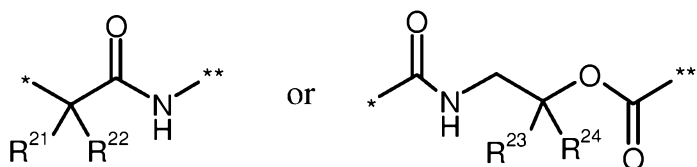
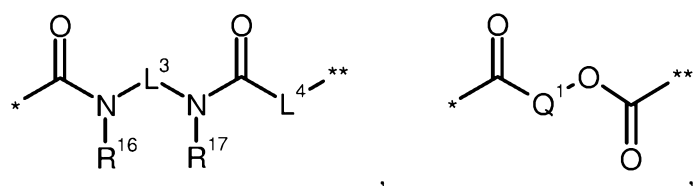
m stands for a number of 2 or 3,

##¹ denotes the linkage site to the group G,

##² denotes the linkage site to the group B,

5 wherein (C₂-C₆)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



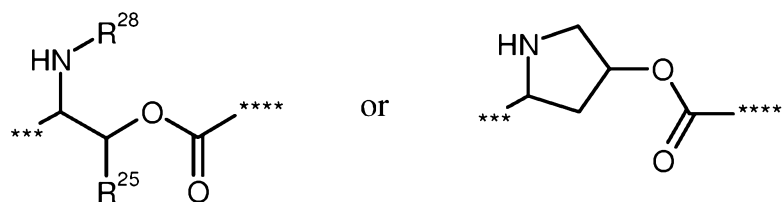
wherein

* denotes the linkage site to L¹,

10 ** denotes the linkage site to L²,

L³ stands for a bond or ethane-1,2-diyl,

L⁴ stands for a bond or a group of the formula



wherein

15 *** denotes the linkage site to the carbonyl group,

**** denotes the linkage site to L^2 ,

R^{25} stands for methyl,

R^{28} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

Q^1 stands for piperidine-1,4-diyl,

5 R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

R^{21} stands for hydrogen or methyl,

10 R^{22} stands for hydrogen or methyl,

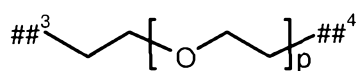
or

R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

R^{23} stands for methyl,

R^{24} stands for hydrogen,

15 L^2 stands for linear (C_2-C_6) -alkanediyl or for a group of the formula



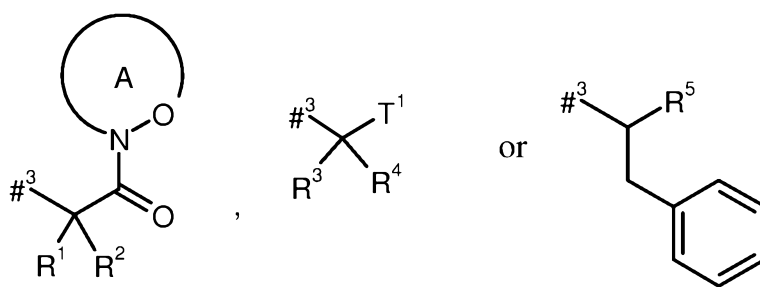
wherein

p stands for a number from 2 to 6,

$##^3$ denotes the linkage site to the group B,

20 $##^4$ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



wherein

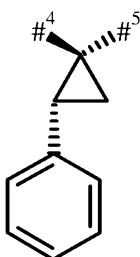
#³ denotes the linkage site to the nitrogen atom,

R¹ stands for hydrogen,

5 R² stands for 1-hydroxyethyl, benzyl, 1-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



10

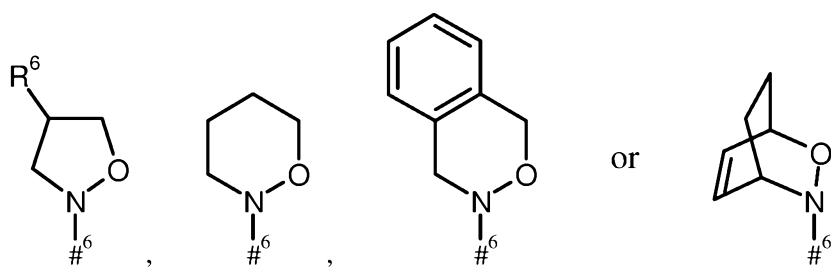
wherein

#⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

15

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

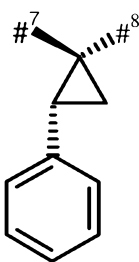
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen,

R⁴ stands for benzyl, 1-hydroxybenzyl, 1-phenylethyl or 1H-indol-3-ylmethyl,

or

R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



10

wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

#⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹ or -CH₂-O-R¹¹,

15

wherein

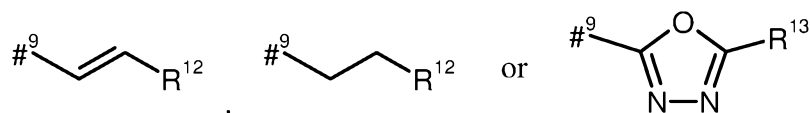
R⁷ stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

5 R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $\text{---CHCH}_2\text{phenyl}$,

10 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $\text{---S(O)}_2\text{OH}$,

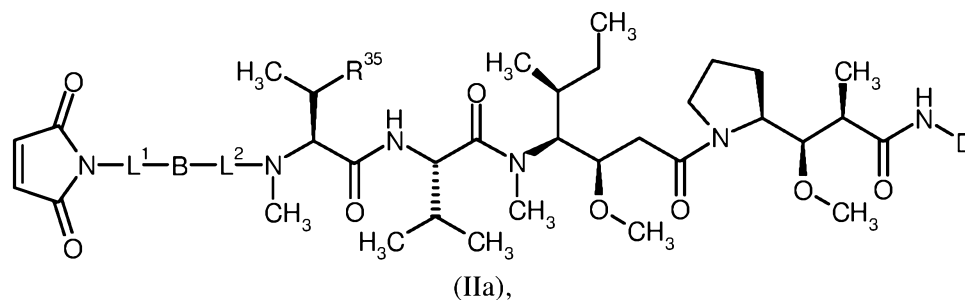
R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts, solvates and solvates of the salts.

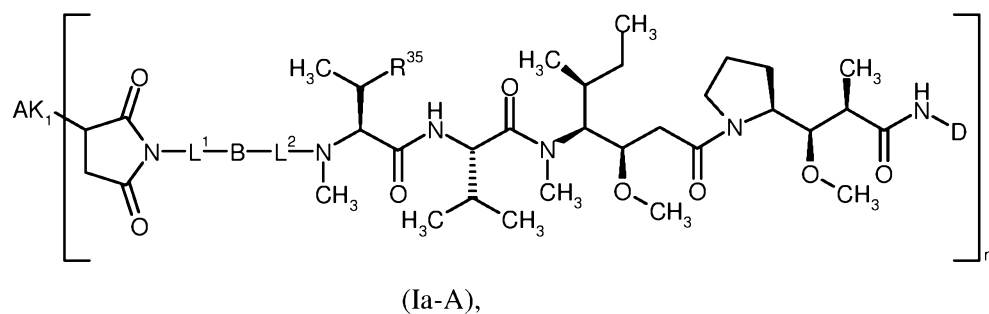
15 51. Method for producing the inventive compounds according to any one of claims 1 to 6 of general formula (Ia), characterized in that a solution of the binder in a buffer

[A] is mixed with a suitable reducing agent such as, for example, dithiothreitol or *tris*-(2-carboxyethyl)phosphine hydrochloride and then is reacted with a compound of formula (IIa)



in which D, L¹, B, L² and R³⁵ each have the meanings given in claims 1 to 6,

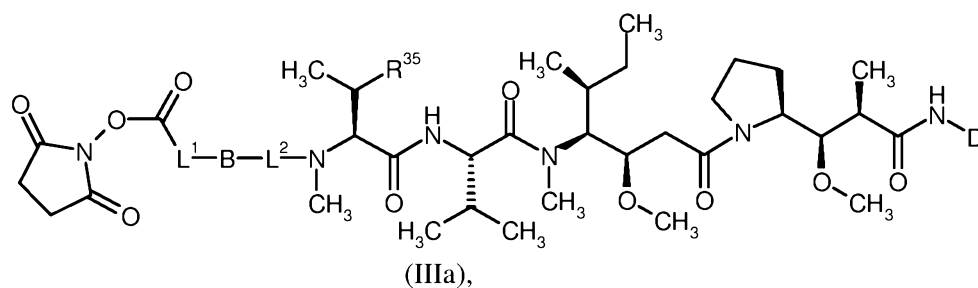
to form a compound of formula (Ia-A)



5 in which n, AK₁, D, L¹, B, L² and R³⁵ each have the meanings given in claims 1 to 6,

or

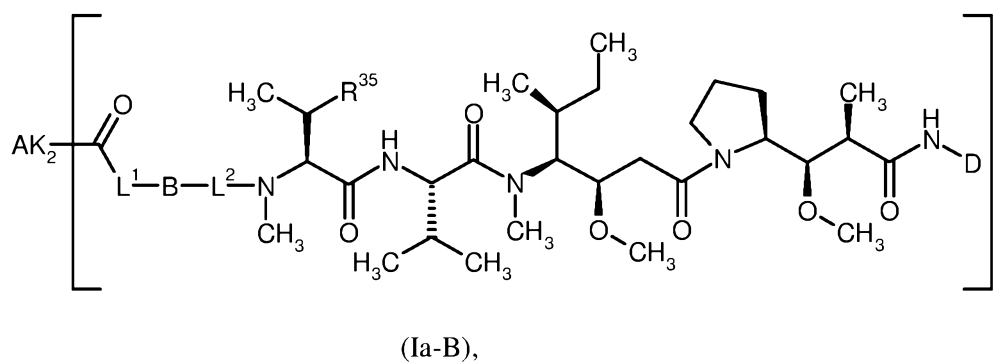
[B] is reacted with a compound of formula (IIIa)



10

in which D, L¹, B, L² and R³⁵ each have the meanings given in claims 1 to 6,

to form a compound of formula (Ia-B)



15 in which n, AK₂, D, L¹, B, L² and R³⁵ each have the meanings given in claims 1 to 6,

52. Compounds produced by the method in claim 51, wherein AK_1 and AK_2 stand for cetuximab, pantitumumab or nimotuzumab as well as their salts, solvates and solvates of the salts.

53. Binder-drug conjugates of general formula (Ia) according to claim 1, wherein

5 n stands for a number from 1 to 50,

AK stands for a bond,

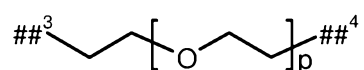
the group $\S-G-L^1-B-\S\S$ stands for a linker,

wherein

$\S$ denotes the linkage site to the group AK and

10 $\S\S$ denotes the linkage site to the nitrogen atom,

L^2 stands for linear (C_2-C_{10}) -alkanediyl or for a group of the formula



wherein

p stands for a number from 2 to 6,

15 $\#\#^3$ denotes the linkage site to the group B,

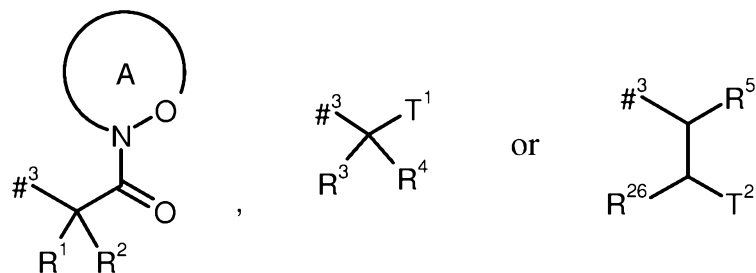
$\#\#^4$ denotes the linkage site to the nitrogen atom,

wherein (C_2-C_{10}) -alkanediyl may be substituted with 1 to 4 substituents selected independently of one another from the group comprising methyl, hydroxyl and benzyl,

20 and

wherein two carbon atoms of the alkanediyl chain may be bridged in 1,2-, 1,3- or 1,4- relation to one another, including the carbon atoms optionally present between them to form a (C_3-C_6) cycloalkyl ring or a phenyl ring,

D stands for a group of the formula



wherein

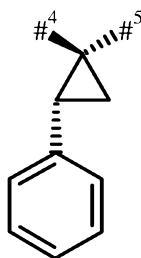
#³ denotes the linkage site to the nitrogen atom,

