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(54) Titre : NOUVEAUX CONJUGUES LIANT-PRINCIPE ACTIF (ADC) ET LEUR UTILISATION
(54) Title: NOVEL BINDER-DRUG CONJUGATES (ADCS) AND THEIR USE

(57) Abrégé/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.

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Abstract

The present patent application relates to novel binder-drug conjugates (ADCs) of N,N-dialkylauristatins directed against the target epidermal growth factor receptor (EGFR, gene ID 1956), effective metabolites of these ADCs, methods for producing these ADCs, use of these ADCs for treatment and or prevention of diseases as well as the use of these ADCs to produce pharmaceutical drugs for treatment and/or prevention of diseases, in particular hyperproliferative and/or angiogenic diseases such as cancer, for example. Such treatments may be administered as monotherapy or in combination with other pharmaceutical drugs or other therapeutic measures.

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Novel Binder-Drug Conjugates (ADCs) and Their Use

The present application relates to novel binder-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 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.

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.

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 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 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 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 (Erbitux) 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. Karkan *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 over-expression 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-

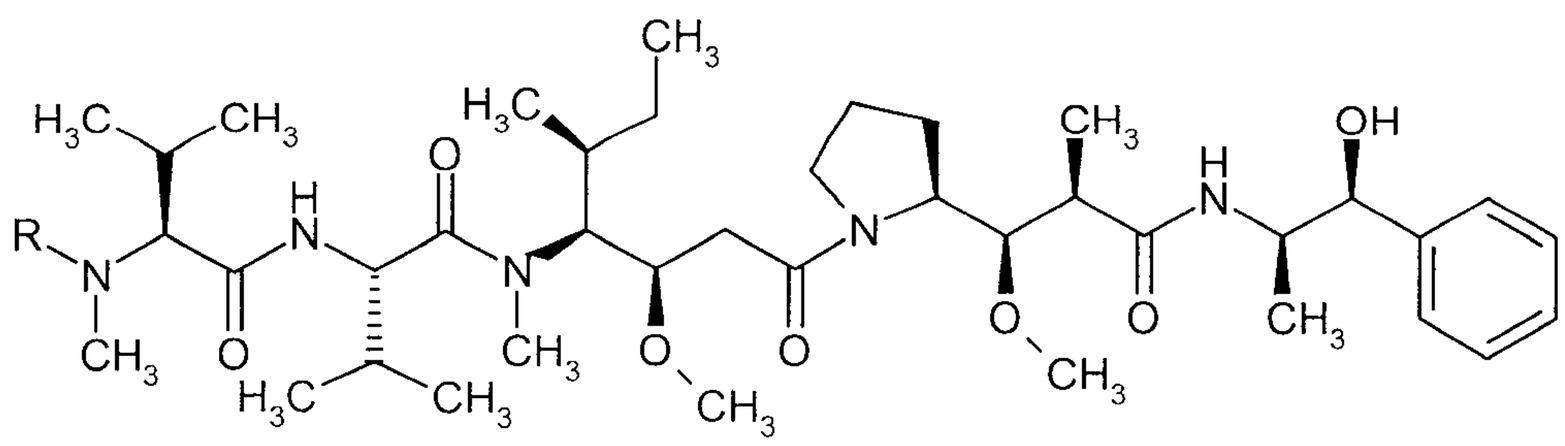
lation and thus activate pro-proliferative signal cascades (including those via mitogen-activated protein kinases (MAPKs) and Akt). These signal cascades regulate the transcription of genes involved in cell growth and cell survival, motility and proliferation.

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.

The following publications describe the EGF receptor and anti-EGFR antibodies in general: WO 00069459 A1, WO 2010145796 A2, WO 02100348 A2, EP 00979246 B1, EP 00531472 B1, 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.

Auristatin E (AE) and monomethyl auristatin 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 Design* **13**, 243-277 (1998)).



Auristatin E (AE): R = CH_3

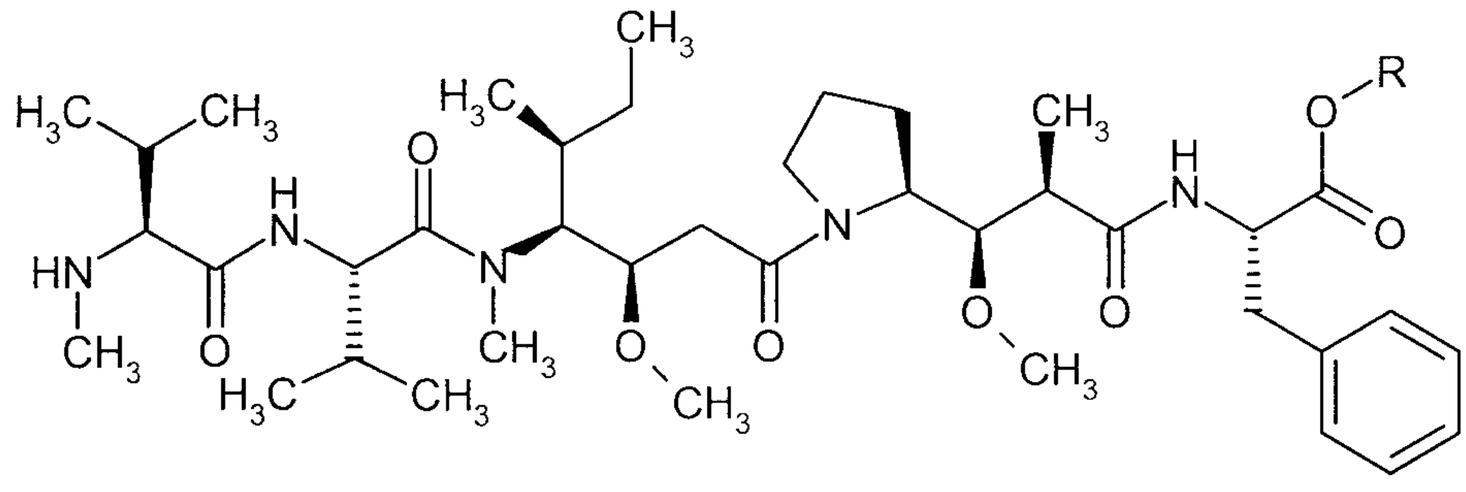
Monomethylauristatin E (MMAE): R = H

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 = CH_3

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.

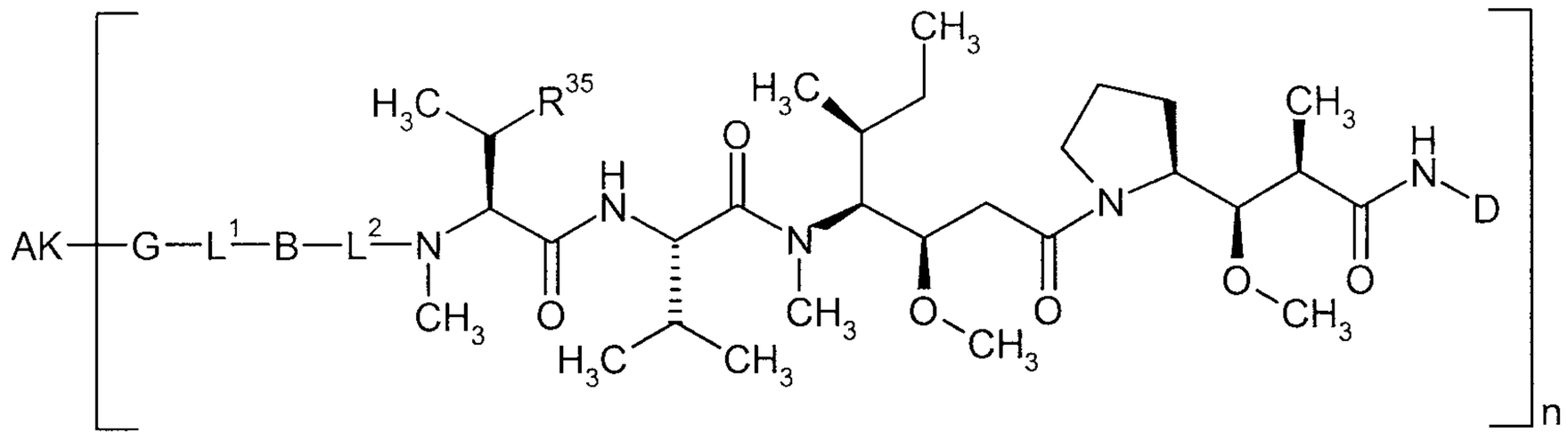
Monomethyl auristatin F (MMAF) as well as various esters and amide derivatives thereof were disclosed in WO 2005/081711 A2. Additional auristatin analogs having a C-terminal amide-substituted phenylalanine unit are described in WO 01/18032 A2. MMAF analogs involving side chain modifications of phenylalanine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

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)



(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,

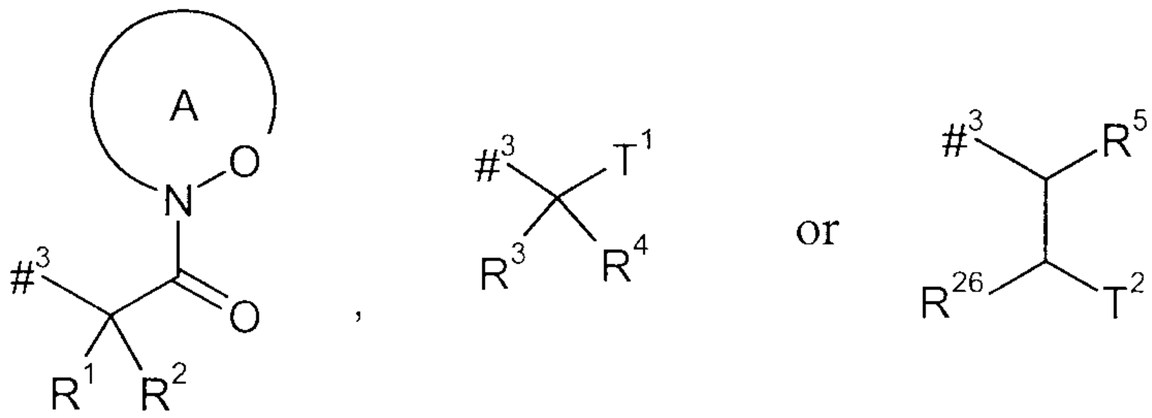
the group §-G-L^1-B-L^2-§§ stands for a linker,

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

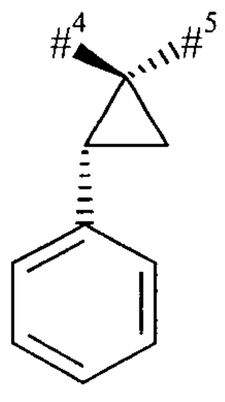
$\#^3$ denotes the linkage site to the nitrogen atom,

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, 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

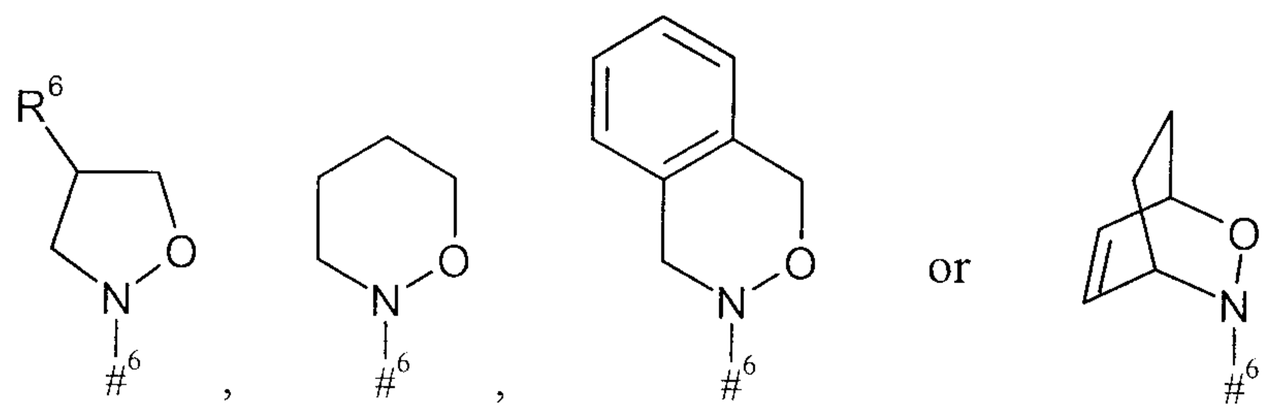


wherein

$\#^4$ 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,

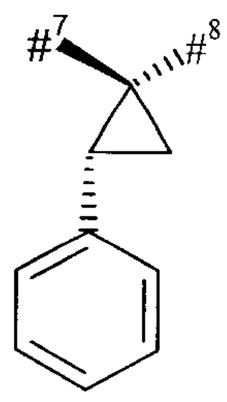
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-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



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,

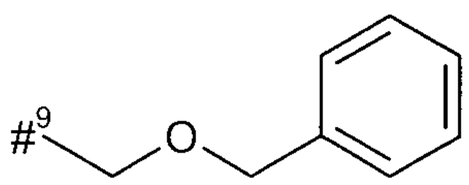
or

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



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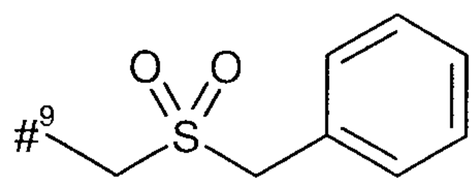

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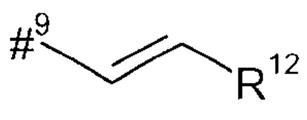

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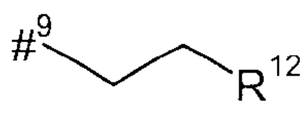

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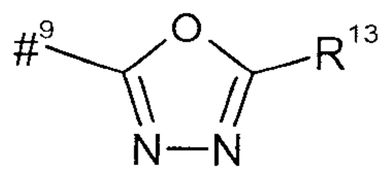


wherein

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

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^{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.

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 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 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 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 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 ^{2}H (deuterium), ^{3}H (tritium), ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{32}P, ^{33}P, ^{33}S, ^{34}S, ^{35}S, ^{36}S, ^{18}F, ^{36}Cl, ^{82}Br, ^{123}I, ^{124}I, ^{129}I and ^{131}I. Certain isotope variants of a compound according to the invention, such as in 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 ^{3}H or ^{14}C 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.

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.

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, 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:

(C$_1$-C$_4$)-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 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).

(C$_3$-C$_7$)-Cycloalkyl and/or three- to seven-membered carbocycle within the scope of the invention 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 R^{19} meaning includes both the side groups of the naturally occurring α-amino acids and the side groups of the homologs and isomers of these 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 (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-carbamoylethyl (glutamine), carboxymethyl (aspartic acid), 2-carboxyethyl (glutamic acid), 4-aminobutan-1-yl (lysine), 4-amino-3-hydroxybutan-1-yl (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 R^{19} 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.

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 SO_2 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 heterocycle with one or two ring heteroatoms from the series of N and/or O is preferred. Examples include: azetidinyl, 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.

In the formula for the group for which A, B, D, G, L^1, L^2, L^4, R^1, R^2, R^3, R^4 and/or R^5 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 CH_2 group but instead is a component of the bond to the respective atom identified, to which A, B, D, G, L^1, L^2, L^4, R^1, R^2, R^3, R^4 and/or R^5 is bound.

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.

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 a drug. The term "linker" is preferably understood to be a series of atoms in the sense of the present invention, which covalently link a binder to a drug. In addition, linkers may be divalent chemical units, such as alkyldiyls, aryldiyls, heteroaryldiyls, heterocyclyldiyls, 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 TNFα of the ligand-receptor pair TNFα/TNFα 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 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 (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 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 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 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 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 Chiota.

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 "multi-functional" 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.

"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).

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.*, 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 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.

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 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 Kd value; i.e., preferably those with a Kd 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 recombinant humanized monoclonal antibody of the IgG1κ type which binds the extracellular domains of human epidermal growth receptor with a high affinity in a cell-based assay (Kd = 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,

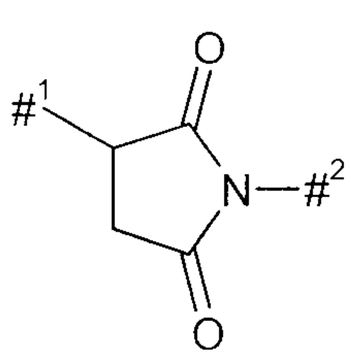
AK stands for AK_1 or AK_2

wherein

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,

AK_2 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 = AK_1 stands for a group of the formula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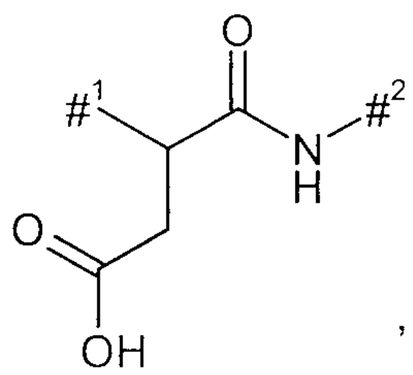
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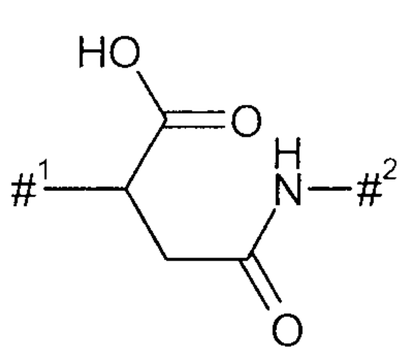

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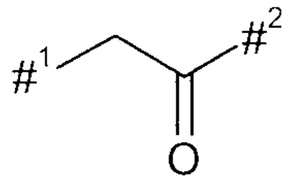


wherein

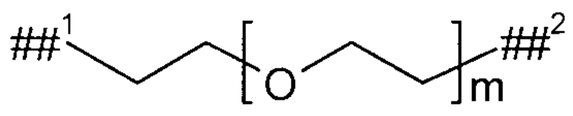
$\#^1$ denotes the linkage site to the sulfur atom of the binder,

$\#^2$ 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_1-C_{10})-alkanediyl, a group of the formula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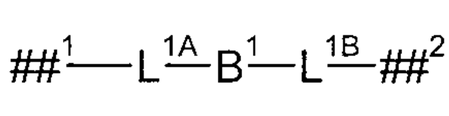


wherein

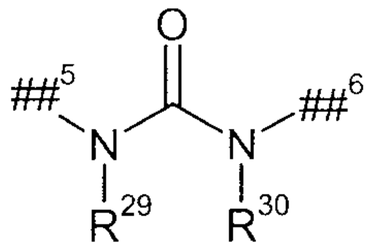
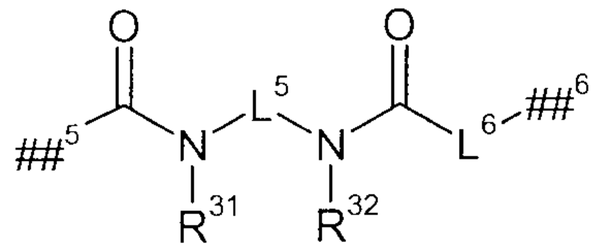
m stands for a number from 2 to 6,

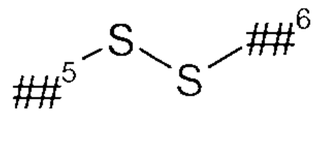
$\#\#^1$ denotes the linkage site to the group G,

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

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

B^1 stands for a group of the formula

or 

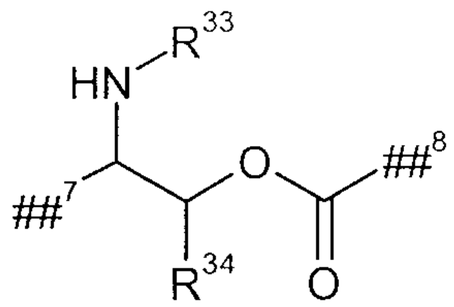
wherein

$##^5$ 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 $(C_2\text{-}C_4)$-alkanediyl,

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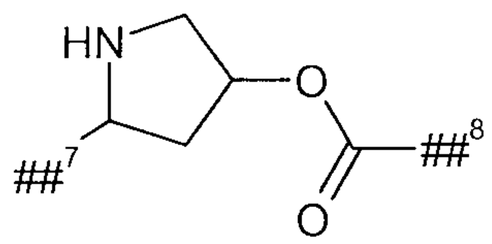


wherein

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

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

R^{33} stands for hydrogen, $(C_1\text{-}C_4)$-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

R^{34} stands for hydrogen or methyl,

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

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

or

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

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,

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

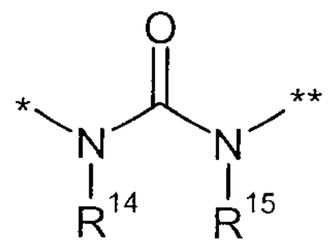
and

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

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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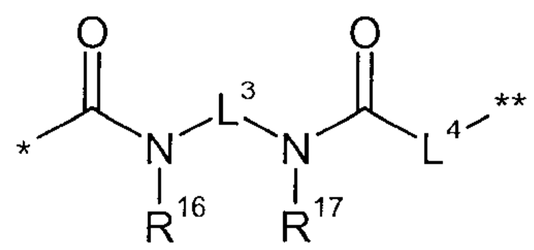
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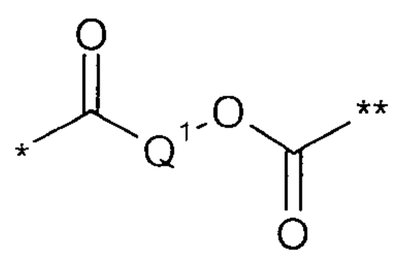
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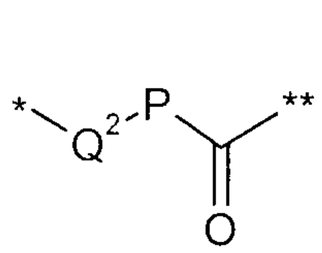
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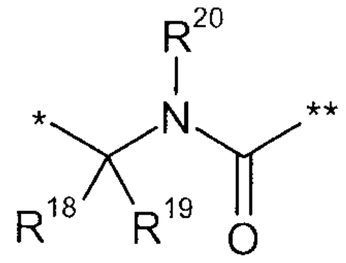
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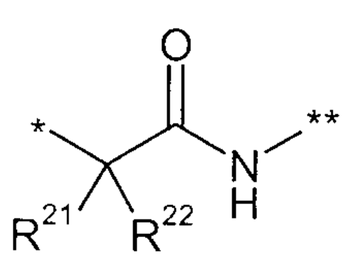
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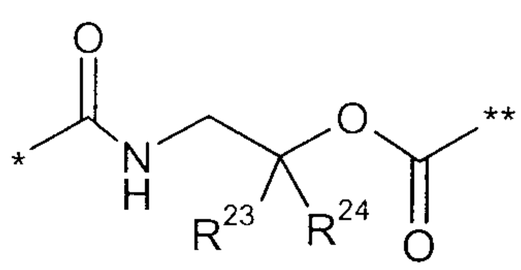
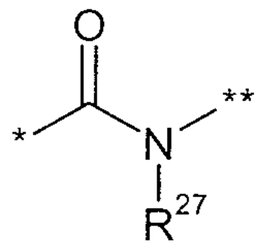
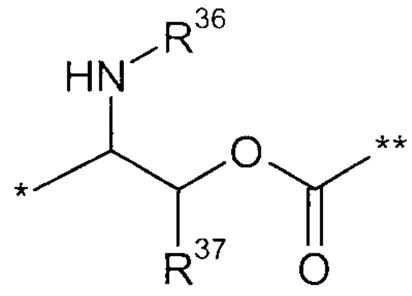
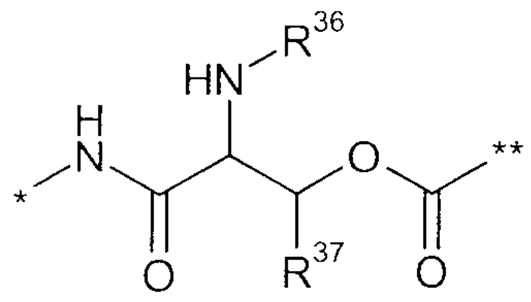





or

wherein

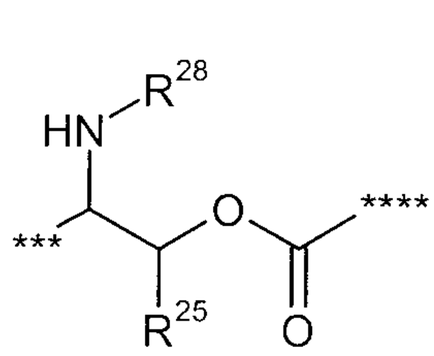
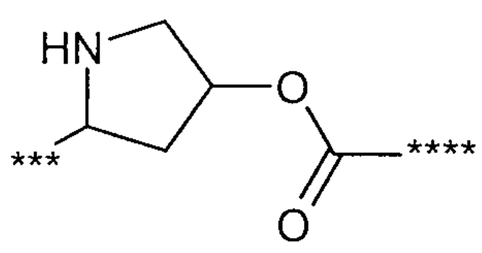
* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

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

or

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, $(C_1$-$C_4)$-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

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 $(C_1$-$C_4)$-alkyl,

R^{15} stands for hydrogen or $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-alkyl,

R^{17} stands for hydrogen or $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-alkyl,

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

R^{20} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

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 $(C_1$-$C_4)$-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,

R^{23} stands for $(C_1$-$C_4)$-alkyl,

R^{24} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

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

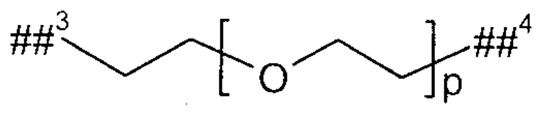
R^{36} stands for hydrogen, $(C_1$-$C_4)$-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

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



wherein

p stands for a number from 2 to 6,

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

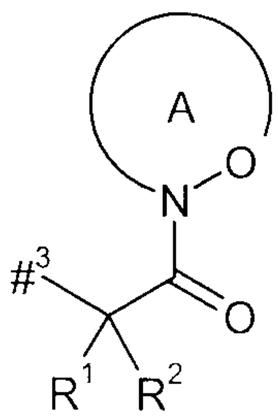
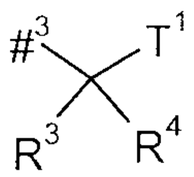
$##^4$ denotes the linkage site to the nitrogen atom,

wherein $(C_2$-$C_{10})$-alkanediyl may be substituted with one 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 $(C_3$-$C_6)$ cycloalkyl ring or a phenyl ring,

D stands for a group of the formula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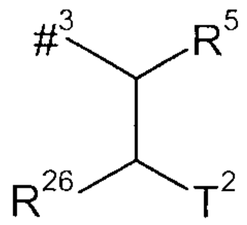


wherein

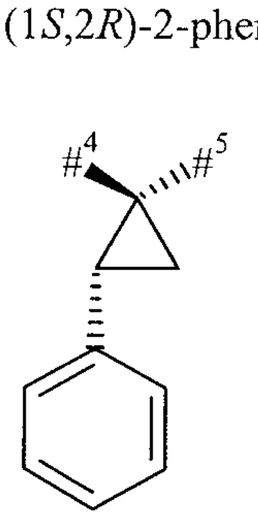
$\#^3$ denotes the linkage site to the nitrogen atom,

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, 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

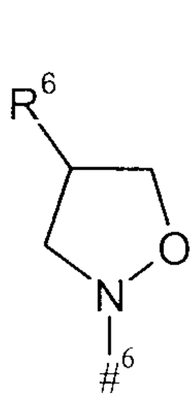


wherein

$\#^4$ 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 grouping contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula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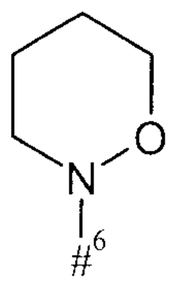
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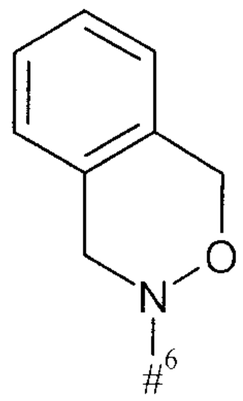
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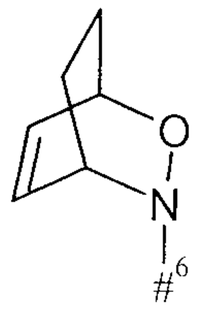


wherein

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

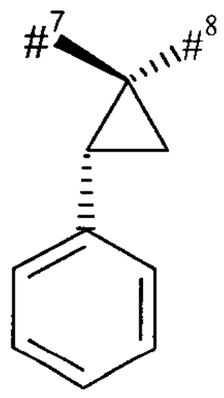
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-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



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,

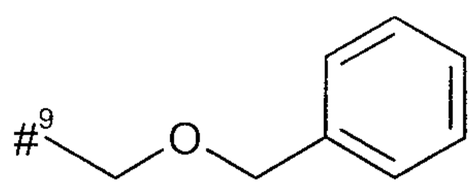
or

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



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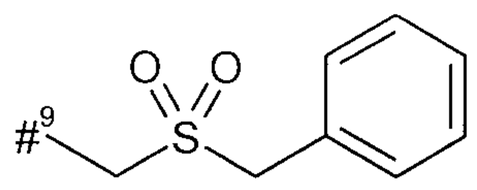

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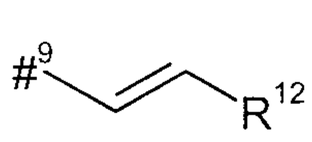

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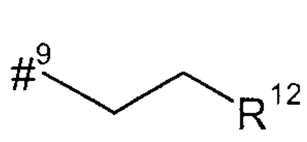

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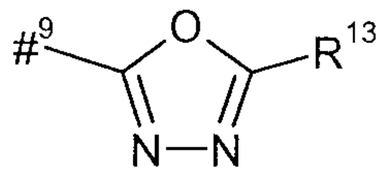


wherein

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

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^{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.

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,

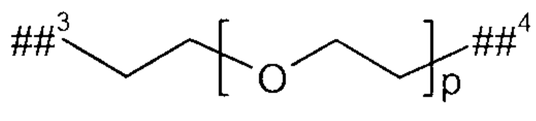
the group §-G-L^1-B-§§ for a linker

wherein

§ denotes the linkage site to the group AK and

§§ denotes the linkage site to the nitrogen atom,

L^2 stands for linear (C_2-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,

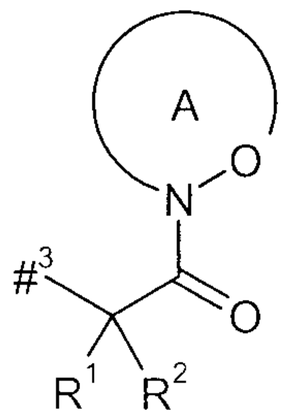
$##^4$ denotes the linkage site to the nitrogen atom,

wherein (C_2-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-C_6) cycloalkyl ring or a phenyl ring, including the carbon atoms optionally situated between them,

D stands for a group of the formula



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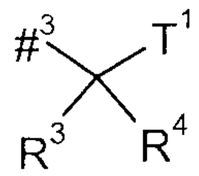

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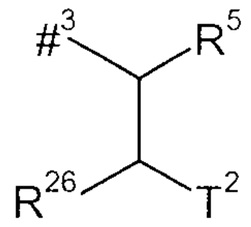


wherein

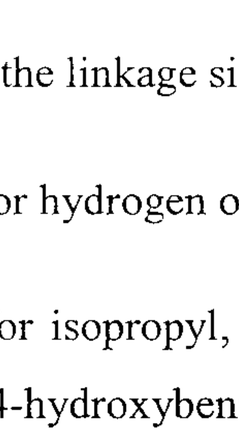
$\#^3$ denotes the linkage site to the nitrogen atom,

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, 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

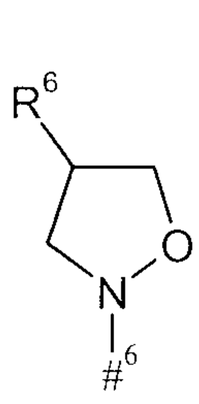
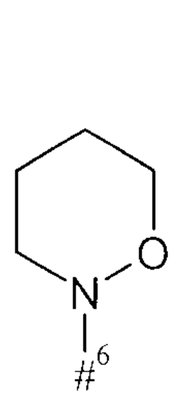
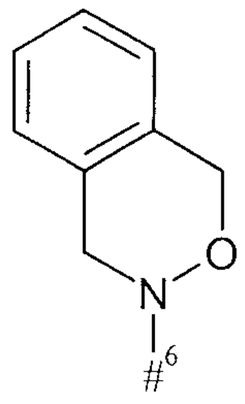


wherein

$\#^4$ 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 monocyclic or bicyclic, optionally substituted heterocycle of the formula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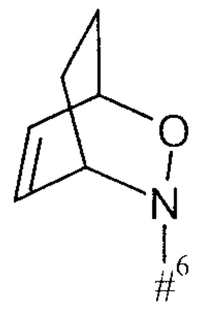


wherein

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

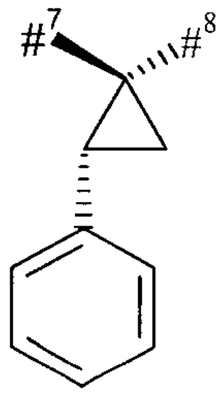
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-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



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,

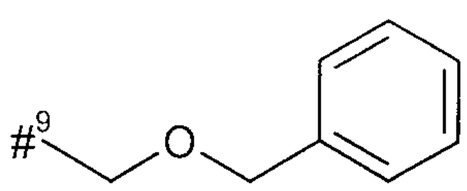
or

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 with methoxycarbonyl or carboxyl in the phenyl group,

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



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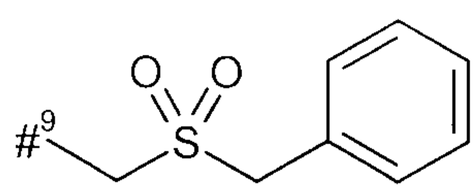

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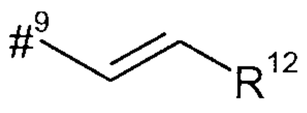

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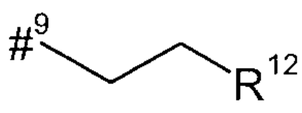

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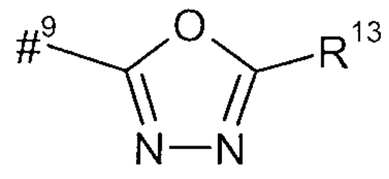


wherein

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

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^{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.

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,

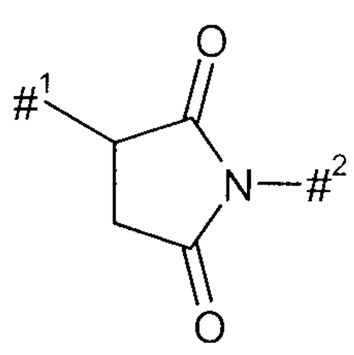
AK stands for AK_1 or AK_2

wherein

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 via a sulfur atom of the binder,

AK_2 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 = AK_1, stands for a group of the formula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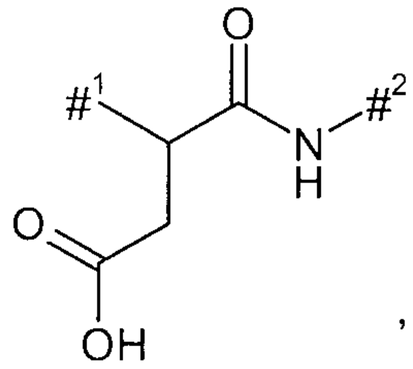
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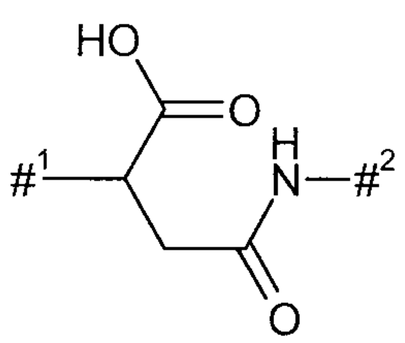

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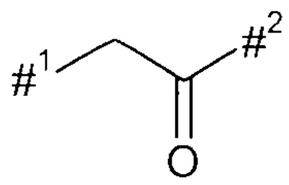


wherein

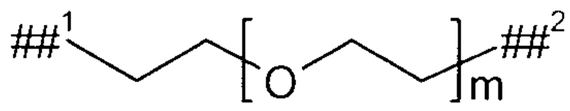
$\#^1$ denotes the linkage site to the sulfur atom of the binder,

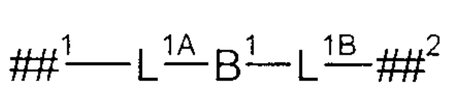
$\#^2$ denotes the linkage site to the group L^1,

or

for the case when AK = AK_2, G stands for carbonyl,

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



or 

wherein

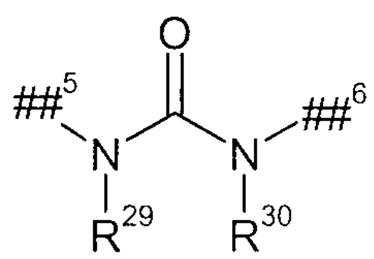
m stands for a number from 2 to 6,

$##^1$ 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_{10})-alkanediyl,

B^1 stands for a group of the formula



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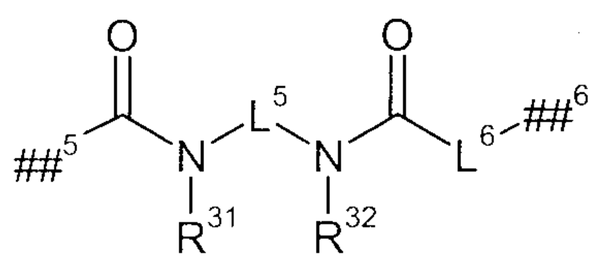
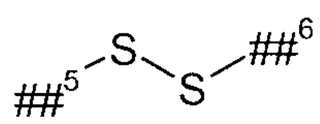


or 

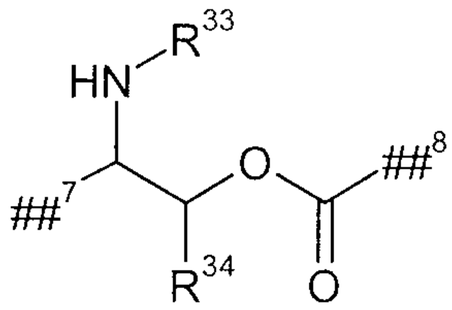
wherein

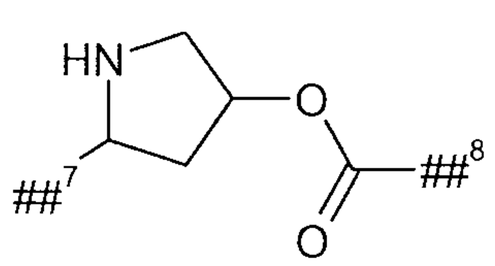
$##^5$ denotes the linkage site to the group L^{1A},

$##^6$ 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 or a group with the formula



or 

wherein

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

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

R^{33} stands for hydrogen, $(C_1\text{-}C_4)$-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

R^{34} stands for hydrogen or methyl,

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

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

or

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

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

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

or

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

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

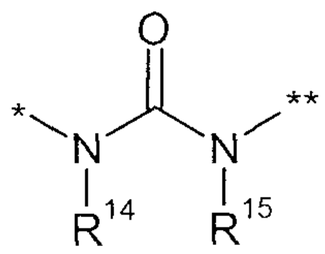
and

wherein $(C_1\text{-}C_{10})$-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 $(C_3\text{-}C_6)$-cycloalkyl ring or a phenyl ring by inclusion of the carbon atoms optionally occurring between them,