5 R¹ stands for hydrogen or methyl,

R² stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

10 or

R¹ and R² together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

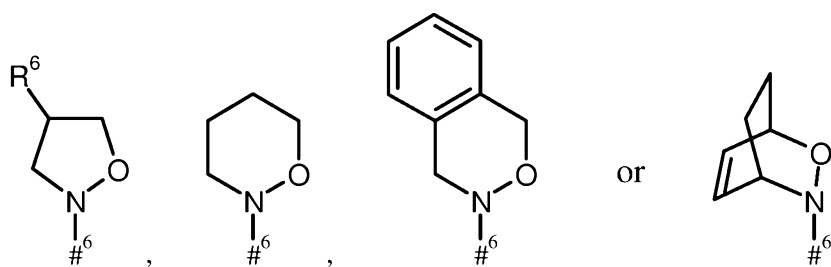


wherein

15 #⁴ denotes the linkage site to the vicinal nitrogen atom,

#⁵ denotes the linkage site to the carbonyl group,

the ring A with the N-O group contained in it stands for a mono- or bicyclic, optionally substituted heterocycle of the formula



wherein

#⁶ denotes the linkage site to the carbonyl group,

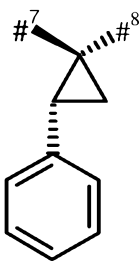
R⁶ stands for hydrogen, hydroxyl or benzyloxy,

5 R³ stands for hydrogen or methyl,

R⁴ stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1H-imidazol-4-ylmethyl or 1H-indol-3-ylmethyl,

or

10 R³ and R⁴ together with the carbon atom to which they are attached form a (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula



wherein

#⁷ denotes the linkage site to the vicinal nitrogen atom,

15 #⁸ denotes the linkage site to the group T¹,

T¹ stands for a group of the formula -C(=O)-OR⁷, -C(=O)-NR⁸R⁹, -C(=O)-NH-NH-R¹⁰ or -CH₂-O-R¹¹,

wherein

R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

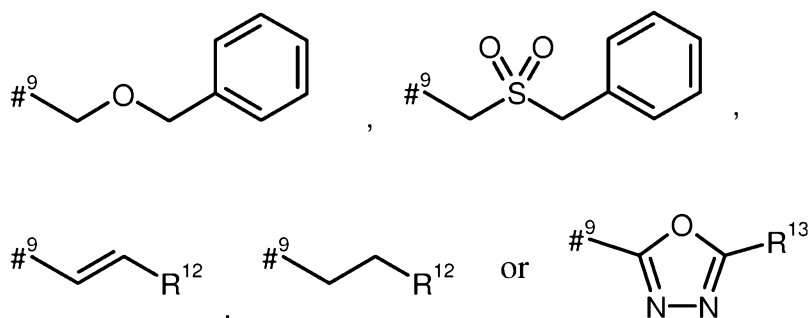
5 or

R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

10 R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

R^5 stands for hydrogen, methyl or a group of the formula



wherein

$\#^9$ denotes the linkage site to $-\text{CHC}(\text{R}^{26})\text{-T}^2$,

15 R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-\text{S}(\text{O})_2\text{OH}$,

R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{26} stands for hydrogen or hydroxyl,

20 T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

as well as their salts, solvates and solvates of the salts.

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Asn Ser Ser Ser Thr Thr Ser Thr Ala Ala Pro Thr Thr Thr Thr Ser
260 265 270

Ile Ile Lys Pro Thr Thr Ala Gln Ala Ser His Thr Ser Pro His Glu
275 280 285

Met Asp Leu Glu Val Ile Gln Glu Glu Gly Ala Ser Leu Ser Gly Gly
290 295 300

Ala Ala Gly His Gly Gly Thr Ala Gly His Gly Gly Ala Ala Gly His
305 310 315 320

Gln Asp Arg Ser Asn Met Glu Lys Tyr Pro Gly Lys Gly Gly Ala Gln
325 330 335

Ile Pro Ala Lys Gly Gly Ser Gly Thr Leu Gly Ser Trp Leu Ser Ala
340 345 350

Val Leu Leu Thr Val Val Ala Gly Ala Met Leu
355 360

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<400> 5

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1 5

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<400> 7

Phe Gly His Tyr Tyr Met Phe Trp Ile

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Phe Ser Ser Asn Tyr Met Ser Trp
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Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
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Val Ser Gly Val Ser Trp Asn Gly Ala Arg Thr His Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
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<400> 11

Val Ser Ala Ile Ser Gly Ser Gly Tyr Ser Thr His Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg

20

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<400> 12

Val Ser Ala Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 13
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<400> 13

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr
 1 5 10

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<400> 14

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ala Tyr Tyr Phe Asp Ser
 1 5 10 15

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 <213> Artificial Sequence

<220>
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<400> 15

Ala Arg Leu Pro Tyr Gly Ser Gln Ser Gly Val Asp Tyr
 1 5 10

<210> 16
 <211> 17
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 <213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 16

Ala Arg Glu Ser Gly Gly Ser Gly Pro Asn Tyr Tyr Tyr Gly Met Asp
1 5 10 15

Val

<210> 17

<211> 14

<212> PRT

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<220>

<223> C4.4a binder

<400> 17

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 18

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

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<400> 18

Ser Gly Ser Ser Ser Asn Val Gly Ser Asn Pro Val Asn
1 5 10

<210> 19

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 19

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

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<400> 20

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

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<400> 21

Asp Asn Asn Lys Arg Pro Ser
 1 5

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Arg Asn Asn Gln Arg Pro Ser
 1 5

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Ser Asn Asn Gln Arg Pro Ser
 1 5

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1

5

10

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<400> 26

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<210> 27

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<400> 27

Cys Gln Ser Tyr Asp Ser Ser His Val Leu
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<210> 28

<211> 12

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<400> 28

Cys Gln Ser Tyr Asp Arg Ser Leu Arg Gly Trp Val
1 5 10

<210> 29

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 29

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
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20 25 30Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe

50

55

60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg
85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln

<210> 30
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<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 30

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Val Gly Ser Asn
20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
85 90 95

Asn Gly Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

<210> 31
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 31

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
Page 12

1 5 10 15
 Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30
 Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45
 Leu Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60
 Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80
 Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
 85 90 95
 His Val Leu Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
 100 105 110
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 <212> PRT
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 Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30
 Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45
 Leu Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60
 Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80
 Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Ser
 85 90 95
 Leu Arg Gly Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110
 Gln

<210> 33
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 33

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110
 Val Thr Val Thr Ser
 115

<210> 34
 <211> 122
 <212> PRT
 <213> Artificial Sequence

<220>
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<400> 34

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
 20 25 30
 Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Val Ser Trp Asn Gly Ala Arg Thr His Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ala Tyr Tyr Phe Asp Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Thr Ser
115 120

<210> 35
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 35

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Gly His Tyr
20 25 30

Tyr Met Phe Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Tyr Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Leu Pro Tyr Gly Ser Gln Ser Gly Val Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Thr Ser
115 120

<210> 36
<211> 124
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 36

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Asn
20 25 30

Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Ser Gly Gly Ser Gly Pro Asn Tyr Tyr Tyr Gly Met Asp
100 105 110

Val Trp Gly Gln Gly Thr Leu Val Thr Val Thr Ser
115 120

<210> 37
<211> 339
<212> DNA
<213> Artificial Sequence

<220>
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ctcccaggaa cggcccccaa actcctcatc tatgacaata ataagcgacc ctcaggggtc 180
cctgaccgat tctctggctc caagtctggc acctcagcct ccctggccat cagtgggctc 240
cgggtccgagg atgaggctga ttattactgt gcagcatggg atgacaggct gaatgggtccg 300
gtgttcggcg gaggaaccaa gttaaccgtc ctaggtcag 339

<210> 38
<211> 336
<212> DNA
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<220>
<223> C4.4a binder

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ccaggaacgg cccccaaact cctcatctat aggaataatc agcggccctc aggggtccct	180
gaccgattct ctgggtccaa gtctggcacc tcagcctccc tggccatcag tgggtcccg	240
tccgaggatg aggtctgatta ttactgtgca gcatgggatg acaggctgaa tggttgggtg	300
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<210> 39
<211> 333
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cctgaccgat tctctggctc caagtctggc acctcagcct ccctggccat cagtgggctc	240
cgggtccgagg atgaggctga ttattactgc cagtcctatg acagcagcca tgttttattc	300
ggcggaggaa ccaagctgac ggtcctaggt cag	333

<210> 40
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ctcccaggaa cggcccccaa actcctcatc tatagtaata atcagcggcc ctcaggggtc	180
cctgaccgat tctctggctc caagtctggc acctcagcct ccctggccat cagtgggctc	240
cgggtccgagg atgaggctga ttattactgc cagtcctatg acagaagcct gcgtgggttg	300
gtgttcggcg gaggaaccaa gctgacggtc ctaggtcag	339

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<211> 351
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ccagggaagg ggctggagtg ggtttcatatc attagtagta gtggtagtag catatactac	180

c4c6aa138cea41cc8ed77499eb64c0e7.TXT
gcagactctg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcaaatga acagcctgag agccgaggac actgccgtgt attactgtgc gagagaaggg 300
ttatgggcct ttgactactg gggccagggt accctgggtca ccgtgactag t 351

<210> 42
<211> 366
<212> DNA
<213> Artificial Sequence

<220>
<223> C4.4a binder

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ccagggaagg ggctggagtg ggtatcgggt gttagttgga atggcgctag gacgcactat 180
gcagactctg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240
ctacaaatga acagcctgag agccgaggac actgccgtgt attactgtgc gaagggcgac 300
tacctggttt actccgcata ctactttgac tcctggggcc aggggtaccct ggtcaccgtg 360
actagt 366

<210> 43
<211> 360
<212> DNA
<213> Artificial Sequence

<220>
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<400> 43
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ccagggaagg ggctggagtg ggtctcagct attagtggta gtgggttatag cacacactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcaaatga acagcctgag agccgaggac actgccgtgt attactgtgc gagactgcca 300
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<213> Artificial Sequence

<220>
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ccagggaagg ggctggagtg ggtctcagct attagtagta gtggtagtag cacatactac 180

c4c6aa138cea41cc8ed77499eb64c0e7.TXT

gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcaaatga acagcctgag agccgaggac actgccgtgt attactgtgc gagagaatct 300
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<210> 45
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<400> 45

Phe Ser Asn Ala Trp Met Ser Trp Val
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<210> 46
<211> 20
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<220>
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<400> 46

Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser
1 5 10 15

Val Lys Gly Arg
20

<210> 47
<211> 10
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<220>
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<400> 47

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr
1 5 10

<210> 48
<211> 14
<212> PRT
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<400> 48

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 49
 <211> 7
 <212> PRT
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<400> 49

Asp Asn Asn Lys Arg Pro Ser
 1 5

<210> 50
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<400> 50

Cys Ala Ala Trp Asp Asp Arg Leu Asn Gly Pro Val
 1 5 10

<210> 51
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<400> 51

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser

115

<210> 52
 <211> 113
 <212> PRT
 <213> Artificial Sequence

<220>
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<400> 52

Glu Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg
 85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

Gln

<210> 53
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
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<400> 53

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cctggcaagg gcctggaatg ggtgtcctac atcagcagca gcggcagcac catctactac	180
gccgacagcg tgaagggccg gttcaccatc agccgggaca acagcaagaa caccctgtac	240
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<211> 339
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 ctgcccggca ccgcccccaa gctgctgata tacgacaaca acaagcggcc tagcggcgtg 180
 cccgacagat tcagcggcag caagagcggc accagcgcca gcctggccat cagcggcctg 240
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<400> 55

Phe Ser Asn Ala Trp Met Ser Trp Val
 1 5

<210> 56
 <211> 20
 <212> PRT
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<220>
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<400> 56

Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 57
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 <212> PRT
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<220>
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<400> 57

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr
 1 5 10

<210> 58

<211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
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<400> 58

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
 1 5 10

<210> 59
 <211> 7
 <212> PRT
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<220>
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<400> 59

Asp Asn Asn Lys Arg Pro Ser
 1 5

<210> 60
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<400> 60

Cys Ala Ala Tyr Asp Asp Ser Leu Ser Gly Pro Val
 1 5 10

<210> 61
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 <212> PRT
 <213> Artificial Sequence

<220>
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<400> 61

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 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr

c4c6aa138cea41cc8ed77499eb64c0e7.TXT
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 62
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 62

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Tyr Asp Asp Ser
85 90 95

Leu Ser Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln

<210> 63
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 63

gaggtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg

60

c4c6aa138cea41cc8ed77499eb64c0e7.TXT
 tcctgcgccg ccagcggctt taccttctcc aacgcctgga tgtcctgggt ccgacaggcc 120
 cctggcaagg gactggaatg ggtgtcctac atctcctcct ccgggtccac catctactac 180
 gccgactccg tgaagggccg gttcaccatc tcccgggaca actccaagaa caccctgtac 240
 ctgcagatga actccctgcg ggccgaggac accgccgtgt actactgcgc ccgagagggc 300
 ctgtgggcct tcgattattg gggccagggc accctggcca ccgtcagctc a 351

<210> 64
 <211> 339
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 64
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 tcttgaccg gtcctccag caacatcggc gctggctacg tgggtgactg gtatcagcag 120
 ctgcccggca ccgccccaa gctgctgac tacgacaaca acaagcggcc ctccggcgtg 180
 cccgacagat tctccggctc caagtccggc acctccgcct ccctggccat ctccggcctg 240
 agatctgagg acgaggccga ctactactgc gccgcctacg acgactccct gtccggccct 300
 gtgttcggcg gaggcacaaa gttaaccgtg ctgggccag 339

<210> 65
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 65

Phe Ser Asn Ala Trp Met Ser Trp Val
 1 5

<210> 66
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 66

Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 67
 <211> 10
 <212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 67

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr
1 5 10

<210> 68

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 68

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 69

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 69

Asp Asn Asn Lys Arg Pro Ser
1 5

<210> 70

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 70

Cys Ala Ala Phe Asp Asp Ser Leu Asn Gly Pro Val
1 5 10

<210> 71

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 71

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala

20

25

30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser
 115

<210> 72

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 72

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Phe Asp Asp Arg
 85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

Gln

<210> 73
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 73
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 tcctgcgccg cctccggctt taccttctcc aacgcctgga tgcctgggt cgcacaggct 120
 cctggcaagg gcctggaatg ggtgtcctac atctcctcct ccggctccac catctactac 180
 gccgactccg tgaagggccg gttcaccatc tcccgggaca actccaagaa caccctgtac 240
 ctgcagatga actccctgcg ggccgaggac accgccgtgt actactgcgc ccgagagggc 300
 ctgtgggcct tcgataagtg gggccagggc accctgggtca ccgtcagctc a 351

<210> 74
 <211> 339
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 74
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 tcctgcaccg gtcctccag caacatcggc gctggctacg tgggtgactg gtatcagcag 120
 ctgcccggca ccgccccaa gctgctgac tacgacaaca acaagcggcc ctccggcgtg 180
 cccgacagat tctccggctc caagtccggc acctccgcct cctggccat ctccggcctg 240
 agatctgagg acgaggccga ctactactgc gccgccttcg acgaccggct gaacggccct 300
 gtgttcggcg gaggcacaaa gttaaccgtg ctgggccag 339

<210> 75
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 75
 Phe Ser Ser Ala Trp Met Ser Trp Val
 1 5

<210> 76
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 76

Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser
1 5 10 15

Val Lys Gly Arg
20

<210> 77
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 77

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr
1 5 10

<210> 78
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 78

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 79
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 79

Asp Asn Asn Lys Arg Pro Ser
1 5

<210> 80
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 80

Cys Ala Ala Tyr Asp Asp Ser Leu Ser Gly Pro Val
1 5 10

<210> 81
<211> 117
<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 81

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 82

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 82

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Tyr Asp Asp Ser
85 90 95

Leu Ser Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln

<210> 83
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 83
gaggtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg 60
tcctgcgccg ccagcggctt taccttctcc agcgcctgga tgcctgggt ccgacaggcc 120
cctggcaagg gactggaatg ggtgtcctac atctcctcct ccggctccac catctactac 180
gccgactccg tgaagggccg gttcaccatc tcccgggaca actccaagaa caccctgtac 240
ctgcagatga actccctgcg ggccgaggac accgccgtgt actactgcgc ccgagagggc 300
ctgtgggcct tcgattattg gggccagggc accctgggtca ccgtcagctc a 351

<210> 84
<211> 339
<212> DNA
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 84
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tcttgaccg gctcctccag caacatcggc gctggctacg tgggtgactg gtatcagcag 120
ctgcccggca ccgccccaa gctgctgac tacgacaaca acaagcggcc ctccggcgtg 180
cccgacagat tctccggctc caagtccggc acctccgcct ccctggccat ctccggcctg 240
agatctgagg acgaggccga ctactactgc gccgcctacg acgactccct gtccggccct 300
gtgttcggcg gaggcacaaa gttaaccgtg ctgggccag 339

<210> 85
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 85

Phe Ser Ser Ala Trp Met Ser Trp Val

1

5

<210> 86
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 86

Val Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 87
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 87

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys
 1 5 10

<210> 88
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 88

Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
 1 5 10

<210> 89
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 89

Asp Asn Asn Gln Arg Pro Ser
 1 5

<210> 90
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 90

Cys Ala Ala Phe Asp Asp Arg Leu Ser Gly Pro Val
1 5 10

<210> 91

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 91

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 92

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 92

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Phe Asp Asp Arg
85 90 95

Leu Ser Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln

<210> 93
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 93
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agctgtgccg ccagcggctt caccttcagc agcgcttga tgagctgggt ccgacaggcc 120
cctggcaagg gcctggaatg ggtgtcctac atcagcagca gcggcagcag cacctactac 180
gccgacagcg tgaagggccg gttcaccatc agccgggaca acagcaagaa caccctgtac 240
ctgcagatga acagcctgcg ggccgaggac accgccgtgt actactgcgc cagagaaggc 300
ctgtgggcct tcgataagtg gggccagggc accctgggtca ccgtcagctc a 351

<210> 94
<211> 339
<212> DNA
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 94
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agctgcagcg gcagcagcag caacatcgga gccggctacg tgggtgactg gtatcagcag 120
ctgcccggca ccgccccaa gctgctgatc tacgacaaca accagcggcc cagcggcgtg 180
cccagacagat tttccggcag caagagcggc accagcgcca gcctggccat cagcggcctg 240
agaagcgagg acgaggccga ctactactgc gccgccttcg acgacagact gagcggccct 300
gtgttcggcg gaggcacaaa gttaaccgtg ctgggccag 339

<210> 95
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 95

Phe Ser Asp Tyr Gln Met Thr Trp Ile
 1 5

<210> 96
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 96

Val Ser Gly Val Ser Trp Asn Gly Ala Arg Thr His Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 97
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 97

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ala Tyr Tyr Phe Asp Ser
 1 5 10 15

<210> 98
 <211> 13
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 98

Ser Gly Ser Ser Ser Asn Val Gly Ser Asn Pro Val Asn
 1 5 10

<210> 99
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 99

Arg Asn Asn Gln Arg Pro Ser
1 5

<210> 100

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 100

Cys Ala Ala Trp Asp Asp Arg Leu Asn Gly Trp Val
1 5 10

<210> 101

<211> 122

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 101

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ala Tyr Tyr Phe Asp Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 102

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 102

Glu Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
85 90 95

Asn Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

<210> 103

<211> 366

<212> DNA

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 103

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tcttgcgccg ccagcggcctt caccttcagc gactaccaga tgacctggat ccgacagacc 120
cctggcaagg gcctggaatg ggtgtccggc atcagctgga acggaggcag caccactac 180
gccgacagcg tgaagggccg gttcaccatc agccgggaca acagcaagaa caccctgtac 240
ctgcagatga acagcctgcg ggccgaggac accgccgtgt actactgcgc caagggcgac 300
tacctggtgt acagcgccta ctacttcgac agctggggcc agggcaccct ggtcaccgtg 360
tctagc 366

<210> 104

<211> 336

<212> DNA

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 104

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agctgctctg gcagcagcag caacatcgga agcaaccccg tgaactggta tcagcagctg	120
cccggcaccg cccccaagct gctgatctac cggaacaacc agcggcctag cggcgtgccc	180
gacagattca gcggcagcaa gagcggcacc agcgccagcc tggccatcag cggcctgaga	240
agcgaggacg aggccgacta ctactgcgcc gcctgggacg acagactgaa cggctggggc	300
ttcggcggag gcaccaagct gaccgtgctg ggacag	336

<210> 105
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 105

Phe Ser Asp Tyr Gln Met Thr Trp Ile
1 5

<210> 106
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 106

Val Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser
1 5 10 15

Val Lys Gly Arg
20

<210> 107
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 107

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser
1 5 10 15

<210> 108
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 108

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn

1

5

10

<210> 109
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 109

Arg Asn Asn Gln Arg Pro Ser
 1 5

<210> 110
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 110

Cys Ala Ala Trp Asp Asp Arg Leu Ser Gly Trp Ala
 1 5 10

<210> 111
 <211> 122
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 111

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
 20 25 30

Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser Trp
 100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 112
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder
<400> 112

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
85 90 95

Ser Gly Trp Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

<210> 113
<211> 366
<212> DNA
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 113
gaggtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg 60
tcctgcgccg cctccggctt caccttctcc gactaccaga tgacctggat cagacagacc 120
cccggcaagg gcctggaatg ggtgtccggc atctcctgga acggcggctc caccactac 180
gccgactctg tgaagggccg gttcaccatc tcccgggaca actccaagaa caccctgtac 240
ctgcagatga actccctgcg ggccgaggac accgccgtgt actactgcgc caagggcgac 300
tacctggtgt actcctccta ctacttcaag tcctggggcc agggcaccct ggtcaccgtc 360
agctca 366

<210> 114

<211> 336
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 114
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 cccggcaccg cccccaagct gctgatctac cggaacaacc agcggccctc cggcgtgccc 180
 gacagattct ccggctcaa gtccggcacc tccgcctccc tggccatctc cggcctgaga 240
 tctgaggacg aggccgacta ctactgcgcc gcctgggacg accggctgtc tggctgggct 300
 tttggcggcg gaacaaagtt aaccgtgctg ggccag 336

<210> 115
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 115
 Phe Ser Asp Tyr Gln Met Thr Trp Ile
 1 5

<210> 116
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 116
 Val Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 117
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 117
 Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser
 1 5 10 15

<210> 118

<211> 13
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 118

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn
 1 5 10

<210> 119
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 119

Arg Asn Asn Gln Arg Pro Ser
 1 5

<210> 120
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 120

Cys Ala Ala Trp Asp Asp Ser Leu Ser Gly Trp Ala
 1 5 10

<210> 121
 <211> 122
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 121

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
 20 25 30

Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr

65

70

75

80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser Trp
 100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 122

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 122

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
 20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
 85 90 95

Ser Gly Trp Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
 100 105 110

<210> 123

<211> 366

<212> DNA

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 123

gaggtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg 60

tcctgcgccg cctccggctt caccttctcc gactaccaga tgacctggat cagacagacc 120

cccggcaagg gcctggaatg ggtgtccggc atctcctgga acggcggctc caccactac 180

c4c6aa138cea41cc8ed77499eb64c0e7.TXT

gccgactctg tgaagggccg gttcaccatc tcccgggaca actccaagaa caccctgtac 240
 ctgcagatga actccctgcg ggccgaggac accgccgtgt actactgcfg caagggcgac 300
 tacctggtgt acaagtccta ctacttcaag tcctggggcc agggcaccct ggtcaccgtc 360
 agtca 366

<210> 124
 <211> 336
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 124
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 tcctgctccg gtcctctc caacatcggc tccaaccccg tgaactggta tcagcagctg 120
 cccggcaccg cccccaagct gctgatctac cggaacaacc agcggccctc cggcgtgccc 180
 gacagattct ccggctcaa gtccggcacc tccgcctccc tggccatctc cggcctgaga 240
 tctgaggacg aggccgacta ctactgcgcc gcctgggacg actccctgtc tggctgggct 300
 tttggcggcg gaacaaagtt aaccgtgctg ggccag 336

<210> 125
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 125

Phe Ser Asp Tyr Gln Met Thr Trp Ile
 1 5

<210> 126
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 126

Val Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 127
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 127

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser
1 5 10 15

<210> 128

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 128

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn
1 5 10

<210> 129

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 129

Arg Asn Asn Gln Arg Pro Ser
1 5

<210> 130

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 130

Cys Ala Ala Trp Asp Asp Arg Leu Ser Gly Trp Gly
1 5 10

<210> 131

<211> 122

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 131

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

c4c6aa138cea41cc8ed77499eb64c0e7.TXT

Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 132
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 132

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
85 90 95

Ser Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

<210> 133
<211> 366
<212> DNA
<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 133

gaggtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg 60
 tcctgcgccg cctccggctt caccttctcc gactaccaga tgacctggat cagacagacc 120
 cccggcaagg gcctggaatg ggtgtccggc atctcctgga acggcggctc caccactac 180
 gccgactctg tgaagggccg gttcaccatc tcccgggaca actccaagaa caccctgtac 240
 ctgcagatga actccctgcg ggccgaggac accgccgtgt actactgcgc caagggcgac 300
 tacctggtgt actcctccta ctacttcaag tcctggggcc agggcacctt ggtcaccgtc 360
 agtca 366

<210> 134

<211> 336

<212> DNA

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 134

cagtccgtgc tgaccagcc tccttccgcc tctggcacc cttggccagag agtgaccatc 60
 tcctgctccg gtcctctc caacatcggc tccaaccccg tgaactggta tcagcagctg 120
 cccggcaccg cccccaagct gctgatctac cggaacaacc agcgccctc cggcgtgccc 180
 gacagattct ccggctcaa gtccggcacc tccgcctccc tggccatctc cggcctgaga 240
 tctgaggacg aggccgacta ctactgcgcc gcctgggacg accggctgtc tggctgggga 300
 tttggcggcg gaacaaagtt aaccgtgctg ggccag 336