B stands for a bond or a group of the formula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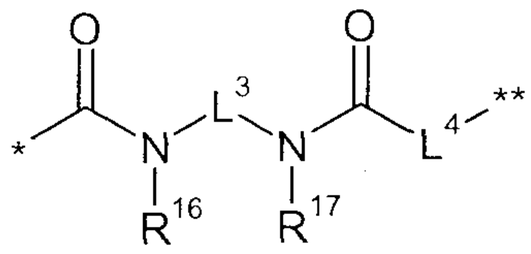
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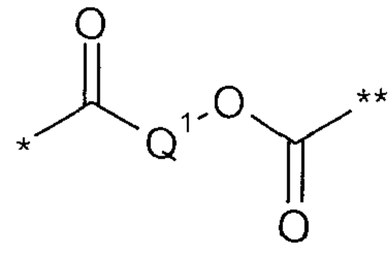
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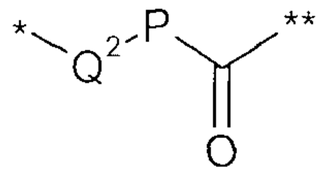
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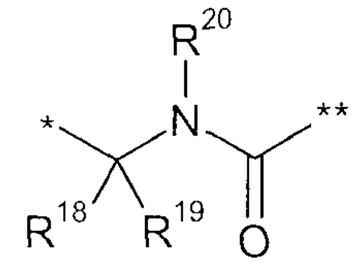
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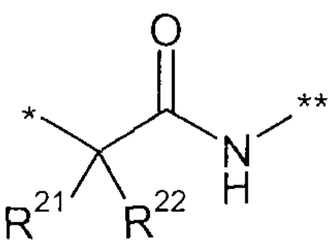
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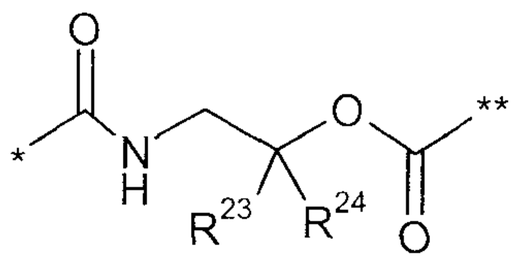
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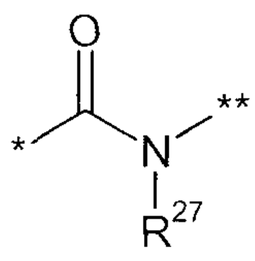
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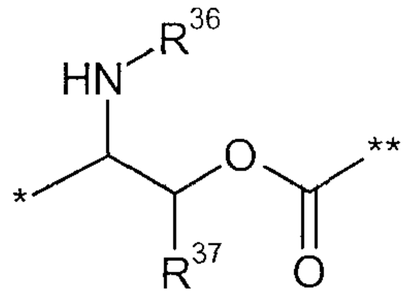


wherein

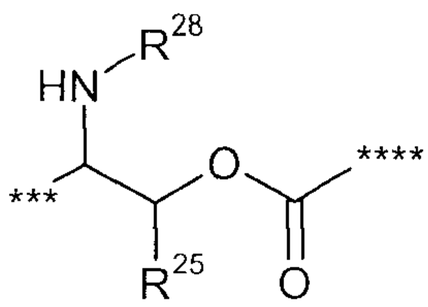
* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

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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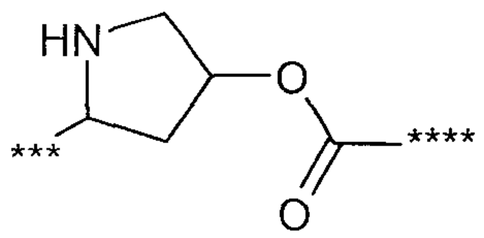


wherein

*** 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, $(C_1$-$C_4)$-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

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 $(C_1$-$C_4)$-alkyl,

R^{15} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

or

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 $(C_1$-$C_4)$-alkyl,

R^{17} stands for hydrogen or $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-alkyl,

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 $(C_1$-$C_4)$-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,

R^{23} stands for $(C_1$-$C_4)$-alkyl,

R^{24} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

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

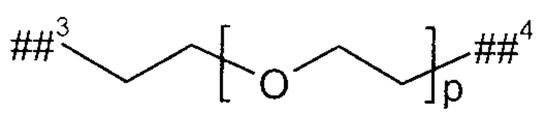
R^{36} stands for hydrogen, $(C_1$-$C_4)$-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxy-carbonyl,

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



wherein

p stands for a number from 2 to 6,

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

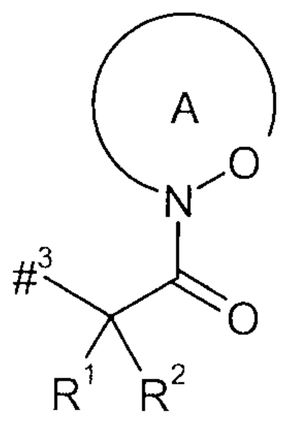
$##^4$ denotes the linkage site to the nitrogen atom,

wherein $(C_2$-$C_{10})$-alkanediyl may be substituted with 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 in 1,2-, 1,3- or 1,4- relation to one another to form a $(C_3$-$C_6)$ cycloalkyl ring or a phenyl ring, including the carbon atoms optionally situated between them,

D stands for a group of the formula



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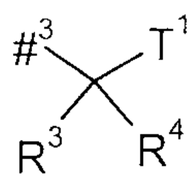

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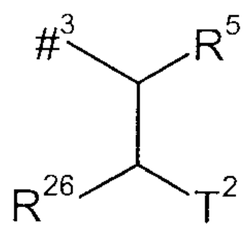


wherein

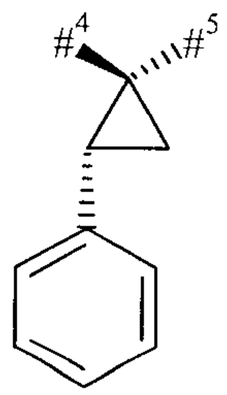
$\#^3$ denotes the linkage site to the nitrogen atom,

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, 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 may form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

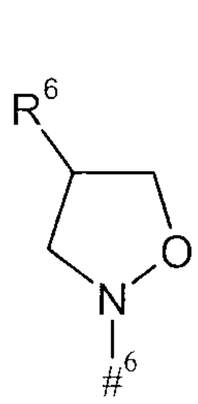
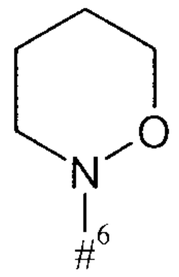
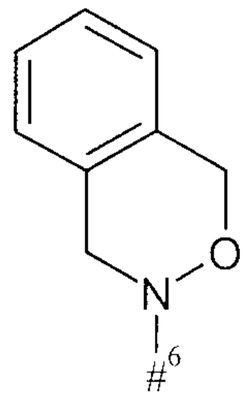


wherein

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

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

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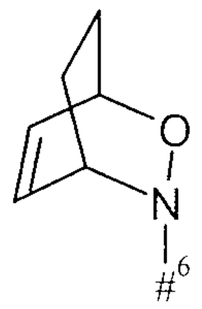


wherein

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

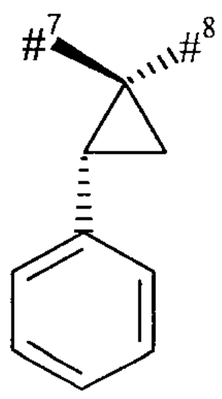
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-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 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,

$\#^8$ 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,

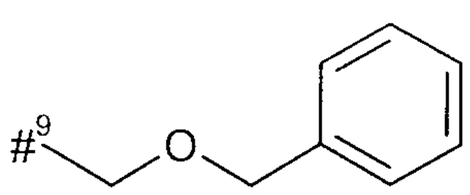
or

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



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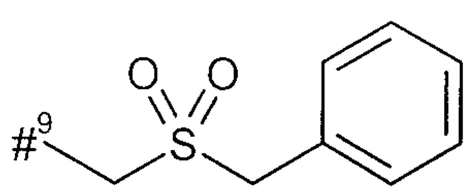

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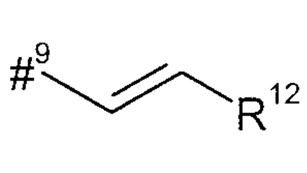

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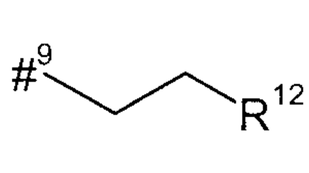

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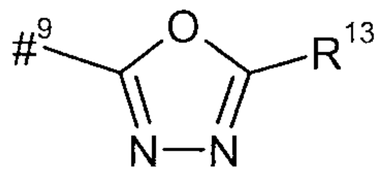


wherein

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

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^{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.

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,

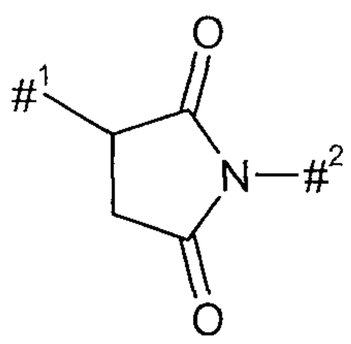
AK stands for AK_1 or AK_2

wherein

AK_1 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,

AK_2 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 = AK_1 stands for a group of the formula



wherein

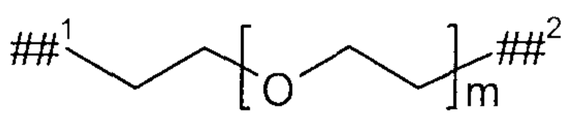
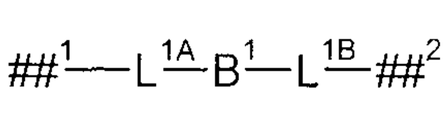
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

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

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

 or 

wherein

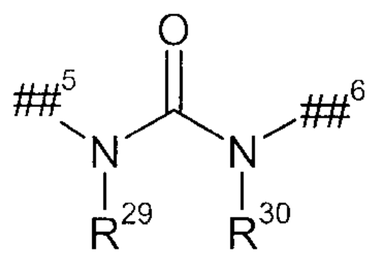
m stands for a number from 2 to 6,

$##^1$ 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,

B^1 stands for a group of the formula



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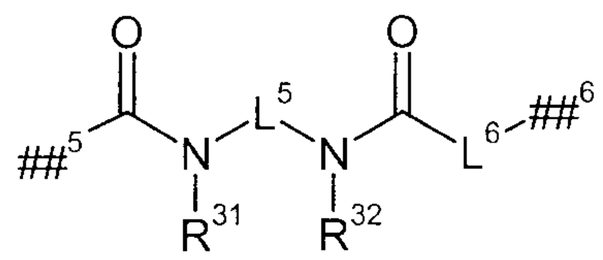
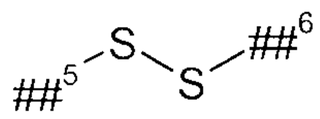


or 

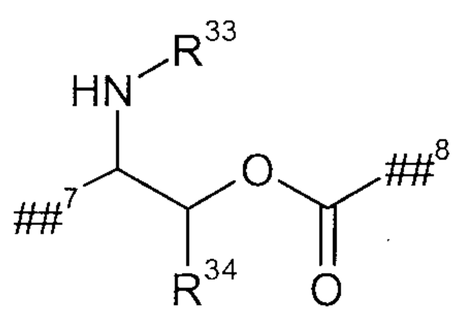
wherein

$##^5$ 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,

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



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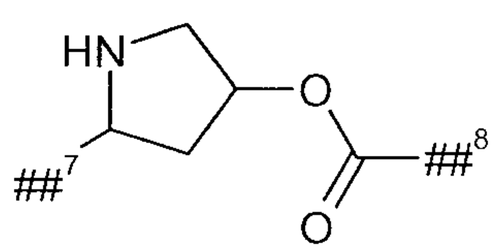


wherein

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

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

R^{33} 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,

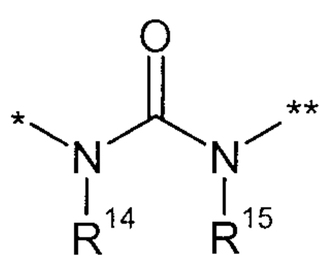
R^{32} stands for hydrogen or methyl,

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

and

wherein $(C_2\text{-}C_6)$-alkanediyl may be substituted with one to two methyl substituents,

B stands for a bond or a group of the formula



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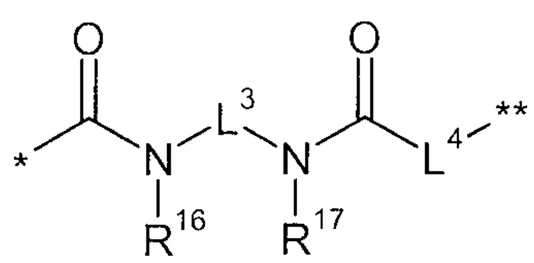

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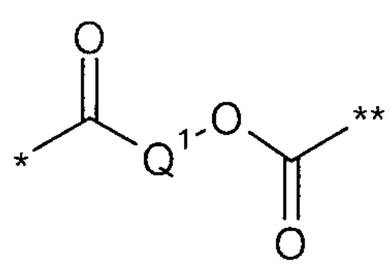

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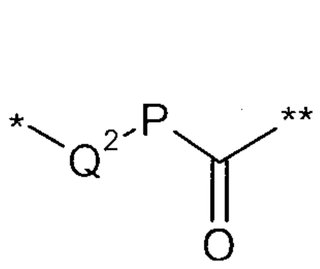

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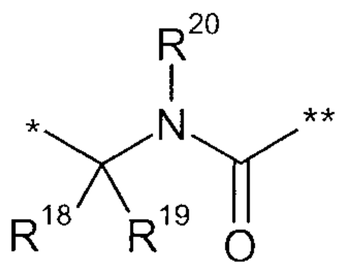

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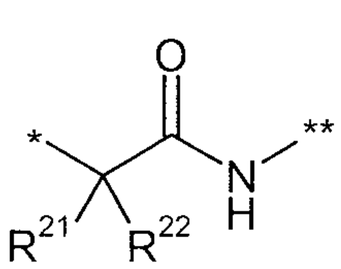

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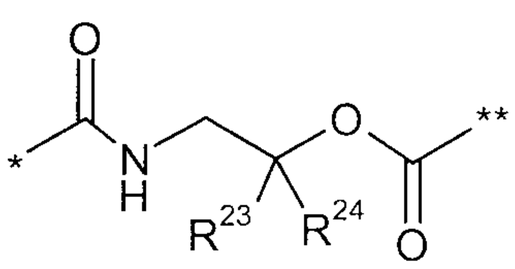

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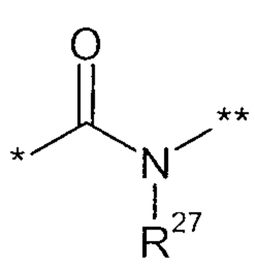

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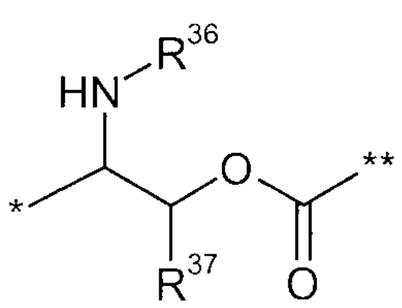


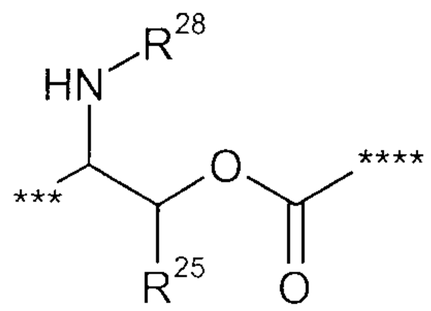
wherein

* 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



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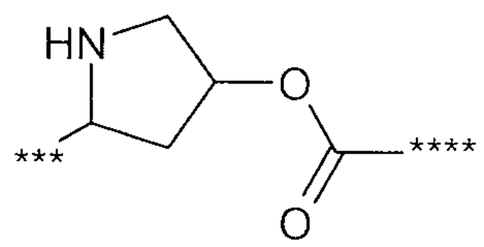


wherein

*** 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,

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,

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 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,

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,

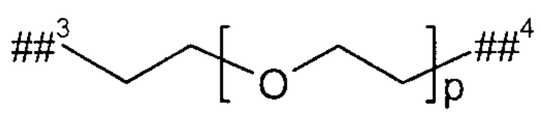
R^{36} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

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,

L^2 stands for linear $(C_2\text{-}C_6)$-alkanediyl or for a group of the formula



wherein

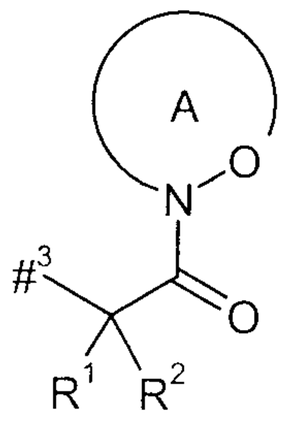
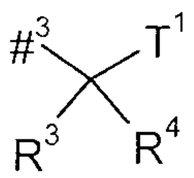
p stands for a number from 2 to 6,

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

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

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

D stands for a group of the formula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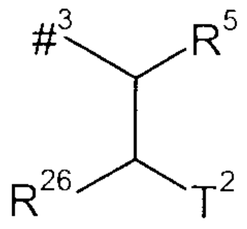


wherein

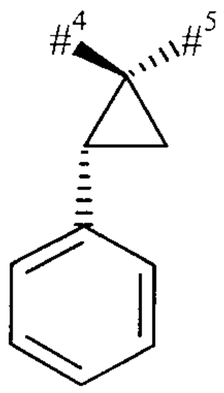
$\#^3$ denotes the linkage site to the nitrogen atom,

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 may form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

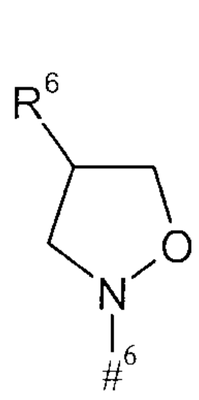
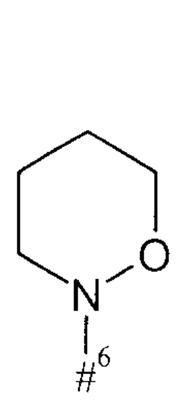
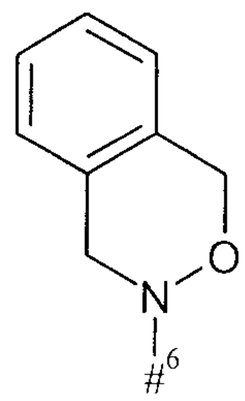


wherein

$\#^4$ 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 therein stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula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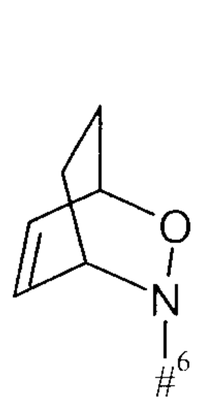


wherein

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

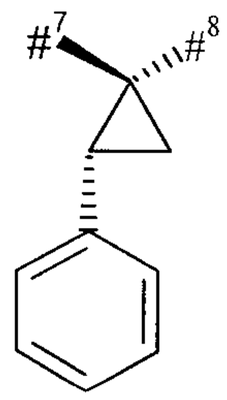
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 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 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 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,

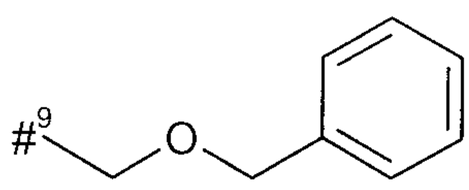
or

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

R^{10} stands for benzoyl,

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



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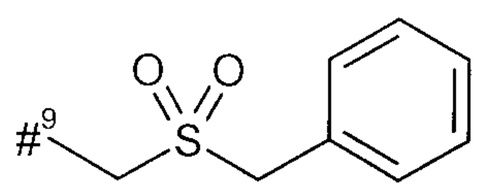

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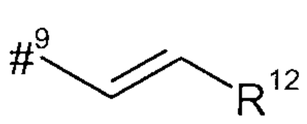

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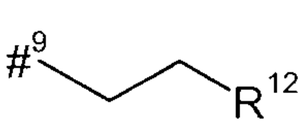

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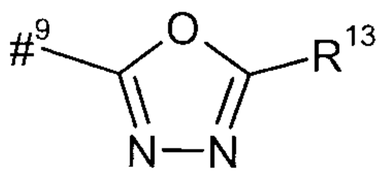


wherein

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

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^{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.

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,

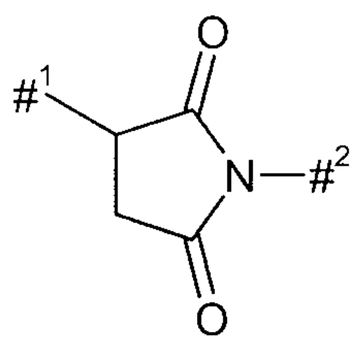
AK stands for AK_1 or AK_2

wherein

AK_1 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,

AK_2 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 = AK_1 stands for the group of the formula



wherein

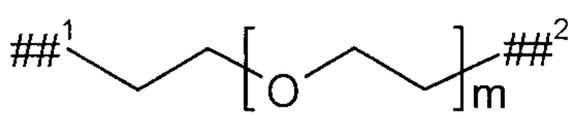
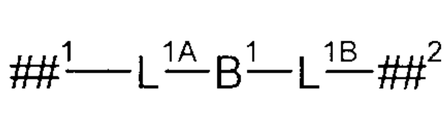
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

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

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

 or 

wherein

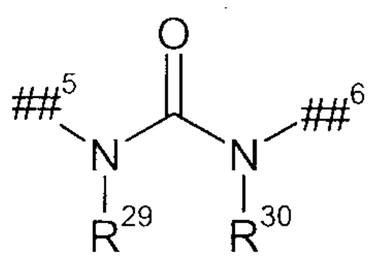
m stands for a number from 2 to 6,

$##^1$ 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,

B^1 stands for a group with the formula



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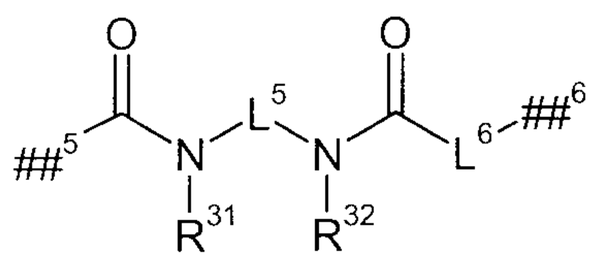
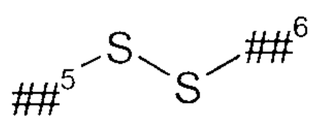


or 

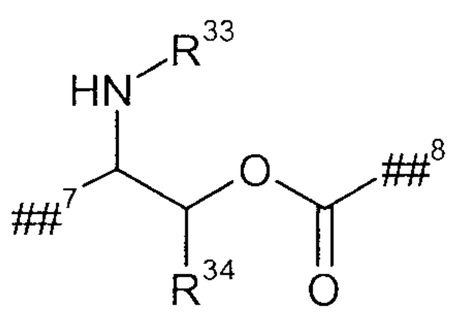
wherein

$##^5$ 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,

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



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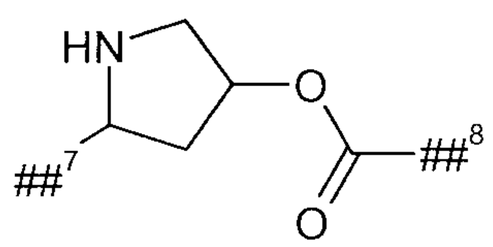


wherein

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

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

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

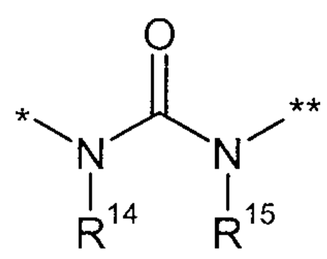
R^{32} stands for hydrogen or methyl,

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

and

wherein $(C_2\text{-}C_6)$-alkanediyl may be substituted with one to two methyl substituents,

B stands for a bond or a group of the formula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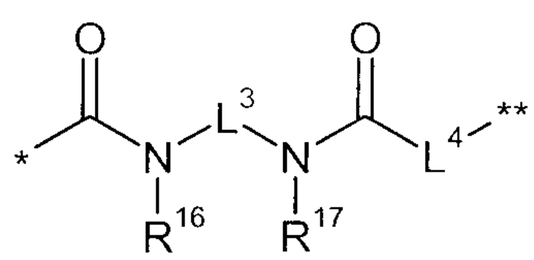
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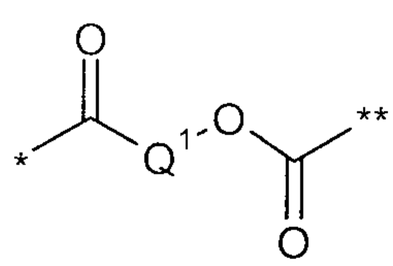
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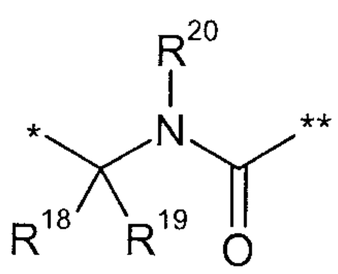
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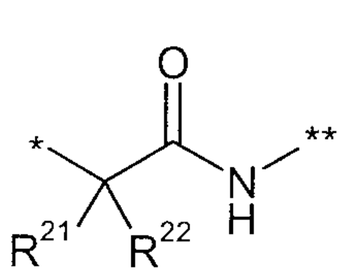
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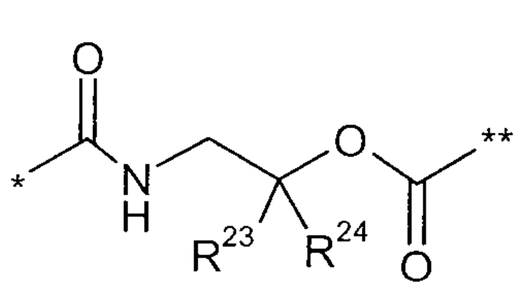
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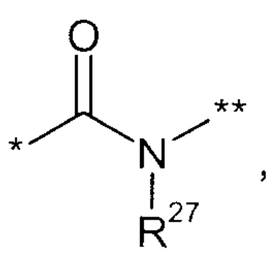
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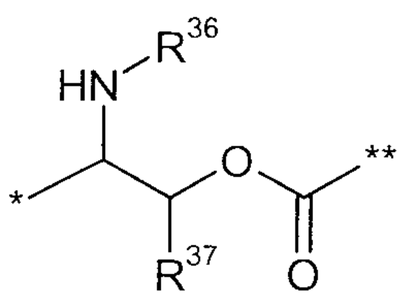


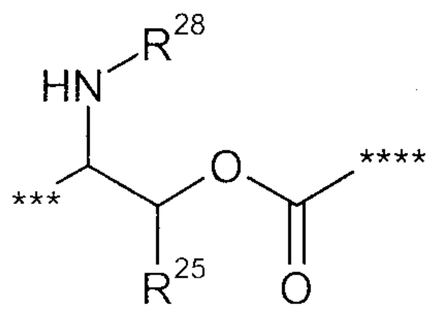
wherein

* 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



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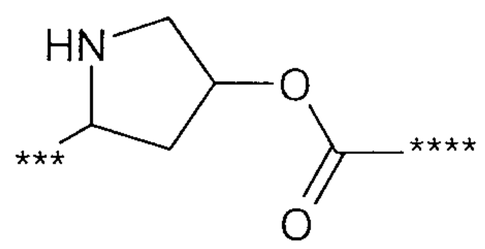


wherein

*** 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 *tert*-butyloxycarbonyl,

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,

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 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,

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,

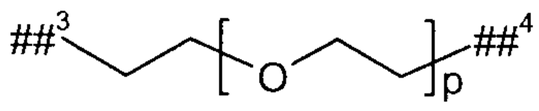
R^{24} stands for hydrogen or methyl,

R^{27} stands for hydrogen,

R^{36} stands for hydrogen, $(C_1$-$C_4)$-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

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

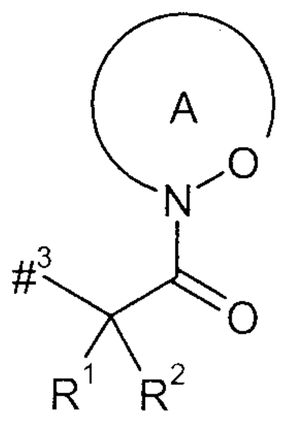
p stands for a number from 2 to 6,

$##^3$ 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,

D stands for a group of the formula



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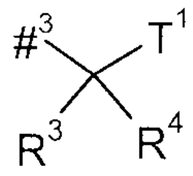

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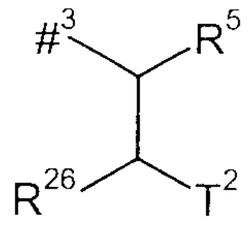


wherein

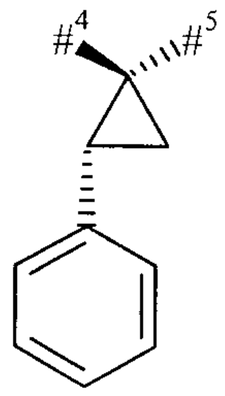
$\#^3$ denotes the linkage site to the nitrogen atom,

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

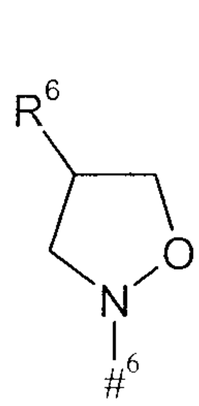
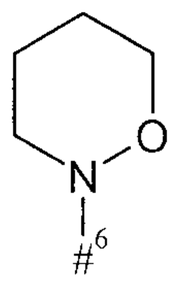
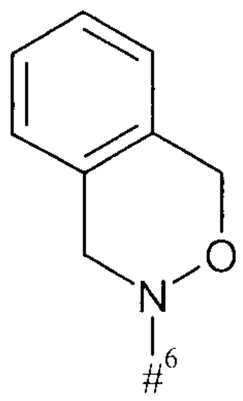


wherein

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

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

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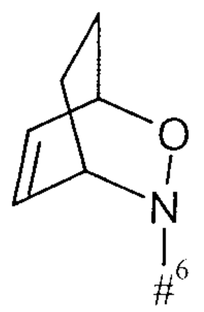


wherein

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

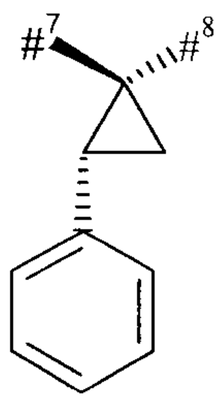
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 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 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 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,

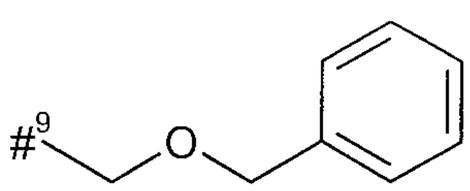
or

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

R^{10} stands for benzoyl,

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



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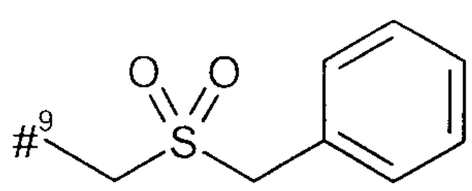

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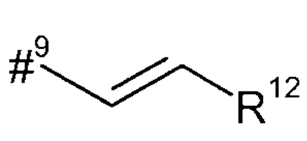

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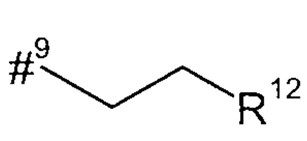

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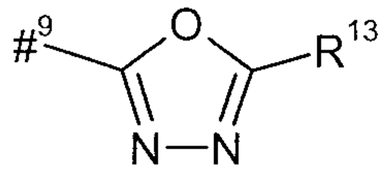


wherein

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

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^{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.

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,

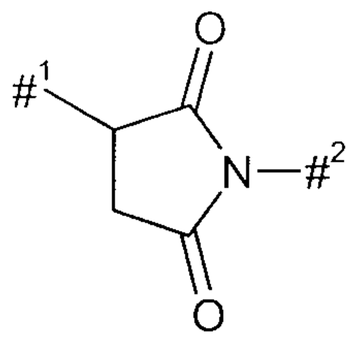
AK stands for AK_1 or AK_2

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,

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

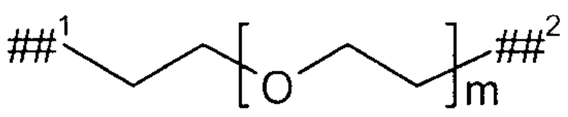
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ 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

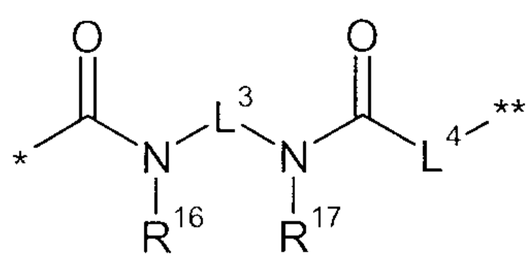
m stands for a number of 2 or 3,

##[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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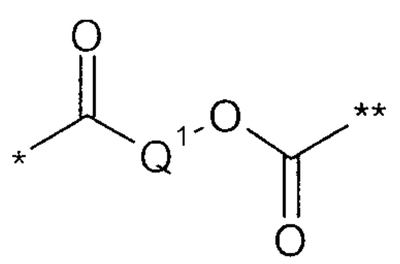
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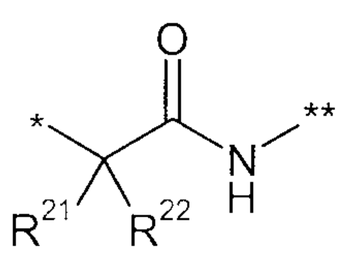
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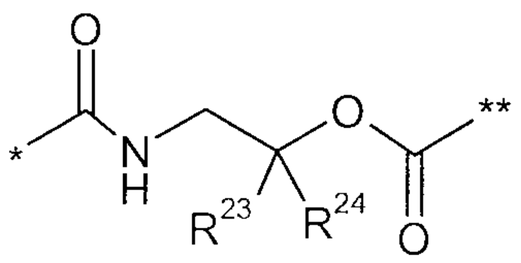
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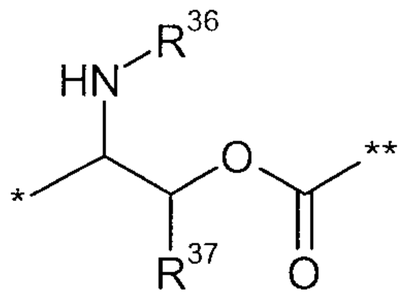
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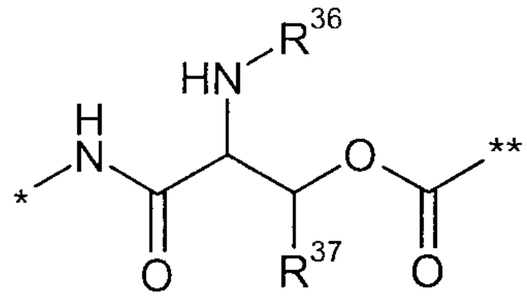


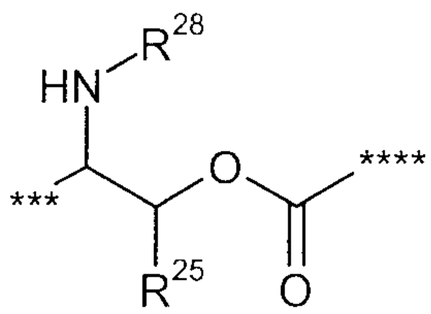
wherein

* 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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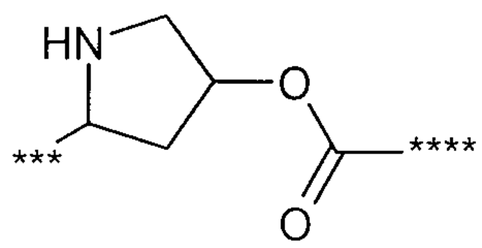


wherein

*** 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,

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

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

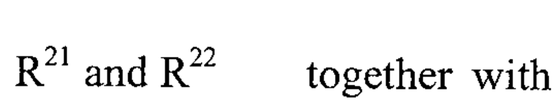
R^{23} stands for methyl,

R^{24} stands for hydrogen,

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

R^{37} stands for hydrogen or methyl,

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



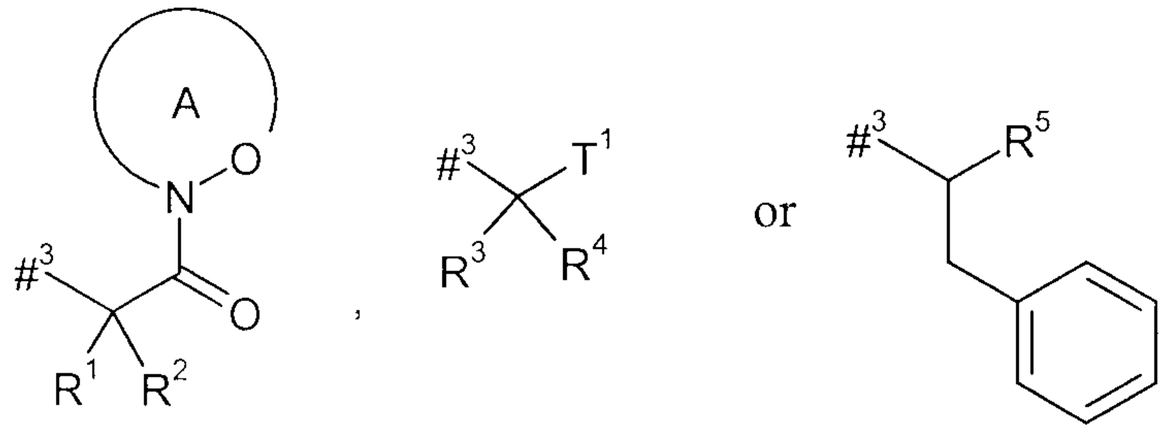
wherein

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

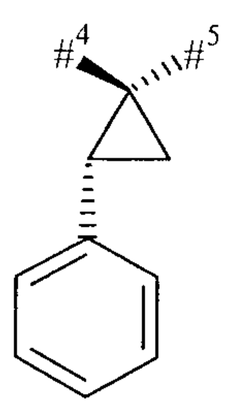
$\#^3$ denotes the linkage site to the nitrogen atom,

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

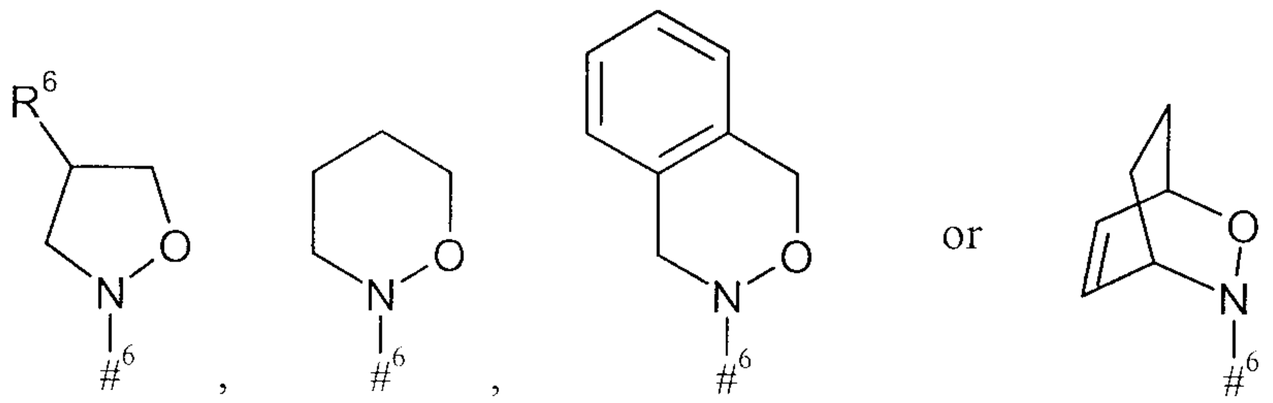


wherein

$\#^4$ 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

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

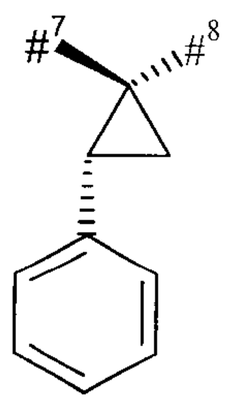
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

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

or

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 $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$ or $-CH_2-O-R^{11}$,

wherein

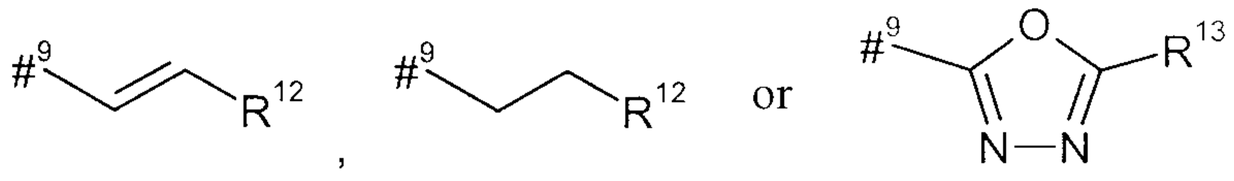
R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

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

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,

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,

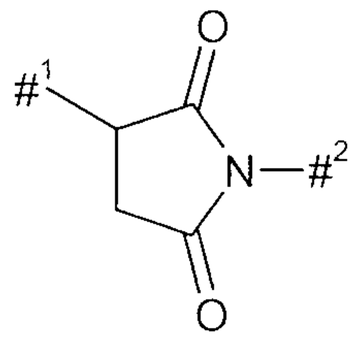
AK stands for AK_1 or AK_2

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,

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

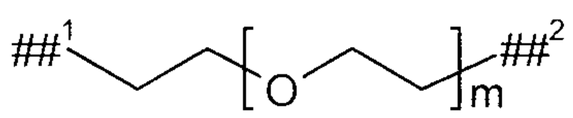
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ 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

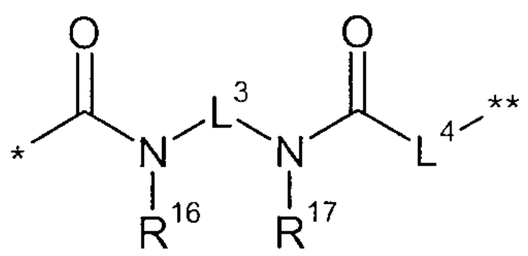
m stands for a number of 2 or 3,

$\#\#^1$ 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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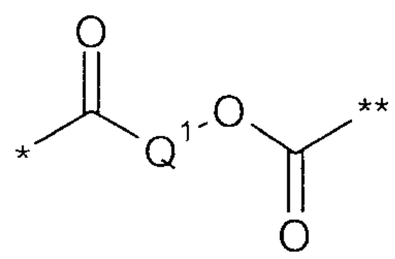
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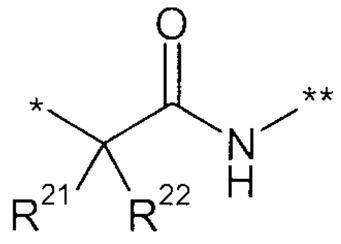
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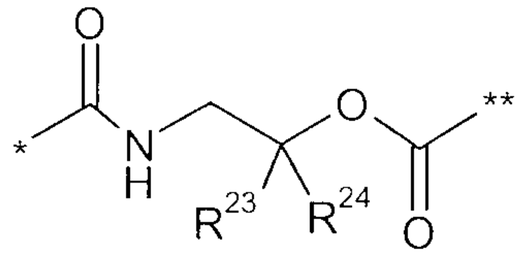


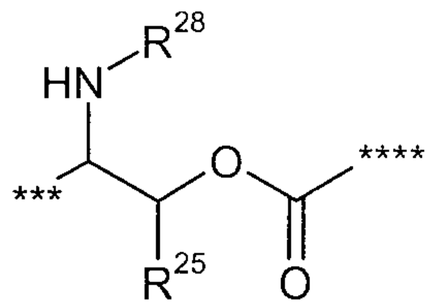
wherein

* 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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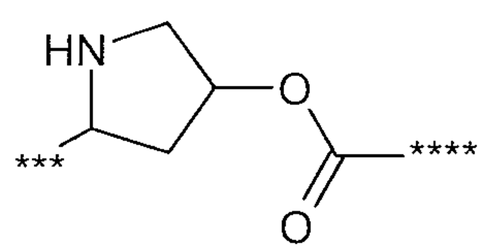


wherein

*** 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,

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,

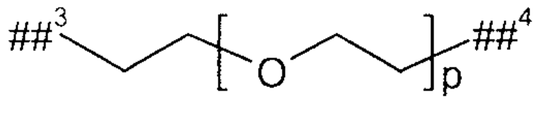
or

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



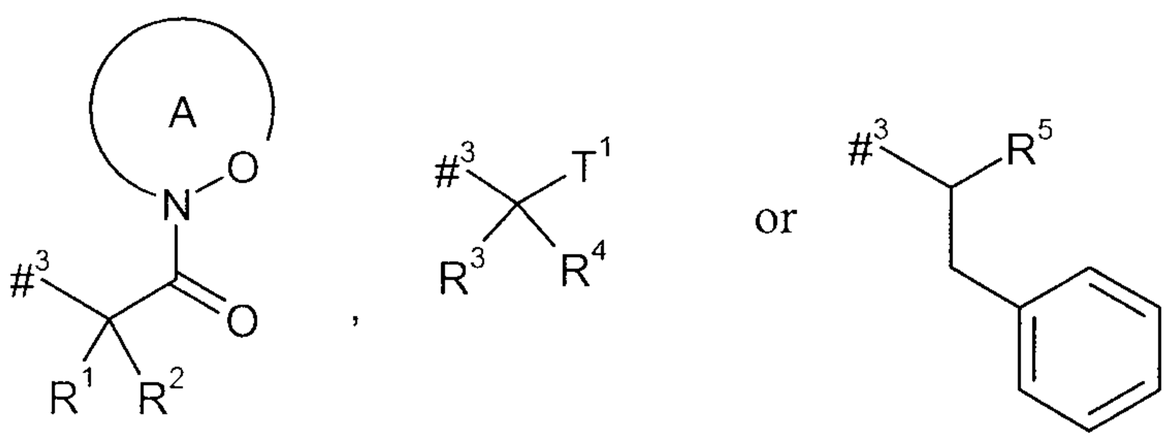
wherein

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

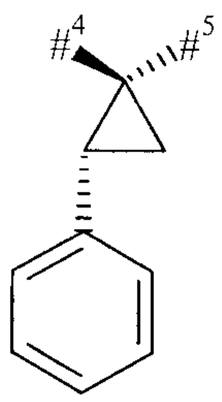
$#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-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 (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

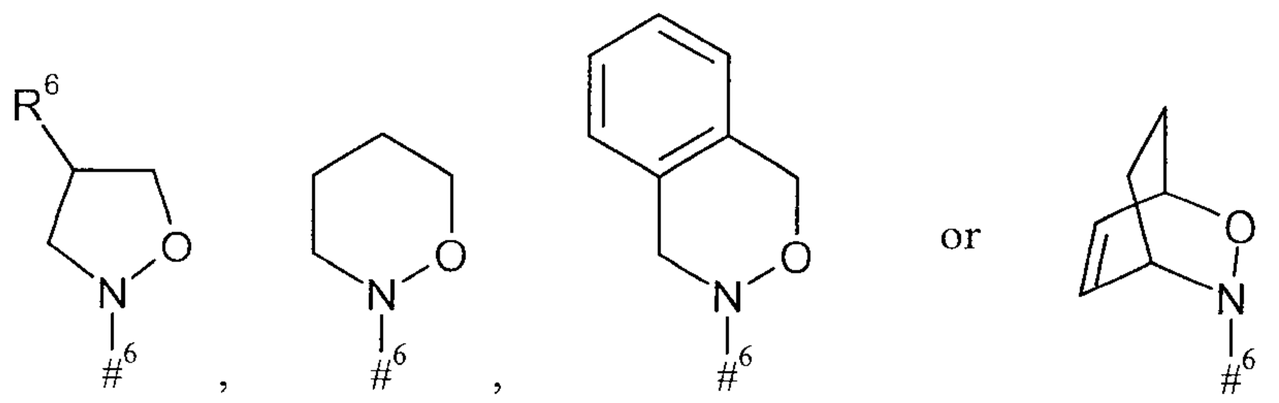


wherein

$\#^4$ 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

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

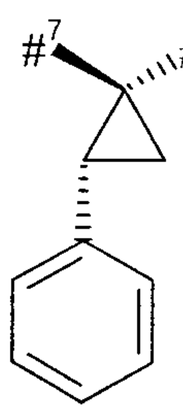
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for benzyl, 1-hydroxybenzyl, 1-phenylethyl or 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



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

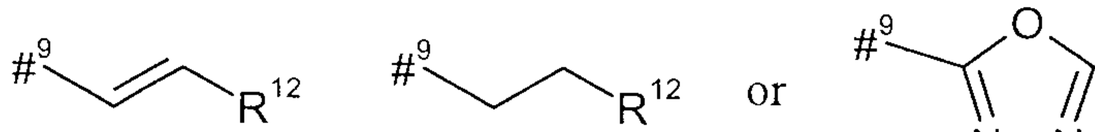
R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

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

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,

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,

AK stands for AK_2

wherein

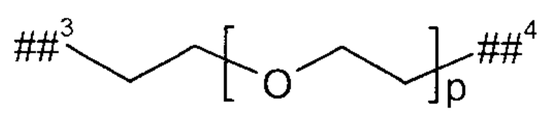
AK_2 stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G stands for carbonyl,

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



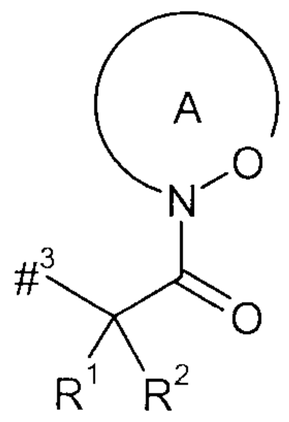
wherein

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



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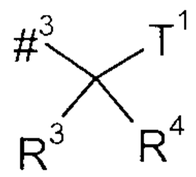


wherein

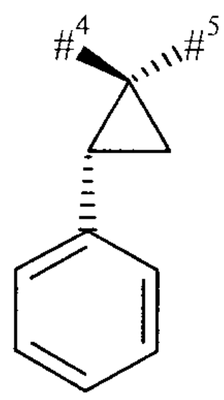
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for benzyl 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,

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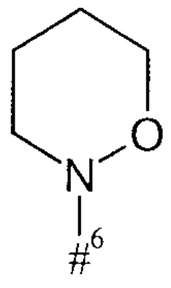


wherein

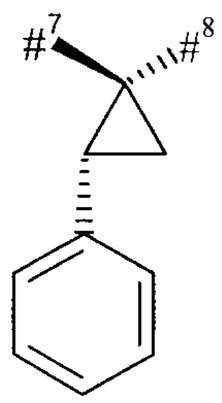
$\#^6$ denotes the linkage site to the carbonyl group,

R^3 stands for hydrogen,

R^4 stands for benzyl 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



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

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

R^8 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.

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_2,

wherein

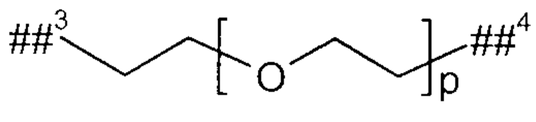
AK_2 stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G stands for carbonyl,

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



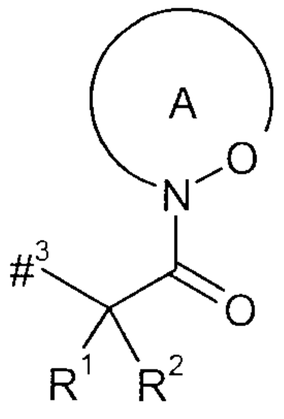
wherein

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



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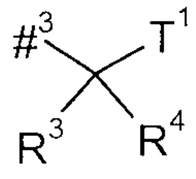


wherein

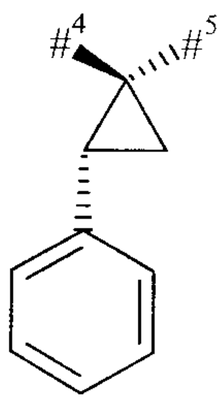
$#^3$ denotes the linkage site to the nitrogen atom,

R^1 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

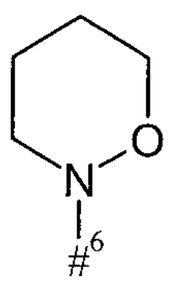


wherein

$\#^4$ 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

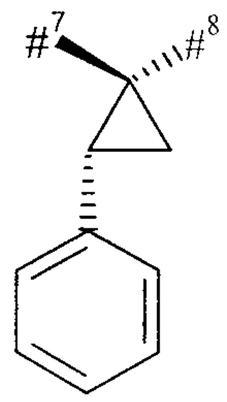
$\#^6$ denotes the linkage site to the carbonyl group,

R^3 stands for hydrogen,

R^4 stands for benzyl, 4-hydroxybenzyl 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



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

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

R^8 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.

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

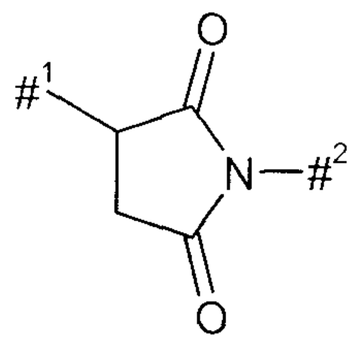
n stands for a number from 1 to 10,

AK stands for AK_1

wherein

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,

G stands for a group of the formula

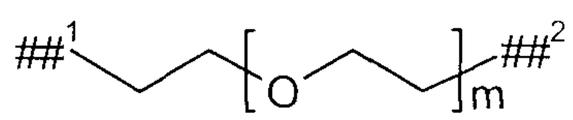


wherein

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

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

L^1 stands for a bond, linear (C_3-C_5)-alkanediyl or a group of the formula



wherein

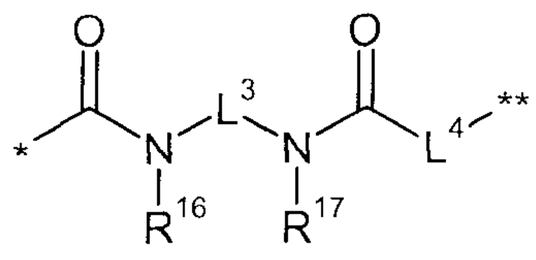
m stands for a number of 2 or 3,

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

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

wherein (C_3-C_5)-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,

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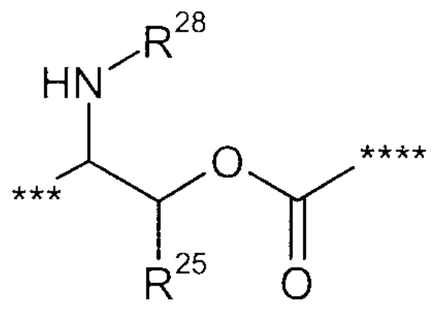
L^3 stands for a bond or ethane-1,2-diyl,

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



wherein

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

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

R^{25} stands for methyl,

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

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

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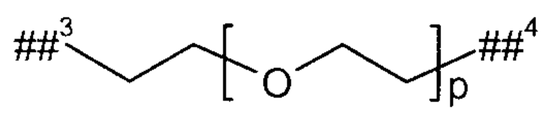
R^{16} and R^{17} together with the atoms to which they are bound form a piperazinyl ring,

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



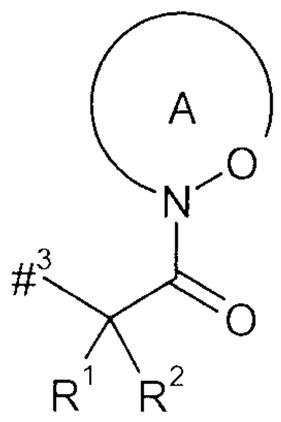
wherein

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,

D stands for a group of the formula



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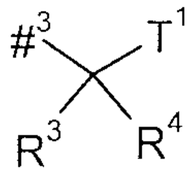

wherein

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

R^1 stands for hydrogen,

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

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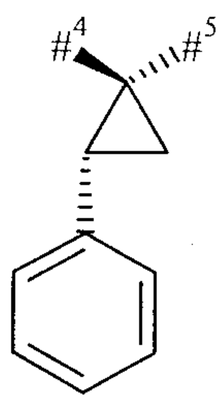

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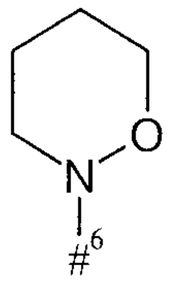

wherein

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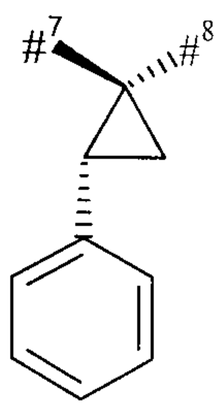

wherein

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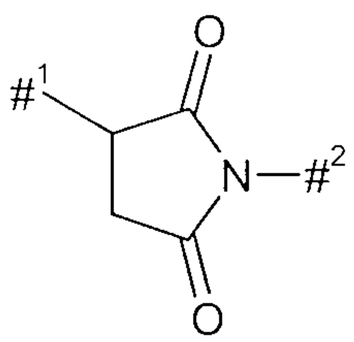

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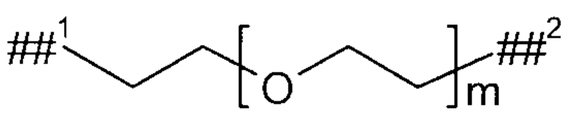

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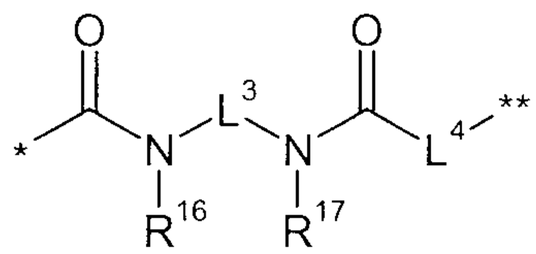
$\#\#^1$ denotes the linkage site to the group G,

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

wherein $(C_3$-$C_5)$-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



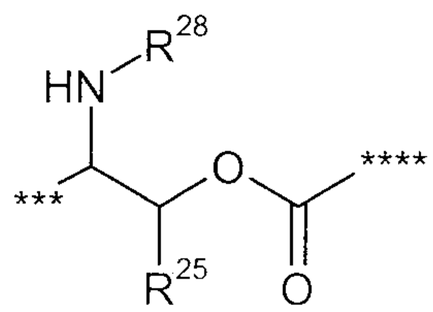
wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

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,

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

R^{25} stands for methyl,

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

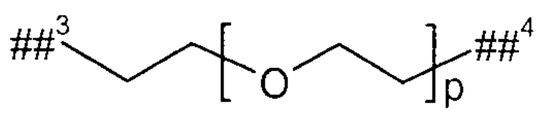
R^{16} stands for hydrogen or methyl,

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 $(C_3\text{-}C_5)$-alkanediyl or for a group of the formula



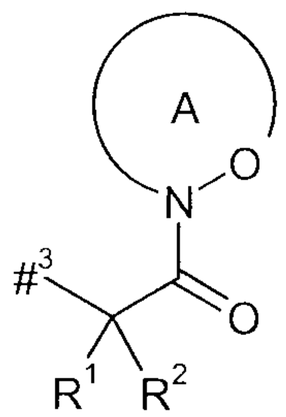
wherein

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,

D stands for a group of the formula



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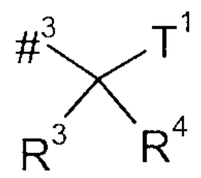


wherein

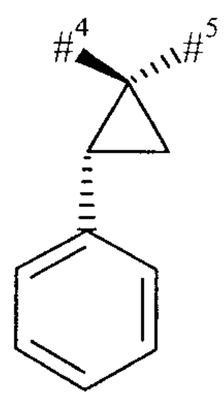
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 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

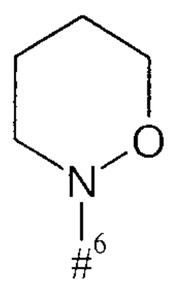


wherein

$\#^4$ 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

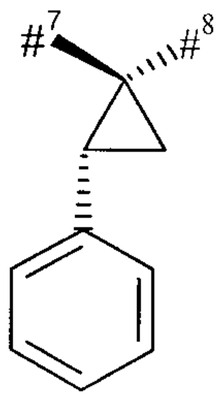
$\#^6$ denotes the linkage site to the carbonyl group,

R^3 stands for hydrogen,

R^4 stands for benzyl, 4-hydroxybenzyl 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



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

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

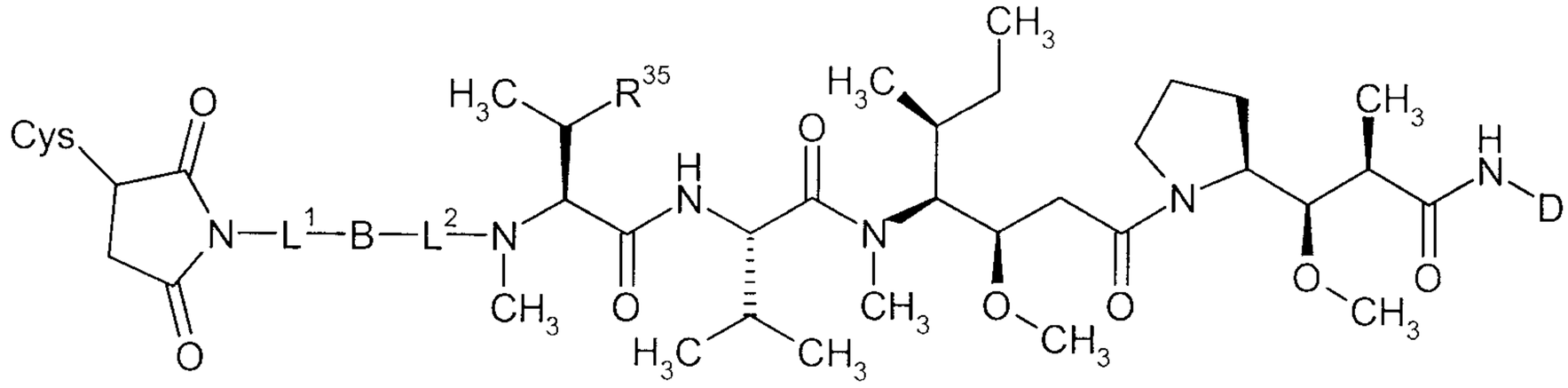
R^8 stands for hydrogen,

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)

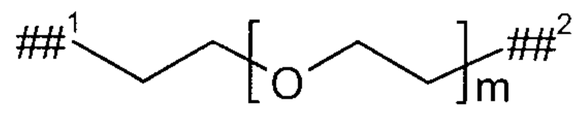


(XXXa),

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,

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



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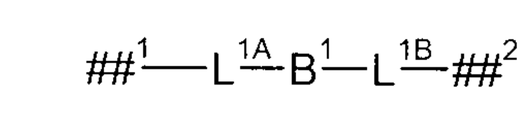


wherein

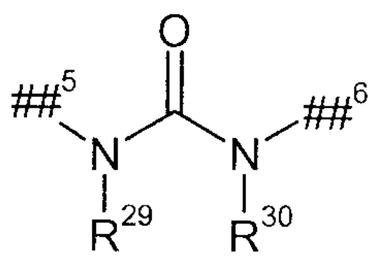
m stands for a number from 2 to 6,

$##^1$ 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_{10})-alkanediyl,

B^1 stands for a group of the formula



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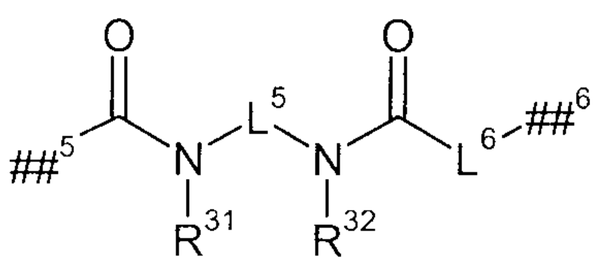


wherein

$##^5$ 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,

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,

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,

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

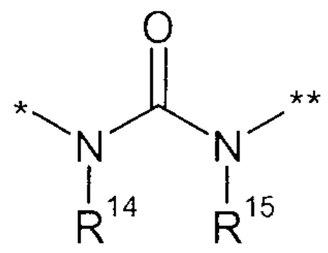
and

wherein $(C_1$-$C_{10})$-alkanediyl may be substituted with one to four substituents selected independently 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 to form a $(C_3$-$C_6)$-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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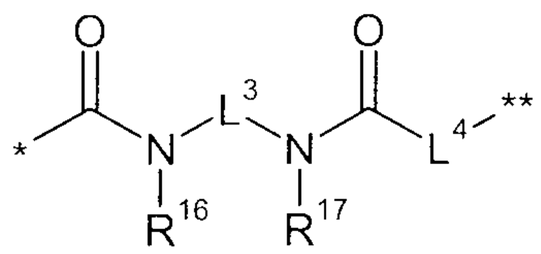
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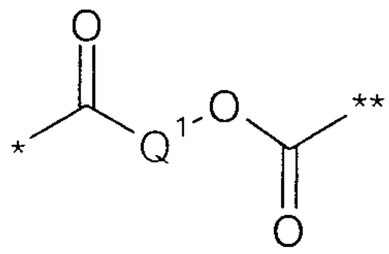
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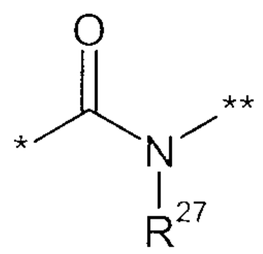


wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

P stands for O or NH,

L^3 stands 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,

R^{14} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

R^{15} stands for hydrogen or $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-alkyl,

R^{17} stands for hydrogen or $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-alkyl,

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 $(C_1$-$C_4)$-alkyl,

R^{22} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

or

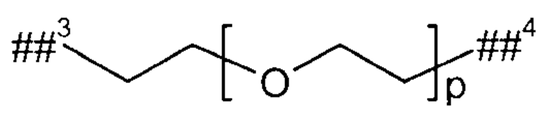
R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{23} stands for $(C_1$-$C_4)$-alkyl,

R^{24} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

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



wherein

p stands for a number from 2 to 6,

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

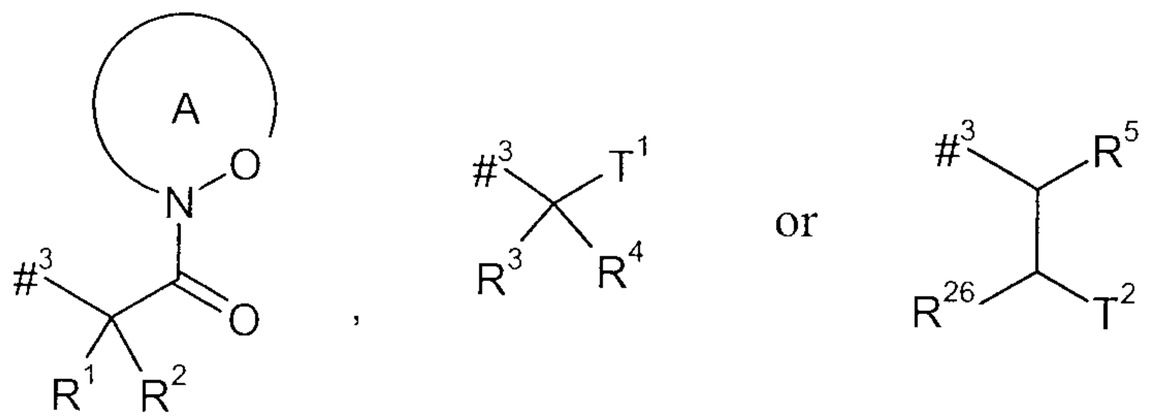
$##^4$ denotes the linkage site to the nitrogen atom,

wherein $(C_2$-$C_{10})$-alkanediyl may be substituted with 1 to 4 substituents selected independently of one another from the group comprised of 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 to form a $(C_3$-$C_6)$ cycloalkyl ring or a phenyl ring, including the carbon atoms optionally situated between them,

D stands for a group of the formula



wherein

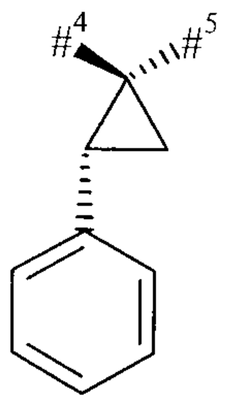
$\#^3$ denotes the linkage site to the nitrogen atom,

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,

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

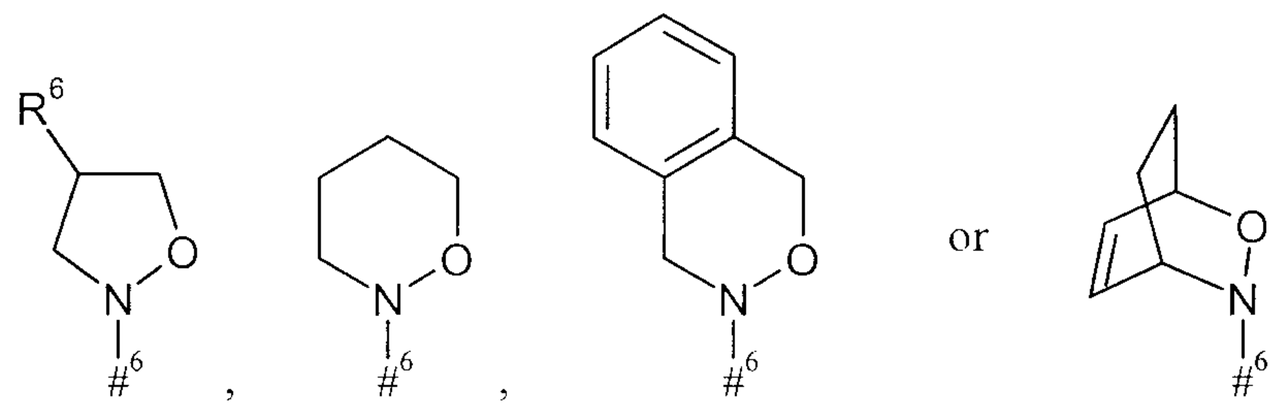


wherein

$\#^4$ 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

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

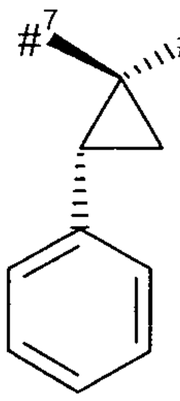
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-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



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,

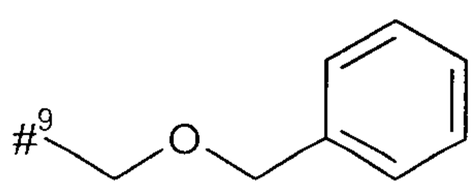
or

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



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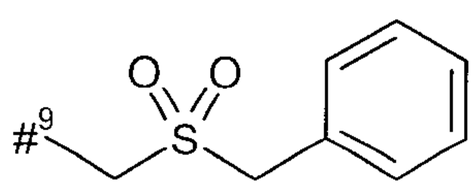

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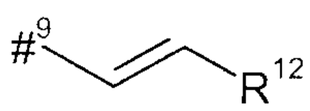

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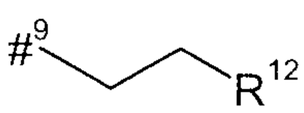

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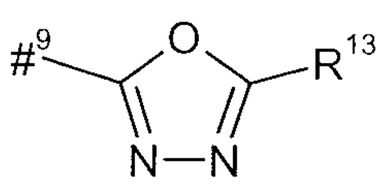


wherein

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

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,

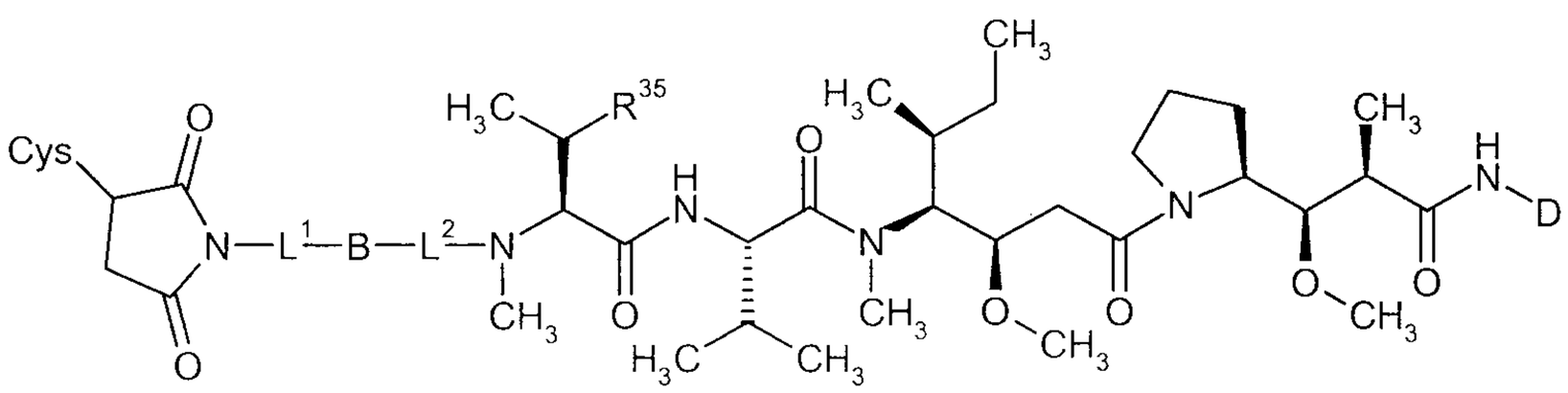
R^{26} stands for hydrogen or hydroxyl,

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.

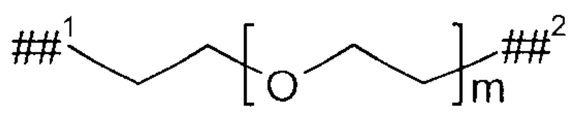
The preferred subject matter of the present invention is compounds of formula (XXXa) as indicated above, wherein



(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 (C_2-C_6)-alkanediyl, a group of the formula



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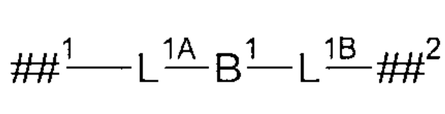


wherein

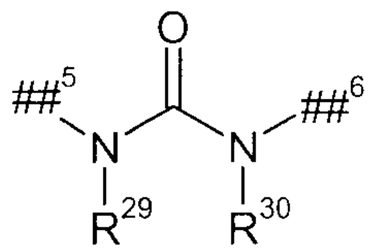
m stands for a number of 2 or 3,

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

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

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

B^1 stands for a group of the formula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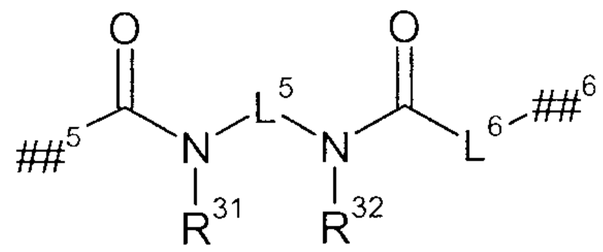


wherein

$##^5$ 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,

L^6 stands for a bond,

R^{29} stands for hydrogen,

R^{30} stands for hydrogen,

R^{31} stands for hydrogen or methyl,

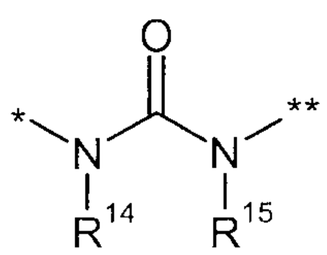
R^{32} stands for hydrogen or methyl,

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

and

wherein $(C_2\text{-}C_6)$-alkanediyl may be substituted with one to two methyl substituents,

B stands for a bond or a group of the formula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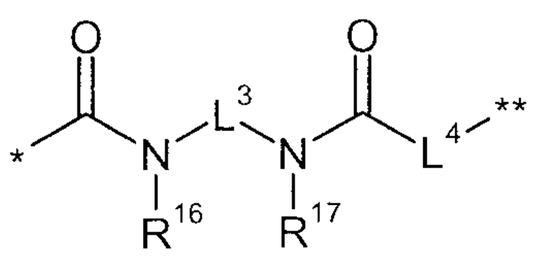

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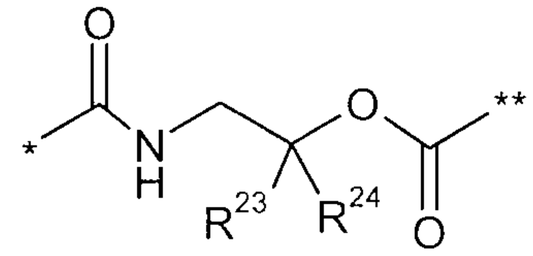


wherein

* 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,

R^{14} stands for hydrogen,

R^{15} stands for hydrogen,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

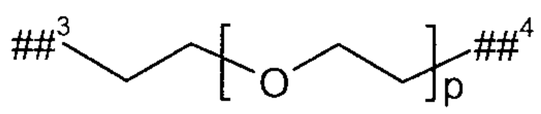
or

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,

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



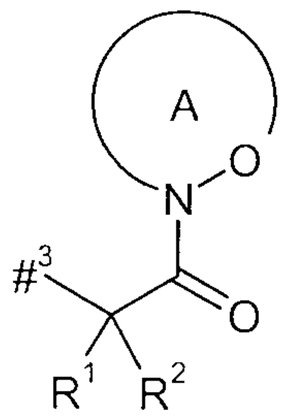
wherein

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



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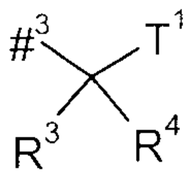

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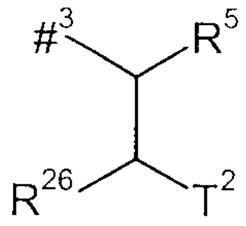


wherein

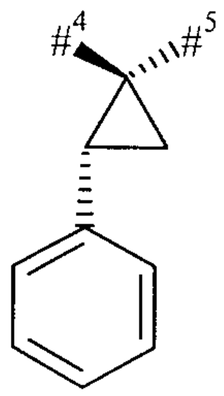
$\#^3$ denotes the linkage site to the nitrogen atom,

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

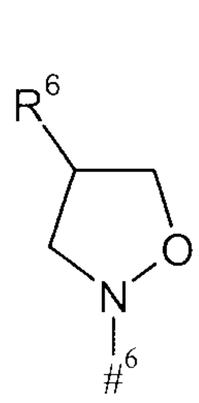
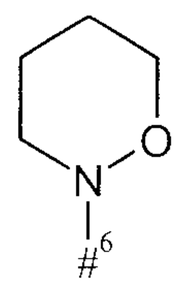
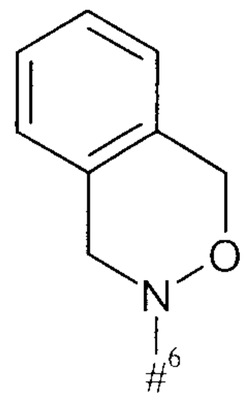
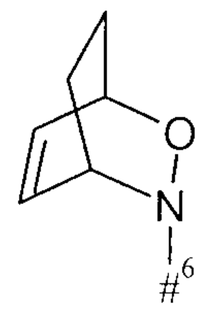


wherein

$\#^4$ 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


, 
, 
or 

wherein

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

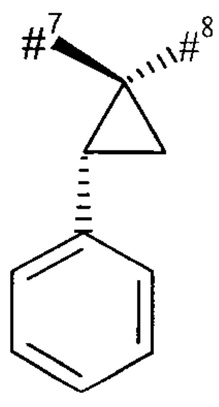
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 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



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,

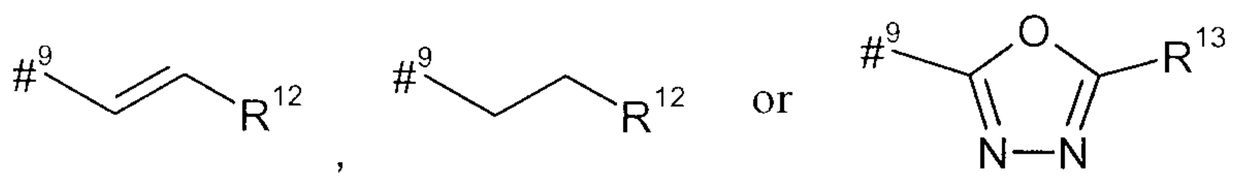
or

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



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,

R^{35} stands for methyl or hydroxyl,

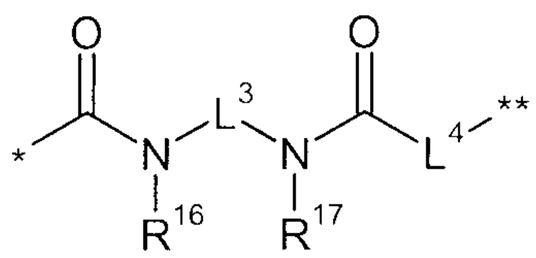
as well as their salts and solvates as well as the solvates of the salts.

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

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



wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

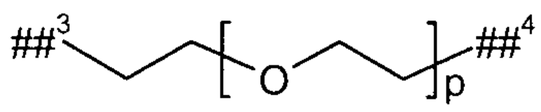
L^3 stands for a bond,

L^4 stands for a bond,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

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



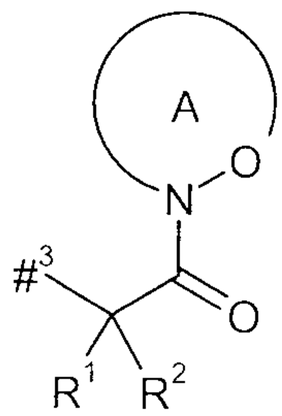
wherein

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,

D stands for a group of the formula



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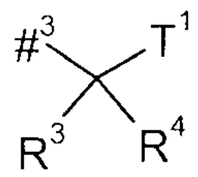


wherein

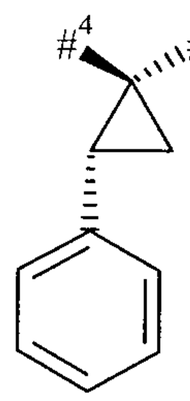
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for benzyl 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

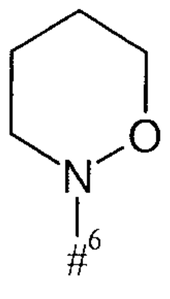


wherein

$\#^4$ 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

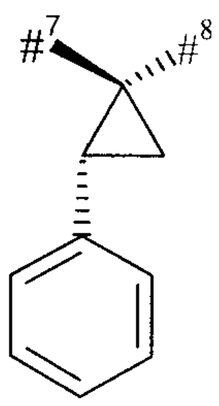
$\#^6$ denotes the linkage site to the carbonyl group,

R^3 stands for hydrogen,

R^4 stands for benzyl 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



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

R^7 stands for hydrogen,

R^8 stands for hydrogen,

R^9 stands for hydrogen,

R^{35} stands for methyl,

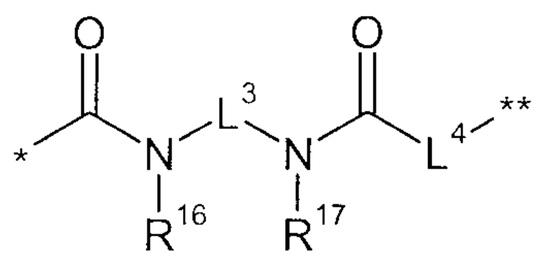
as well as their salts and solvates as well as the solvates of the salts.