<210> 135

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 135

Phe Ser Ser Tyr Gln Met Thr Trp Ile
 1 5

<210> 136

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 136

Val Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
20

<210> 137
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 137

Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser
1 5 10 15

<210> 138
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 138

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn
1 5 10

<210> 139
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 139

Arg Asn Asn Gln Arg Pro Ser
1 5

<210> 140
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 140

Cys Ala Ala Trp Asp Asp Ser Leu Ser Gly Trp Ala
1 5 10

<210> 141
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 141

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gln Met Thr Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 142

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 142

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
85 90 95

Ser Gly Trp Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

<210> 143
<211> 366
<212> DNA
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 143
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agctgtgccg ccagcggcctt caccttcagc agctaccaga tgacctggat cagacaggcc 120
cctggcaagg gcctggaatg ggtgtccggc atcagctgga acggcggcag caccactac 180
gccgacagcg tgaagggccg gttcaccatc agccgggaca acagcaagaa caccctgtac 240
ctgcagatga acagcctgcg ggccgaggac accgccgtgt actactgcgc caagggcgac 300
tacctggtgt acaagagcta ctacttcaag agctggggcc agggcacact ggtcaccgtc 360
agctca 366

<210> 144
<211> 336
<212> DNA
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 144
cagagcgtgc tgaccagcc tcctagcgcc tctggcacc ctggccagag agtgaccatc 60
agctgcagcg gcagcagcag caacatcggc agcaaccccg tgaactggta tcagcagctg 120
cccggcaccg cccccaagct gctgatctac cggaacaacc agcggcccag cggcgtgccc 180
gacagatttt ccggcagcaa gagcggcacc agcggccagc tggccatcag cggcctgaga 240
agcgaggacg aggccgacta ctactgcgcc gcctgggacg atagcctgag cggctggggc 300
tttggcggcg gaacaaagtt aaccgtgctg ggccag 336

<210> 145
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 145
Phe Ser Asn Ala Trp Met Ser Trp Val
1 5

<210> 146
<211> 20
<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 146

Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser
1 5 10 15

Val Lys Gly Arg
20

<210> 147

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 147

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys
1 5 10

<210> 148

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 148

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 149

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 149

Asp Asn Asn Lys Arg Pro Ser
1 5

<210> 150

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 150

Cys Ala Ala Tyr Asp Asp Ser Leu Lys Gly Pro Val

1

5

10

<210> 151
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 151

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Thr Ser
 115

<210> 152
 <211> 113
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 152

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe

50

55

60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Tyr Asp Asp Ser
 85 90 95

Leu Lys Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

Gln

<210> 153
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 153

Phe Ser Asn Ala Trp Met Ser Trp Val
 1 5

<210> 154
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 154

Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 155
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 155

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys
 1 5 10

<210> 156
 <211> 14
 <212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 156

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 157

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 157

Asp Asn Asn Lys Arg Pro Ser
1 5

<210> 158

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 158

Cys Ala Ala Phe Asp Asp Ser Leu Asn Gly Pro Val
1 5 10

<210> 159

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 159

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Thr Ser
115

<210> 160
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 160

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Phe Asp Asp Ser
85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln

<210> 161
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 161

Phe Ser Asn Ala Trp Met Ser Trp Val
1 5

<210> 162
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 162

Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 163
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 163

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys
 1 5 10

<210> 164
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 164

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
 1 5 10

<210> 165
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 165

Asp Asn Asn Lys Arg Pro Ser
 1 5

<210> 166
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 166

Cys Ala Ala Tyr Asp Asp Ser Leu Ser Gly Pro Val
1 5 10

<210> 167

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 167

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Thr Ser
115

<210> 168

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 168

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu

35

40

45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Phe Asp Asp Ser
 85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

Gln

<210> 169
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 169

Phe Ser Ser Ala Trp Met Ser Trp Val
 1 5

<210> 170
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 170

Val Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 171
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 171

Ala Arg Glu Gly Leu Trp Ala Phe Asp Trp
 1 5 10

<210> 172
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 172

Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
 1 5 10

<210> 173
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 173

Asp Asn Asn Gln Arg Pro Ser
 1 5

<210> 174
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 174

Cys Ala Ala Tyr Asp Asp Ser Leu Ser Gly Pro Val
 1 5 10

<210> 175
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 175

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Ala
 20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Trp Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Thr Ser
115

<210> 176
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 176

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Tyr Asp Asp Ser
85 90 95

Leu Ser Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln

<210> 177
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 177

Phe Ser Ser Ala Trp Met Ser Trp Val
1 5

<210> 178
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 178

Val Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
1 5 10 15

Val Lys Gly Arg
20

<210> 179
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 179

Ala Arg Glu Gly Leu Trp Ala Phe Asp Asn
1 5 10

<210> 180
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 180

Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 181
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 181

Asp Asn Asn Gln Arg Pro Ser
1 5

<210> 182
<211> 12
<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 182

Cys Ala Ala Tyr Asp Asp Ser Leu Asn Gly Pro Val
1 5 10

<210> 183

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 183

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Thr Ser
115

<210> 184

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 184

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly

20

25

30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45

Leu Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Tyr Asp Asp Ser
 85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

Gln

<210> 185
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 185

Phe Ser Ser Ala Trp Met Ser Trp Val
 1 5

<210> 186
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 186

Val Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 187
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 187

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr
1 5 10

<210> 188
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 188

Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 189
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 189

Asp Asn Asn Gln Arg Pro Ser
1 5

<210> 190
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 190

Cys Ala Ala Trp Asp Asp Arg Leu Asn Gly Pro Val
1 5 10

<210> 191
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 191

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 192
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 192

Glu Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg
85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln

<210> 193
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 193

Phe Thr Phe Ser Asn Ala Trp Met Ser Trp Val
1 5 10

<210> 194

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 194

Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser
1 5 10 15

Val Lys Gly Arg
20

<210> 195

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 195

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr
1 5 10

<210> 196

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 196

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 197

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 197

Asp Asn Asn Lys Arg Pro Ser
1 5

<210> 198
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 198

Cys Ala Ala Tyr Asp Asp Arg Leu Asn Gly Pro Val
 1 5 10

<210> 199
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 199

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser
 115

<210> 200
 <211> 113
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 200

Glu Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln

1 5 10 15
 Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30
 Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45
 Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60
 Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80
 Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Tyr Asp Asp Arg
 85 90 95
 Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

Gln

<210> 201
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 201

Phe Thr Phe Ser Ser Ala Trp Met Ser Trp Val
 1 5 10

<210> 202
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 202

Val Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 203
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 203

Ala Arg Glu Gly Leu Trp Ala Phe Asp Gly
1 5 10

<210> 204

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 204

Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 205

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 205

Asp Asn Asn Gln Arg Pro Ser
1 5

<210> 206

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 206

Cys Ala Ala Tyr Asp Asp Ser Leu Asn Arg Pro Val
1 5 10

<210> 207

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 207

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Gly Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 208
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 208

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Tyr Asp Asp Ser
85 90 95

Leu Asn Arg Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln

<210> 209

<211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 209

Phe Thr Phe Ser Asn Ala Trp Met Ser Trp Val
 1 5 10

<210> 210
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 210

Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 211
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 211

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr
 1 5 10

<210> 212
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 212

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
 1 5 10

<210> 213
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 213

Asp Asn Asn Lys Arg Pro Ser
1 5

<210> 214
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 214

Cys Ala Ala Phe Asp Asp Ser Leu Asn Gly Pro Val
1 5 10

<210> 215
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 215

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 216
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 216

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30
 Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45
 Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60
 Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80
 Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Phe Asp Asp Ser
 85 90 95
 Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110
 Gln

<210> 217

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 217

Phe Ser Ser Ala Trp Met Ser Trp Val
 1 5

<210> 218

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 218

Val Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 219

<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 219

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys
1 5 10

<210> 220
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 220

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 221
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 221

Asp Asn Asn Lys Arg Pro Ser
1 5

<210> 222
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 222

Cys Ala Ala Phe Asp Asp Ser Leu Asn Gly Pro Val
1 5 10

<210> 223
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 223

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Thr Ser
115

<210> 224
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 224

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Phe Asp Asp Ser
85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln

<210> 225
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 225

Phe Thr Phe Ser Ser Ala Trp Met Ser Trp Val
 1 5 10

<210> 226
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 226

Val ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 227
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 227

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys
 1 5 10

<210> 228
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 228

Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
 1 5 10

<210> 229
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 229

Asp Asn Asn Gln Arg Pro Ser
1 5

<210> 230

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 230

Cys Ala Ala Phe Asp Asp Ser Leu Asn Gly Pro Val
1 5 10

<210> 231

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 231

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 232

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 232

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Phe Asp Asp Ser
85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln

<210> 233

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 233

Phe Thr Phe Ser Ser Ala Trp Met Ser Trp Val
1 5 10

<210> 234

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 234

Val Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
1 5 10 15

Val Lys Gly Arg

<210> 235
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 235

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys
 1 5 10

<210> 236
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 236

Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
 1 5 10

<210> 237
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 237

Asp Asn Asn Gln Arg Pro Ser
 1 5

<210> 238
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 238

Cys Ala Ala Phe Asp Asp Arg Leu Ser Gly Pro Val
 1 5 10

<210> 239
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 239

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 240
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 240

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Phe Asp Asp Arg
85 90 95

Leu Ser Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly

100

105

110

Gln

<210> 241
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 241

Phe Ser Asp Tyr Gln Met Thr Trp Ile
 1 5

<210> 242
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 242

Val Ser Gly Val Ser Trp Asn Gly Ala Arg Thr His Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 243
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 243

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser
 1 5 10 15

<210> 244
 <211> 13
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 244

Ser Gly Ser Ser Ser Asn Val Gly Ser Asn Pro Val Asn
 1 5 10

<210> 245

<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 245

Arg Asn Asn Gln Arg Pro Ser
1 5

<210> 246
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 246

Cys Ala Ala Trp Asp Asp Arg Leu Asn Gly Trp Thr Gly
1 5 10

<210> 247
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 247

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Val Ser Trp Asn Gly Ala Arg Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Thr Ser
115 120

<210> 248
 <211> 112
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 248

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Val Gly Ser Asn
 20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
 85 90 95

Asn Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
 100 105 110

<210> 249
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 249

Phe Thr Phe Ser Ser Tyr Gln Met Thr Trp Ile
 1 5 10

<210> 250
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 250

Val Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg

20

<210> 251
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 251

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ala Tyr Tyr Phe Asp Ser
 1 5 10 15

<210> 252
 <211> 13
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 252

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn
 1 5 10

<210> 253
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 253

Arg Asn Asn Gln Arg Pro Ser
 1 5

<210> 254
 <211> 12
 <212> PRT
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<220>
 <223> C4.4a binder

<400> 254

Cys Ala Ala Trp Asp Asp Arg Leu Asn Gly Trp Gly
 1 5 10

<210> 255
 <211> 122
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 255

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ala Tyr Tyr Phe Asp Ser Trp
 100 105 110
 Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 256
 <211> 112
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 256

Glu Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
 20 25 30
 Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80
 Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
 85 90 95
 Asn Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln

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105 110

<210> 257
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
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<400> 257

Phe Thr Phe Ser Asp Tyr Gln Met Thr Trp Ile
1 5 10

<210> 258
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 258

Val Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser
1 5 10 15

Val Lys Gly Arg
20

<210> 259
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 259

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Tyr
1 5 10 15

<210> 260
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 260

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn
1 5 10

<210> 261
<211> 7
<212> PRT
<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 261

Arg Asn Asn Gln Arg Pro Ser
1 5

<210> 262

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 262

Cys Ala Ala Trp Asp Asp Arg Leu Asn Gly Trp Ala
1 5 10

<210> 263

<211> 122

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 263

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 264

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 264

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
85 90 95

Asn Gly Trp Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

<210> 265

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 265

Phe Thr Phe Ser Asp Tyr Gln Met Thr Trp Ile
1 5 10

<210> 266

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 266

Val Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser
1 5 10 15

Val Lys Gly Arg
20

<210> 267

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<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 267

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser
1 5 10 15

<210> 268
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 268

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn
1 5 10

<210> 269
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 269

Arg Asn Asn Gln Arg Pro Ser
1 5

<210> 270
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 270

Cys Ala Ala Trp Asp Asp Ser Leu Asn Gly Trp Gly
1 5 10

<210> 271
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 271

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 272
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder
<400> 272