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 (C_2-C_6)-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,

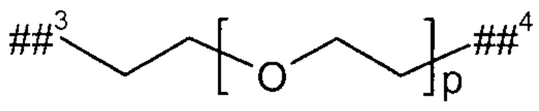
L^3 stands for a bond,

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



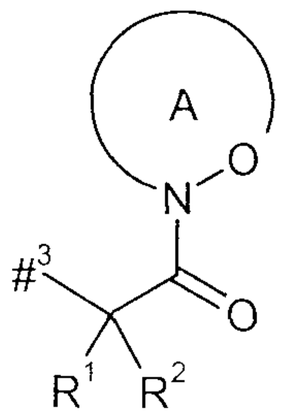
wherein

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



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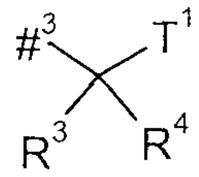


wherein

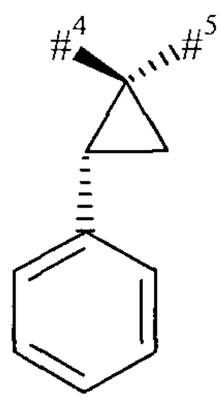
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 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

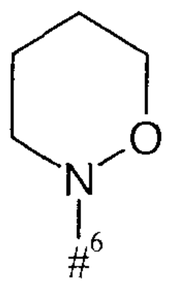


wherein

$\#^4$ 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

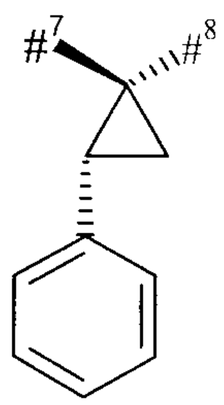
#6 denotes the linkage site to the carbonyl group,

R^3 stands for hydrogen,

R^4 stands for benzyl, 4-hydroxybenzyl 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



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

R^7 stands for hydrogen,

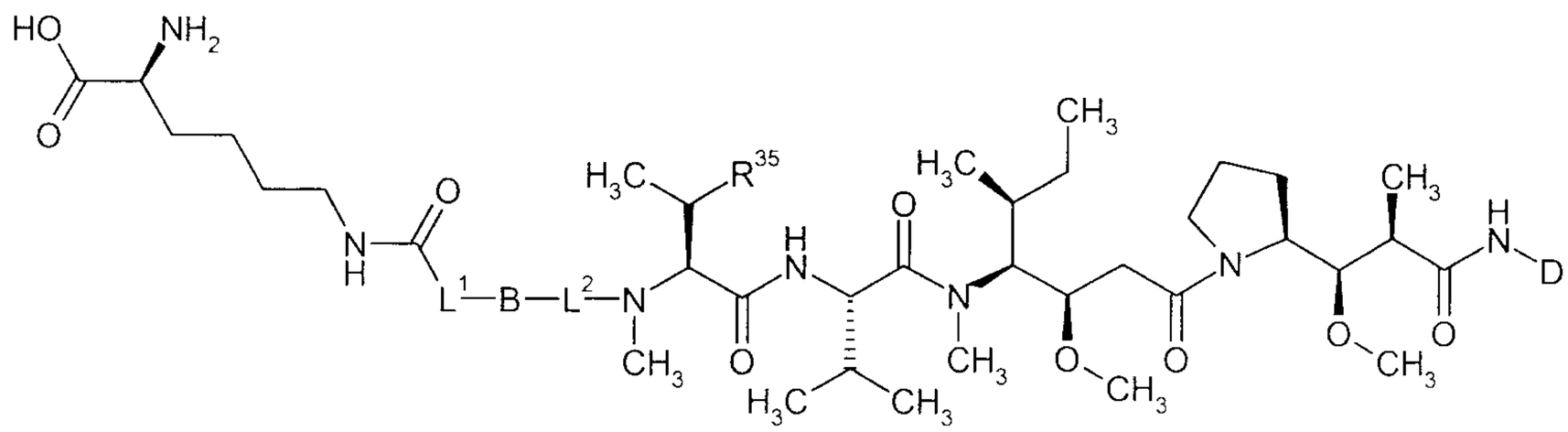
R^8 stands for hydrogen,

R^9 stands for hydrogen,

R^{35} stands for methyl,

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

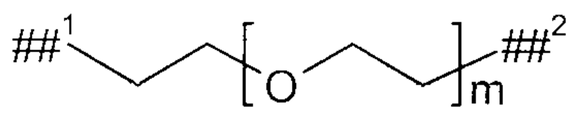
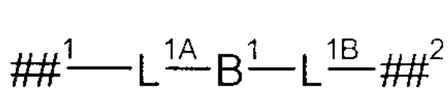
Another subject matter of the present invention relates to compounds of formula (XXXI)



(XXXI),

in which

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

 or 

wherein

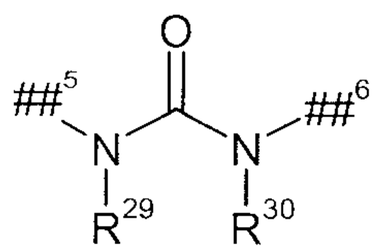
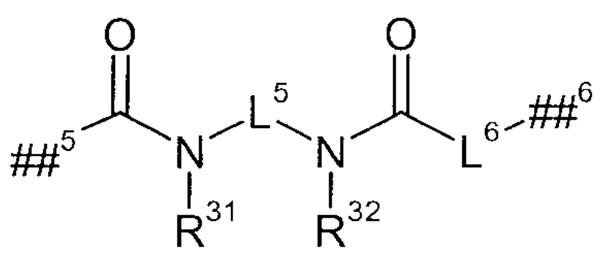
m stands for a number from 2 to 6,

$##^1$ 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_{10})-alkanediyl,

B^1 stands for a group of the formula

 or 

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,

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,

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,

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

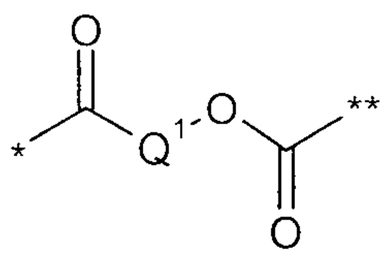
and

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

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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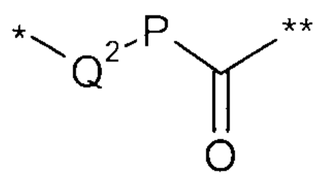
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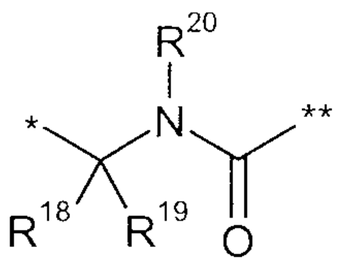
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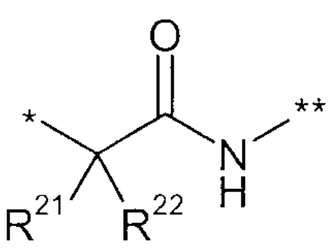
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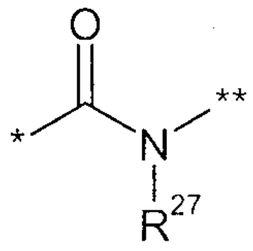


wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

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 (C_1-C_4)-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 (C_1-C_4)-alkyl,

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 (C_1-C_4)-alkyl,

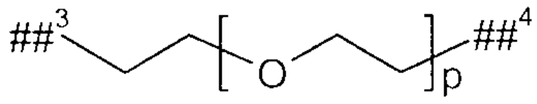
R^{22} stands for hydrogen or (C_1-C_4)-alkyl,

or

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

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



wherein

p stands for a number from 2 to 6,

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

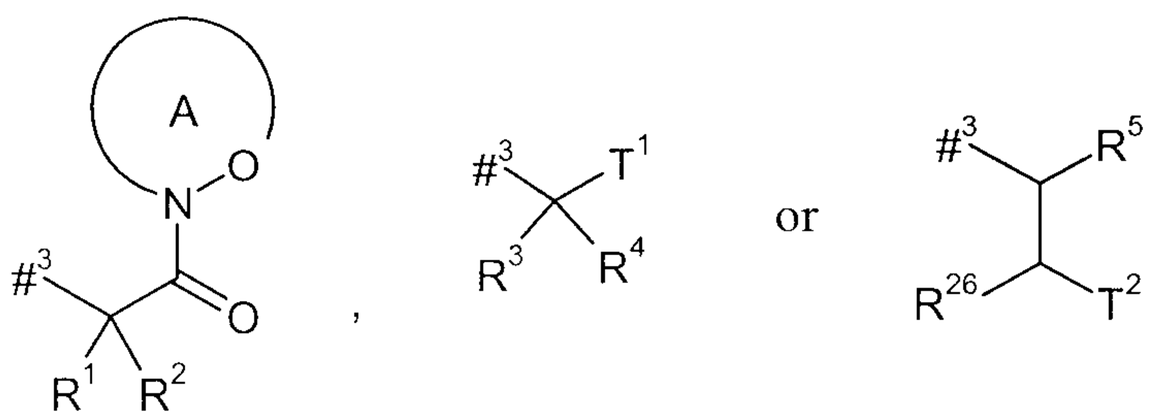
$\#\#^4$ denotes the linkage site to the nitrogen atom,

Wherein $(C_2$-$C_{10})$-alkanediyl may be substituted with 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,

D stands for a group of the formula



wherein

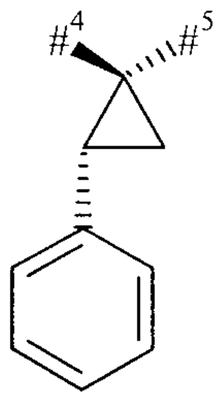
$\#^3$ denotes the linkage site to the nitrogen atom,

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, 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

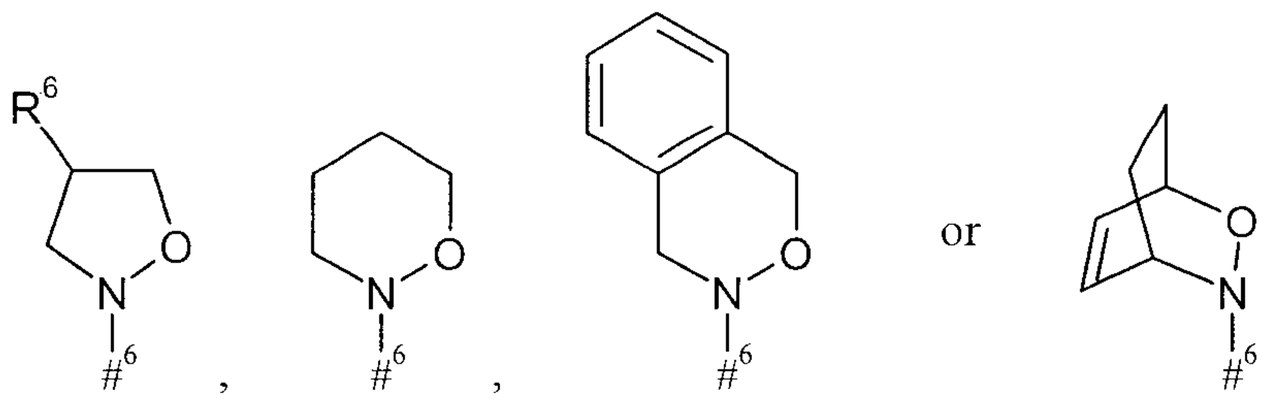


wherein

$\#^4$ 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,

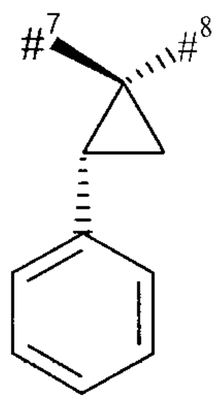
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-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



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,

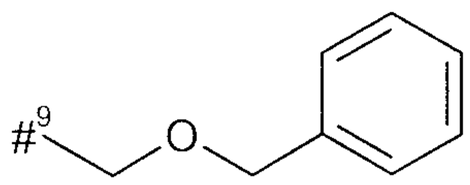
or

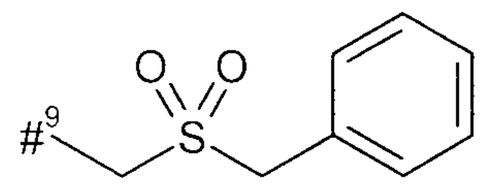
R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

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





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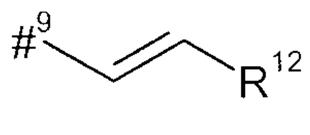

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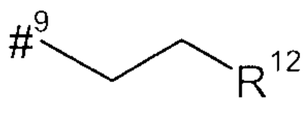
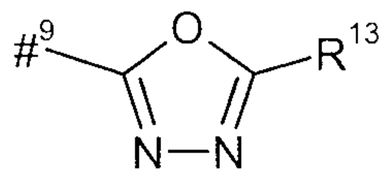


or



wherein

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

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^{26} stands for hydrogen or hydroxyl,

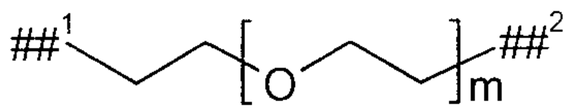
T^2 stands for phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

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

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

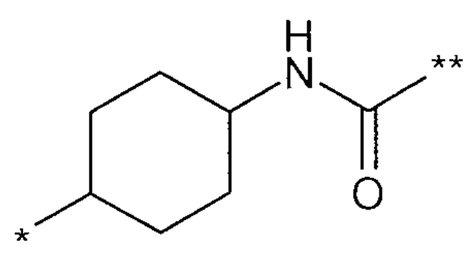
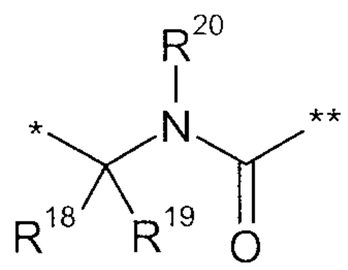
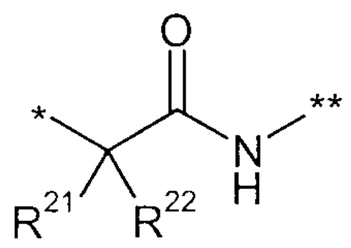
m stands for a number of 2 or 3,

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

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

wherein (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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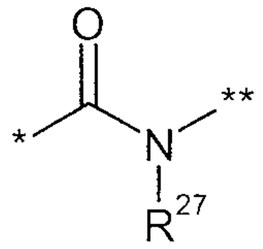


wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

R^{18} stands for hydrogen,

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

R^{20} stands for hydrogen or (C_1-C_4)-alkyl,

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,

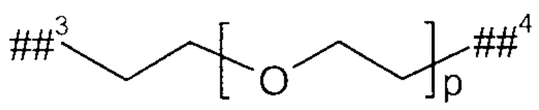
R^{22} stands for hydrogen or methyl,

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,

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,

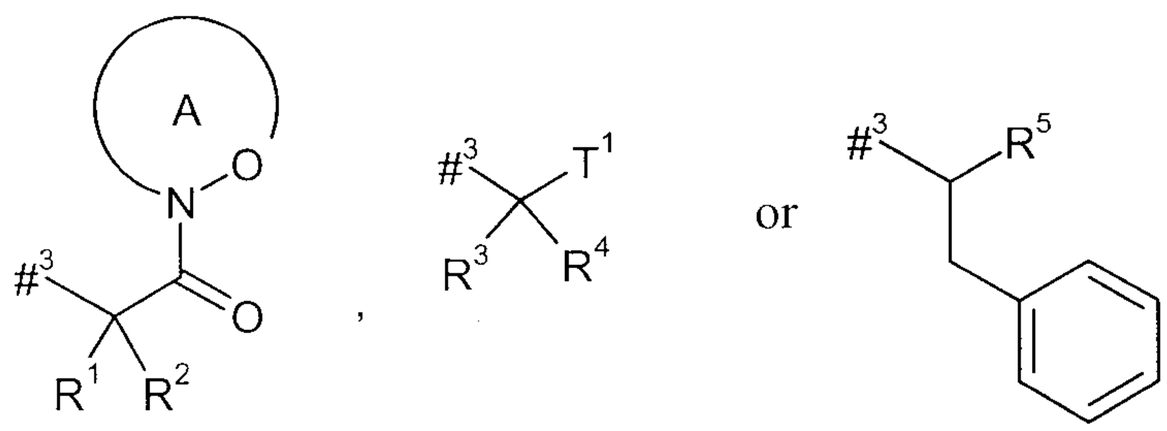
$\#\#^4$ denotes the linkage site to the nitrogen atom,

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,

D stands for a group of the formula



wherein

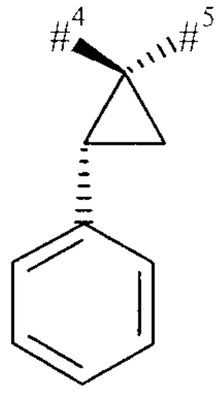
$\#^3$ denotes the linkage site to the nitrogen atom,

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

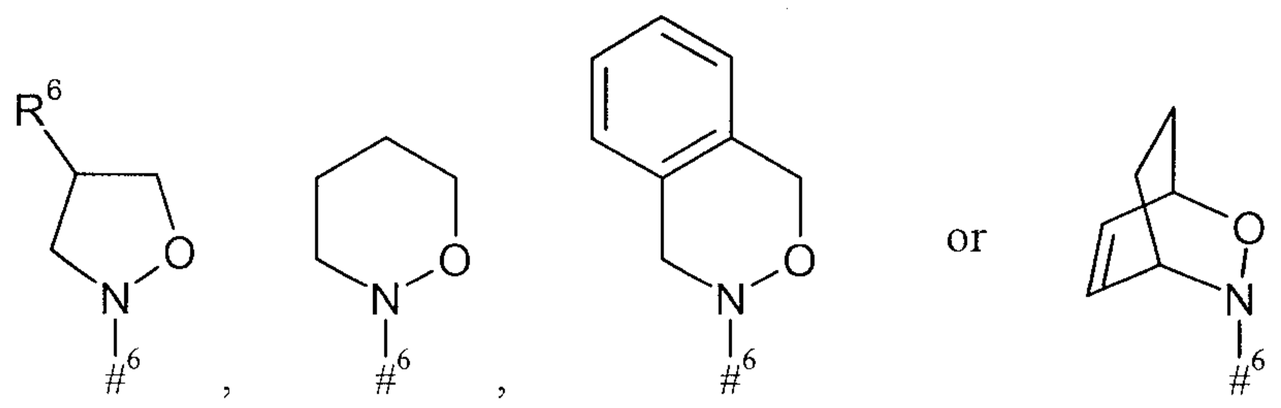


wherein

$\#^4$ 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

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

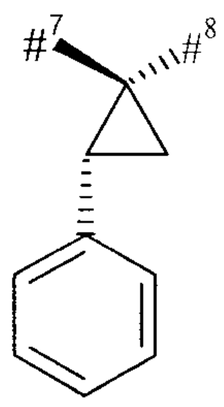
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 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



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,

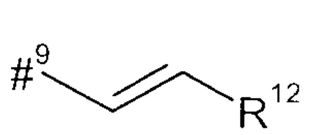
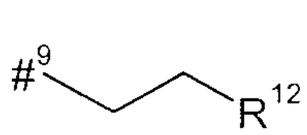
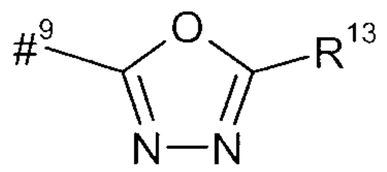
or

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

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,

R^{35} stands for methyl or hydroxyl,

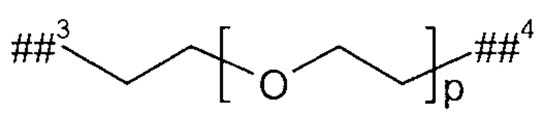
as well as their salts and solvates as well as the solvates of the salts.

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

L^1 stands for a bond,

B stands for a bond,

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



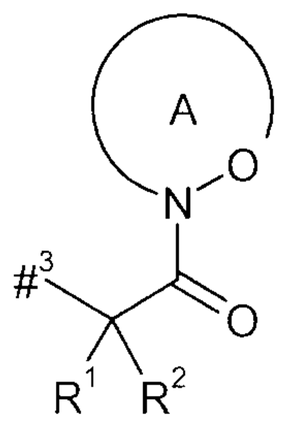
wherein

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



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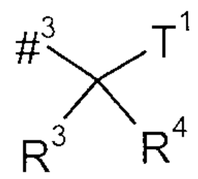


wherein

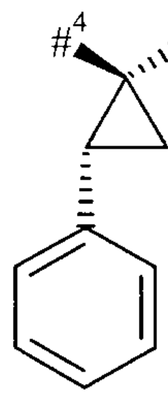
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for benzyl 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

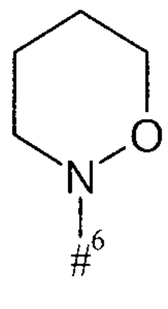


wherein

$\#^4$ 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

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

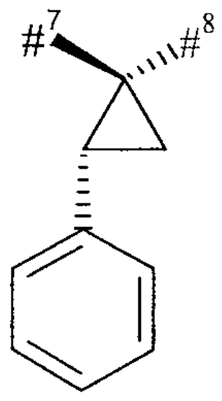
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for benzyl 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



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

R^7 stands for hydrogen,

R^8 stands for hydrogen,

R^9 stands for hydrogen,

R^{35} stands for methyl,

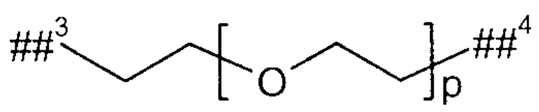
as well as their salts and solvates as well as the solvates of the salts.

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,

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



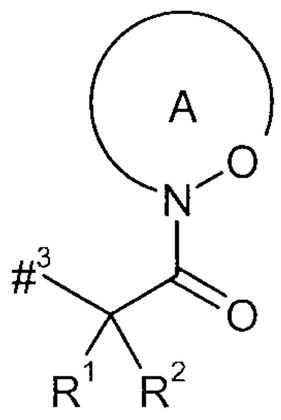
wherein

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



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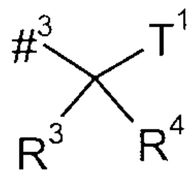


wherein

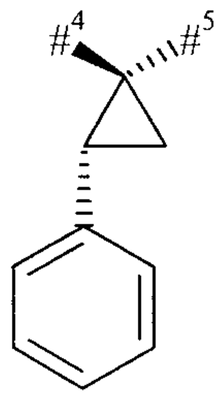
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 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

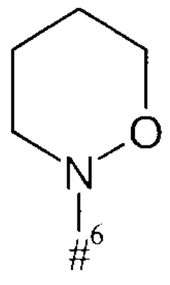


wherein

$\#^4$ 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,

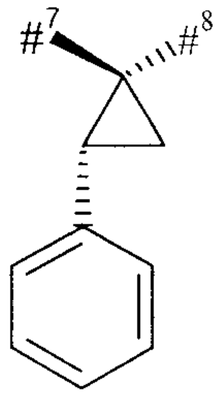
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for benzyl, 4-hydroxybenzyl 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



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

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 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:

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,

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,

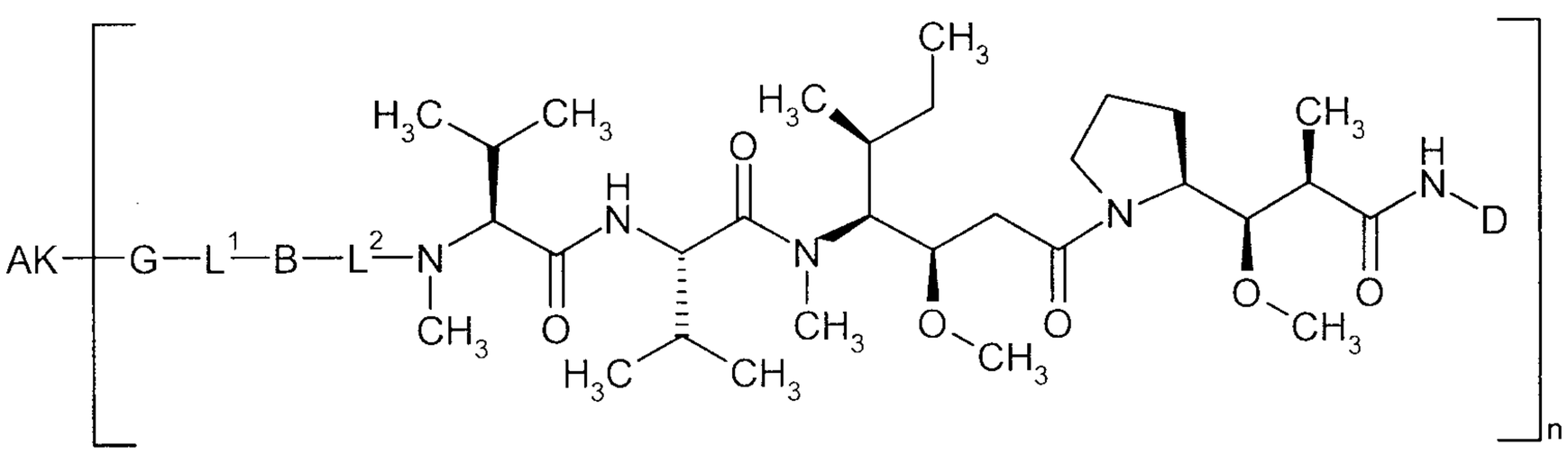
N-(6-{[(5*S*)-5-amino-5-carboxypentyl]amino}-6-oxohexyl)-N-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-

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-{[(5S)-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)



(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,

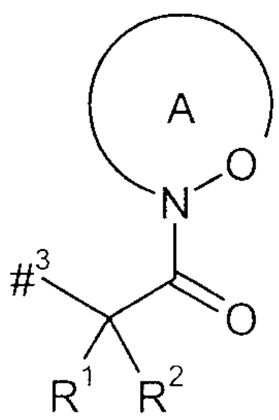
the group §-G-L1-B-L2-§§ stands for a linker,

wherein

§ denotes the linkage site to the group AK and

§§ denotes the linkage site to the nitrogen atom,

D stands for a group of the formula



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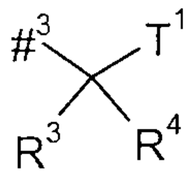

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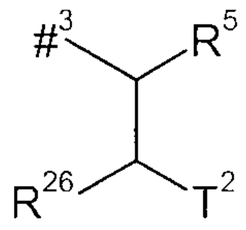


wherein

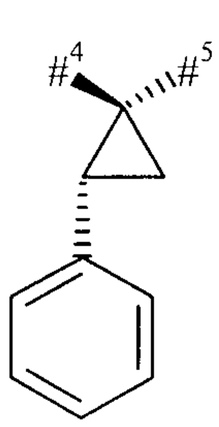
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl, 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

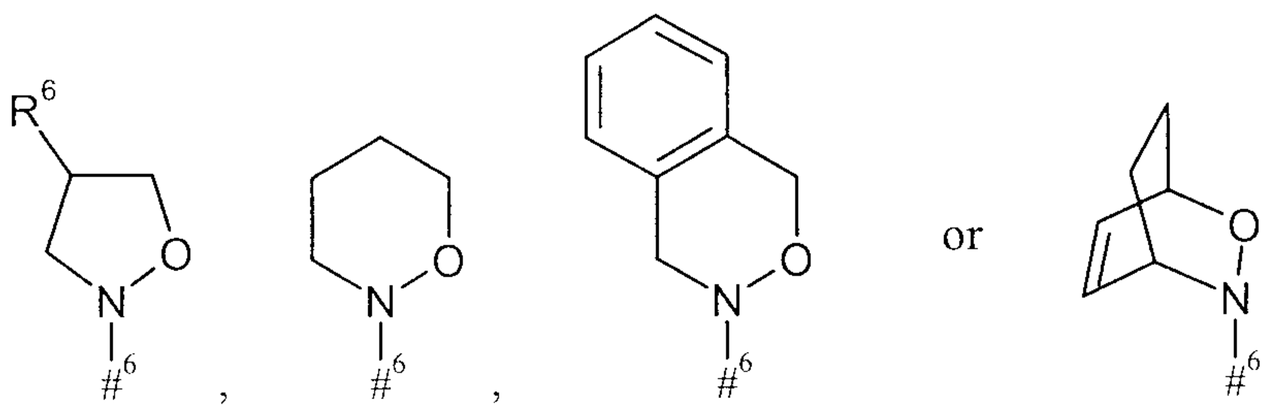


wherein

$\#^4$ 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,

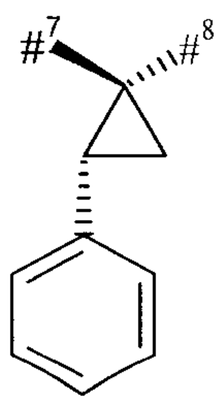
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl, 4-hydroxybenzyl 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



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,

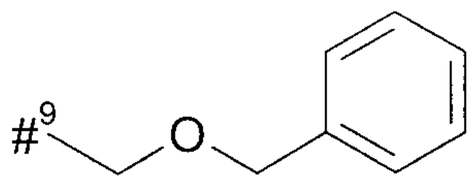
or

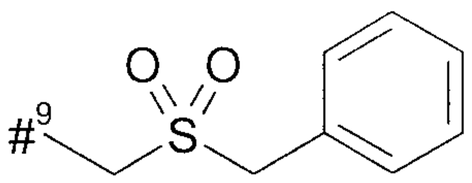
R^8 and R^9 together with the nitrogen atom to which they are attached form a four- to seven-membered heterocycle,

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





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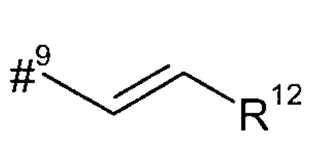

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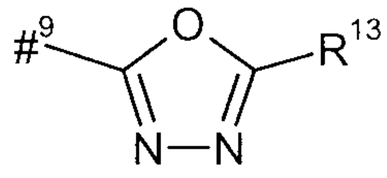


wherein

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

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^{26} stands for hydrogen or hydroxyl,

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,

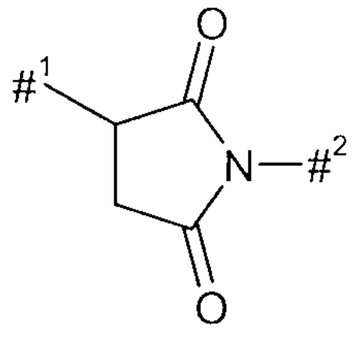
AK stands for AK_1 or AK_2

wherein

AK_1 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,

AK_2 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 $AK = AK_1$ stands for a group of the formula



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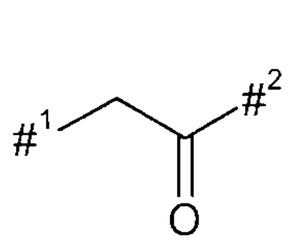


wherein

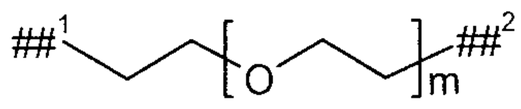
$\#^1$ denotes the linkage site to the sulfur atom of the binder,

$\#^2$ 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_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,

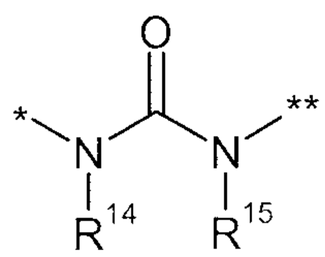
##[2] denotes the linkage site to the group B,

wherein (C_1-C_{10})-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another 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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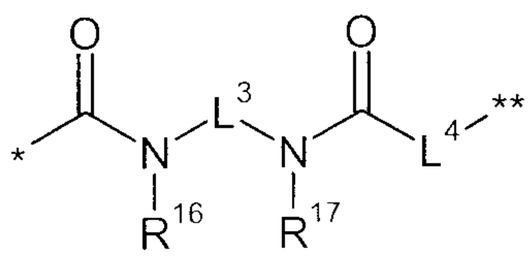
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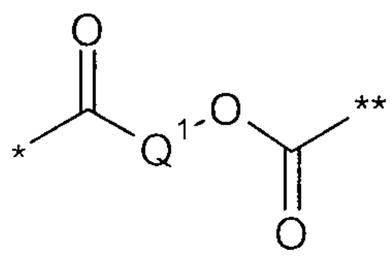
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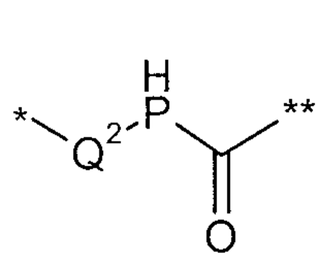
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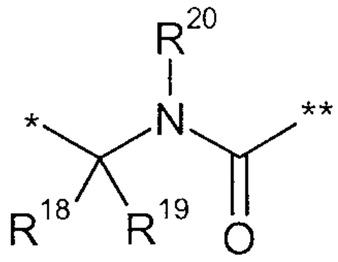
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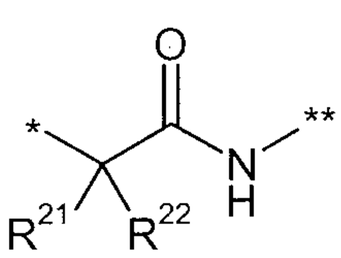
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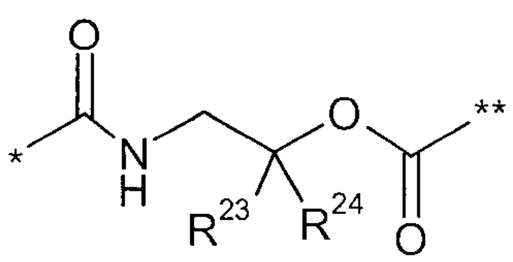
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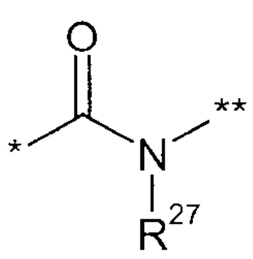


wherein

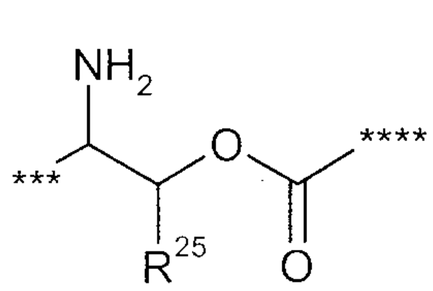
* denotes the linkage site to L^1,

** 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 or a group of the formula



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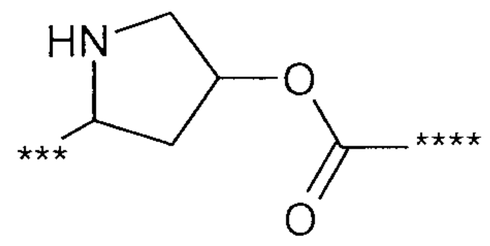


wherein

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

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

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 (C_1-C_4)-alkyl,

R^{15} stands for hydrogen or (C_1-C_4)-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 (C_1-C_4)-alkyl,

R^{17} stands for hydrogen or (C_1-C_4)-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 (C_1-C_4)-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 (C_1-C_4)-alkyl,

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 (C_1-C_4)-alkyl,

R^{22} stands for hydrogen or (C_1-C_4)-alkyl,

or

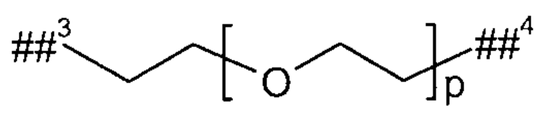
R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{23} stands for (C_1-C_4)-alkyl,

R^{24} stands for hydrogen or (C_1-C_4)-alkyl,

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



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 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 (C_3-C_6)-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

n stands for a number from 1 to 50,

AK stands for AK_1 or AK_2

wherein

AK_1 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,

AK_2 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, L^1, B, L^2 and D have 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 present invention is binder-drug conjugates of general formula (I)

in which

n stands for a number from 1 to 20,

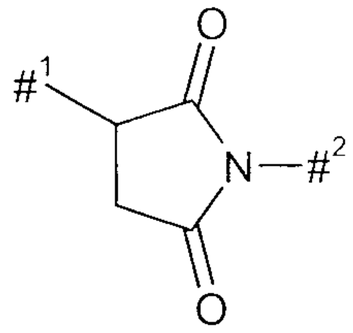
AK stands for AK_1 or AK_2

wherein

AK_1 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,

AK_2 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 $AK = AK_1$ stands for a group of the formula



wherein

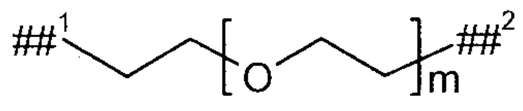
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

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

or

for the case when $AK = AK_2$, G stands for carbonyl,

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



wherein

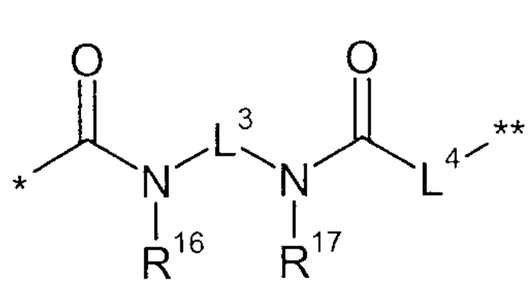
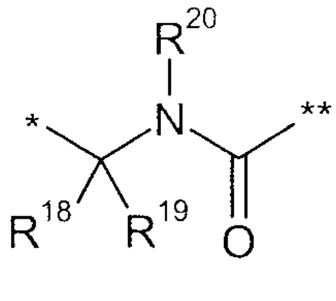
m stands for a number from 2 to 6,

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

$\#\#^2$ 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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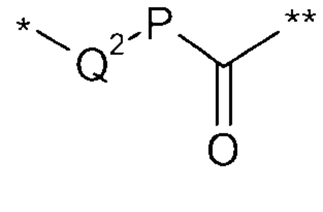


wherein

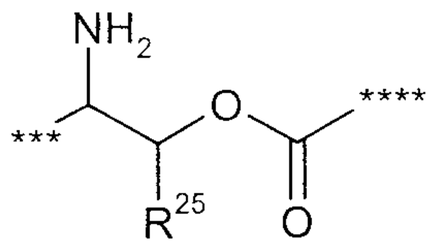
* denotes the linkage site to L^1,

** 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



wherein

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

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

R^{25} stands for methyl,

Q^2 stands for cyclopentyl or cyclohexyl,

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^{18} stands for hydrogen,

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

R^{20} stands for hydrogen or methyl,

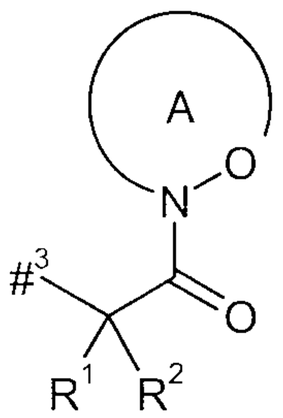
or

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

L^2 stands for linear $(C_2\text{-}C_6)$-alkanediyl,

wherein $(C_2\text{-}C_6)$-alkanediyl may be substituted with one or two methyl substituents,

D stands for a group of the formula



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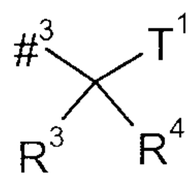

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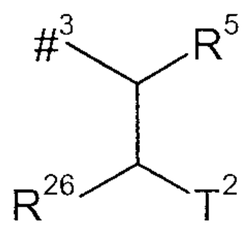


wherein

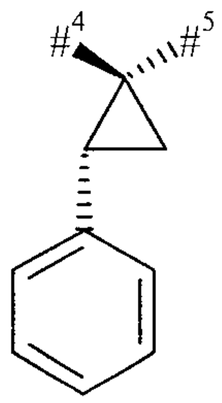
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

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

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

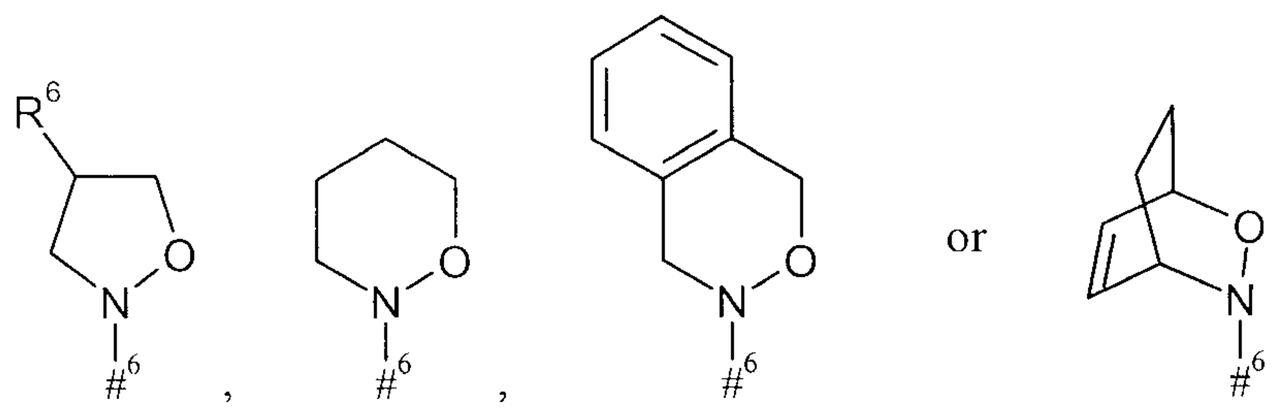


wherein

$\#^4$ 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



wherein

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

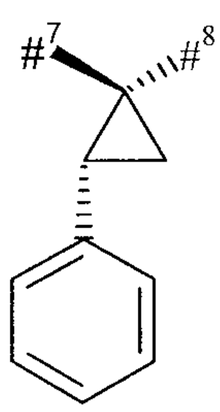
R^6 stands for hydrogen, hydroxyl or benzyloxy,

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



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,

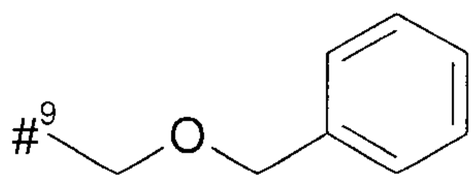
or

R^8 and R^9 together with the nitrogen atom to which they are attached 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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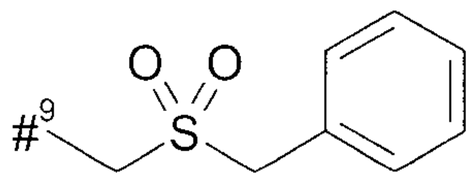
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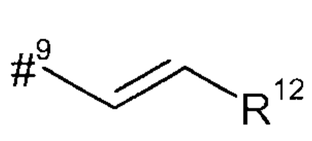
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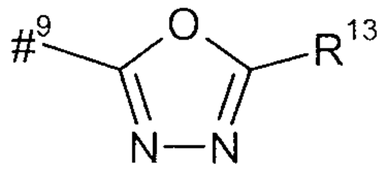


wherein

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

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^{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 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,

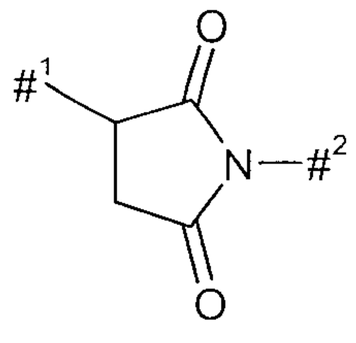
AK stands for AK_1 or AK_2

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,

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

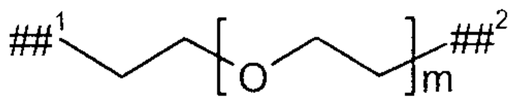
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ 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

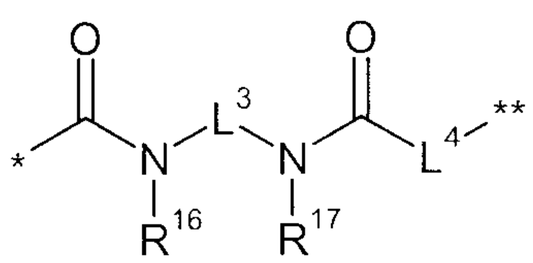
m stands for a number of 2 or 3,

$##^1$ 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



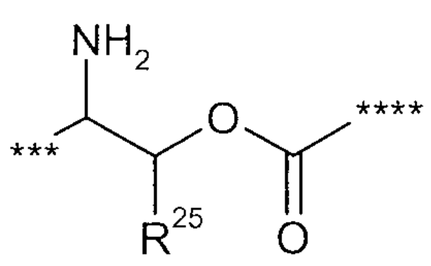
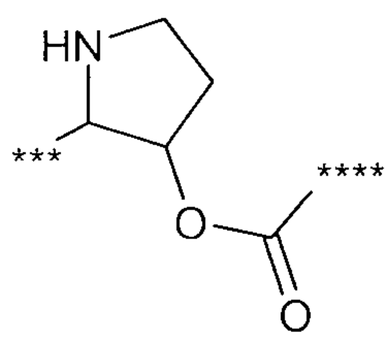
wherein

* 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

 or 

wherein

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

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

R^{25} stands for methyl,

R^{16} stands for hydrogen or methyl,

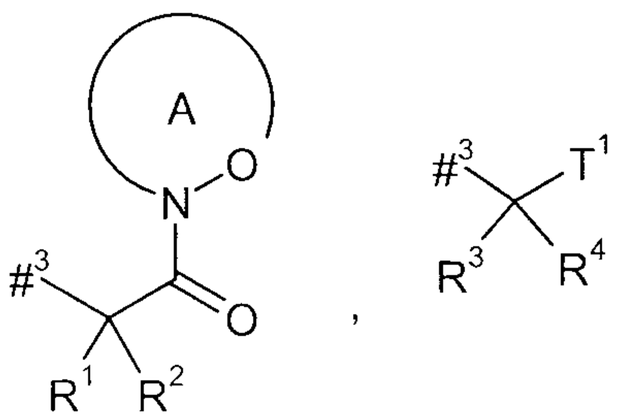
R^{17} stands for hydrogen or methyl,

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_2-C_6)-alkanediyl,

D stands for a group of the formula



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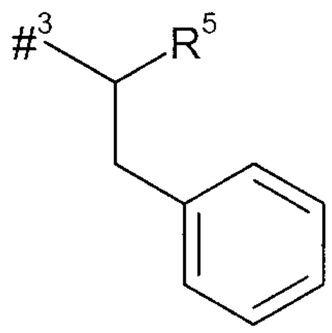


wherein

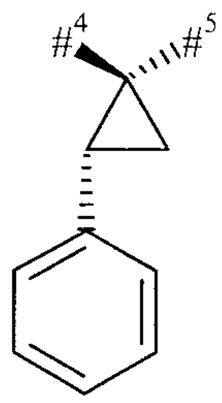
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

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

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

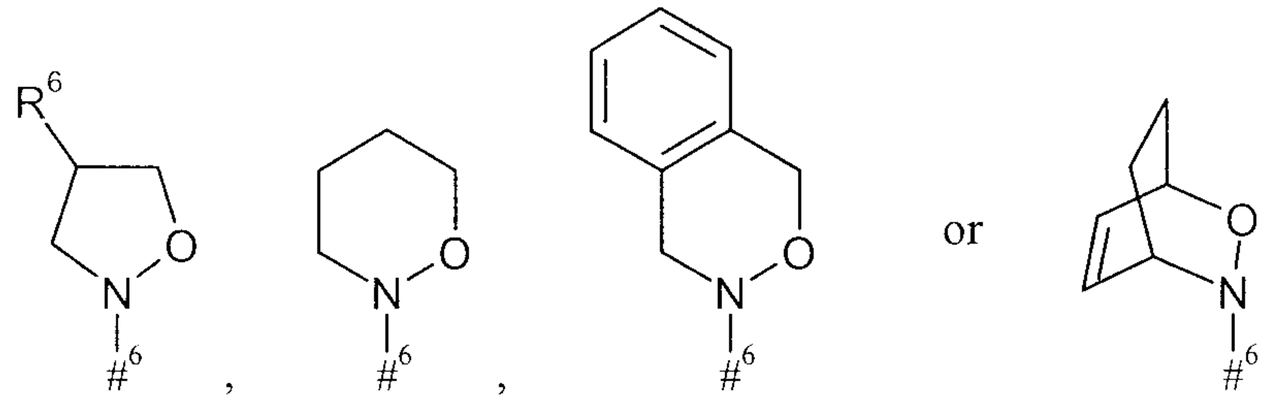


wherein

$\#^4$ 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



wherein

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

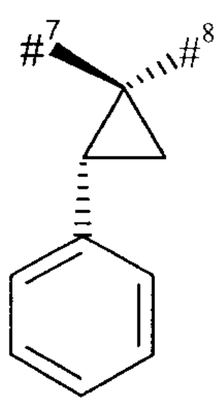
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 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

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

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

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

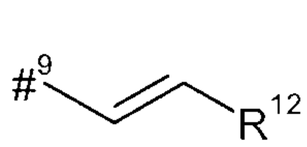
R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, benzyl or adamantylmethyl,

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,

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



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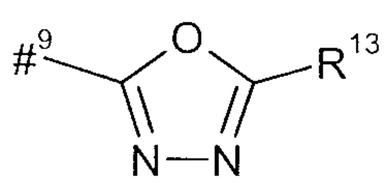


wherein

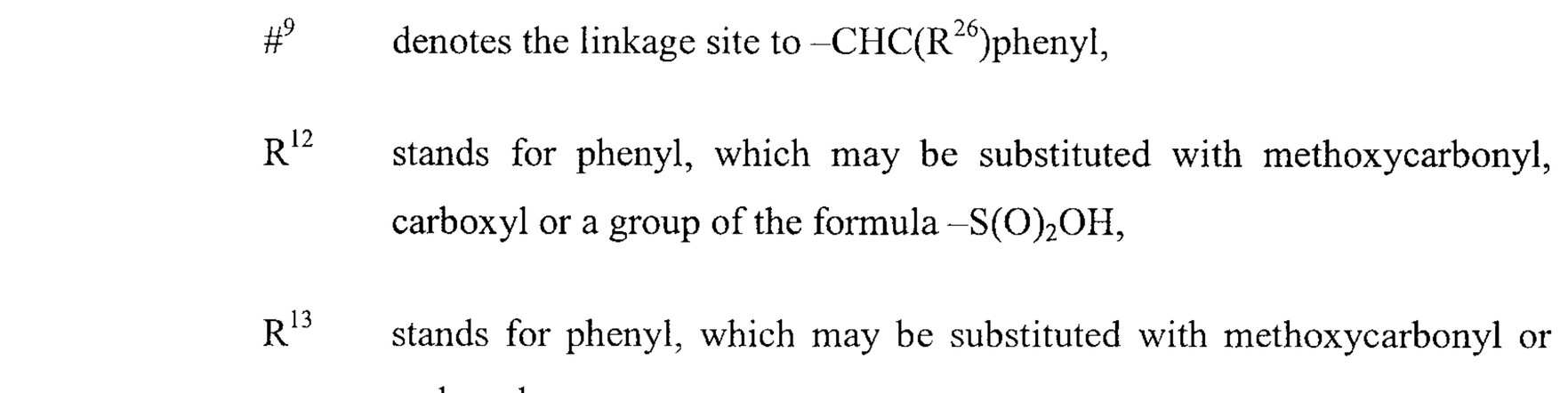
$\#^9$ denotes the linkage site to $-CHC(R^{26})$phenyl,

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,

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)

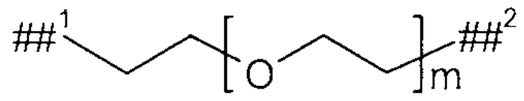


(XXX),

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,

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,

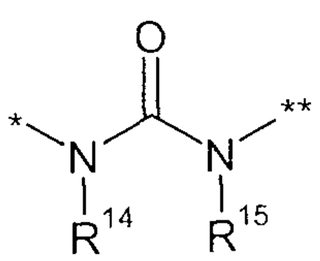
$##^2$ denotes the linkage site to the group B,

wherein (C_1-C_{10})-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another 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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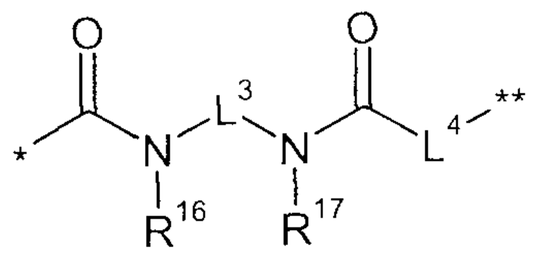
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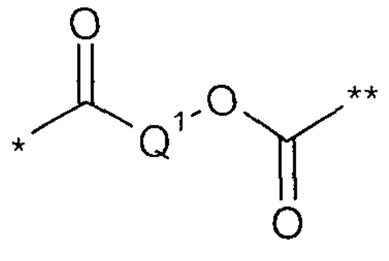
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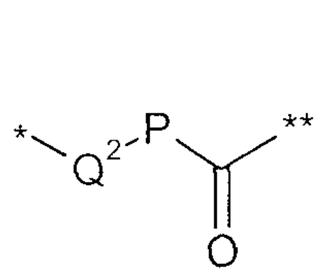
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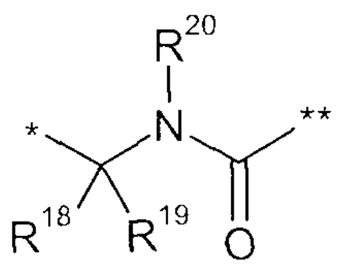
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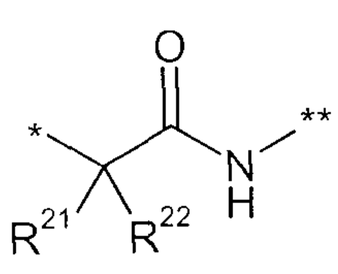
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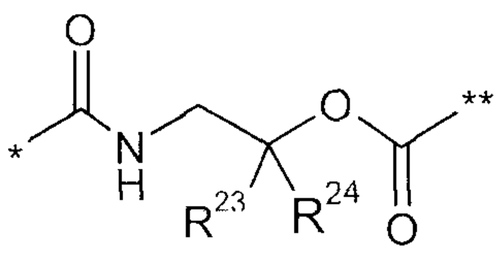

,

wherein

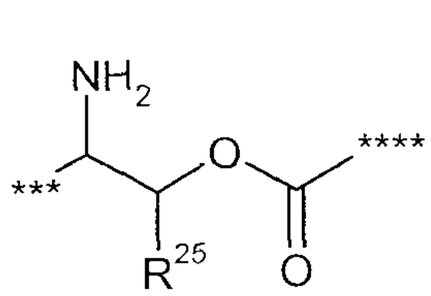
* denotes the linkage site to L^1,

** 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 or a group of the formula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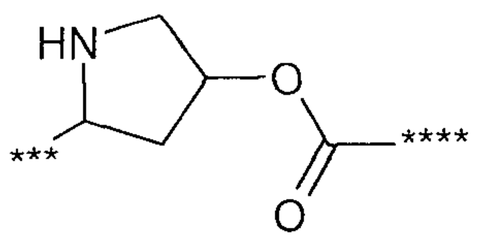


wherein

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

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

R^{25} stands for hydrogen or methyl,

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 (C_1-C_4)-alkyl,

R^{15} stands for hydrogen or (C_1-C_4)-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 (C_1-C_4)-alkyl,

R^{17} stands for hydrogen or (C_1-C_4)-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 (C_1-C_4)-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 (C_1-C_4)-alkyl,

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 (C_1-C_4)-alkyl,

R^{22} stands for hydrogen or (C_1-C_4)-alkyl,

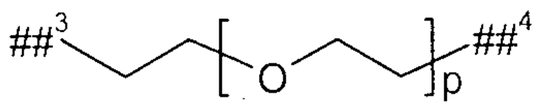
or

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

R^{23} stands for $(C_1\text{-}C_4)$-alkyl,

R^{24} stands for hydrogen or $(C_1\text{-}C_4)$-alkyl,

L^2 stands for linear $(C_2\text{-}C_{10})$-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,

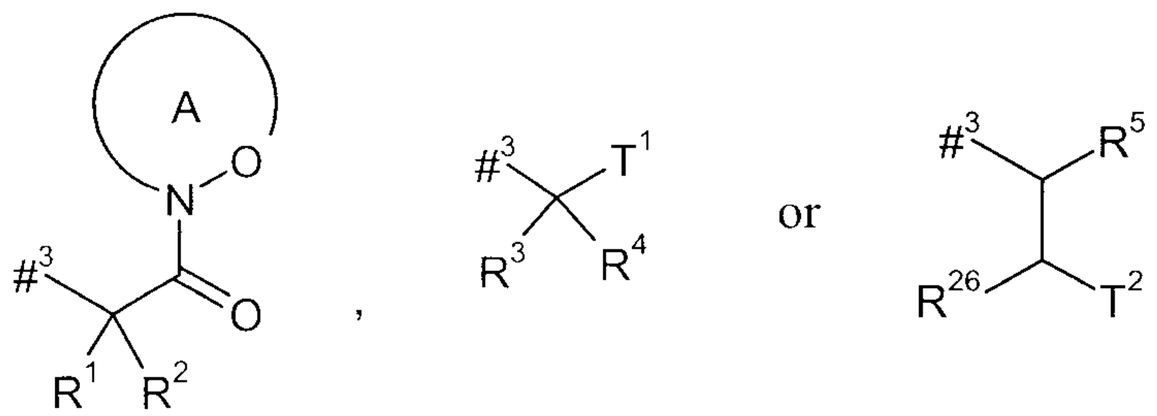
$\#\#^4$ denotes the linkage site to the nitrogen atom,

wherein $(C_2\text{-}C_{10})$-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 $(C_3\text{-}C_6)$ cycloalkyl ring or a phenyl ring,

D stands for a group of the formula



wherein

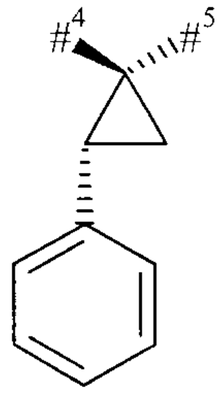
$\#^3$ denotes the linkage site to the nitrogen atom,

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

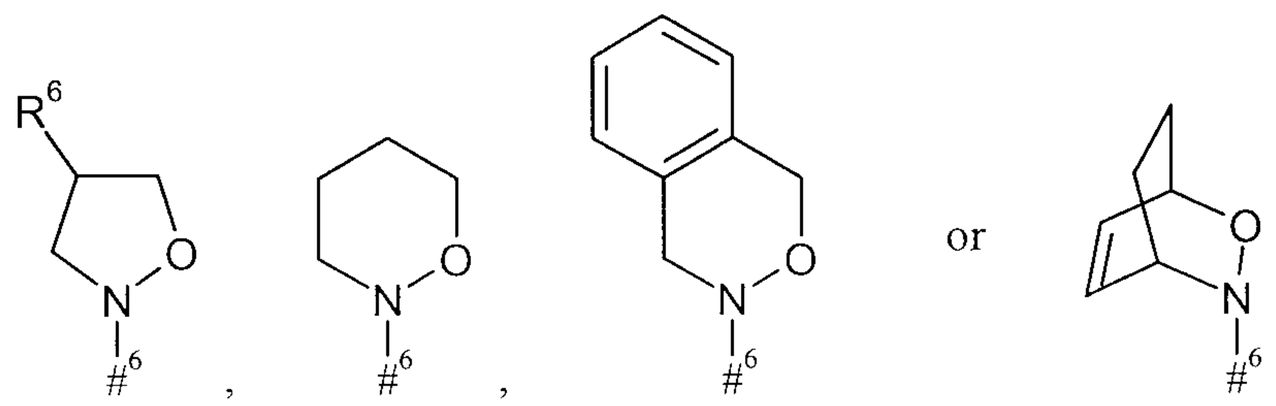


wherein

$\#^4$ 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 monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

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

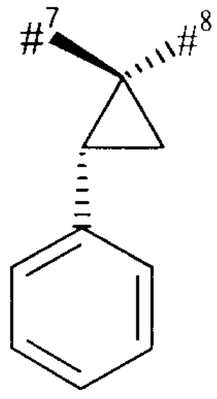
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

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

$\#^7$ 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,

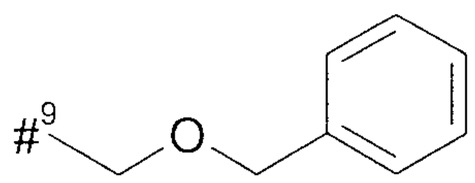
or

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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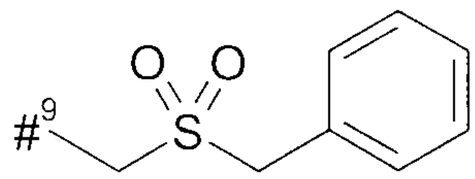
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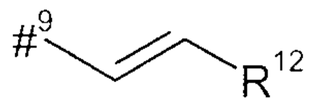
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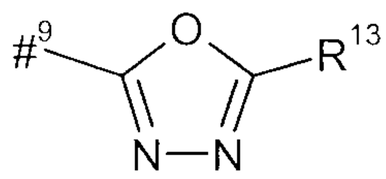


wherein

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

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^{26} stands for hydrogen or hydroxyl,

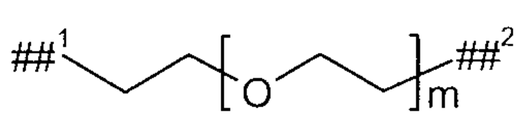
T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

as well as their salts 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,

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



wherein

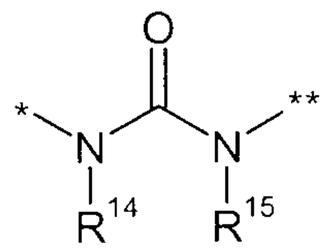
m stands for a number from 2 to 6,

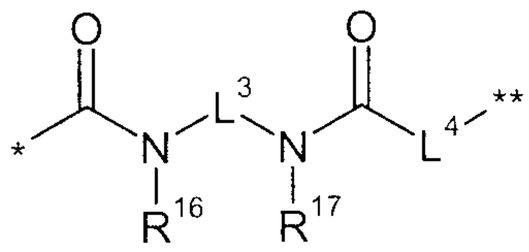
$\#\#^1$ denotes the linkage site to the group G,

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

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

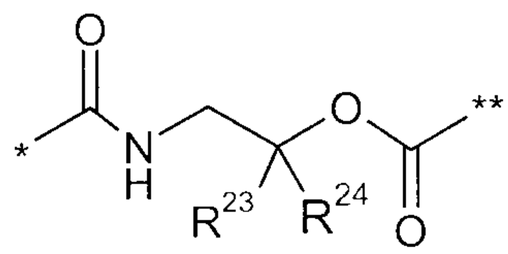
B stands for a bond or a group of the formula





,

or



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,

R^{14} stands for hydrogen,

R^{15} stands for hydrogen,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

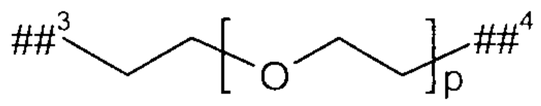
or

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,

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



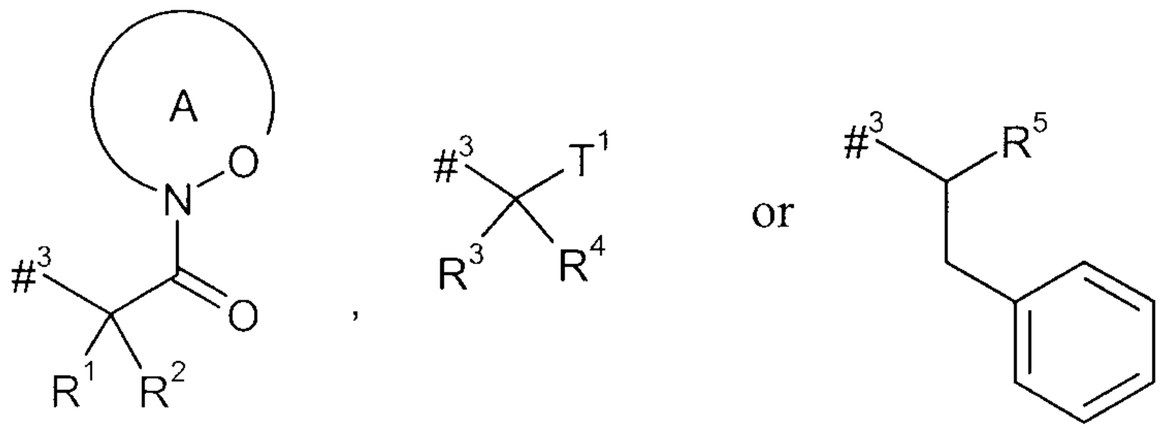
wherein

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



wherein

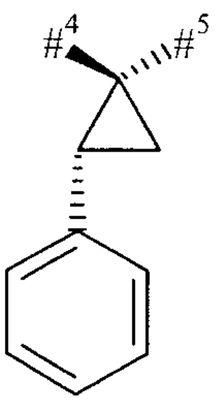
$#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

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

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

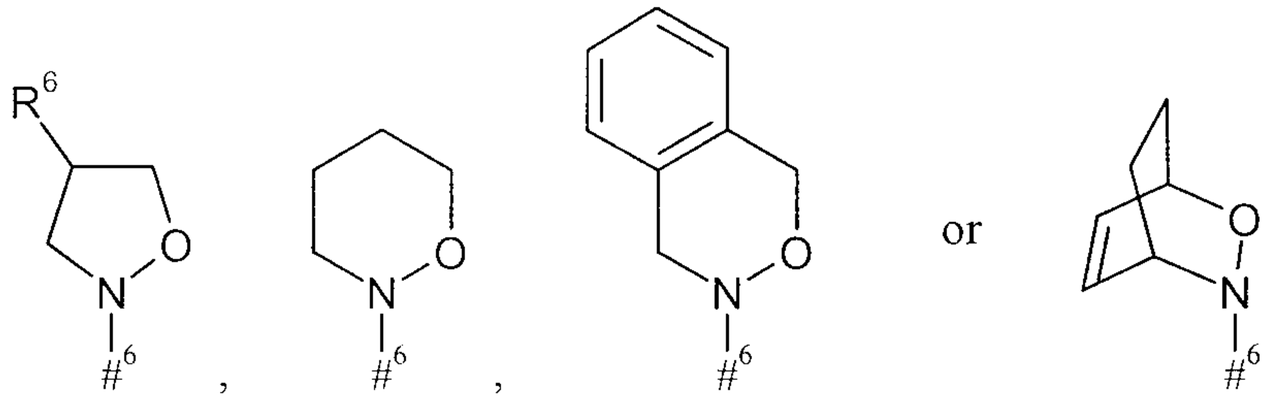


wherein

$\#^4$ 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



wherein

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

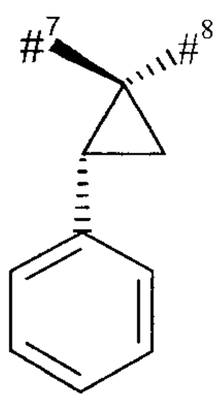
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 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

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

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

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, benzyl or adamantylmethyl,

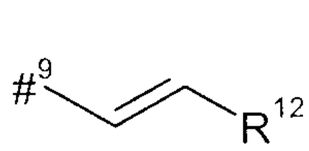
R^8 stands for hydrogen or methyl,

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

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 or a group of the formula



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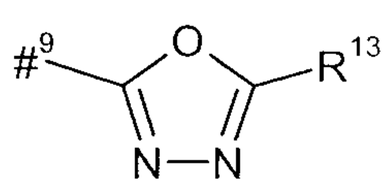


wherein

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

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

n = 1-20, especially preferably n = 1-10 and most especially preferably n = 2-8.

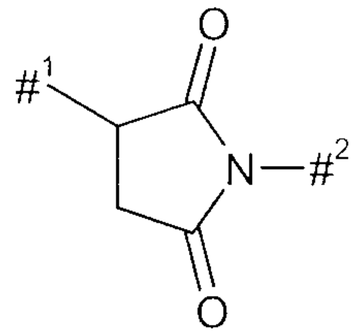
Within the scope of the present invention, compounds of formula (Ia) are preferred, in which

AK stands for AK_1

wherein

AK_1 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



wherein

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

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

and

n, 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), wherein

AK stands for AK_2

wherein

AK_2 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

n, 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.

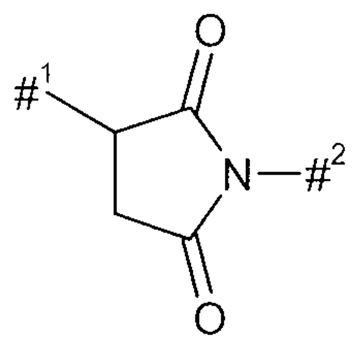
Also preferred within the scope of the present invention are compounds of formula (Ia), in which

AK stands for AK_1

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,

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,

and

n, 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), in which

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,

and

n, 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 general formula (Ia), in which

AK stands for AK_2

wherein

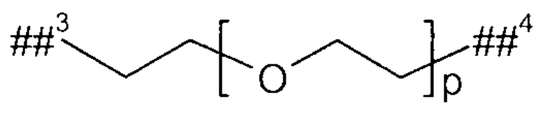
AK_2 stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G stands for carbonyl,

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

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, D and R^{35} have the meanings given above

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

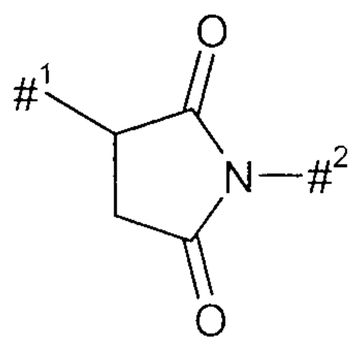
Also preferred within the scope of the present invention are compounds of general formula (Ia), in which

AK stands for AK_1

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,

G stands for a group of the formula

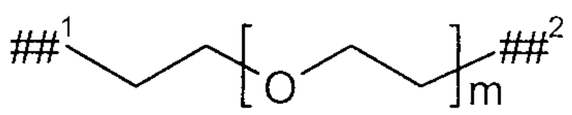


wherein

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

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

L^1 stands for a bond, linear (C_3-C_5)-alkanediyl, a group of the formula



wherein

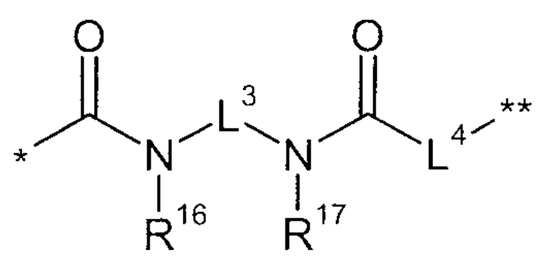
m stands for a number of 2 or 3,

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

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

wherein (C_3-C_5)-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



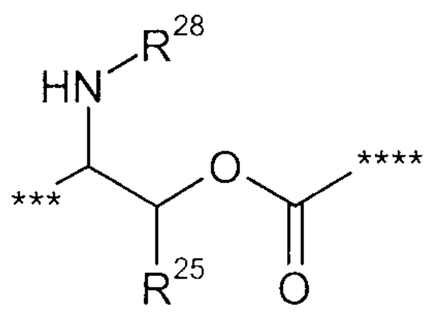
wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

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,

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

R^{25} stands for methyl,

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

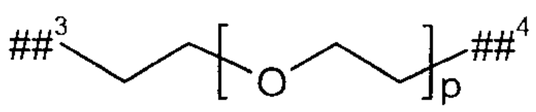
R^{16} stands for hydrogen or methyl,

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,

and

n, D and R^{35} have the meanings given above

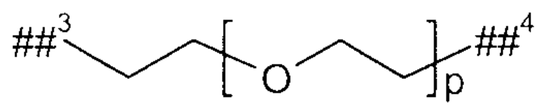
as well as their salts and solvates as well as the solvates of the salts.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

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

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,

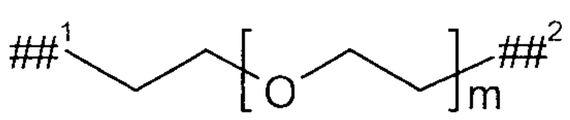
and

n, 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.

Also preferred within the scope of the present invention are compounds of formula (Ia), in which

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



wherein

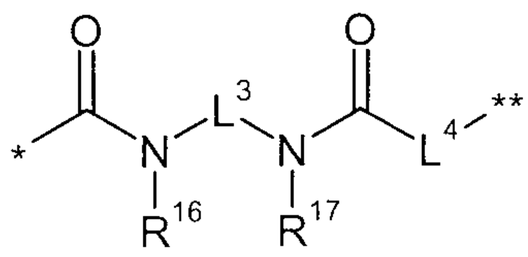
m stands for a number from 2 to 6,

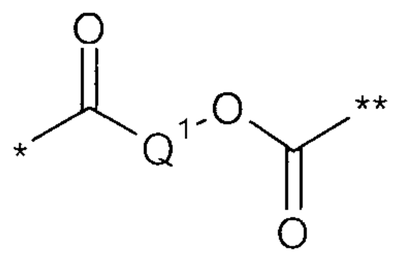
$##^1$ denotes the linkage site to the group G,

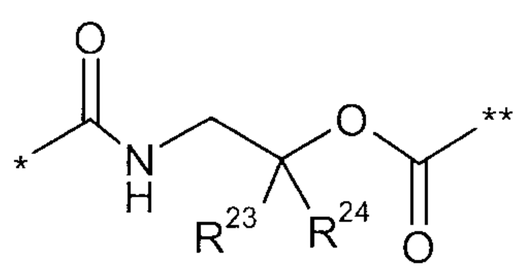
$##^2$ denotes the linkage site to the group B,

wherein (C_1-C_{10})-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







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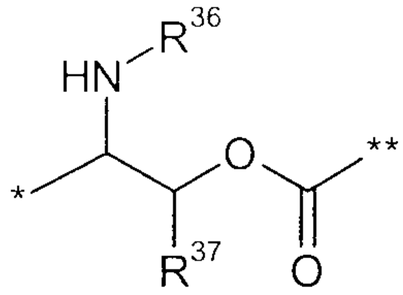


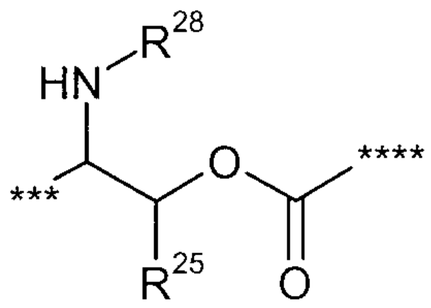
wherein

* denotes the linkage site to L^1,

** 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



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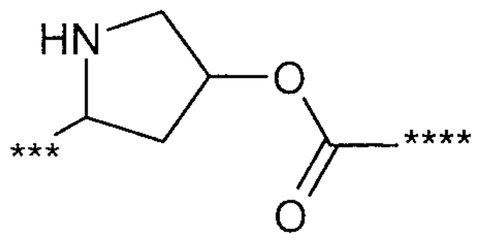


wherein

*** 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, $(C_1$-$C_4)$-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

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 (C_1-C_4)-alkyl,

or

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

R^{23} stands for (C_1-C_4)-alkyl,

R^{24} stands for hydrogen or (C_1-C_4)-alkyl,

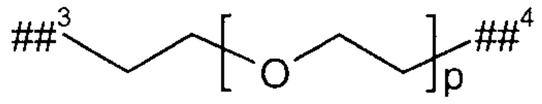
R^{36} stands for hydrogen, (C_1-C_4)-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxy-carbonyl,

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



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 1 to 4 substituents selected independently of one another from the group comprising methyl, hydroxyl and benzyl,

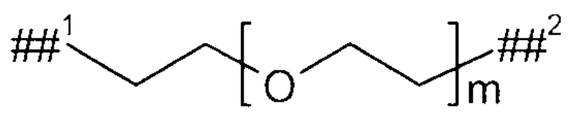
and

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



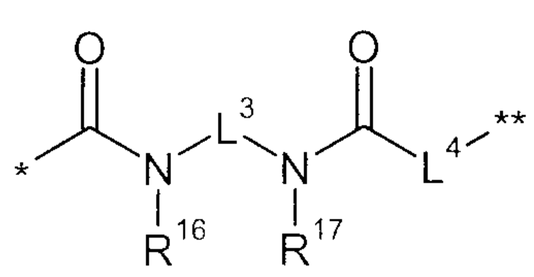
wherein

m stands for a number of 2 or 3,

$\#\#^1$ 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



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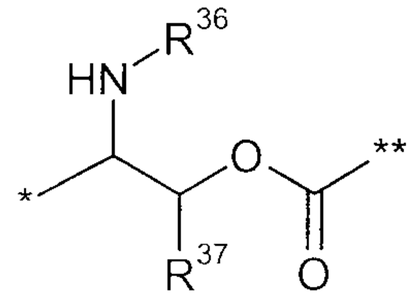


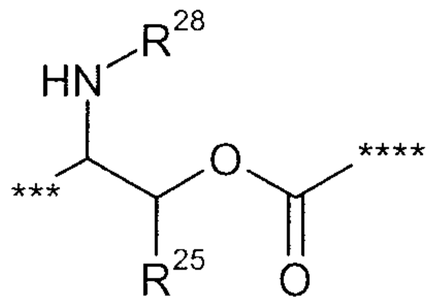
wherein

* 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



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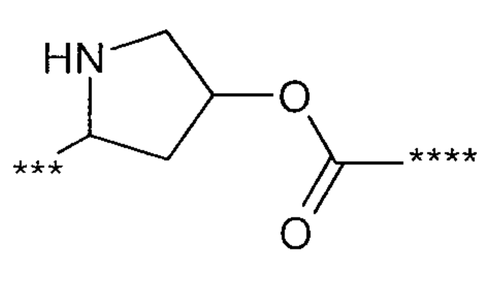


wherein

*** 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,

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,

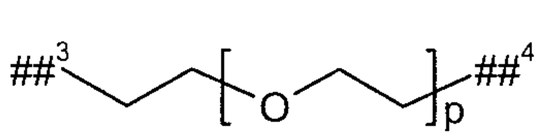
R^{36} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

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,

L^2 stands for linear $(C_2\text{-}C_6)$-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,

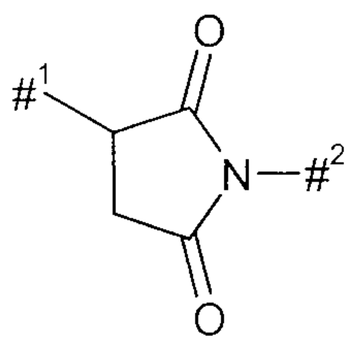
and

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) and (XXXa), in which

G stands for a group of the formula

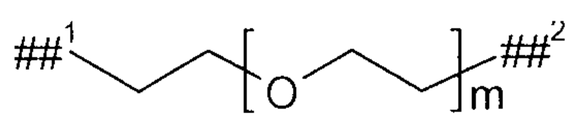


wherein

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

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

L^1 stands for linear $(C_3$-$C_5)$-alkanediyl or for a group of the formula



wherein

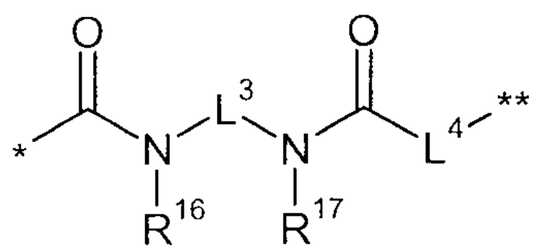
m stands for a number of 2 or 3,

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

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

wherein $(C_3$-$C_5)$-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



wherein

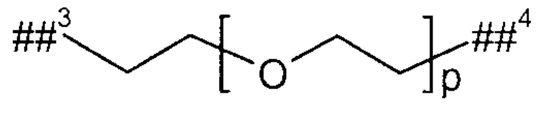
* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

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

L^4 stands for a bond,

L^2 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,

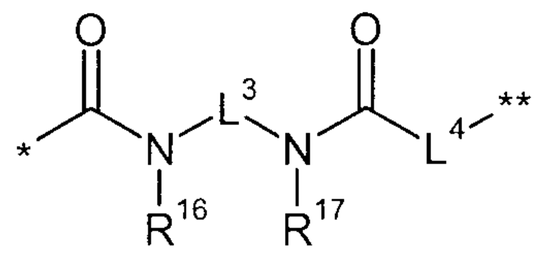
and

n, AK_I, 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.

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

* 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,

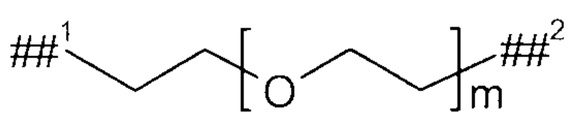
L^4 stands for a bond,

n, AK, Cys, G, L^1, L^2, D, R^{16}, R^{17} and R^{35} have the meanings given above

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 (C_3-C_5)-alkanediyl, a group of the formula



wherein

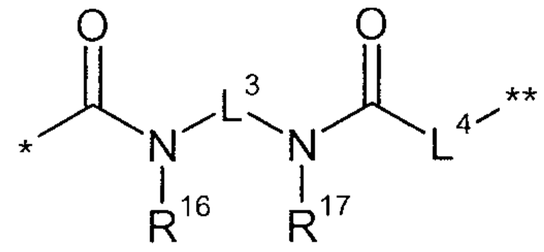
m stands for a number of 2 or 3,

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

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

wherein (C_3-C_5)-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,

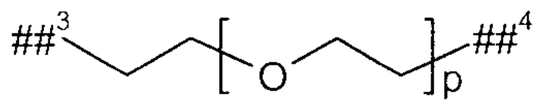
L^3 stands for a bond,

L^4 stands for a bond

R^{16} stands for hydrogen,

R^{17} stands for hydrogen,

L^2 stands for linear (C_3-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,

n, AK, Cys, G, D and R^{35} have the meanings given above

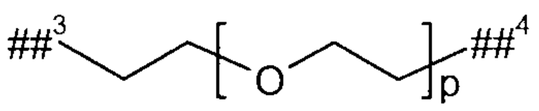
as well as their salts and solvates as well as the solvates of the salts.

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,

B stands for a bond,

L^2 stands for linear $(C_3$-$C_6)$-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,

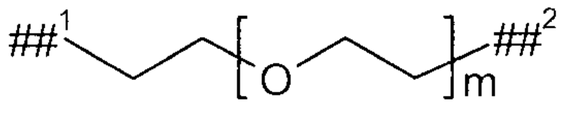
$##^4$ denotes the linkage site to the nitrogen atom,

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.

Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

L^1 stands for linear $(C_3$-$C_5)$-alkanediyl or for a group of the formula



wherein

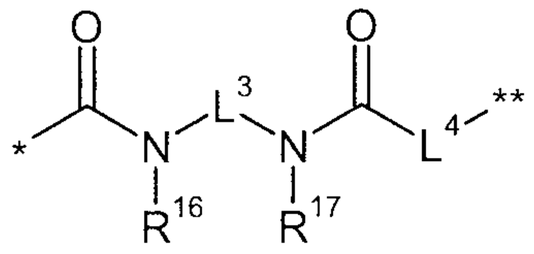
m stands for a number of 2 or 3,

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

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

wherein $(C_3\text{-}C_5)$-alkanediyl may be substituted with one or two methyl substituents,

B stands for a group of the formula



wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

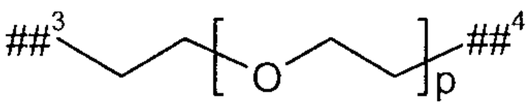
L^3 stands for a bond,

L^4 stands for a bond

R^{16} stands for hydrogen,

R^{17} stands for hydrogen,

L^2 stands for linear $(C_3\text{-}C_6)$-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,

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.

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,

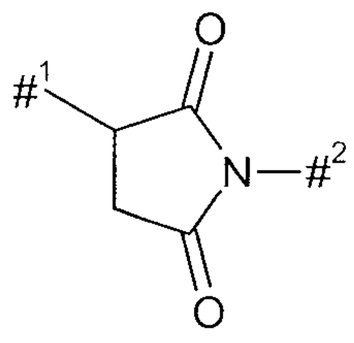
AK stands for AK_1 or AK_2

wherein

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,

G for the case when $AK = AK_1$ stands for a group of the formula



wherein

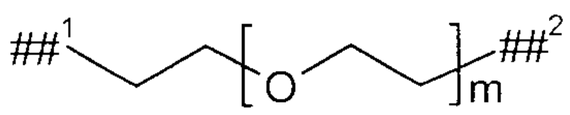
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

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

or

for the case when $AK = AK_2$, G stands for carbonyl,

L^1 stands for a bond, linear $(C_3\text{-}C_5)$-alkanediyl, a group of the formula



wherein

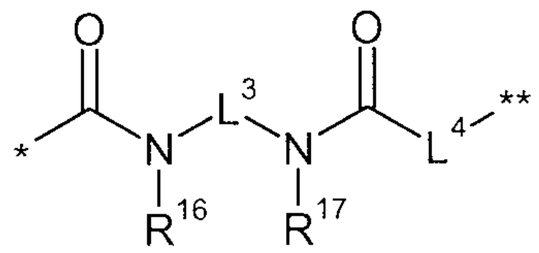
m stands for a number of 2 or 3,

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

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

wherein (C_3-C_5)-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,

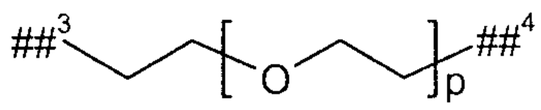
L^3 stands for a bond,

L^4 stands for a bond

R^{16} stands for hydrogen,

R^{17} stands for hydrogen,

L^2 stands for linear (C_3-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,

and

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

n stands for a number from 2 to 8, preferably 2 to 5,

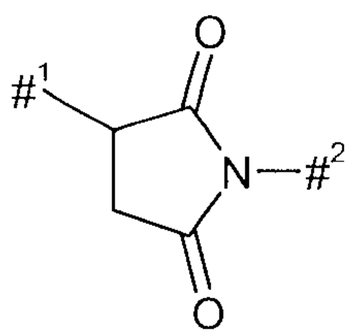
AK stands for AK_1 or AK_2

wherein

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,

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,

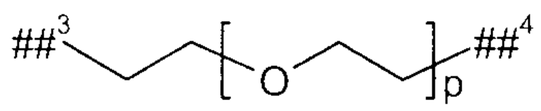
or

for the case when AK = AK_2, G stands for carbonyl,

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

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 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

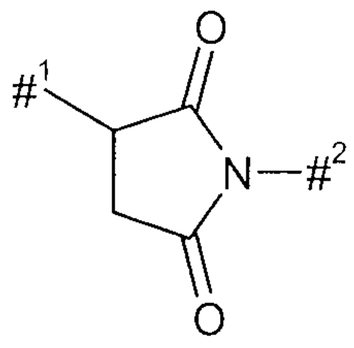
n stands for a number from 2 to 8, preferably 2 to 5,

AK stands for AK_1,

wherein

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,

G stands for a group of the formula

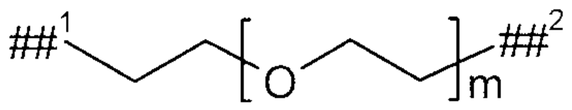


wherein

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

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

L^1 stands for linear $(C_3$-$C_5)$-alkanediyl or for a group of the formula



wherein

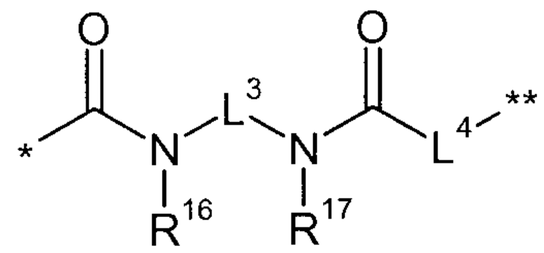
m stands for a number of 2 or 3,

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

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

wherein $(C_3$-$C_5)$-alkanediyl may be substituted with one or two methyl substituents,

B stands for a group of the formula



wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

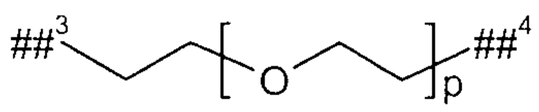
L^3 stands for a bond,

L^4 stands for a bond

R^{16} stands for hydrogen,

R^{17} stands for hydrogen,

L^2 stands for linear $(C_3$-$C_6)$-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,

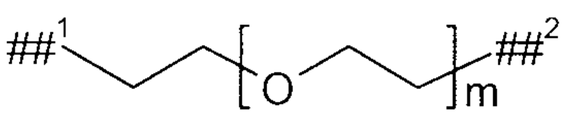
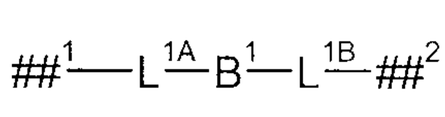
$##^4$ denotes the linkage site to the nitrogen atom,

D 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 a bond, linear (C_3-C_5)-alkanediyl, a group of the formula

 or 

wherein

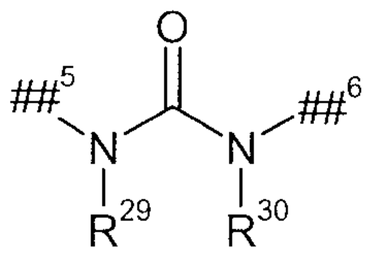
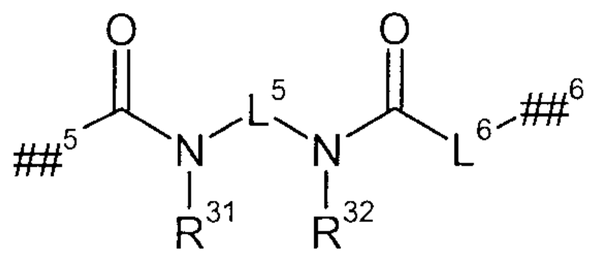
m stands for a number of 2 or 3,

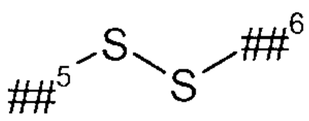
$##^1$ denotes the linkage site to the group G,

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

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

B^1 stands for a group of the formula

 , 

or 

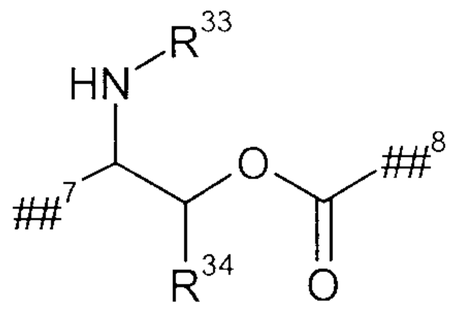
wherein

$##^5$ 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 ethane-1,2-diyl,

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



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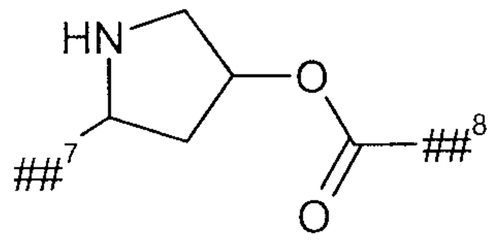


wherein

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

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

R^{33} stands for hydrogen, $(C_1$-$C_4)$-alkylcarbonyl or *tert*-butyloxycarbonyl,

R^{34} stands for hydrogen or methyl,

R^{29} stands for hydrogen or methyl,

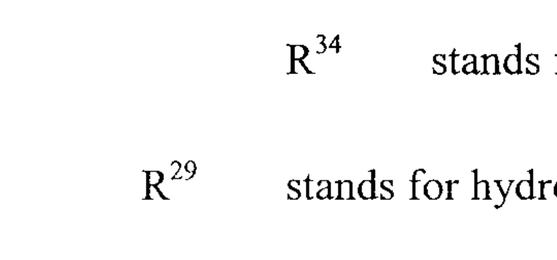
R^{30} stands for hydrogen or methyl,

R^{31} stands for hydrogen or methyl,

R^{32} stands for hydrogen or methyl,

L^{1B} stands for linear $(C_3$-$C_6)$-alkanediyl,

B stands for a bond or a group of the formula



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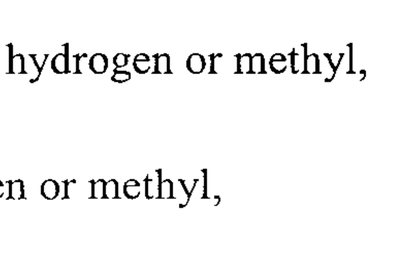

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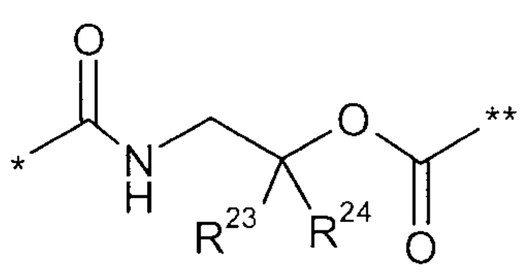

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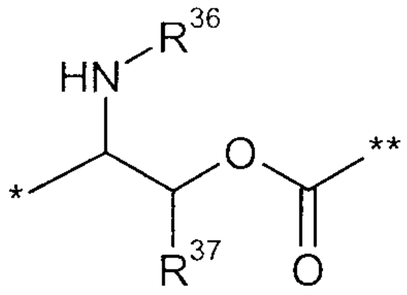

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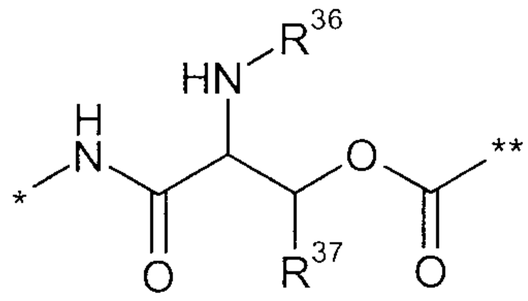


wherein

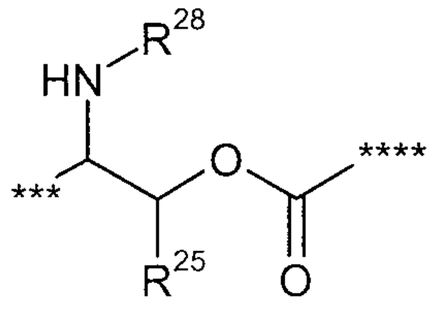
* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

P stands for O,

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

L^4 stands for a group of the formula



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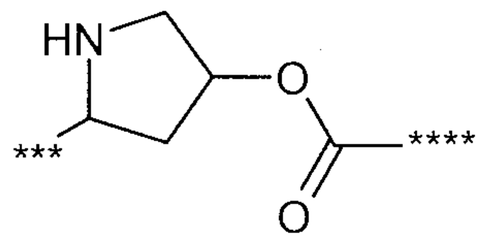


wherein

*** 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, $(C_1\text{-}C_4)$-alkylcarbonyl or *tert*-butyloxycarbonyl,

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^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

R^{23} stands for $(C_1\text{-}C_4)$-alkyl,

R^{24} stands for hydrogen or $(C_1\text{-}C_4)$-alkyl,

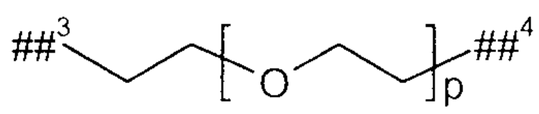
R^{36} stands for hydrogen, $(C_1\text{-}C_4)$-alkylcarbonyl or *tert*-butyloxycarbonyl,

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 $(C_2\text{-}C_6)$-alkanediyl or for a group of the formula



wherein

p stands for a number of 2 or 3,

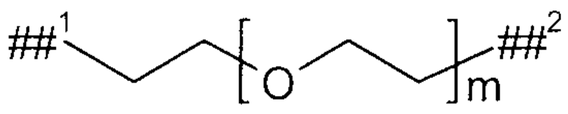
$##^3$ denotes the linkage site to the group B,

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

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 $(C_3\text{-}C_5)$-alkanediyl or for a group of the formula



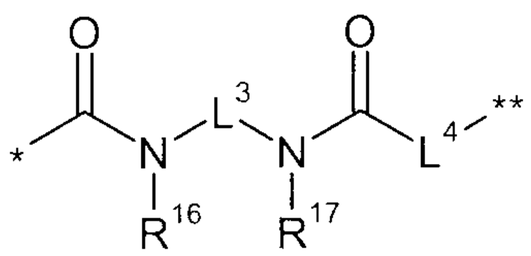
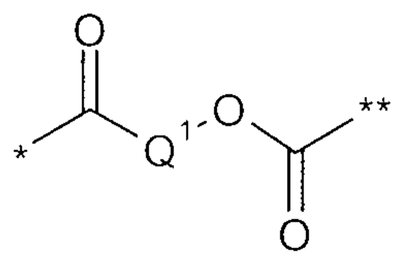
wherein

m stands for a number of 2 or 3,

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

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

B stands for a group of the formula

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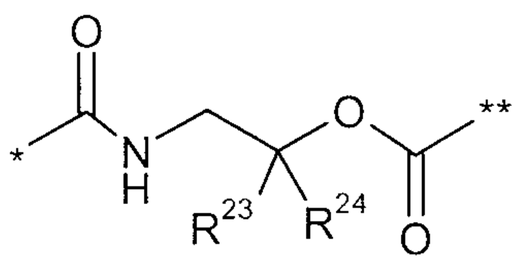
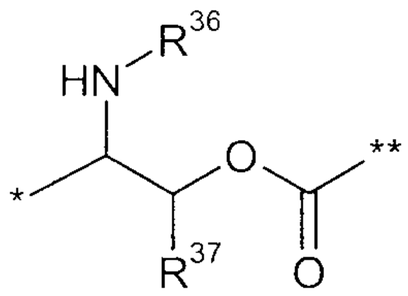

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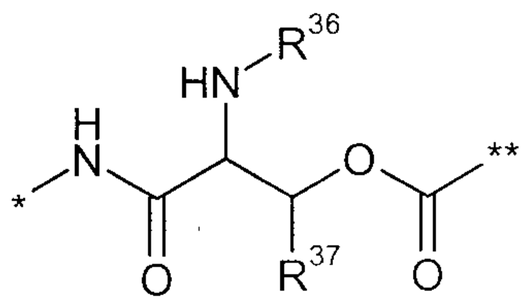
or 

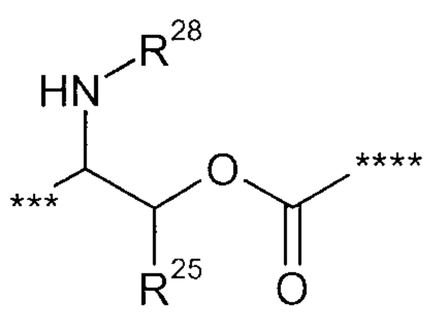
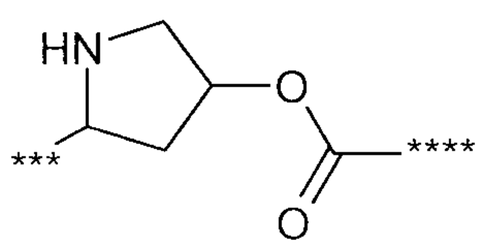
wherein

* 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

 or 

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,

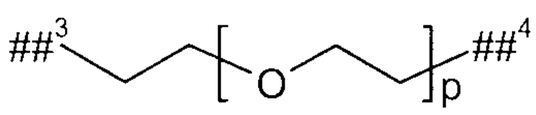
R^{23} stands for methyl,

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,

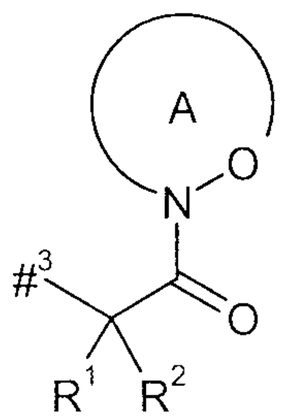
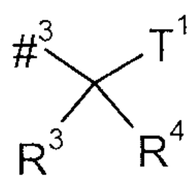
$##^3$ denotes the linkage site to the group B,

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

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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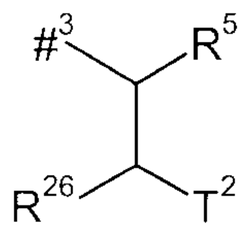


wherein

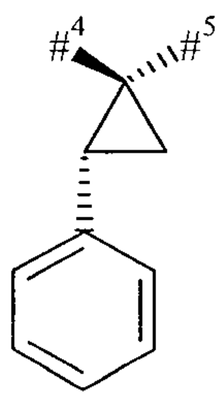
$\#^3$ denotes the linkage site to the nitrogen atom,

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 (1S,2R)-2- phenylcyclopropane-1,1-diyl group of the formula

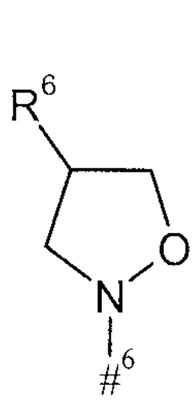
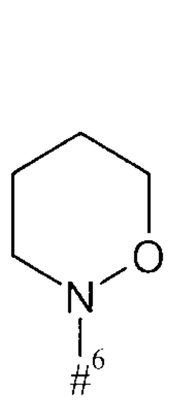
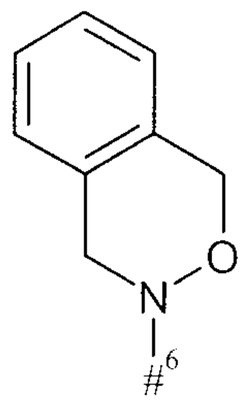


wherein

$\#^4$ 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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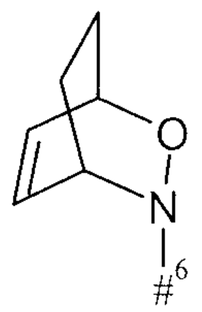


wherein

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

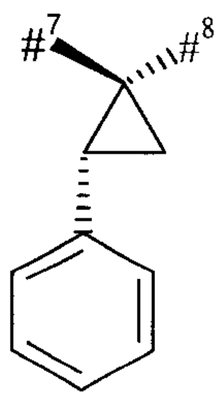
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-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^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 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,

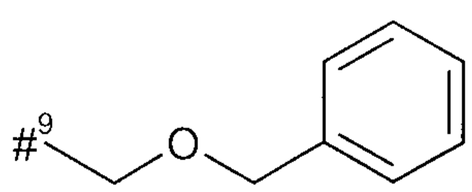
or

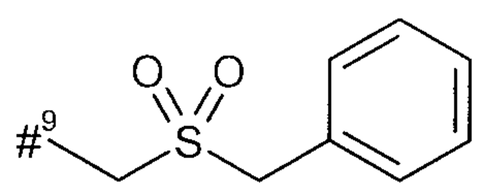
R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

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





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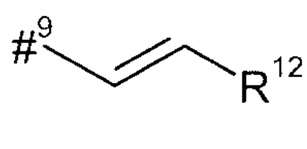

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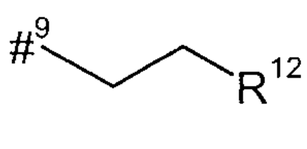

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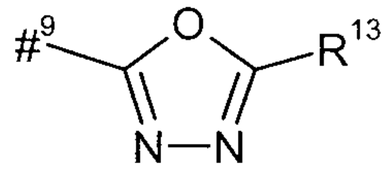


wherein

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

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^{26} stands for hydrogen,

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

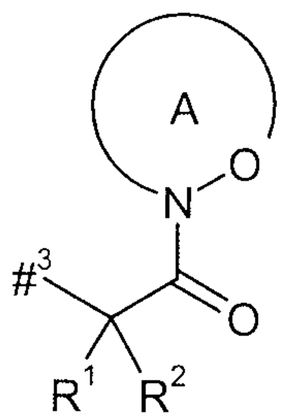
and

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



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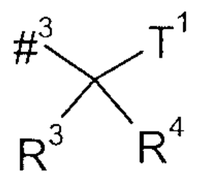


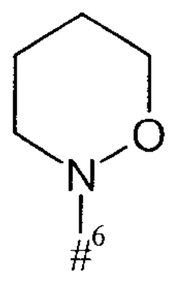
wherein

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

R^1 stands for hydrogen,

R^2 stands for benzyl, 4-hydroxybenyl, 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

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

R^3 stands for hydrogen,

R^4 stands for benzyl, 4-hydroxybenzyl, 1-phenylethyl 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,

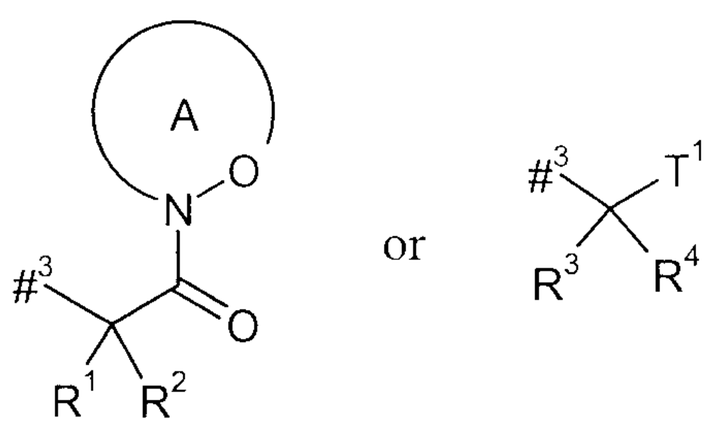
R^9 stands for hydrogen,

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



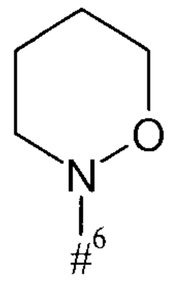
wherein

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

R^1 stands for hydrogen,

R^2 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



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n, AK, Cys, G, L^1, B, L^2, D and R^{35} have the meanings given above

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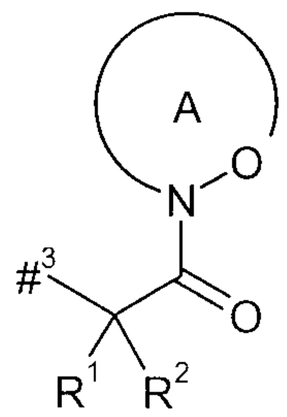

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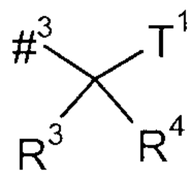


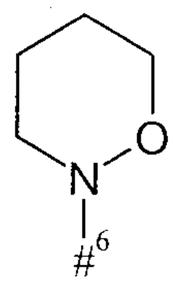
wherein

$\#^3$ 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 in it stands for a heterocycle of the formula



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,

R^9 stands for hydrogen,

n, AK, Cys, G, L^1, B, L^2, D and R^{35} have the meanings given above

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Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

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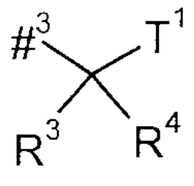

wherein

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

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 $-C(=O)-OR^7$ or $-C(=O)-NR^8R^9$,

wherein

R^7 stands for hydrogen,

R^8 stands for hydrogen,

R^9 stands for hydrogen,

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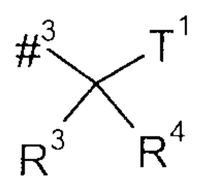
Also preferred within the scope of the present invention are compounds of formula (Ia), (XXXa) and (XXXI), in which

D stands for a group of the formula



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

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R^8 stands for hydrogen,

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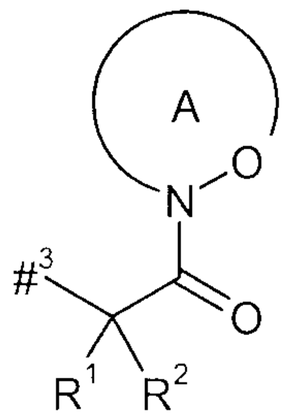


wherein

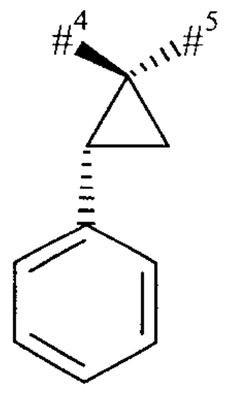
$\#^3$ denotes the linkage site to the nitrogen atom,

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 (1S,2R)-2- phenylcyclopropane-1,1-diyl group of the formula



wherein

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

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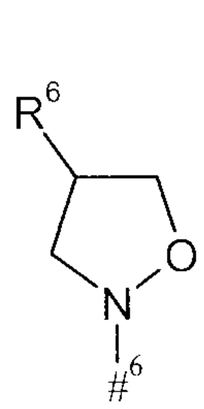
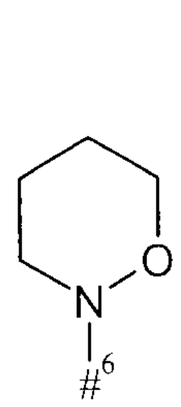
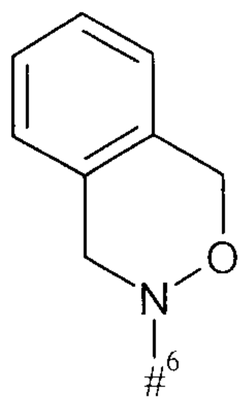
the ring A with the N-O group contained in it stands for a monocyclic or bicyclic, optionally substituted heterocycle of the formula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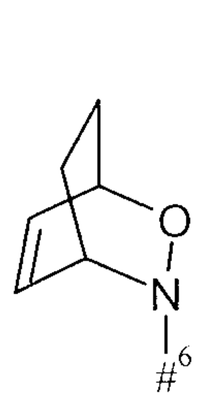

wherein

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

R^6 stands for hydrogen, hydroxyl or benzyloxy,

and

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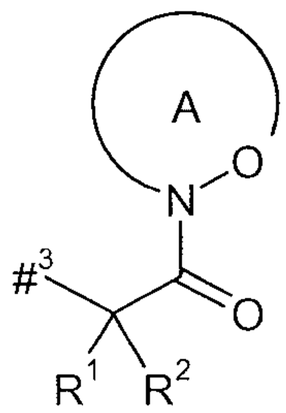
as well as their salts and solvates as well as the solvates of the salts.

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

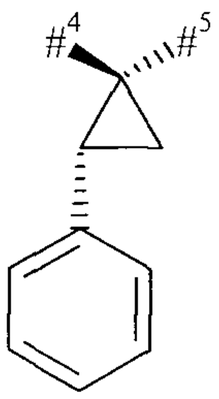
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 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

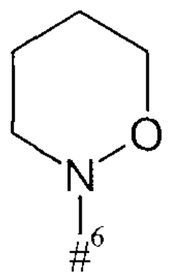


wherein

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

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

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,

and

n, AK, Cys, G, L^1, B, L^2 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

R^{35} stands for hydroxyl,

and

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

R^{35} stands for methyl,

and

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.

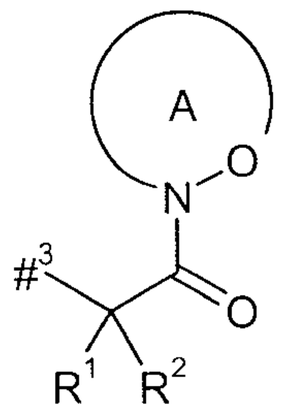
Also especially preferred within the scope of the present invention are compounds of formula (XXXa), in which

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,

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



wherein

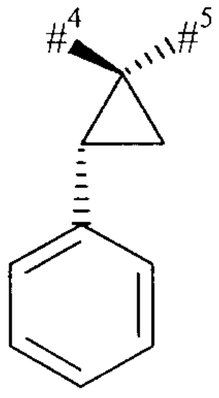
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 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

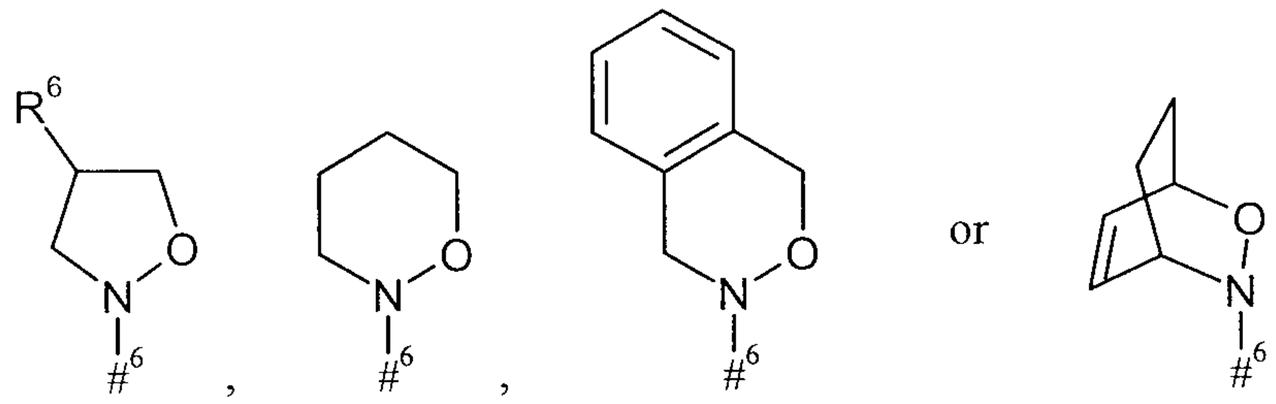


wherein

$\#^4$ 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 monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

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

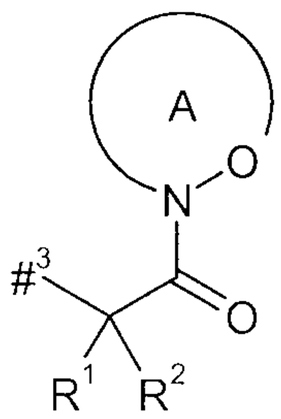
R^6 stands for hydrogen, hydroxyl or benzyloxy,

n, AK, Cys, G, L^1, L^2 and B 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 (Ia) and (XXXa), in which

D stands for a group of the formula



wherein

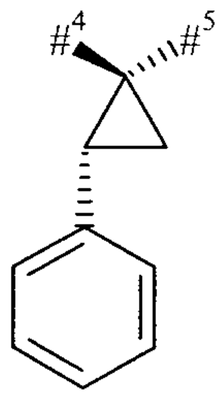
$\#^3$ denotes the linkage site to the nitrogen atom,

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

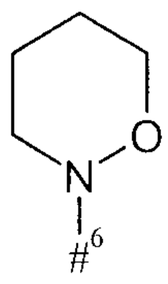


wherein

$\#^4$ 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



wherein

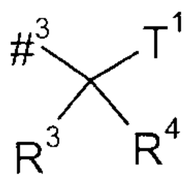
$\#^6$ denotes the linkage site to the carbonyl group,

n, AK, Cys, G, L^1, L^2 and B have the meanings given above

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



wherein

T^1 stands for -C(=O)-OH or $-C(=O)-NH_2$ and

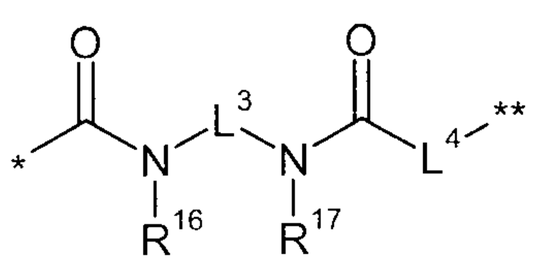
n, AK, G, L^1, B, L^2, $\#^3$, R^3 and R^4 have the meanings given above.

Also preferred within the scope of the present invention are compounds of formula (I), in which

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



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,

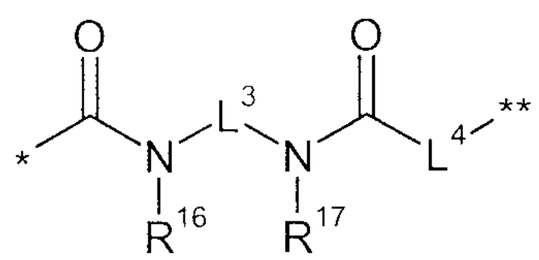
L^4 stands for a bond,

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,

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.

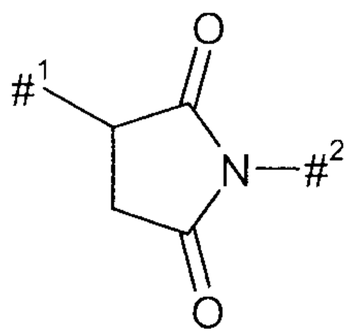
Also preferred within the scope of the present invention are binder-drug conjugates of general formula (I), in which

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,

G stands for a group of the formula

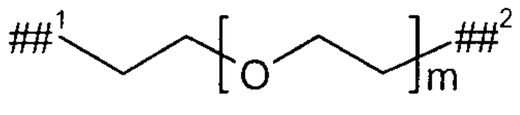


wherein

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

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

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,

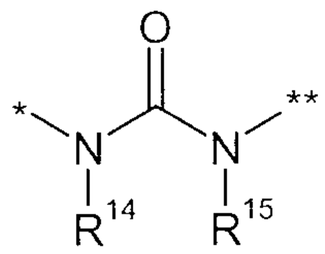
$\#\#^2$ denotes the linkage site to the group B,

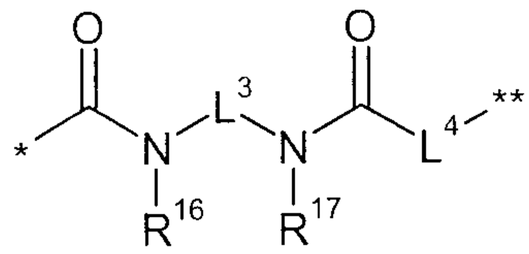
wherein (C_1-C_{10})-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another 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





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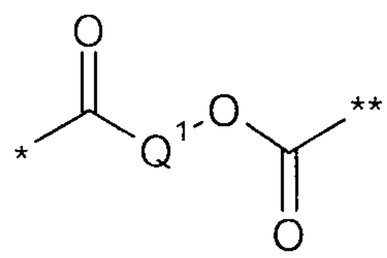


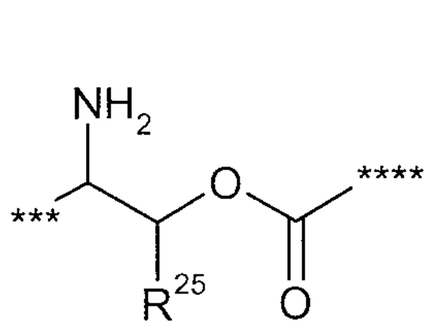
wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

L^3 for a bond or $(C_2$-$C_4)$-alkanediyl,

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



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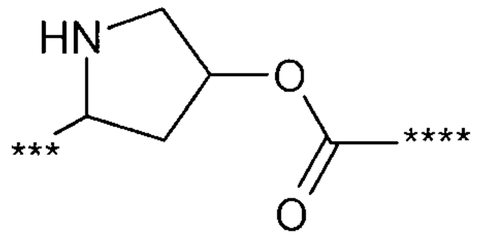


wherein

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

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

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

R^{14} and R^{15} together with the atoms to which they are bound form a five- or six-membered heterocycle,

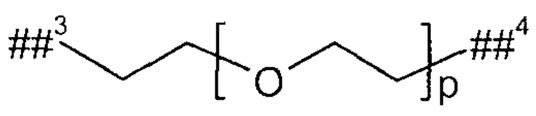
R^{16} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

R^{17} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

or

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

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

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.