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
85 90 95

Asn Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

<210> 273

<211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 273

Phe Thr Phe Ser Asp Tyr Gln Met Thr Trp Ile
 1 5 10

<210> 274
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 274

Val Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser
 1 5 10 15

Val Lys Gly Arg
 20

<210> 275
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 275

Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser
 1 5 10 15

<210> 276
 <211> 13
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 276

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn
 1 5 10

<210> 277
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 277

Arg Asn Asn Gln Arg Pro Ser
1 5

<210> 278
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder
<400> 278

Cys Ala Ala Trp Asp Asp Arg Leu Ser Gly Trp Gly
1 5 10

<210> 279
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder
<400> 279

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 280
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 280

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
 20 25 30
 Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80
 Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
 85 90 95
 Ser Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
 100 105 110

<210> 281
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 281

Phe Thr Phe Ser Asp Tyr Gln Met Thr Trp Ile
 1 5 10

<210> 282
 <211> 20
 <212> PRT
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<220>
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<400> 282

Val Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser
 1 5 10 15
 Val Lys Gly Arg
 20

<210> 283
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 283

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser
1 5 10 15

<210> 284

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 284

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn
1 5 10

<210> 285

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 285

Arg Asn Asn Gln Arg Pro Ser
1 5

<210> 286

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 286

Cys Ala Ala Trp Asp Asp Arg Leu Ser Gly Trp Gly
1 5 10

<210> 287

<211> 122

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 287

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 288
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 288

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
85 90 95

Ser Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

<210> 289
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 289

Phe Thr Phe Ser Asp Tyr Gln Met Thr Trp Ile
1 5 10

<210> 290

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 290

Val Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser
1 5 10 15

Val Lys Gly Arg
20

<210> 291

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 291

Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser
1 5 10 15

<210> 292

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 292

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn
1 5 10

<210> 293

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> C4.4a binder

<400> 293

Arg Asn Asn Gln Arg Pro Ser
1 5

<210> 294
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 294

Cys Ala Ala Trp Asp Asp Ser Leu Asn Gly Trp Gly
 1 5 10

<210> 295
 <211> 122
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 295

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
 20 25 30

Gln Met Thr Trp Ile Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser Trp
 100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 296
 <211> 112
 <212> PRT
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<400> 296

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln

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1					5					10					15				
Arg	Val	Thr	Ile	Ser	Cys	Ser	Gly	Ser	Ser	Ser	Asn	Ile	Gly	Ser	Asn				
			20							25				30					
Pro	Val	Asn	Trp	Tyr	Gln	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu	Leu				
		35					40					45							
Ile	Tyr	Arg	Asn	Asn	Gln	Arg	Pro	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser				
		50					55					60							
Gly	Ser	Lys	Ser	Gly	Thr	Ser	Ala	Ser	Leu	Ala	Ile	Ser	Gly	Leu	Arg				
65							70				75								
Ser	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Ala	Ala	Trp	Asp	Asp	Ser	Leu				
						85				90				95					
Asn	Gly	Trp	Gly	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Thr	Val	Leu	Gly	Gln				
			100							105				110					

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<210> 297
<211> 9
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<223> consensus sequence for M20 D02 S-A derived CDR H1
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<220>
<221>  VARIANT
<222>  (3)..(3)
<223>  X = D or S
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<400> 297

Phe Ser Xaa Tyr Gln Met Thr Trp Ile
1 5

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<210> 298
<211> 20
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<213> Artificial sequence

<220>
<223> consensus sequence for M20 D02 S-A derived CDRH2
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<220>
<221>  VARIANT
<222>  (4)..(4)
<223>  X = Y or I

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<220>
<221>  VARIANT
<222>  (9)..(9)
<223>  X = A or G
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<220>
<221> VARIANT

<222> (10)..(10)

<223> X = R or S

<400> 298

Val	Ser	Gly	Xaa	Ser	Trp	Asn	Gly	Xaa	Xaa	Thr	His	Tyr	Ala	Asp	Ser
1				5				10						15	

Val	Lys	Gly	Arg
			20

<210> 299

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> consensus for M20 D02 S-A derived CDRH3

<220>

<221> VARIANT

<222> (9)..(9)

<223> X = S, K, or R

<220>

<221> VARIANT

<222> (10)..(10)

<223> X = A, S or R

<220>

<221> VARIANT

<222> (14)..(14)

<223> X = D, K, E or R

<220>

<221> VARIANT

<222> (15)..(15)

<223> X = S or Y

<400> 299

Ala	Lys	Gly	Asp	Tyr	Leu	Val	Tyr	Xaa	Xaa	Tyr	Tyr	Phe	Xaa	Xaa
1				5				10					15	

<210> 300

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> consensus sequence derived from M20 D02 S-A CDRL1

<220>

<221> VARIANT

<222> (7)..(7)

<223> X = V or I

<400> 300

Ser	Gly	Ser	Ser	Ser	Asn	Xaa	Gly	Ser	Asn	Pro	Val	Asn
1				5				10				

<210> 301
 <211> 12
 <212> PRT
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<220>
 <223> consensus sequence derived from M20 D02 S-A derived CDR L3

<220>
 <221> VARIANT
 <222> (3)..(3)
 <223> X = A, Q or R

<220>
 <221> VARIANT
 <222> (5)..(5)
 <223> X = D or G

<220>
 <221> VARIANT
 <222> (7)..(7)
 <223> X = R or S

<220>
 <221> VARIANT
 <222> (9)..(9)
 <223> X = N, W or S

<220>
 <221> VARIANT
 <222> (12)..(12)
 <223> X = V, A or G

<400> 301

Cys Ala Xaa Trp Xaa Asp Xaa Leu Xaa Gly Trp Xaa
 1 5 10

<210> 302
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> consensus sequence derived from M31 B01 CDR H1

<220>
 <221> VARIANT
 <222> (3)..(3)
 <223> X = N or S

<220>
 <221> VARIANT
 <222> (4)..(4)
 <223> X = A or Y

<400> 302

Phe Ser Xaa Xaa Trp Met Ser Trp Val
 1 5

<210> 303
 <211> 20
 <212> PRT

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<213> Artificial Sequence

<220>

<223> consensus sequence derived from M31 B01 CDR H2

<220>

<221> VARIANT

<222> (10)..(10)

<223> X = T or S

<220>

<221> VARIANT

<222> (11)..(11)

<223> X = I or T

<400> 303

Val Ser Tyr Ile Ser Ser Ser Gly Ser Xaa Xaa Tyr Tyr Ala Asp Ser
1 5 10 15

Val Lys Gly Arg
20

<210> 304

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> consensus sequence derived from M31 B01 CDR H3

<220>

<221> VARIANT

<222> (10)..(10)

<223> X = Y, K, G, W or N

<400> 304

Ala Arg Glu Gly Leu Trp Ala Phe Asp Xaa
1 5 10

<210> 305

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> consensus sequence derived from M31 B01 CDR L1

<220>

<221> VARIANT

<222> (1)..(1)

<223> X = T or S

<400> 305

Xaa Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 306

<211> 7

<212> PRT
<213> Artificial Sequence

<220>
<223> consensus sequence derived from M31 B01 CDR L2

<220>
<221> VARIANT
<222> (4)..(4)
<223> X = K or Q

<400> 306

Asp Asn Asn Xaa Arg Pro Ser
1 5

<210> 307
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> consensus sequence derived from M31 B01 CDR L3

<220>
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<222> (4)..(4)
<223> X = W, E, F or Y

<220>
<221> VARIANT
<222> (7)..(7)
<223> X = R, S or M

<220>
<221> VARIANT
<222> (9)..(9)
<223> X = N, K or S

<220>
<221> VARIANT
<222> (10)..(10)
<223> X = G or R

<220>
<221> VARIANT
<222> (11)..(11)
<223> X = P or A

<400> 307

Cys Ala Ala Xaa Asp Asp Xaa Leu Xaa Xaa Xaa Val
1 5 10

<210> 308
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> C4.4a binder

<400> 308
gaggtgcagc tgctggagag cggggggggg ctggtgcagc cggggggggag cctgcgcctg

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ccgggggaaag	ggctggagt	ggtgagctat	attagcagca	gcgggagcac	catttattat	180
gcggatagcg	tgaaagggcg	ctttaccatt	agccgcgata	acagcaaaaa	caccctgtat	240
ctgcagatga	acagcctgcg	cgcgaggat	accgcggtgt	attattgcgc	gcgcgagggg	300
ctgtgggcgt	ttgataaatg	ggggcagggg	accctggtga	ccgtgaccag	c	351

<210> 309
 <211> 339
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 309	
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agctgcaccg	ggagcagcag caacattggg gcggggatatg tgggtgcattg gtatcagcag 120
ctgccgggga	ccgcgccgaa actgctgatt tatgataaca acaaacgccc gagcgggggtg 180
ccggatcgct	ttagcgggag caaaagcggg accagcgcga gcctggcgat tagcgggctg 240
cgagcgcagg	atgaggcgga ttattattgc gcggcgatatg atgatagcct gaaagggccg 300
gtgtttgggg	gggggaccaa actgaccgtg ctggggcag 339

<210> 310
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 310	
gaggtgcagc	tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgcctg 60
agctgcgcg	cgagcgggtt tacctttagc aacgcgtgga tgagctgggt gcgccaggcg 120
ccgggggaaag	ggctggagt ggtgagctat attagcagca gcgggagcac catttattat 180
gcggatagcg	tgaaagggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
ctgcagatga	acagcctgcg cgcgaggat accgcggtgt attattgcgc gcgcgagggg 300
ctgtgggcgt	ttgataaatg ggggcagggg accctggtga ccgtgaccag c 351

<210> 311
 <211> 339
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 311	
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agctgcaccg	ggagcagcag caacattggg gcggggatatg tgggtgcattg gtatcagcag 120

ctgccgggga ccgcgccgaa actgctgatt tatgataaca acaaacgccc gagcggggtg 180
 ccggatcgct ttagcgggag caaaagcggg accagcgca gcctggcgat tagcgggctg 240
 cgcagcgagg atgaggcgga ttattattgc gcggcgtttg atgatagcct gaacgggccc 300
 gtgtttgggg gggggaccaa actgaccgtg ctggggcag 339

<210> 312
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 312
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgccctg 60
 agctgcgcgg cgagcggggt taccttttagc aacgcgtgga tgagctgggt gcgccaggcg 120
 ccggggaaag ggctggagtg ggtgagctat attagcagca gcgggagcac catttattat 180
 gcggatagcg tgaaaggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcggaggat accgcggtgt attattgcgc gcgcgagggg 300
 ctgtgggcgt ttgataaatg ggggcagggg accctggtga ccgtgaccag c 351

<210> 313
 <211> 339
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 313
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 agctgcaccg ggagcagcag caacattggg gcggggatatg tgggtgcattg gtatcagcag 120
 ctgccgggga ccgcgccgaa actgctgatt tatgataaca acaaacgccc gagcggggtg 180
 ccggatcgct ttagcgggag caaaagcggg accagcgca gcctggcgat tagcgggctg 240
 cgcagcgagg atgaggcgga ttattattgc gcggcgtttg atgatagcct gaacgggccc 300
 gtgtttgggg gggggaccaa actgaccgtg ctggggcag 339

<210> 314
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 314
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgccctg 60
 agctgcgcgg cgagcggggt taccttttagc agcgcgtgga tgagctgggt gcgccaggcg 120
 ccggggaaag ggctggagtg ggtgagctat attagcagca gcgggagcag cacctattat 180

gcggatagcg tgaaaggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcgaggat accgcggtgt attattgcgc gcgcgagggg 300
 ctgtgggcgt ttgattggtg ggggcagggg accctggtga ccgtgaccag c 351

<210> 315
 <211> 339
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 315
 cagagcgtgc tgaccagcc gccgagcgcg agcgggaccc cggggcagcg cgtgaccatt 60
 agctgcagcg ggagcagcag caacattggg gcggggtatg tggatgcattg gtatcagcag 120
 ctgccgggga ccgcgccgaa actgctgatt tatgataaca accagcgcgc gagcgggggtg 180
 ccggatcgct ttagcgggag caaaagcggg accagcgcga gcctggcgat tagcgggctg 240
 cgagcagagg atgaggcgga ttattattgc gcggcgatg atgatagcct gagcggggccg 300
 gtgtttgggg gggggaccaa actgaccgtg ctggggcag 339

<210> 316
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 316
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgcctg 60
 agctgcgcgg cgagcggggt tacctttagc aacgcgtgga tgagctgggt gcgccaggcg 120
 ccggggaaag ggctggagt ggtgagctat attagcagca gcgggagcac catttattat 180
 gcggatagcg tgaaaggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcgaggat accgcggtgt attattgcgc gcgcgagggg 300
 ctgtgggcgt ttgattattg ggggcagggg accctggtga ccgtgaccag c 351

<210> 317
 <211> 339
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 317
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 agctgcagcg ggagcagcag caacattggg gcggggtatg tggatgcattg gtatcagcag 120
 ctgccgggga ccgcgccgaa actgctgatt tatgataaca accagcgcgc gagcgggggtg 180
 ccggatcgct ttagcgggag caaaagcggg accagcgcga gcctggcgat tagcgggctg 240

cgagcgagg atgaggcgga ttattattgc gcggcgatg atgatagcct gaacgggccg 300
 gtgtttgggg gggggaccaa actgaccgtg ctggggcag 339