Also preferred within the scope of the present invention are binder-drug conjugates of general formula (I), in which

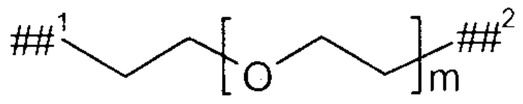
AK stands for AK_2

wherein

AK_2 stands for a binder that is bound to the group G via the NH side group of a lysine radical of the binder,

G stands for carbonyl,

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,

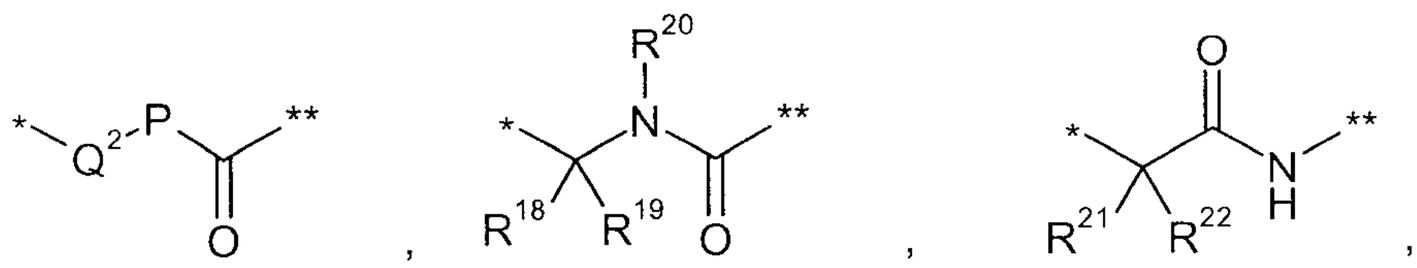
$##^2$ denotes the linkage site to the group B,

wherein (C_1-C_{10})-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

wherein two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4- relation to one another 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



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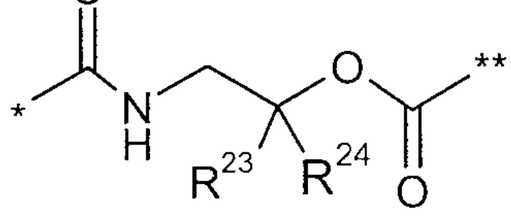


wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

P stands for O or NH,

Q^2 stands for a three- to seven-membered carbocycle or a four- to seven-membered heterocycle,

R^{18} stands for hydrogen or (C_1-C_4)-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 (C_1-C_4)-alkyl,

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 (C_1-C_4)-alkyl,

R^{22} stands for hydrogen or (C_1-C_4)-alkyl,

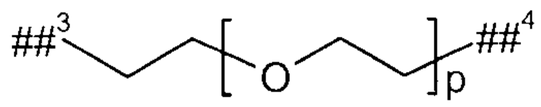
or

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

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

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

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 formula (Ia) as indicated above, in which

n stands for a number from 1 to 20,

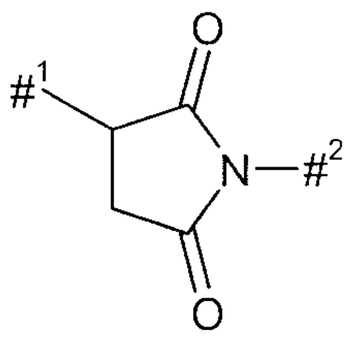
AK stands for AK_1 or AK_2

wherein

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,

AK_2 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 = AK_1 stands for a group of the formula



wherein

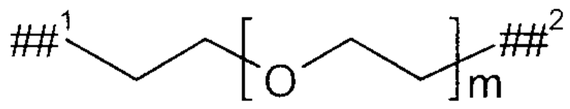
#[1] denotes the linkage site to the cysteine radical of the binder,

#[2] denotes the linkage site to the group L^1,

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



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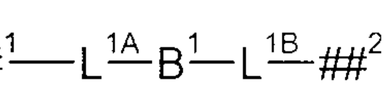


wherein

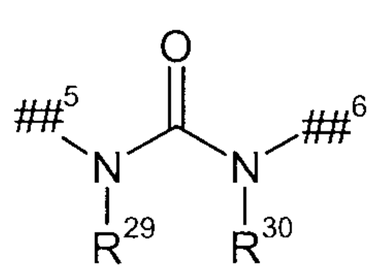
m stands for a number from 2 to 6,

$##^1$ 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,

B^1 stands for a group of the formula



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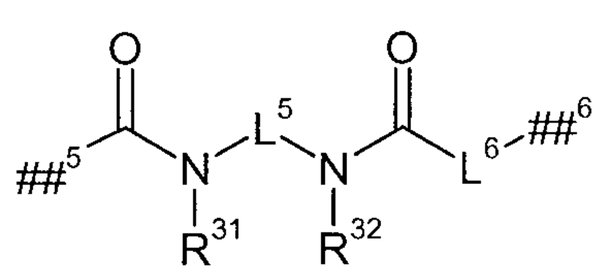

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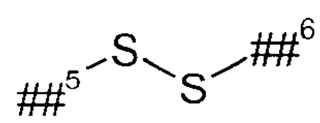


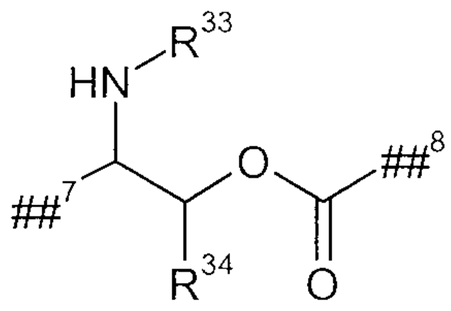
wherein

$##^5$ 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,

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



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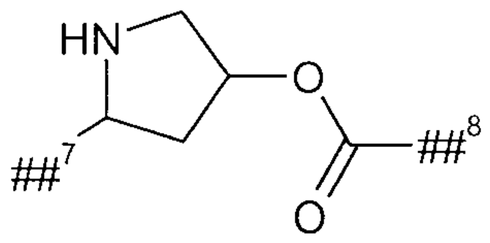


wherein

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

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

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

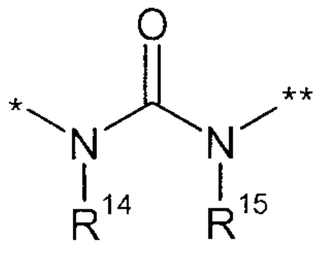
R^{32} stands for hydrogen or methyl,

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

and

wherein $(C_2\text{-}C_6)$-alkanediyl may be substituted with one to two methyl substituents,

B stands for a bond or a group of the formula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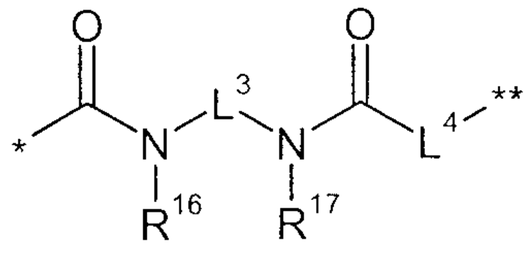
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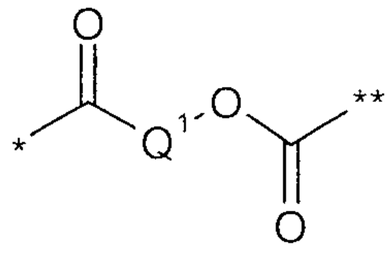
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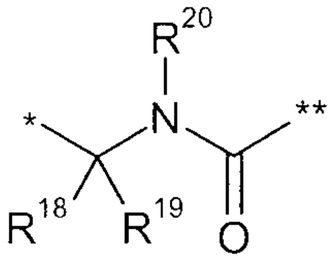
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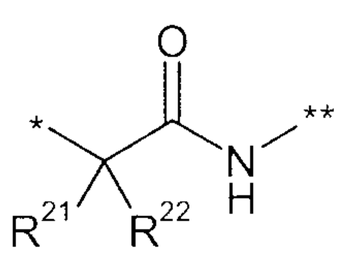
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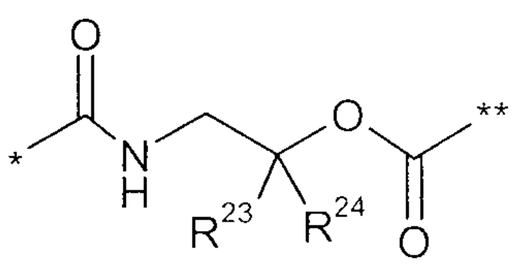
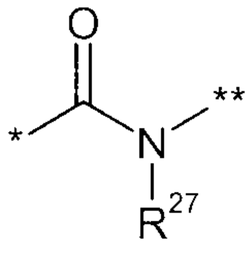


or 

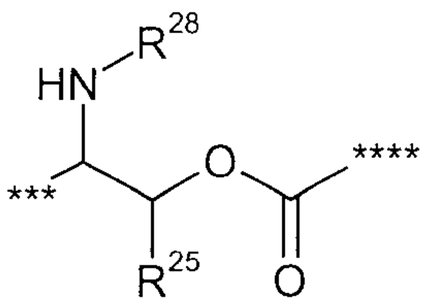
wherein

* 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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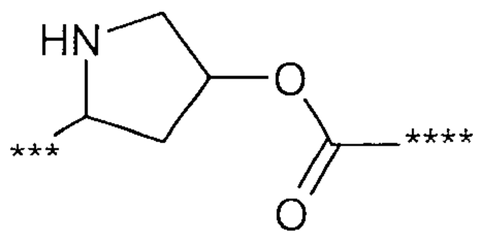


wherein

*** 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,

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,

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 1-methyl-propan-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,

R^{22} stands for hydrogen or methyl,

or

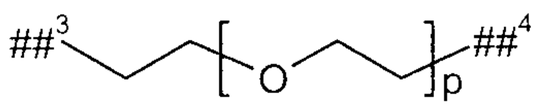
R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

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



wherein

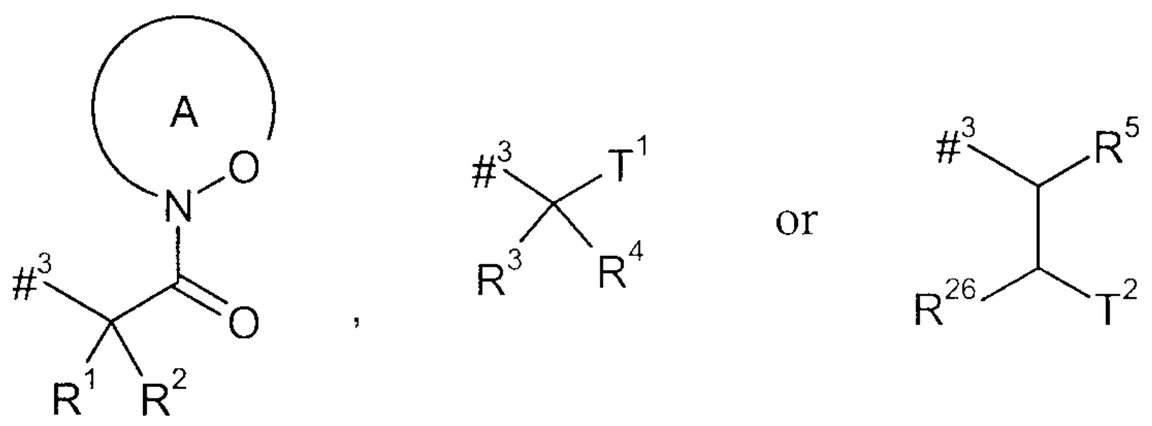
p stands for a number from 2 to 6,

$\#^3$ 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,

D stands for a group of the formula



wherein

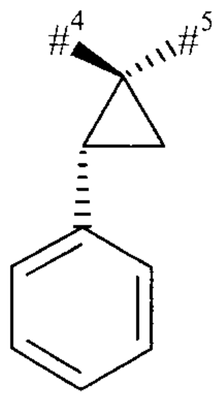
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 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

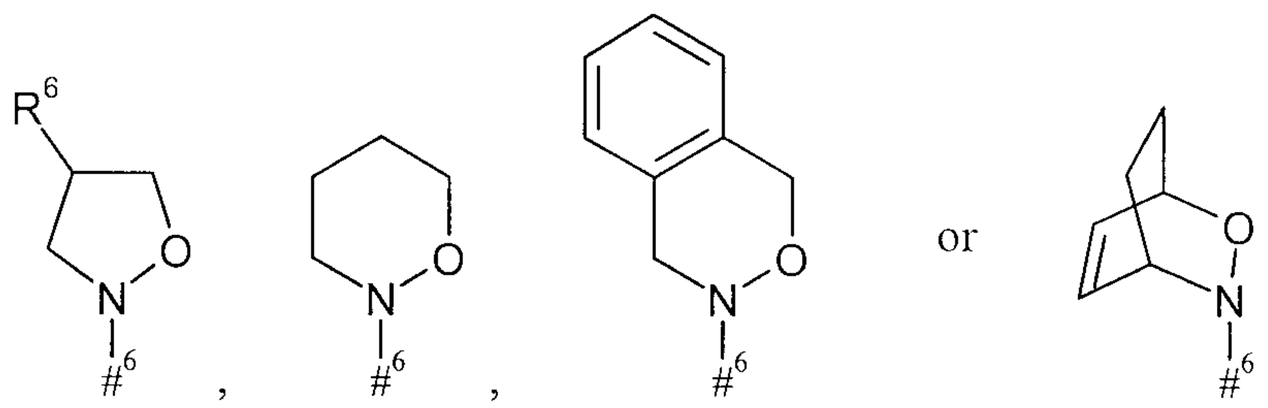


wherein

$\#^4$ 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 monocyclic or bicyclic, optionally substituted heterocycle of the formula



wherein

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

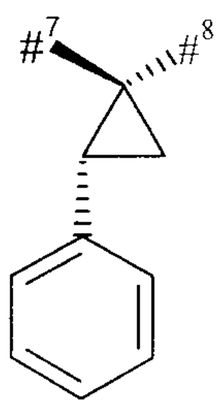
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands 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 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,

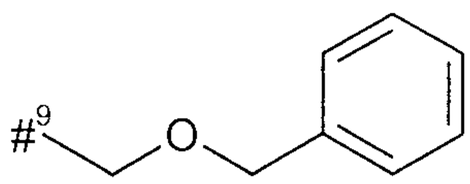
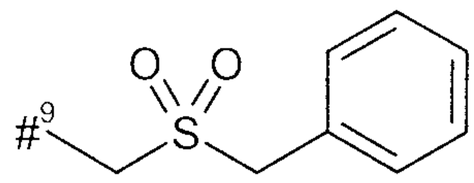
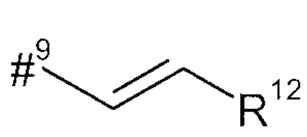
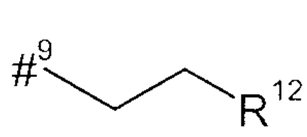
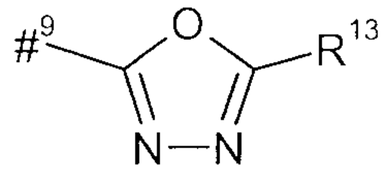
or

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

wherein

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

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^{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 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,

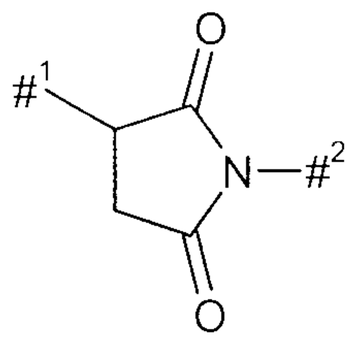
AK stands for AK_1 or AK_2

wherein

AK_1 stands for a binder bound to the group G via a sulfur atom of the binder,

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

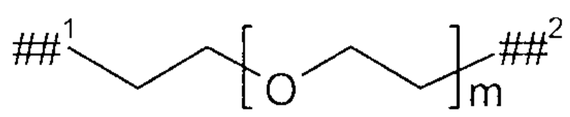
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ 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

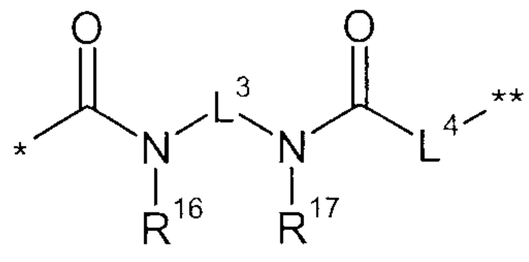
m stands for a number of 2 or 3,

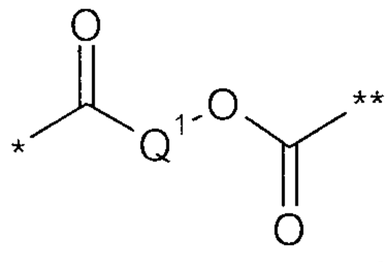
$\#\#^1$ denotes the linkage site to the group G,

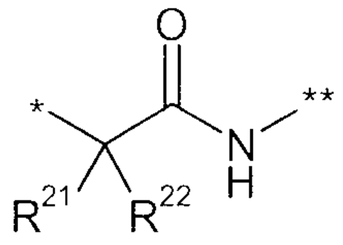
$\#\#^2$ denotes the linkage site to the group B,

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







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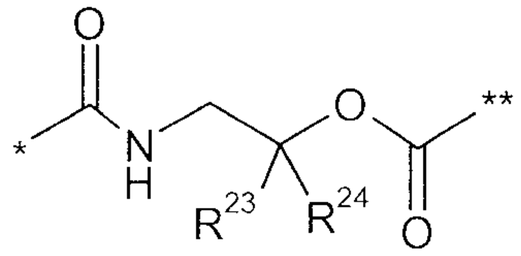


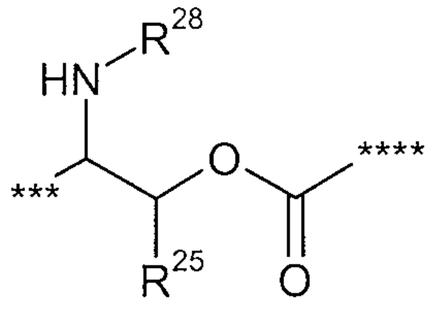
wherein

* 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



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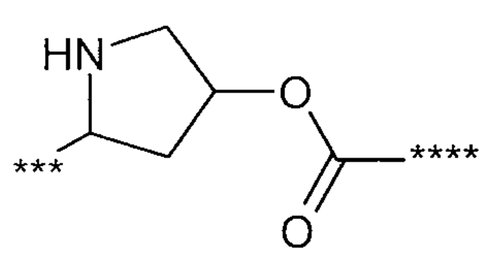


wherein

*** 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,

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,

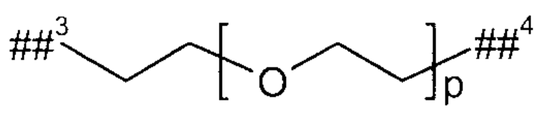
or

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



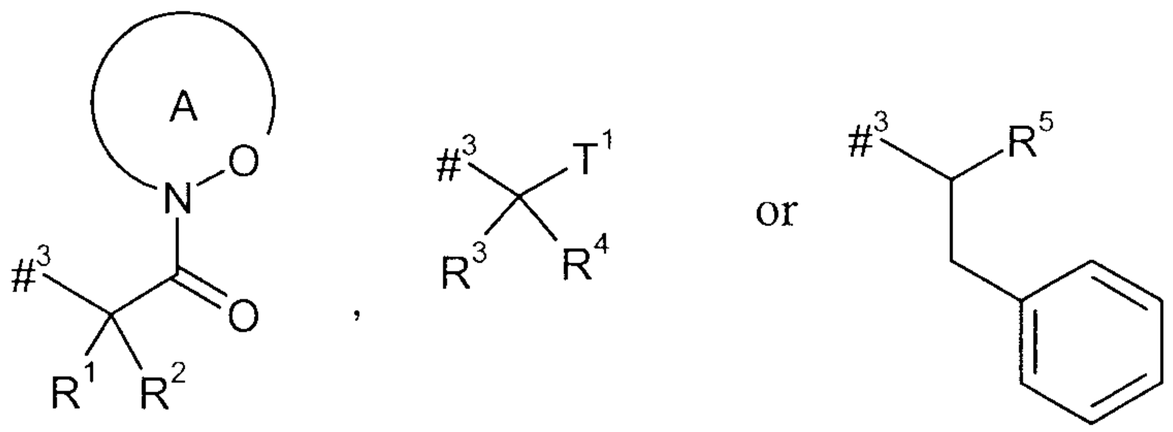
wherein

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

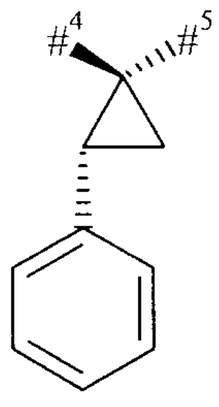
$#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-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

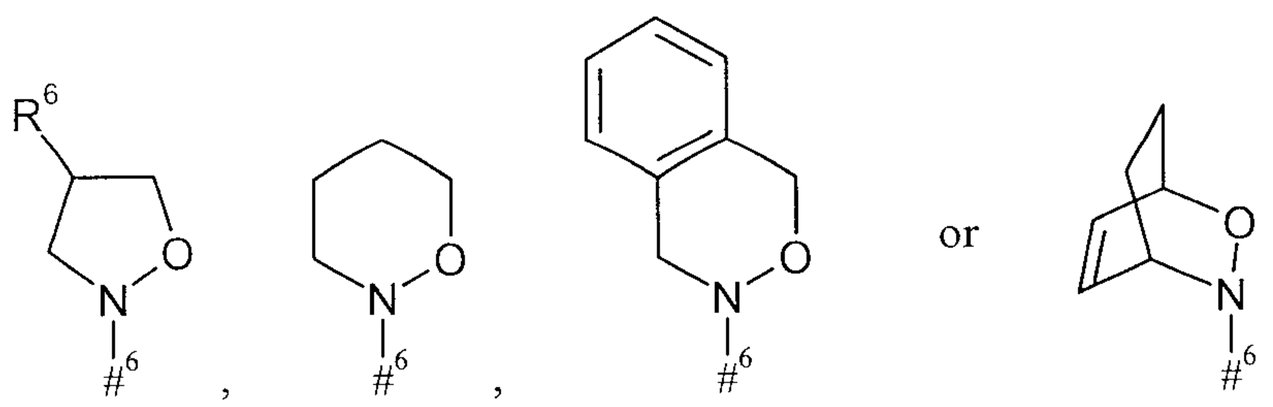


wherein

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

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

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,

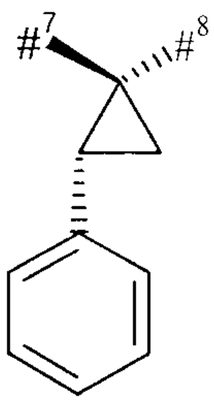
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

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



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

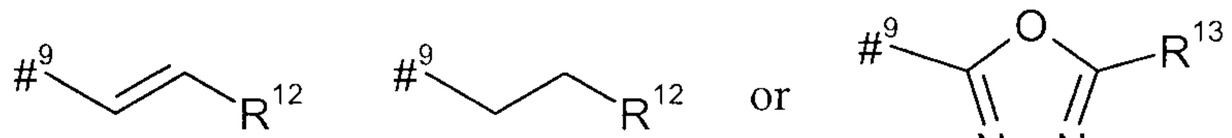
R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

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

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,

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,

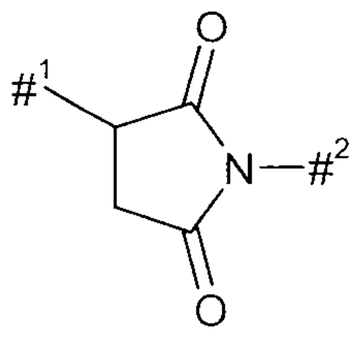
AK stands for AK_1 or AK_2,

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,

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



wherein

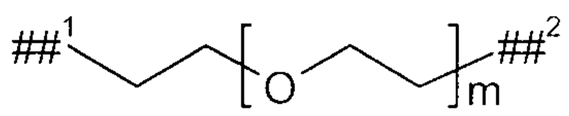
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ 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

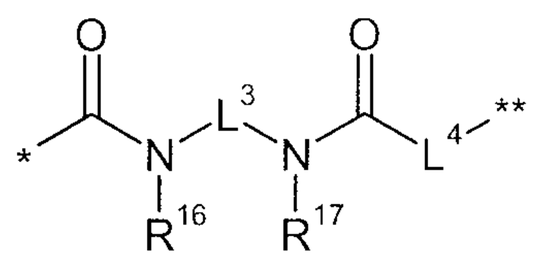
m stands for a number of 2 or 3,

$##^1$ 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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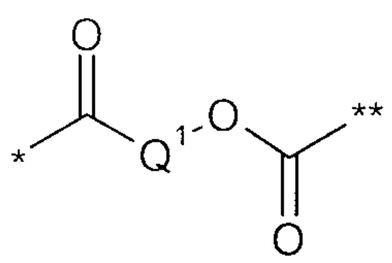
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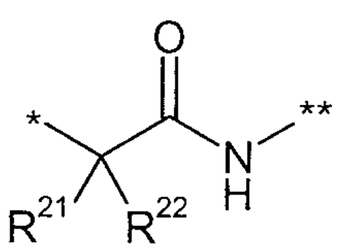
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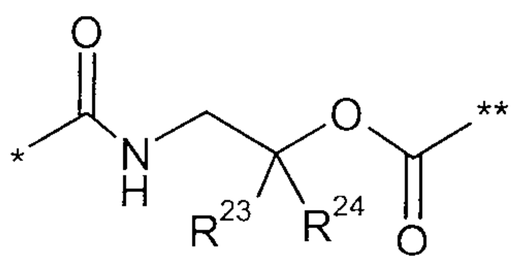


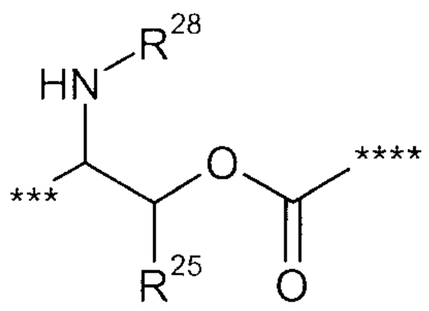
wherein

* denotes the linkage site to L^1,

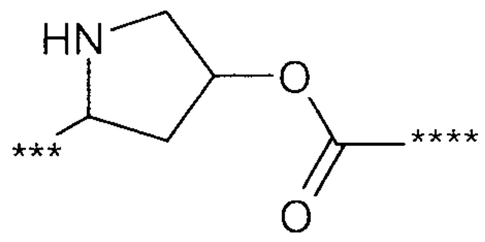
** denotes the linkage site to L^2,

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

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



or



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,

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,

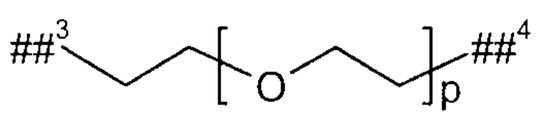
or

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



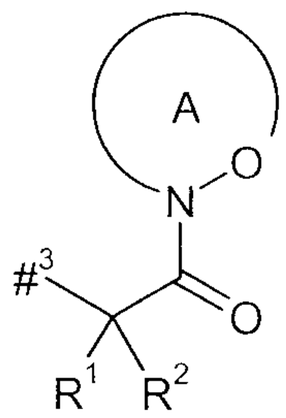
wherein

p stands for a number from 2 to 6,

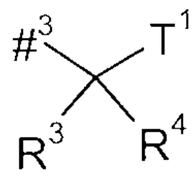
$\#^3$ denotes the linkage site to the group B,

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

D stands for a group of the formula



or



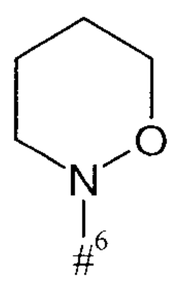
wherein

$\#^3$ 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



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 $-C(=O)-NR^8R^9$,

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.

Especially preferred within the scope of the present invention are binder-drug conjugates of formula (Ia), in which

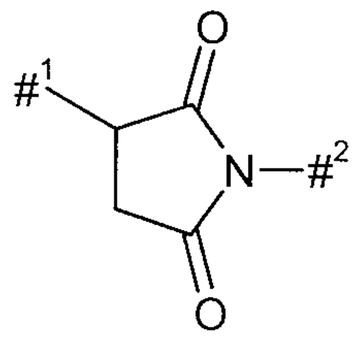
n stands for a number from 2 to 8, preferably 2 to 5,

AK stands for AK_1,

wherein

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



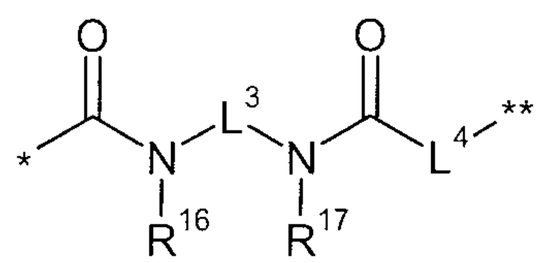
wherein

$\#^1$ 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,

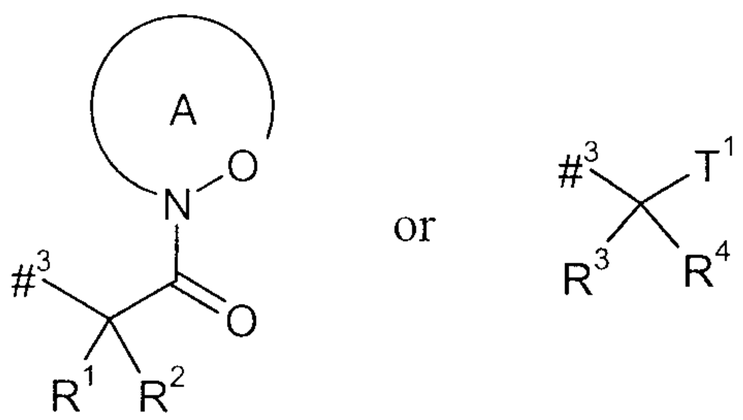
L^4 stands for a bond,

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



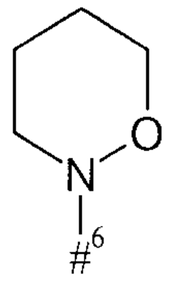
wherein

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

R^1 stands for hydrogen,

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

the ring A with the N-O group contained therein stands for



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 $-C(=O)-NR^8R^9$,

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 formula (Ia), in which

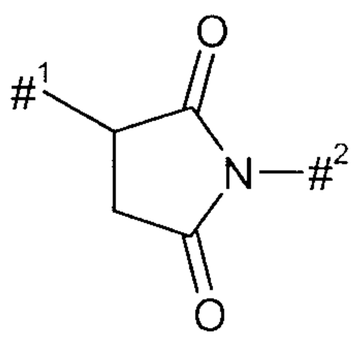
n stands for a number from 2 to 8, preferably 2 to 5,

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 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



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 a bond,

B stands for a bond,

L^2 stands for hexane-1,6-diyl,

and D has the meaning 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 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 AK_2,

wherein

AK_2 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,

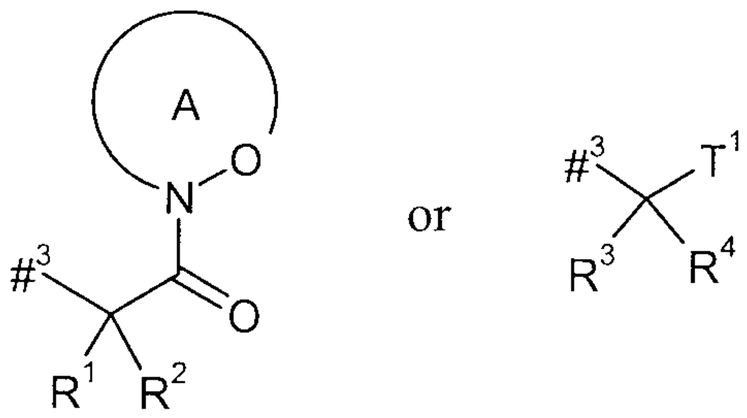
G stands for carbonyl,

L^1 stands for a bond,

B stands for a bond,

L^2 stands for pentane-1,5-diyl,

D stands for a group of the formula



or

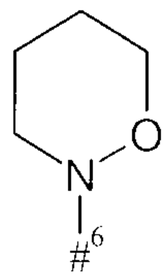
wherein

$\#^3$ 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



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 $-C(=O)-NR^8R^9$,

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 formula (Ia), in which

n stands for a number from 2 to 8, preferably 2 to 5,

AK stands for AK_2,

wherein

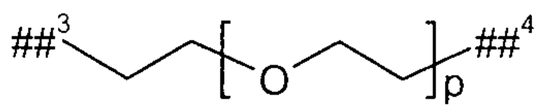
AK_2 stands for a binder that is bound to the group G via a nitrogen atom of the lysine radical of the binder, preferably a chimeric, humanized or human antibody, especially preferably an anti-EGFR antibody,

G stands for carbonyl,

L^1 stands for a bond,

B stands for a bond,

L^2 stands for a group of the formula



wherein

p stands for the number 3,

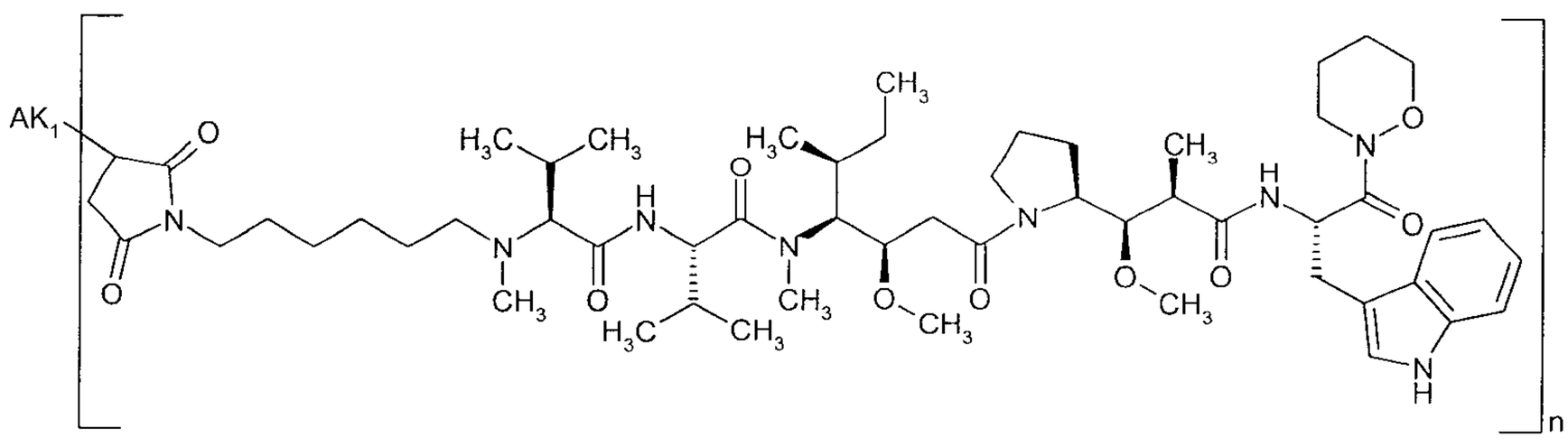
$##^3$ denotes the linkage site to the group B,

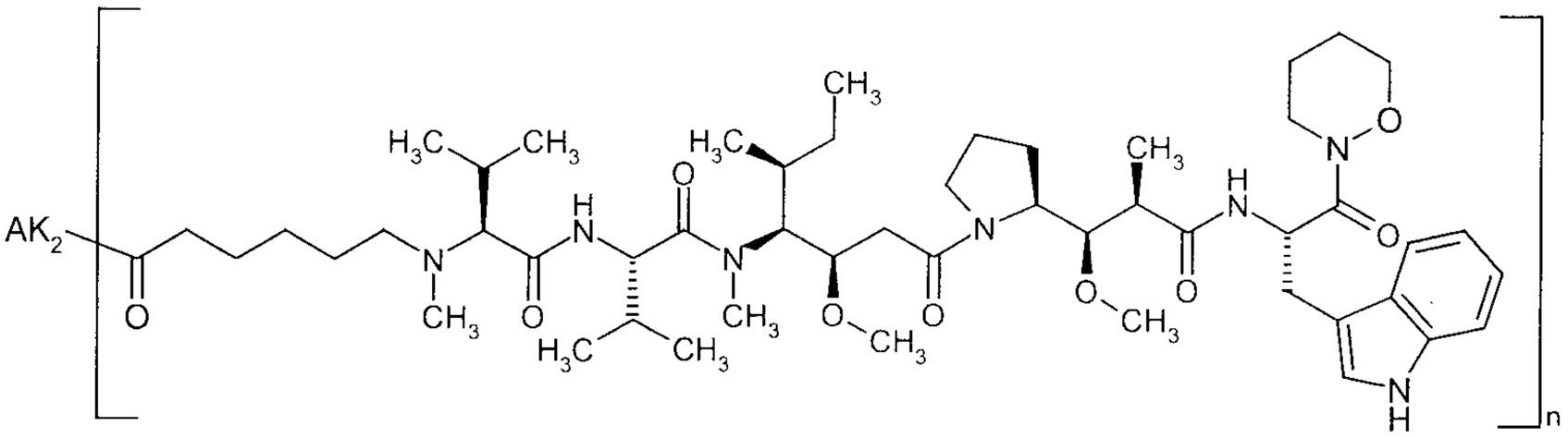
$##^4$ denotes the linkage site to the nitrogen atom,

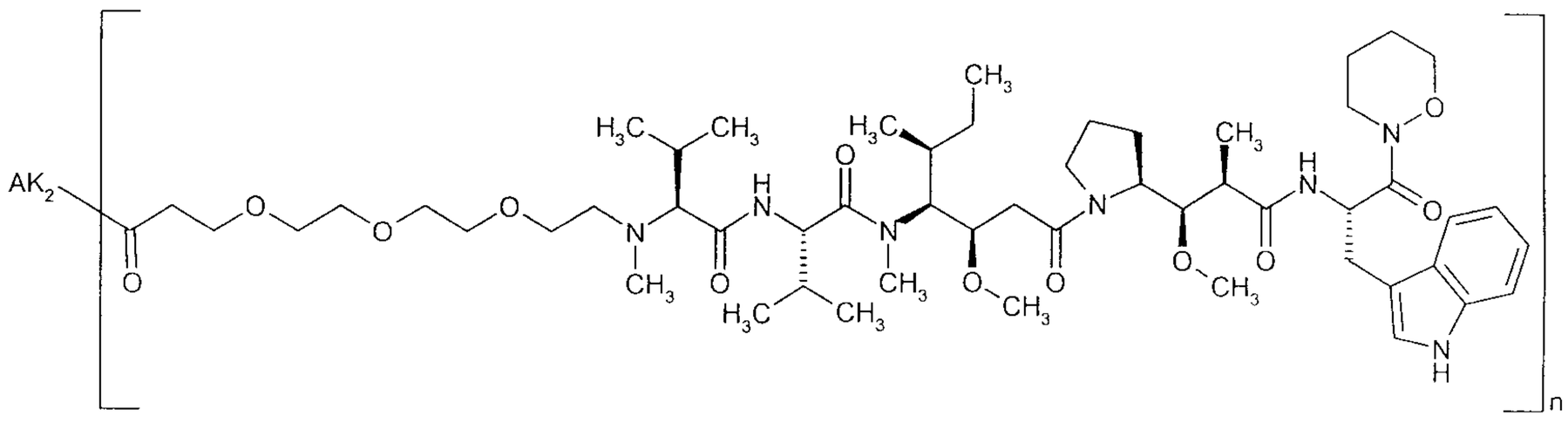
and D has the meaning given above,

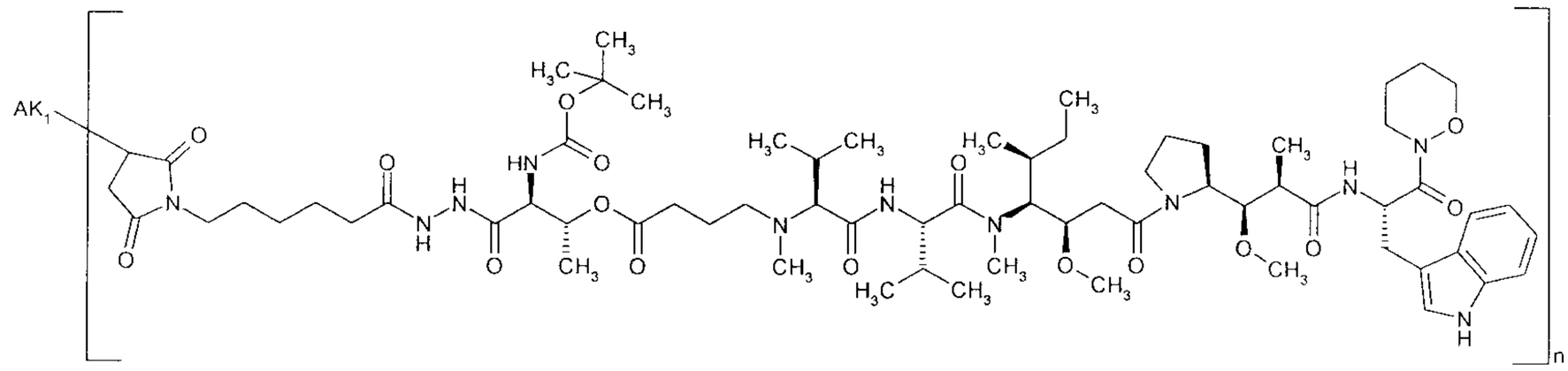
as well as their salts and solvates as well as the solvates of the salts.