<210> 318
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 318
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgccctg 60
 agctgcgcgg cgagcggggt taccttttagc agcgcgtgga tgagctgggt gcgccaggcg 120
 ccggggaaag ggctggagt ggtgagctat attagcagca gcgggagcag cacctattat 180
 gcggatagcg tgaaagggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcggaggat accgcggtgt attattgcgc gcgcgagggg 300
 ctgtgggcgt ttgattattg ggggcagggg accctggtga ccgtgagcag c 351

<210> 319
 <211> 339
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 319
 gagagcgtgc tgaccagcc gccgagcgcg agcgggaccc cggggcagcg cgtgaccatt 60
 agctgcagcg ggagcagcag caacattggg gcggggatg tggtgcattg gtatcagcag 120
 ctgccgggga ccgcgccgaa actgctgatt tatgataaca accagcggcc gagcgggggtg 180
 ccggatcgct ttagcgggag caaaagcggg accagcgcga gcctggcgat tagcgggctg 240
 cgagcgagg atgaggcgga ttattattgc gcggcgatg atgatgcct gaacgggccg 300
 gtgtttgggg gggggaccaa actgaccgtg ctggggcag 339

<210> 320
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 320
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgccctg 60
 agctgcgcgg cgagcggggt taccttttagc aacgcgtgga tgagctgggt gcgccaggcg 120
 ccggggaaag ggctggagt ggtgagctat attagcagca gcgggagcac catttattat 180
 gcggatagcg tgaaagggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcggaggat accgcggtgt attattgcgc gcgcgagggg 300

ctgtgggcgt ttgattattg ggggcagggg accctggtga ccgtgagcag c 351

<210> 321
 <211> 339
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 321
 gagagcgtgc tgaccagcc gccgagcgtg agcggggcgc cggggcagcg cgtgaccatt 60
 agctgcaccg ggagcagcag caacattggg gcggggatatg tggatgcattg gtatcagcag 120
 ctgccgggga ccgcgccgaa actgctgatt tatgataaca acaaacgccc gagcgggggtg 180
 ccggatcgct ttagcgggag caaaagcggg accagcgcga gcctggcgat tagcgggctg 240
 cgcagcgagg atgaggcgga ttattattgc gcggcgatatg atgatgcct gaacggggccg 300
 gtgtttgggg gggggaccaa actgaccgtg ctggggcag 339

<210> 322
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 322
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgcctg 60
 agctgcgcgg cgagcgggtt tacctttagc agcgcgtgga tgagctgggt gcgccaggcg 120
 ccggggaaag ggctggagt ggtgagctat attagcagca gcgggagcag cacctattat 180
 gcggatagcg tgaaagggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcggaggat accgcggtgt attattgcgc gcgcgagggg 300
 ctgtgggcgt ttgatgggtg ggggcagggg accctggtga ccgtgagcag c 351

<210> 323
 <211> 339
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 323
 cagagcgtgc tgaccagcc gccgagcgcg agcgggaccc cggggcagcg cgtgaccatt 60
 agctgcagcg ggagcagcag caacattggg gcggggatatg tggatgcattg gtatcagcag 120
 ctgccgggga ccgcgccgaa actgctgatt tatgataaca accagcgccc gagcgggggtg 180
 ccggatcgct ttagcgggag caaaagcggg accagcgcga gcctggcgat tagcgggctg 240
 cgcagcgagg atgaggcgga ttattattgc gcggcgatatg atgatgcct gaaccgcccg 300
 gtgtttgggg gggggaccaa actgaccgtg ctggggcag 339

<210> 324
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 324
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgccctg 60
 agctgcgcg cgagcgggtt taccttttagc aacgcgtgga tgagctgggt gcgccaggcg 120
 ccgggggaaag ggctggagtg ggtgagctat attagcagca gcgggagcac catttattat 180
 gcggatagcg tgaaagggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcggaggat accgcggtgt attattgcgc gcgcgagggg 300
 ctgtgggcgt ttgattattg ggggcagggg accctggtga ccgtgagcag c 351

<210> 325
 <211> 339
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 325
 cagagcgtgc tgaccagcc gccgagcgtg agcggggcgc cggggcagcg cgtgaccatt 60
 agctgcaccg ggagcagcag caacattggg gcgggggatg tggtgcattg gtatcagcag 120
 ctgccgggga ccgcgccgaa actgctgatt tatgataaca acaaacgcc gagcgggggtg 180
 ccggatcgct ttagcgggag caaaagcggg accagcgcga gcctggcgat tagcgggctg 240
 cgcagcgagg atgaggcgga ttattattgc gcggcgtttg atgatagcct gaacggggccg 300
 gtgtttgggg gggggaccaa actgaccgtg ctggggcag 339

<210> 326
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 326
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgccctg 60
 agctgcgcg cgagcgggtt taccttttagc agcgcgtgga tgagctgggt gcgccaggcg 120
 ccgggggaaag ggctggagtg ggtgagctat attagcagca gcgggagcag cacctattat 180
 gcggatagcg tgaaagggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcggaggat accgcggtgt attattgcgc gcgcgagggg 300
 ctgtgggcgt ttgataaatg ggggcagggg accctggtga ccgtgaccag c 351

<210> 327
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 327
 cagagcgtgc tgaccagcc gccgagcgcg agcgggaccc cggggcagcg cgtgaccatt 60
 agctgcaccg ggagcagcag caacattggg gcggggatatg tgggtgcattg gtatcagcag 120
 ctgccgggga ccgcgccgaa actgctgatt tatgataaca acaaacgccc gagcgggggtg 180
 ccggatcgct ttagcgggag caaaagcggg accagcgcga gcctggcgat tagcgggctg 240
 cgcagcgagg atgaggcgga ttattattgc gcggcgtttg atgatagcct gaacggggccg 300
 gtgtttgggg gggggaccaa actgaccgtg ctggggcag 339

<210> 328
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 328
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgcctg 60
 agctgcgcgg cgagcgggtt tacctttagc agcgcgtgga tgagctgggt gcgccaggcg 120
 ccggggaaag ggctggagtg ggtgagctat attagcagca gcgggagcag cacctattat 180
 gcggatagcg tgaaagggag ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcggaggat accgcggtgt attattgcgc gcgcgagggg 300
 ctgtgggcgt ttgataaatg ggggcagggg accctggtga ccgtgagcag c 351

<210> 329
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a

<400> 329
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 ctgccgggga ccgcgccgaa actgctgatt tatgataaca accagcgcgc gagcgggggtg 180
 ccggatcgct ttagcgggag caaaagcggg accagcgcga gcctggcgat tagcgggctg 240
 cgcagcgagg atgaggcgga ttattattgc gcggcgtttg atgatagcct gaacggggccg 300
 gtgtttgggg gggggaccaa actgaccgtg ctggggcag 339

<210> 330
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<212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 330
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgccctg 60
 agctgcgcgg cgagcggggtt taccttttagc agcgcgtgga tgagctgggt gcgccaggcg 120
 ccgggggaaag ggctggagtg ggtgagctat attagcagca gcgggagcag cacctattat 180
 gcggatagcg tgaaaggggc ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcggaggat accgcggtgt attattgcgc gcgcgagggg 300
 ctgtgggcgt ttgataaatg ggggcagggg accctggtga ccgtgagcag c 351

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<220>
 <223> C4.4a binder

<400> 331
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 ctgccgggga ccgcgccgaa actgctgatt tatgataaca accagcgccc gagcgggggtg 180
 ccggatcgct ttagcgggag caaaagcggg accagcgcgga gcctggcgat tagcgggctg 240
 cgagcgagg atgaggcgga ttattattgc gcggcgtttg atgatcgctt gagcggggccg 300
 gtgtttgggg gggggaccaa actgaccgtg ctggggcag 339

<210> 332
 <211> 366
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 332
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 agctgcgcgg cgagcggggtt taccttttagc gattatcaga tgacctggat tcgccagacc 120
 ccgggggaaag ggctggagtg ggtgagcggg gtgagctgga acggggcgcg caccattat 180
 gcggatagcg tgaaaggggc ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcggaggat accgcggtgt attattgcgc gaaaggggat 300
 tatctggtgt atagcagcta ttattttaaa agctgggggc aggggaccct ggtgaccgtg 360
 accagc 366

<210> 333
 <211> 336

<212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 333
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 agctgcagcg ggagcagcag caacgtgggg agcaaccgg tgaactggta tcagcagctg 120
 ccggggaccg cgccgaaact gctgatttat cgcaacaacc agcgcccgag cggggtgccg 180
 gatcgcttta gcgggagcaa aagcgggacc agcgcgagcc tggcgattag cgggctgcgc 240
 agcgaggatg aggcggatta ttattgcgcg gcgtgggatg atcgctgaa cgggtggggg 300
 tttggggggg ggaccaaact gaccgtgctg gggcag 336

<210> 334
 <211> 363
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 334
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 tgcgcggcga gcgggtttac ctttagcagc tatcagatga cctggattcg ccagaccccg 120
 gggaaagggc tggagtgggt gagcgggatt agctggaacg gggggagcac ccattatgcg 180
 gatagcgtga aagggcgctt taccattagc cgcgataaca gcaaaaacac cctgtatctg 240
 cagatgaaca gcctgcgcgc ggaggatacc gcggtgtatt attgcgcgaa aggggattat 300
 ctggtgtata gcgcgtatta ttttgatagc tgggggcagg ggaccctggt gaccgtgagc 360
 agc 363

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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

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 tgcagcggga gcagcagcaa cattgggagc aaccgggtga actggtatca gcagctgccg 120
 gggaccgcgc cgaaactgct gatttatcgc aacaaccagc gcccagcgcg ggtgccggat 180
 cgcttttagcg ggagcaaaag cgggaccagc gcgagcctgg cgattagcgg gctgcgcagc 240
 gaggatgagg cggattatta ttgcgcggcg tgggatgatc gcctgaacgg gtggggggtt 300
 ggggggggga ccaaactgac cgtgctgggg cag 333

<210> 336
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<212> DNA
 <213> Artificial Sequence
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 <223> C4.4a binder
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 agctgcgcgg cgagcggggtt taccttttagc gattatcaga tgacctggat tcgccagacc 120
 ccgggggaaag ggctggagtg ggtgagcggg attagctgga acgggggggag caccattat 180
 gcggatagcg tgaaagggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcgaggat accgcggtgt attattgcgc gaaaggggat 300
 tatctggtgt atagcagcta ttattttaaa tattgggggc aggggaccct ggtgaccgtg 360
 agcagc 366

<210> 337
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 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> C4.4a binder
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 agctgcagcg ggagcagcag caacattggg agcaaccgag tgaactggta tcagcagctg 120
 ccggggaccg cgccgaaact gctgatttat cgcaacaacc agcgcccgag cggggtgccg 180
 gatcgcttta gcgggagcaa aagcgggacc agcgcgagcc tggcgattag cgggctgcgc 240
 agcgaggatg aggcggatta ttattgcgcg gcgtgggatg atcgctgaa cgggtgggag 300
 tttggggggg ggaccaaact gaccgtgctg gggcag 336

<210> 338
 <211> 366
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> C4.4a binder
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 agctgcgcgg cgagcggggtt taccttttagc gattatcaga tgacctggat tcgccagacc 120
 ccgggggaaag ggctggagtg ggtgagcggg attagctgga acgggggggag caccattat 180
 gcggatagcg tgaaagggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcgaggat accgcggtgt attattgcgc gaaaggggat 300
 tatctggtgt atagcagcta ttattttaaa agctgggggc aggggaccct ggtgaccgtg 360
 agcagc 366

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 <211> 336
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 339
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 agctgcagcg ggagcagcag caacattggg agcaaccggg tgaactggta tcagcagctg 120
 ccggggaccg cgccgaaact gctgatttat cgcaacaacc agcgcccgag cggggtgccg 180
 gatcgcttta gcgggagcaa aagcgggacc agcgcgagcc tggcgattag cgggctgcgc 240
 agcgaggatg aggcggatta ttattgcgcg gcgtgggatg atagcctgaa cgggtggggg 300
 tttggggggg ggaccaaact gaccgtgctg gggcag 336

<210> 340
 <211> 366
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 340
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cgggggggag cctgcgcctg 60
 agctgcgcgg cgagcgggtt taccttttagc gattatcaga tgacctggat tcgccagacc 120
 ccgggggaaag ggctggagtg ggtgagcggg attagctgga acgggggggag caccattat 180
 gcggatagcg tgaaagggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcgaggat accgcggtgt attattgcgc gaaaggggat 300
 tatctggtgt ataaaagcta ttatttttaa agctgggggc aggggaccct ggtgaccgtg 360
 agcagc 366

<210> 341
 <211> 336
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 341
 cagagcgtgc tgaccagcc gccgagcgcg agcgggaccc cggggcagcg cgtgaccatt 60
 agctgcagcg ggagcagcag caacattggg agcaaccggg tgaactggta tcagcagctg 120
 ccggggaccg cgccgaaact gctgatttat cgcaacaacc agcgcccgag cggggtgccg 180
 gatcgcttta gcgggagcaa aagcgggacc agcgcgagcc tggcgattag cgggctgcgc 240
 agcgaggatg aggcggatta ttattgcgcg gcgtgggatg atcgctgag cgggtggggg 300
 tttggggggg ggaccaaact gaccgtgctg gggcag 336

<210> 342
 <211> 366
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 342
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cggggggggag cctgcgcttg 60
 agctgcgcgg cgagcgggtt tacctttagc gattatcaga tgacctggat tcgccagacc 120
 ccggggaaag ggctggagtg ggtgagcggg attagctgga acggggggag caccattat 180
 gcggatagcg tgaaagggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcggaggat accgcggtgt attattgcgc gaaaggggat 300
 tatctggtgt atagcagcta ttattttaaa agctgggggc aggggaccct ggtgaccgtg 360
 agcagc 366

<210> 343
 <211> 336
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 343
 cagagcgtgc tgaccagcc gccgagcgcg agcgggaccc cggggcagcg cgtgaccatt 60
 agctgcagcg ggagcagcag caacattggg agcaaccggg tgaactggta tcagcagctg 120
 ccggggaccg cgccgaaact gctgatttat cgcaacaacc agcgcccgag cggggtgccg 180
 gatcgcttta gcgggagcaa aagcgggacc agcgcgagcc tggcgattag cgggctgcgc 240
 agcgaggatg aggcggatta ttattgcgcg gcgtgggatg atcgctgag cgggtggggg 300
 tttggggggg ggaccaaact gaccgtgctg gggcag 336

<210> 344
 <211> 366
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> C4.4a binder

<400> 344
 gaggtgcagc tgctggagag cggggggggg ctggtgcagc cggggggggag cctgcgcttg 60
 agctgcgcgg cgagcgggtt tacctttagc gattatcaga tgacctggat tcgccagacc 120
 ccggggaaag ggctggagtg ggtgagcggg attagctgga acggggggag caccattat 180
 gcggatagcg tgaaagggcg ctttaccatt agccgcgata acagcaaaaa caccctgtat 240
 ctgcagatga acagcctgcg cgcggaggat accgcggtgt attattgcgc gaaaggggat 300
 tatctggtgt ataaaagcta ttattttaaa agctgggggc aggggaccct ggtgaccgtg 360
 agcagc 366

<210> 345
 <211> 336
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> c4.4a binder

<400> 345
 cagagcgtgc tgaccagcc gccgagcgcg agcgggaccc cggggcagcg cgtgaccatt 60
 agctgcagcg ggagcagcag caacattggg agcaaccgg tgaactggta tcagcagctg 120
 ccgggggaccg cgccgaaact gctgatttat cgcaacaacc agcgcccgag cggggtgccg 180
 gatcgcttta gcgggagcaa aagcgggacc agcgcgagcc tggcgattag cgggctgcgc 240
 agcgaggatg aggcggatta ttattgcgcg gcgtgggatg atagcctgaa cgggtggggg 300
 tttggggggg ggaccaaact gaccgtgctg gggcag 336

<210> 346
 <211> 217
 <212> PRT
 <213> Homo Sapiens

<400> 346
 Asp Ile Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30
 Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45
 Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60
 Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80
 Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg
 85 90 95
 Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110
 Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
 115 120 125
 Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
 130 135 140
 Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Gly Asp Ser Ser Pro Val

145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
165 170 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
180 185 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
195 200 205

Lys Thr Val Ala Pro Thr Glu Cys Ser
210 215

<210> 347
<211> 447
<212> PRT
<213> Homo sapiens

<400> 347

Gln Val Glu Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
 180 185 190
 Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
 195 200 205
 Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
 210 215 220
 Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
 225 230 235 240
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 245 250 255
 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 260 265 270
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 275 280 285
 Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 290 295 300
 Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 305 310 315 320
 Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
 325 330 335
 Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 340 345 350
 Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 355 360 365
 Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 370 375 380
 Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 385 390 395 400
 Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 405 410 415
 Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 420 425 430
 His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

<210> 348
 <211> 217
 <212> PRT
 <213> Homo Sapiens

<400> 348

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Glu Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1      5      10      15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
      20      25      30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
      35      40      45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
      50      55      60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65      70      75      80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg
      85      90      95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
      100      105      110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
      115      120      125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
      130      135      140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
145      150      155      160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
      165      170      175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
      180      185      190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
      195      200      205

Lys Thr Val Ala Pro Thr Glu Cys Ser
      210      215
    
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<210> 349
 <211> 446
 <212> PRT

<213> Homo Sapiens

<400> 349

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110
 Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125
 Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 130 135 140
 Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
 145 150 155 160
 Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
 165 170 175
 Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
 180 185 190
 Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
 195 200 205
 Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
 210 215 220
 Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
 225 230 235 240
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 350
<211> 217
<212> PRT
<213> Homo Sapiens

<400> 350

Glu Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
Page 120

50

55

60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg
85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
115 120 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
130 135 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
165 170 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
180 185 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
195 200 205

Lys Thr Val Ala Pro Thr Glu Cys Ser
210 215

<210> 351
<211> 446
<212> PRT
<213> Homo Sapiens

<400> 351

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser₈₅ Leu Arg Ala Glu Asp₉₀ Thr Ala Val Tyr Tyr₉₅ Cys
 Ala Arg Glu Gly₁₀₀ Leu Trp Ala Phe Asp₁₀₅ Tyr Trp Gly Gln Gly₁₁₀ Thr Leu
 Val Thr Val₁₁₅ Ser Ser Ala Ser Thr₁₂₀ Lys Gly Pro Ser Val₁₂₅ Phe Pro Leu
 Ala Pro₁₃₀ Ser Ser Lys Ser Thr₁₃₅ Ser Gly Gly Thr Ala₁₄₀ Ala Leu Gly Cys
 Leu Val₁₄₅ Lys Asp Tyr Phe₁₅₀ Pro Glu Pro Val₁₅₅ Thr Val Ser Trp Asn Ser₁₆₀
 Gly Ala Leu Thr Ser₁₆₅ Gly Val His Thr Phe₁₇₀ Pro Ala Val Leu Gln Ser₁₇₅
 Ser Gly Leu Tyr₁₈₀ Ser Leu Ser Ser Val₁₈₅ Val Thr Val Pro Ser₁₉₀ Ser Ser
 Leu Gly Thr₁₉₅ Gln Thr Tyr Ile Cys₂₀₀ Asn Val Asn His Lys₂₀₅ Pro Ser Asn
 Thr Lys₂₁₀ Val Asp Lys Lys Val₂₁₅ Glu Pro Lys Ser Cys₂₂₀ Asp Lys Thr His
 Thr₂₂₅ Cys Pro Pro Cys Pro₂₃₀ Ala Pro Glu Leu Leu₂₃₅ Gly Gly Pro Ser Val₂₄₀
 Phe Leu Phe Pro₂₄₅ Lys Pro Lys Asp Thr₂₅₀ Leu Met Ile Ser Arg₂₅₅ Thr
 Pro Glu Val Thr₂₆₀ Cys Val Val Val Asp₂₆₅ Val Ser His Glu Asp₂₇₀ Pro Glu
 Val Lys Phe₂₇₅ Asn Trp Tyr Val Asp₂₈₀ Gly Val Glu Val His₂₈₅ Asn Ala Lys
 Thr Lys₂₉₀ Pro Arg Glu Glu Gln₂₉₅ Tyr Asn Ser Thr Tyr₃₀₀ Arg Val Val Ser
 Val₃₀₅ Leu Thr Val Leu His₃₁₀ Gln Asp Trp Leu Asn₃₁₅ Gly Lys Glu Tyr Lys₃₂₀
 Cys Lys Val Ser Asn₃₂₅ Lys Ala Leu Pro Ala₃₃₀ Pro Ile Glu Lys Thr₃₃₅ Ile
 Ser Lys Ala Lys₃₄₀ Gly Gln Pro Arg Glu₃₄₅ Pro Gln Val Tyr Thr₃₅₀ Leu Pro

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 352
<211> 217
<212> PRT
<213> Homo Sapiens

<400> 352

Glu Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg
85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
115 120 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
130 135 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
165 170 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
180 185 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
195 200 205

Lys Thr Val Ala Pro Thr Glu Cys Ser
210 215

<210> 353
<211> 446
<212> PRT
<213> Homo sapiens

<400> 353

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
 165 170 175
 Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
 180 185 190
 Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
 195 200 205
 Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
 210 215 220
 Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
 225 230 235 240
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 245 250 255
 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 260 265 270
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 275 280 285
 Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 290 295 300
 Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 305 310 315 320
 Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
 325 330 335
 Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 340 345 350
 Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 355 360 365
 Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 370 375 380
 Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 385 390 395 400
 Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 405 410 415
 Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 435 440 445

<210> 354
 <211> 217
 <212> PRT
 <213> Homo Sapiens

<400> 354

Glu Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Tyr Asp Asp Arg
 85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
 115 120 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
 130 135 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
 145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
 165 170 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
 180 185 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
 195 200 205

Lys Thr Val Ala Pro Thr Glu Cys Ser
 210 215

<210> 355

<211> 446
 <212> PRT
 <213> Homo Sapiens

<400> 355

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
 145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
 165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
 180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
 195 200 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
 210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
 225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 356
<211> 217
<212> PRT
<213> Homo Sapiens

<400> 356

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Tyr Asp Asp Ser
85 90 95

Leu Ser Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
115 120 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
130 135 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
165 170 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
180 185 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
195 200 205

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Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
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Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
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Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
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Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
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Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
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Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
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Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
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Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
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Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
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Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
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Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
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Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
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Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
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Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
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Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
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Leu Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
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Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Tyr Asp Asp Ser
 85 90 95

Leu Asn Arg Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
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Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
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Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
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 Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
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 Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
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 Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
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 Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
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Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
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Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
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Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
 165 170 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
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Lys Thr Val Ala Pro Thr Glu Cys Ser
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 35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
 145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
 165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
 180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
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Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
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305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
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Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
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Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
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His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

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Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
Page 136

35

40

45

Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
 65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Phe Asp Asp Ser
 85 90 95

Leu Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
 115 120 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
 130 135 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
 145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
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Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
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Lys Thr Val Ala Pro Thr Glu Cys Ser
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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 Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
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 Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125
 Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 130 135 140
 Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
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 Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
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 Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
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 Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
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 Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
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 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 260 265 270
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
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 Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
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 Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
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 Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
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Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
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Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
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Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
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Leu Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
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Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
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Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Tyr Asp Asp Ser
85 90 95

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145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
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Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
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Lys Thr Val Ala Pro Thr Glu Cys Ser
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50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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85 90 95

Ala Arg Glu Gly Leu Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
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Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
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Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
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Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
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Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
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Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
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Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
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Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
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Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
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Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
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Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
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Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
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Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
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Leu Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
 50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
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 100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
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Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
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Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
 145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
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Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
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Lys Thr Val Ala Pro Thr Glu Cys Ser

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Ala Arg Glu Gly Leu Trp Ala Phe Asp Lys Trp Gly Gln Gly Thr Leu
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Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
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Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
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Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
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Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
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Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
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Val³⁰⁵ Leu Thr Val³¹⁰ Leu His³¹⁵ Gln Asp Trp Leu Asn³²⁰ Gly Lys Glu Tyr Lys³²⁵

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
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Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
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Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
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Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
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Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
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Leu Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe
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Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
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Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
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Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
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Lys Thr Val Ala Pro Thr Glu Cys Ser
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 Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
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 145 150 155 160
 Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
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 Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
 180 185 190
 Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
 195 200 205
 Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
 210 215 220
 Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
 225 230 235 240
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 245 250 255
 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 260 265 270
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 275 280 285
 Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
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 Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
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His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

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Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
85 90 95

Asn Gly Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
Page 147

115

120

125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
 130 135 140

Pro Gly Ala Val Thr Val Ala Trp Lys Gly Asp Ser Ser Pro Val Lys
 145 150 155 160

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
 165 170 175

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
 180 185 190

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
 195 200 205

Thr Val Ala Pro Thr Glu Cys Ser
 210 215

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Gln Val Glu Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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 35 40 45

Ser Gly Val Ser Trp Asn Gly Ala Arg Thr His Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ala Tyr Tyr Phe Asp Ser Trp
 100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 260 265 270
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 290 295 300
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 305 310 315 320
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 325 330 335
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 340 345 350
 Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 355 360 365
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 370 375 380
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 385 390 395 400
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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Ser Pro Gly Lys
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<211> 216
<212> PRT
<213> Homo Sapiens

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35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
85 90 95

Asn Gly Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
115 120 125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
130 135 140

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
145 150 155 160

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
165 170 175

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
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Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
195 200 205

Thr Val Ala Pro Thr Glu Cys Ser
210 215

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<211> 451
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35 40 45

Ser Gly Val Ser Trp Asn Gly Ala Arg Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ala Tyr Tyr Phe Asp Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 305 310 315 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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Ser Pro Gly
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 35 40 45
 Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80
 Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
 85 90 95
 Asn Gly Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
 100 105 110
 Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
 115 120 125
 Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
 130 135 140
 Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
 145 150 155 160
 Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
 165 170 175
 Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
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 Thr Val Ala Pro Thr Glu Cys Ser
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 35 40 45
 Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ala Tyr Tyr Phe Asp Ser Trp
 100 105 110
 Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 260 265 270
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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435 440 445

Ser Pro Gly
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<211> 216
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Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
85 90 95

Asn Gly Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
115 120 125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
130 135 140

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
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Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
165 170 175

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
180 185 190

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
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Thr Val Ala Pro Thr Glu Cys Ser
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<212> PRT
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Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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Gly Gln Gly Thr 115 Leu Val Thr Val 120 Ser Ser Ala Ser Thr 125 Lys Gly Pro

Ser Val 130 Phe Pro Leu Ala Pro 135 Ser Ser Lys Ser Thr 140 Ser Gly Gly Thr

Ala Ala Leu Gly Cys 145 Leu Val Lys Asp Tyr Phe 155 Pro Glu Pro Val Thr 160

Val Ser Trp Asn 165 Ser Gly Ala Leu Thr Ser 170 Gly Val His Thr Phe 175 Pro

Ala Val Leu Gln 180 Ser Ser Gly Leu Tyr 185 Ser Leu Ser Ser Val 190 Val Thr

Val Pro Ser 195 Ser Ser Leu Gly Thr 200 Gln Thr Tyr Ile Cys 205 Asn Val Asn

His Lys 210 Pro Ser Asn Thr Lys 215 Val Asp Lys Lys Val 220 Glu Pro Lys Ser

Cys Asp Lys Thr His 225 Thr Cys Pro Pro Cys Pro 235 Ala Pro Glu Leu Leu 240

Gly Gly Pro Ser Val 245 Phe Leu Phe Pro Pro 250 Lys Pro Lys Asp Thr 255 Leu

Met Ile Ser Arg 260 Thr Pro Glu Val Thr 265 Cys Val Val Val Asp 270 Val Ser

His Glu Asp 275 Pro Glu Val Lys Phe 280 Asn Trp Tyr Val Asp 285 Gly Val Glu

Val His 290 Asn Ala Lys Thr Lys 295 Pro Arg Glu Glu Gln 300 Tyr Asn Ser Thr

Tyr Arg Val Val Ser 305 Val Leu Thr Val Leu His 315 Gln Asp Trp Leu Asn 320

Gly Lys Glu Tyr Lys 325 Cys Lys Val Ser Asn 330 Lys Ala Leu Pro Ala 335 Pro

Ile Glu Lys Thr 340 Ile Ser Lys Ala Lys 345 Gly Gln Pro Arg Glu 350 Pro Gln

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
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Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
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Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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Ser Pro Gly
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 35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
 85 90 95

Asn Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
 100 105 110

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
 115 120 125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr

130

135

140

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
145 150 155 160

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
165 170 175

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
180 185 190

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
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Thr Val Ala Pro Thr Glu Cys Ser
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Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ala Tyr Tyr Phe Asp Ser Trp
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Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
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Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
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 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
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 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
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 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
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 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
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 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
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 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
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 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
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 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
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 Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
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 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
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 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
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 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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Ser Pro Gly
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Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
85 90 95

Asn Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
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Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
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Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
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Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
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Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
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Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
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Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
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Thr Val Ala Pro Thr Glu Cys Ser
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Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ala Tyr Tyr Phe Asp Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
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His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
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Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
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Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
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Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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Ser Pro Gly
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<210> 382
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<212> PRT
<213> Homo sapiens

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Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
Page 163

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 Ser Gly Trp Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
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 Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
 115 120 125
 Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
 130 135 140
 Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
 145 150 155 160
 Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
 165 170 175
 Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
 180 185 190
 Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
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 Thr Val Ala Pro Thr Glu Cys Ser
 210 215

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 35 40 45
 Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser Trp
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 Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 260 265 270
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 305 310 315 320
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 325 330 335
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 340 345 350
 Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 355 360 365
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 370 375 380
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 385 390 395 400
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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 Ser Pro Gly
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 20 25 30
 Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
85 90 95

Ser Gly Trp Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
115 120 125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
130 135 140

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
145 150 155 160

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
165 170 175

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
180 185 190

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
195 200 205

Thr Val Ala Pro Thr Glu Cys Ser
210 215

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<400> 385

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35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser Trp
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 Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
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 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 260 265 270
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 290 295 300
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 305 310 315 320
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 325 330 335
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 340 345 350
 Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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Ser Pro Gly
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 35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
 85 90 95

Asn Gly Trp Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
 100 105 110

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
 115 120 125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
 130 135 140

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
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145 150 155 160

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
165 170 175

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
180 185 190

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
195 200 205

Thr Val Ala Pro Thr Glu Cys Ser
210 215

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35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 260 265 270
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 290 295 300
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 305 310 315 320
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 325 330 335
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 340 345 350
 Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 355 360 365
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 370 375 380
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 385 390 395 400
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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Ser Pro Gly
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35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
85 90 95

Asn Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
115 120 125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
130 135 140

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
145 150 155 160

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
165 170 175

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
180 185 190

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
195 200 205

Thr Val Ala Pro Thr Glu Cys Ser
210 215

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 35 40 45
 Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser Trp
 100 105 110
 Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
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Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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Ser Pro Gly
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Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
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Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
Page 174

20

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30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
 85 90 95

Ser Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
 100 105 110

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
 115 120 125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
 130 135 140

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
 145 150 155 160

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
 165 170 175

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
 180 185 190

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
 195 200 205

Thr Val Ala Pro Thr Glu Cys Ser
 210 215

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 <211> 451
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<400> 391

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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 35 40 45

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 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
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 Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser Trp
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 Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
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 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 260 265 270
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 290 295 300
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 305 310 315 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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Ser Pro Gly
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35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
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Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Arg Leu
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Ser Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
100 105 110

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
115 120 125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
130 135 140

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
145 150 155 160

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
165 170 175

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
180 185 190

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
195 200 205

Thr Val Ala Pro Thr Glu Cys Ser
210 215

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35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Ser Ser Tyr Tyr Phe Lys Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
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 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 260 265 270
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 290 295 300
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 305 310 315 320
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 325 330 335
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 340 345 350
 Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 355 360 365
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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Ser Pro Gly
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 35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
 85 90 95

Asn Gly Trp Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
 100 105 110

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
 115 120 125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
 130 135 140

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
 145 150 155 160

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
 Page 180

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180 185 190

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Thr Val Ala Pro Thr Glu Cys Ser
210 215

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Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
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 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 260 265 270
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 275 280 285
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 290 295 300
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 305 310 315 320
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
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 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 340 345 350
 Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 355 360 365
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 370 375 380
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 385 390 395 400
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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 35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
 85 90 95

Ser Gly Trp Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
 100 105 110

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
 115 120 125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
 130 135 140

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
 145 150 155 160

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
 165 170 175

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
 180 185 190

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
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Thr Val Ala Pro Thr Glu Cys Ser
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 35 40 45

Ser Gly Ile Ser Trp Asn Gly Gly Ser Thr His Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Asp Tyr Leu Val Tyr Lys Ser Tyr Tyr Phe Lys Ser Trp
 100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255

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Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
325 330 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
435 440 445

Ser Pro Gly
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<213> Homo sapiens

<400> 398

Gly Tyr Ser Phe Thr Ser Tyr Trp Ile Gly
1 5 10

<210> 399
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<213> Homo sapiens

<400> 399

Trp Met Gly Ile Ile Asp Pro Gly Asp Ser Arg Thr Arg Tyr Ser Pro
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Ser Phe Gln Gly
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Gly Gln Leu Tyr Gly Gly Thr Tyr Met Asp Gly
1 5 10

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<400> 401

Thr Gly Thr Ser Ser Asp Ile Gly Gly Tyr Asn Ser Val Ser
1 5 10

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Leu Met Ile Tyr Gly Val Asn Asn Arg Pro Ser
1 5 10

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<400> 403

Ser Ser Tyr Asp Ile Glu Ser Ala Thr Pro
1 5 10

<210> 404
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<212> PRT
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<400> 404

Gln Val Glu Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
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Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Tyr
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Trp Ile Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Ile Ile Asp Pro Gly Asp Ser Arg Thr Arg Tyr Ser Pro Ser Phe
50 55 60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Gly Gln Leu Tyr Gly Gly Thr Tyr Met Asp Gly Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 405
<211> 113
<212> PRT
<213> Homo sapiens

<400> 405

Asp Ile Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1 5 10 15

Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Ile Gly Gly Tyr
20 25 30

Asn Ser Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
35 40 45

Met Ile Tyr Gly Val Asn Asn Arg Pro Ser Gly Val Ser Asn Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Asp Ile Glu
85 90 95

Ser Ala Thr Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln

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<211> 360
<212> DNA
<213> Homo sapiens

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cctgggaagg gtctcgagt gatgggcatt atcgatccgg gtgatagccg taccggttat 180
tctccgagct ttcagggcca ggtgaccatt agcgcggaata aaagcattag caccgcgtat 240
cttcaatgga gcagcctgaa agcgagcgat acggccatgt attattgcgc gcgtgggtcag 300
ctttatggtg gtacttatat ggatggttgg ggccaaggca ccctggtgac ggtagctca 360

<210> 407
<211> 339
<212> DNA
<213> Homo sapiens

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catcccgga aggcgccgaa acttatgatt tatggtgtta ataatcgctc ctcaggcgtg 180
agcaaccgtt ttagcggatc caaaagcggc aacaccgcga gcctgaccat tagcggcctg 240
caagcgggaag acgaagcggg ttattattgc tcttcttatg atattgagtc tgctactcct 300
gtgtttggcg gcggcacgaa gttaaccgtc ctaggtcag 339

<210> 408
<211> 450
<212> PRT
<213> Homo sapiens

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Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Tyr
20 25 30
Trp Ile Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Ile Ile Asp Pro Gly Asp Ser Arg Thr Arg Tyr Ser Pro Ser Phe
50 55 60
Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80
Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95
Ala Arg Gly Gln Leu Tyr Gly Gly Thr Tyr Met Asp Gly Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
 115 120 125
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
 130 135 140
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
 145 150 155 160
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
 165 170 175
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
 180 185 190
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
 195 200 205
 Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
 210 215 220
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
 225 230 235 240
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
 245 250 255
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
 260 265 270
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
 275 280 285
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
 290 295 300
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
 305 310 315 320
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
 325 330 335
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
 340 345 350
 Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
 355 360 365
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
 370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly Lys
450

<210> 409
<211> 217
<212> PRT
<213> Homo sapiens
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Asp Ile Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1 5 10 15

Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Ile Gly Gly Tyr
20 25 30

Asn Ser Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
35 40 45

Met Ile Tyr Gly Val Asn Asn Arg Pro Ser Gly Val Ser Asn Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Asp Ile Glu
85 90 95

Ser Ala Thr Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
115 120 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
130 135 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Gly Asp Ser Ser Pro Val
145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
 165 170 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
 180 185 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
 195 200 205

Lys Thr Val Ala Pro Thr Glu Cys Ser
 210 215

<210> 410
 <211> 1353
 <212> DNA
 <213> Homo sapiens

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 cctgggaagg gtctcgagt gatgggcatt atcgatccgg gtgatagccg taccggttat 180
 tctccgagct ttcagggcca ggtgaccatt agcgcggata aaagcattag caccgcgtat 240
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 ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccctt 780
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 agcacgtacc ggggtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 960
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 ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 1260
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 cagaagagcc tctccctgtc tccgggtaaa tga 1353

<210> 411
 <211> 654
 <212> DNA
 <213> Homo sapiens

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catcccggga aggcgccgaa acttatgatt tatggtgtta ataatcgccc ctcaggcgtg      180
agcaaccgtt ttagcggatc caaaagcggc aacaccgcga gcctgaccat tagcggcctg      240
caagcgggaag acgaagcgga ttattattgc tcttcttatg atattgagtc tgctactcct      300
gtgtttggcg gcggcacgaa gttaaccgtc ctaggtcagc ccaaggctgc cccctcggtc      360
actctgttcc cgccctcctc tgaggagctt caagccaaca aggccacact ggtgtgtctc      420
ataagtgact tctaccggg agccgtgaca gtggcctgga agggagatag cagccccgtc      480
aaggcgggag tggagaccac cacaccctcc aaacaaagca acaacaagta cgcggccagc      540
agctatctga gcctgacgcc tgagcagtg aagtcccaca gaagctacag ctgccaggtc      600
acgcatgaag ggagcaccgt ggagaagaca gtggccccta cagaatgttc atag          654
    
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