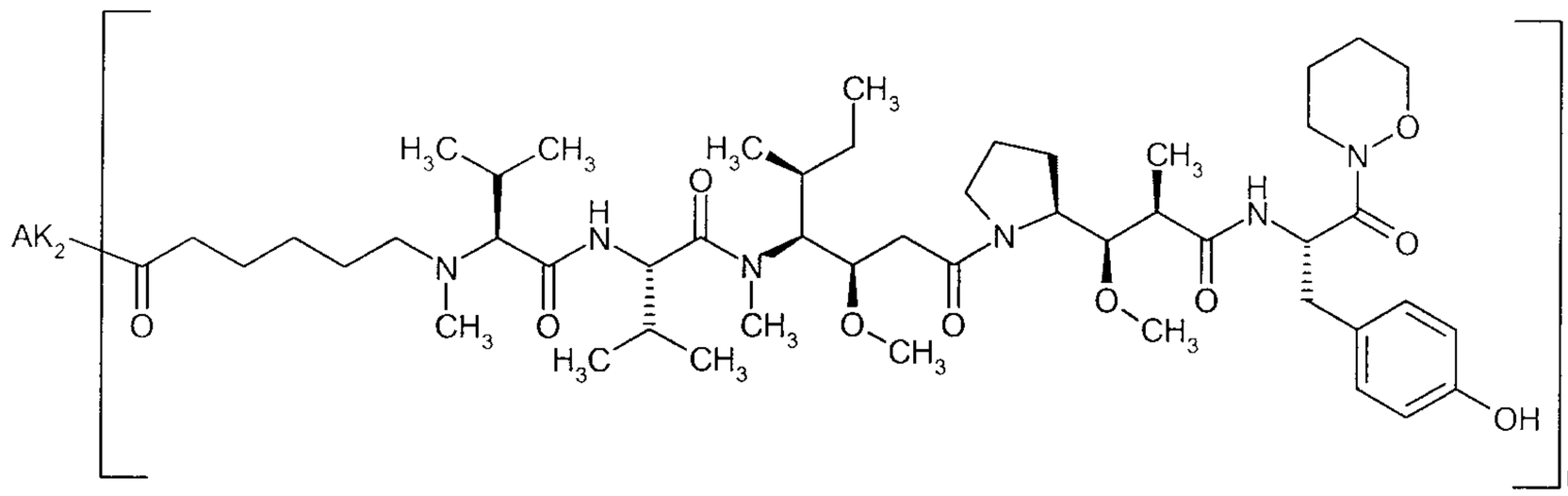
According to the invention, the drug-binder conjugate preferably comprises the following compounds in particular, where n stands for a number from 2 to 8, preferably 2 to 8, and AK stands for a chimeric, human or humanized antibody or an antigen binding antibody fragment which binds to mesothelin, C4.4a or EGFR:

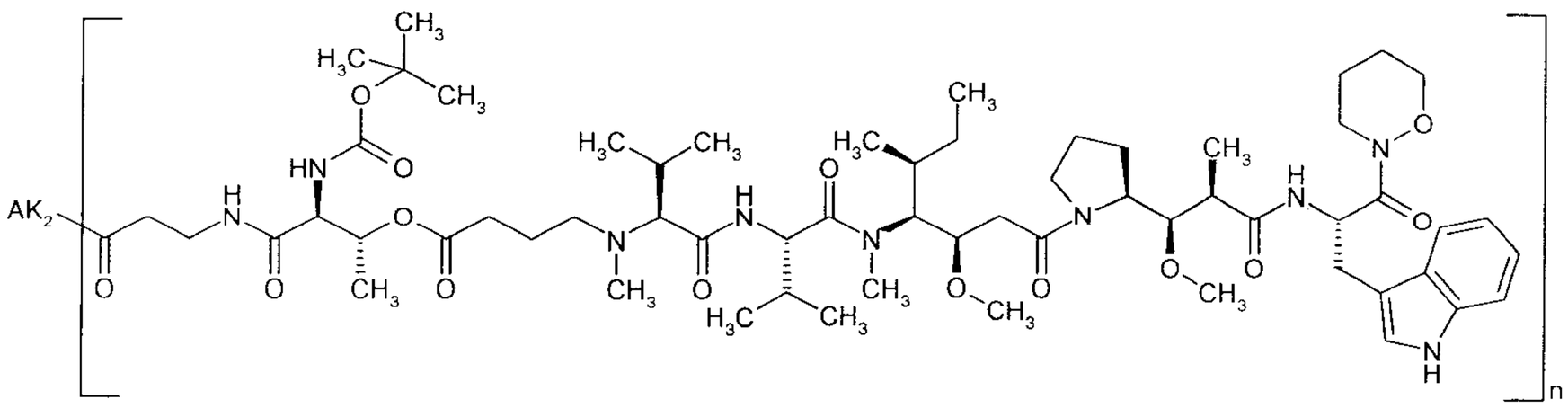




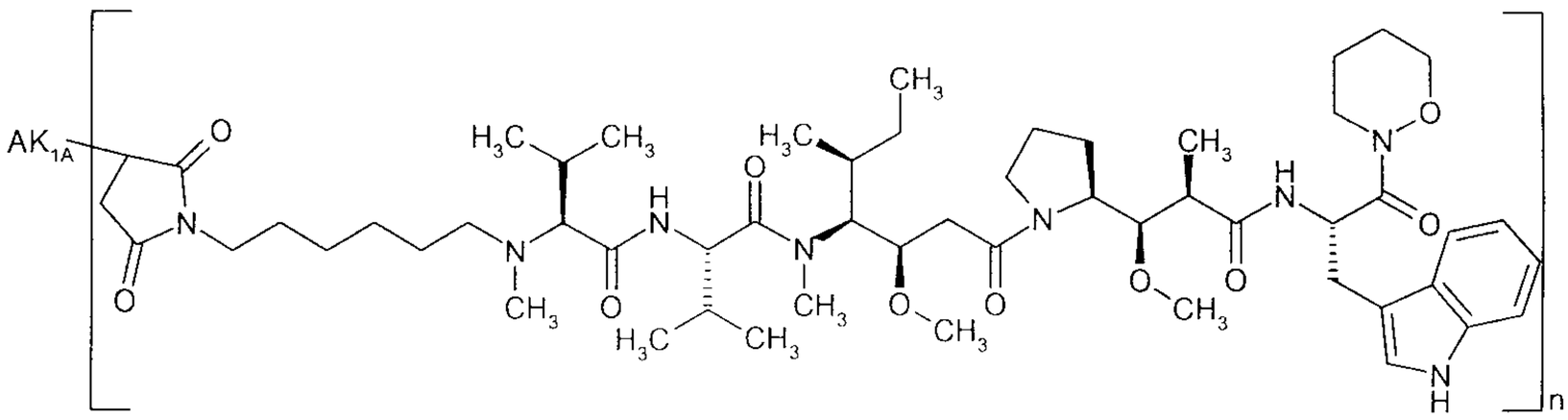


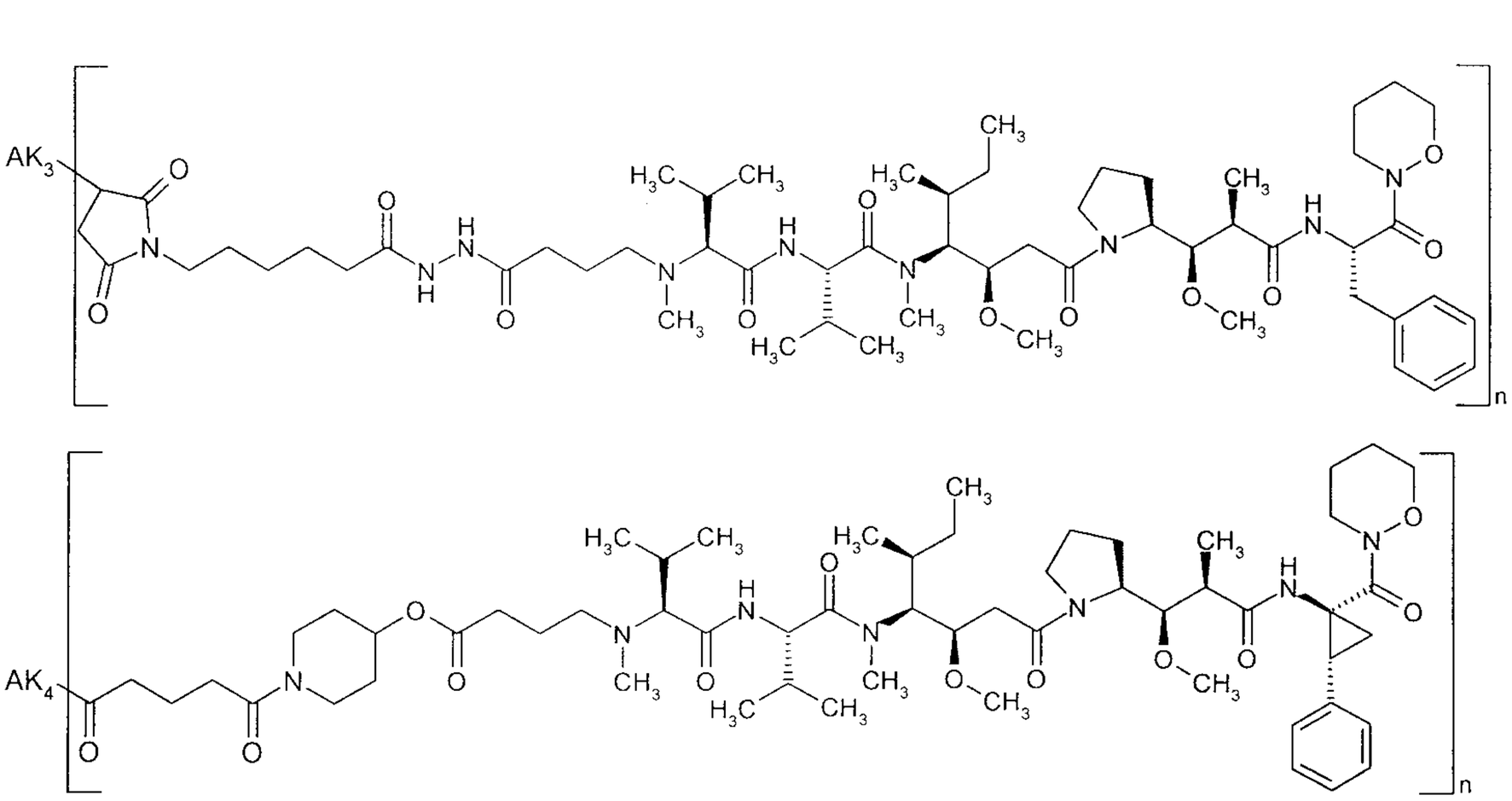


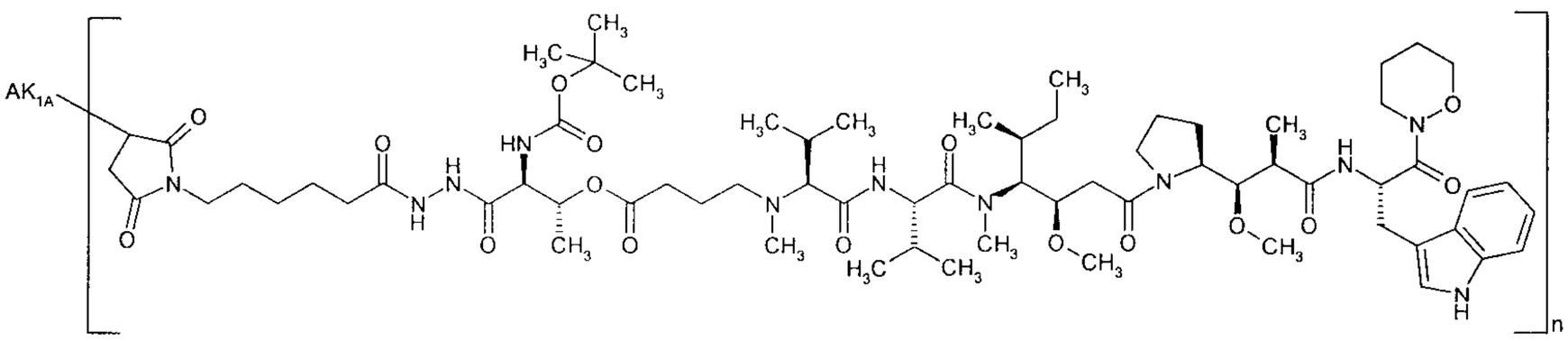


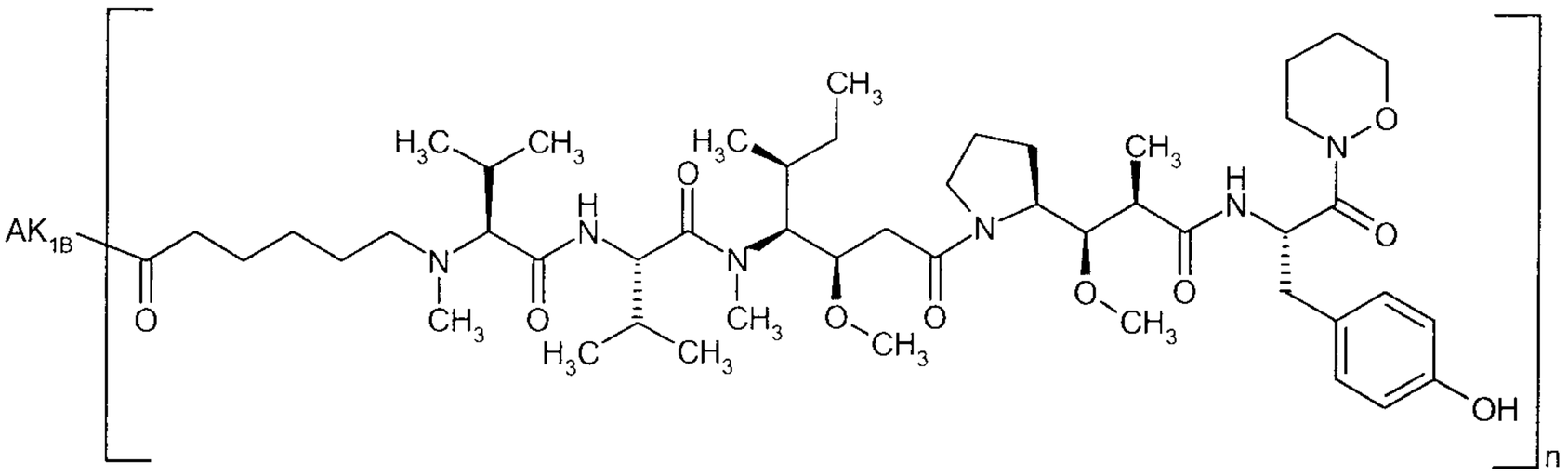


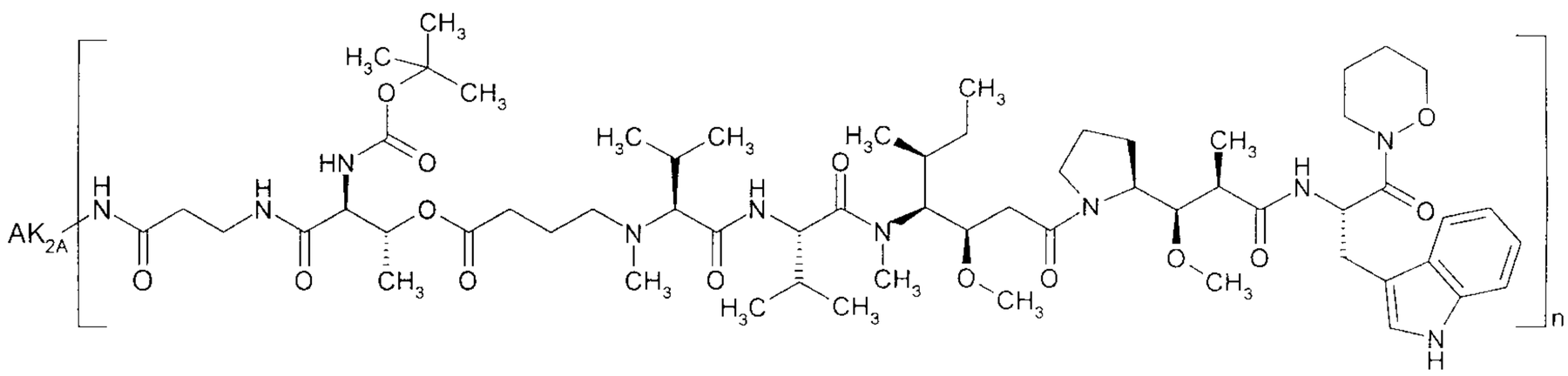
In addition, according to the invention the drug-binder conjugate is especially preferably selected from the following compounds:











in which

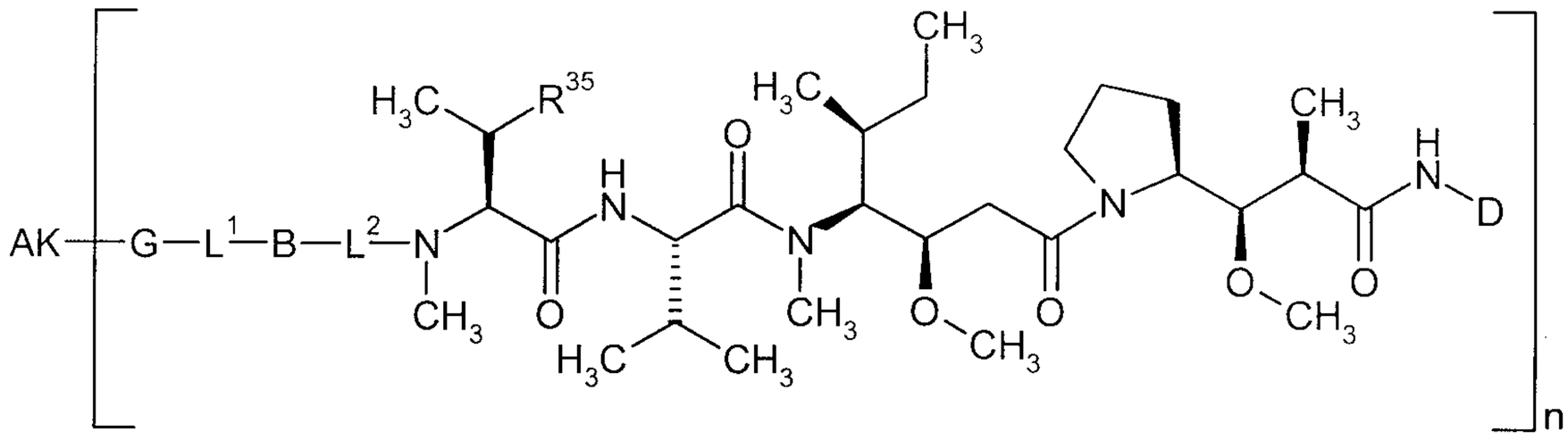
n stands for a number from 2 to 8, preferably 2 to 5,

and

AK_{1A}, AK_{1B}, AK_{2A}, A_{K3} and AK_4 stand for the antibodies indicated.

AK

binder-drug conjugate of the following formula Ia



wherein

n stands for a number from 2 to 8;

AK stands for AK_1 or AK_2

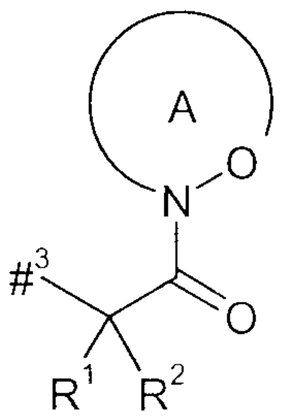
wherein

AK_1 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,

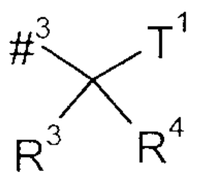
AK_2 stands for a chimeric, human or humanized antibody or an antigen binding antibody fragment which is bound to mesothelin, EGFR or C4.4a and is bound to the group G via the NH side group of a lysine radical of the binder,

R^{35} stands for methyl;

D stands for a group of the formula



or



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

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 $-C(=O)-NR^8R^9$,

R^8 stands for hydrogen or methyl,

R^9 stands for hydrogen, methyl or ethyl,

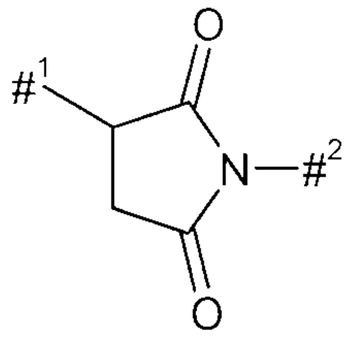
the group §-G-L1-B-L2-§§ stands for a linker,

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



wherein

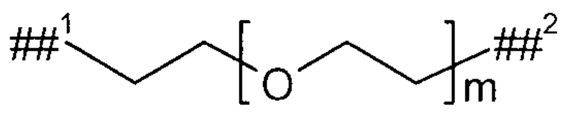
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ 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

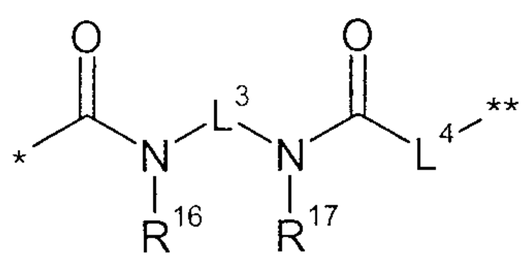
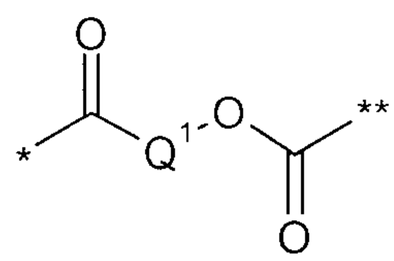
m stands for a number of 2 or 3,

$\#\#^1$ 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

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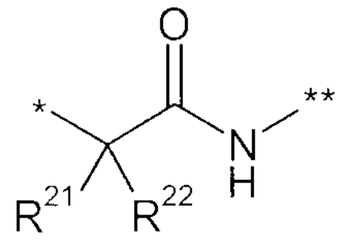

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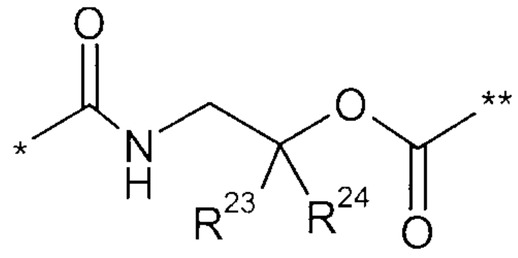


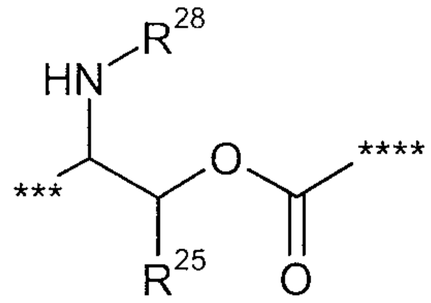
wherein

* 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



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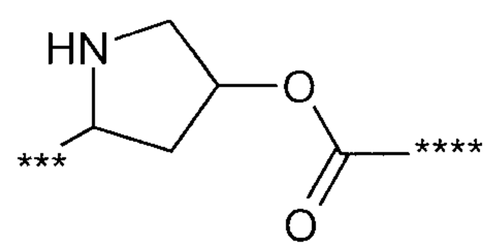


wherein

*** 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,

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,

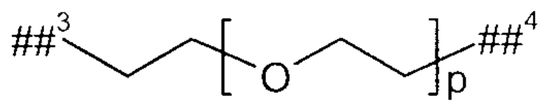
or

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



wherein

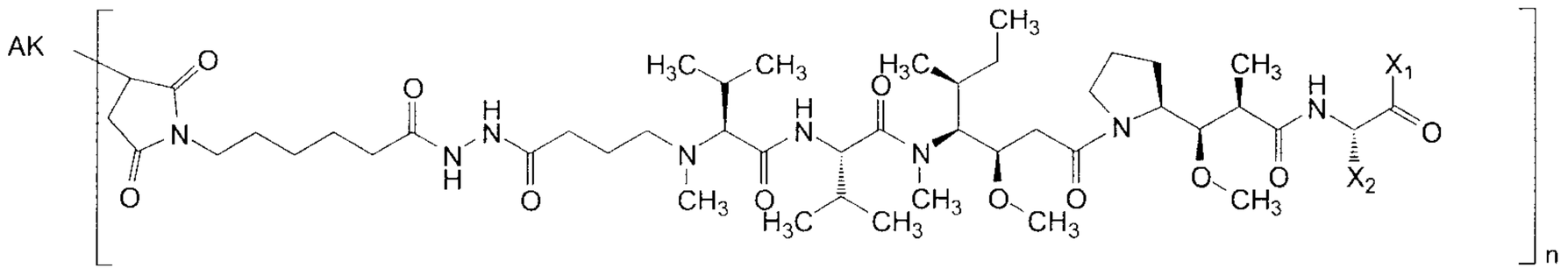
p stands for a number from 2 to 6,

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

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

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

Especially preferred are conjugates of the following formula,

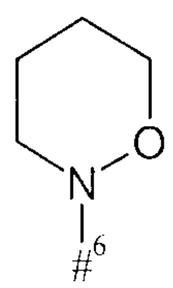


wherein

n stands for a number from 2 to 8, preferably 2 to 5;

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,

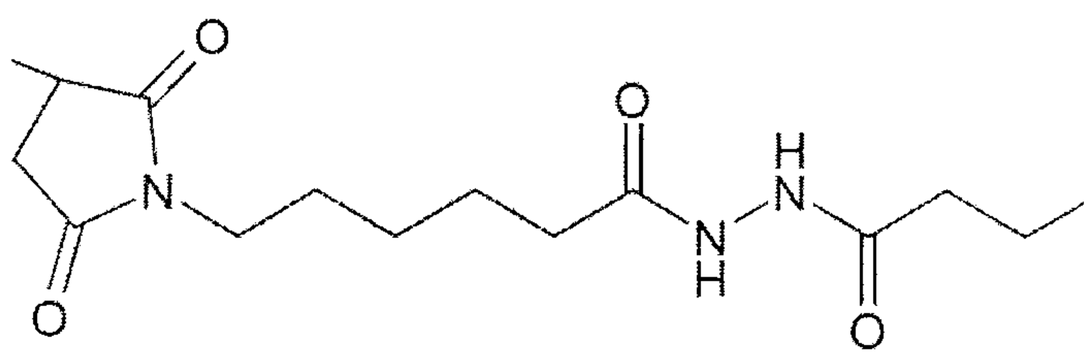
X_1 stands for NH_2 or



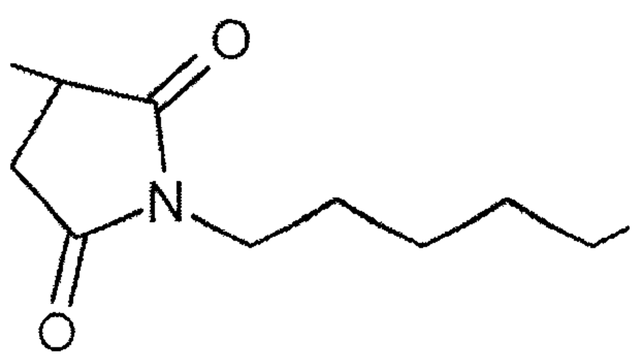
and

X_2 stands for 4-hydroxybenzyl or 1H-indol-3-ylmethyl.

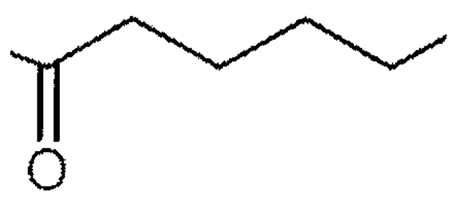
When the toxophore is bound to a cysteine radical of the antibody, the linker

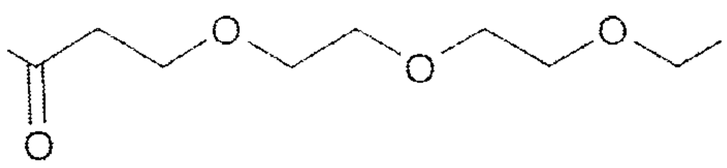


may be replaced by the following linker, for example:

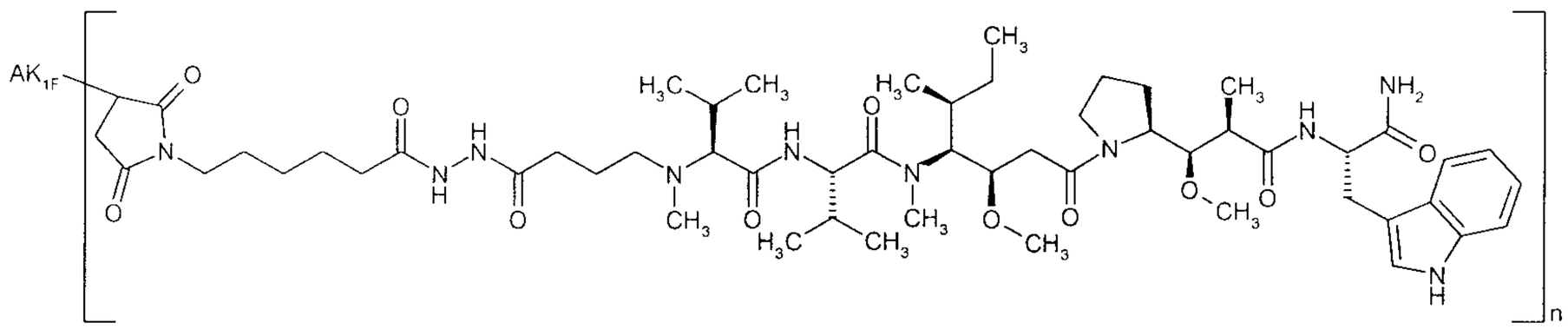


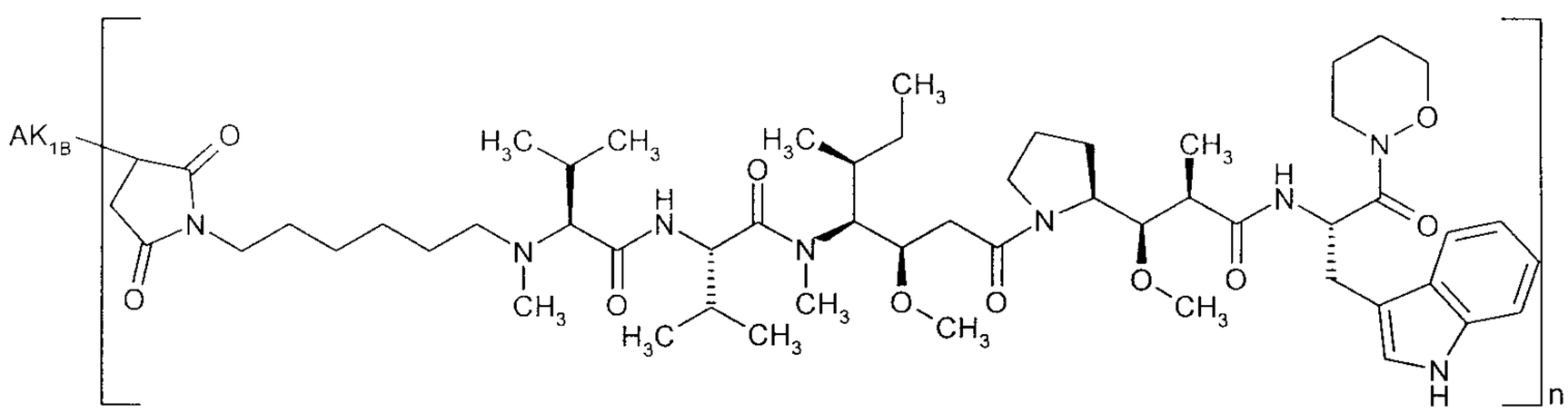
When the toxophore is bound to an NH group of the lysine radical of the antibody, the linker may be replaced by the following:

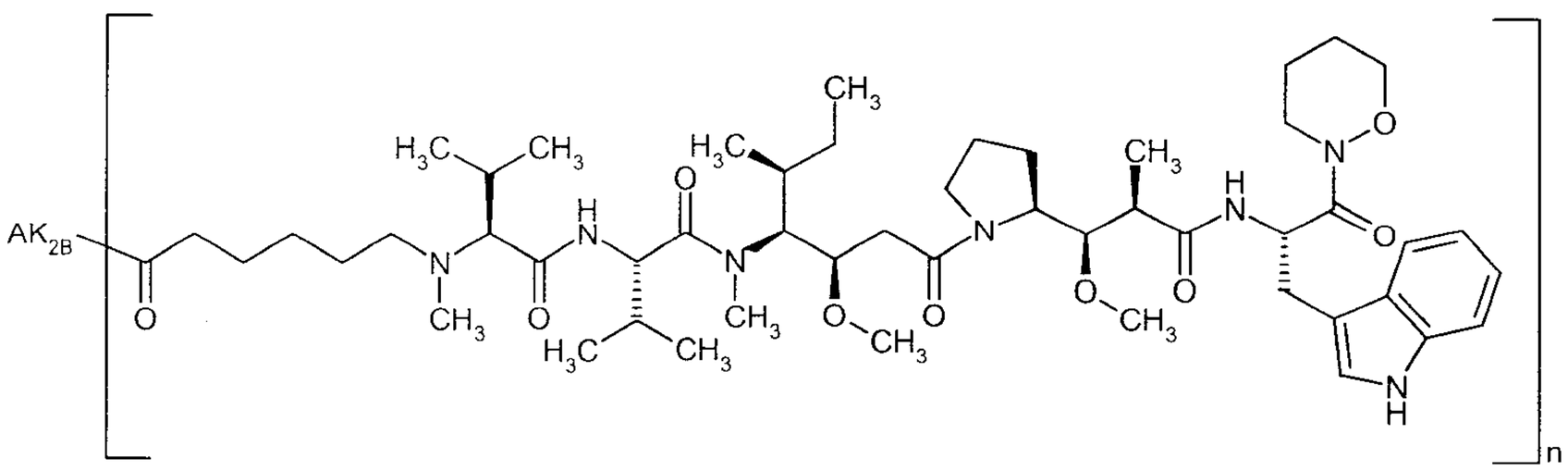


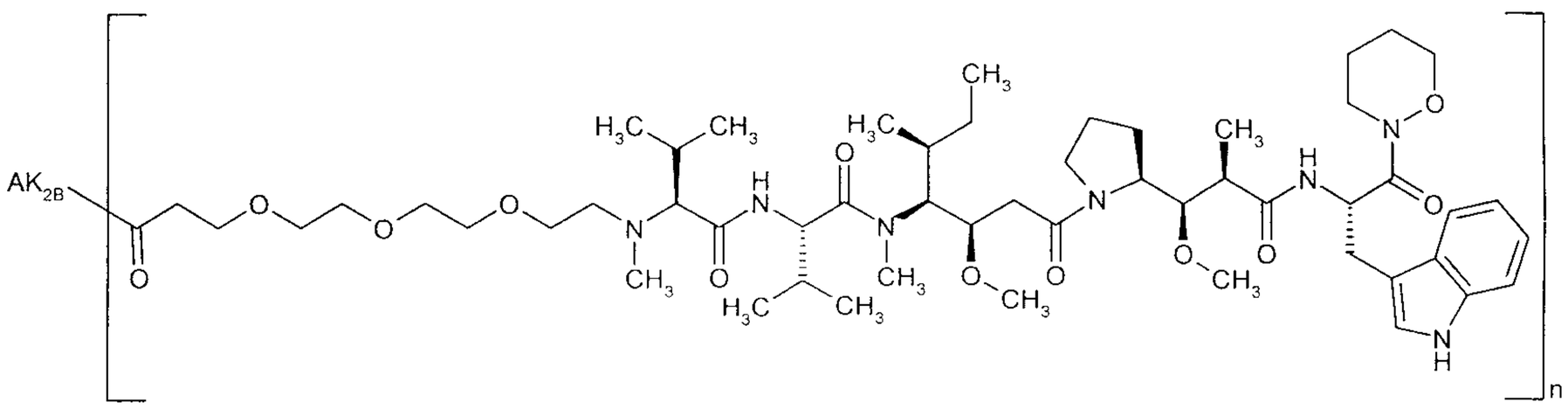


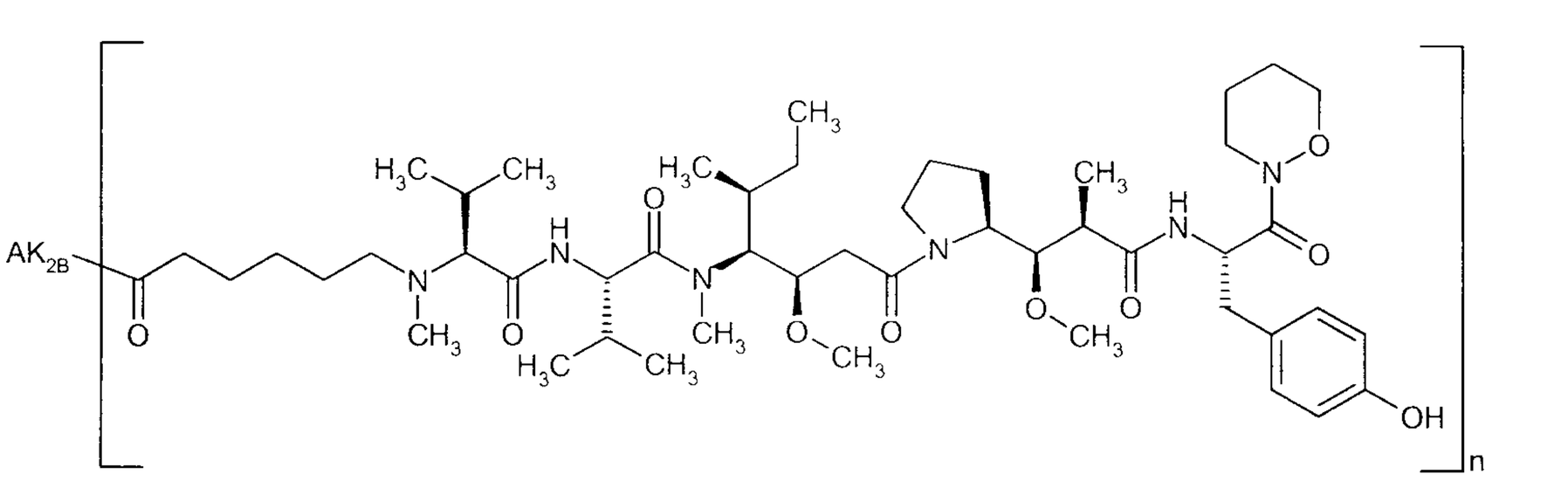
According to the invention the drug-binder conjugate is especially comprised of the following compounds, where n stands for a number from 2 to 8, preferably 2 to 5, and AK stands for a chimeric, human or humanized antibody or an antigen binding antibody fragment which binds to mesothelin, EGFR or C4.4a:

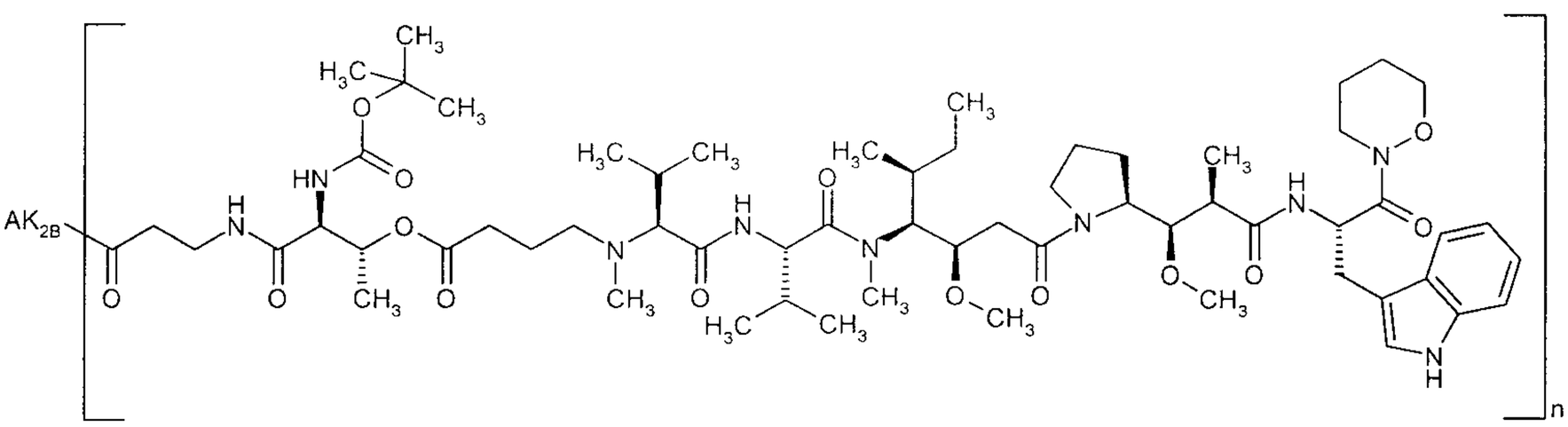












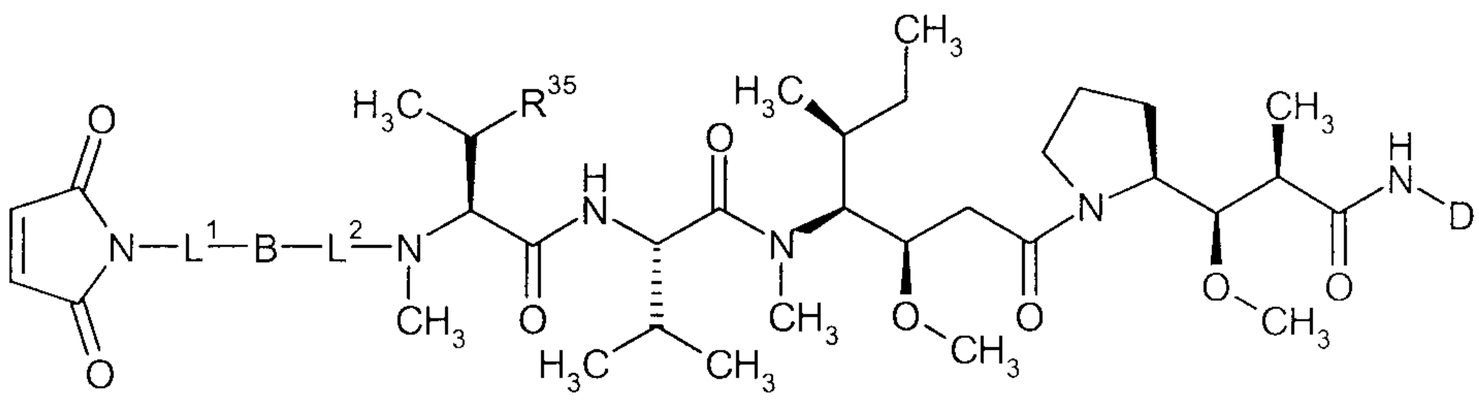
In these formulas, AK_{1F}, AK_{1B} and AK_{2B} may be replaced by other chimeric, human or humanized anti-C4.4a antibodies, anti-EGFR antibodies or anti-mesothelin antibodies.

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.

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

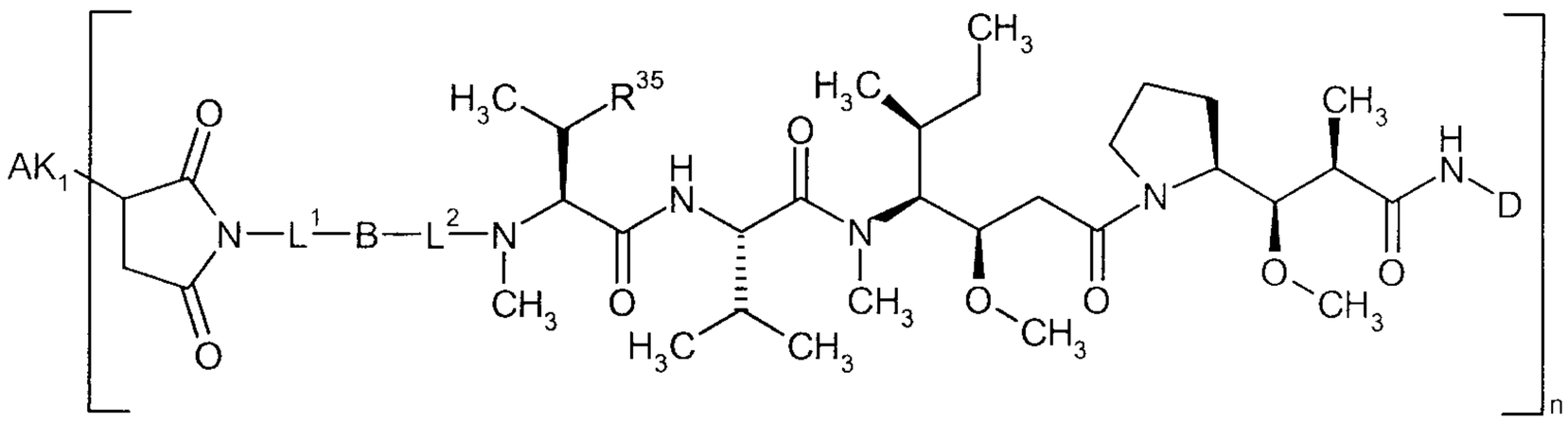
[A] is mixed with a suitable reducing agent such as, for example, dithiothreitol or *tris*-(2-carboxyethyl)phosphine hydrochloride and then is reacted with a compound of formula (IIa)



(IIa),

in which D, L^1, B, L^2 and R^{35} each have the meanings given above,

to form a compound of formula (I-A)

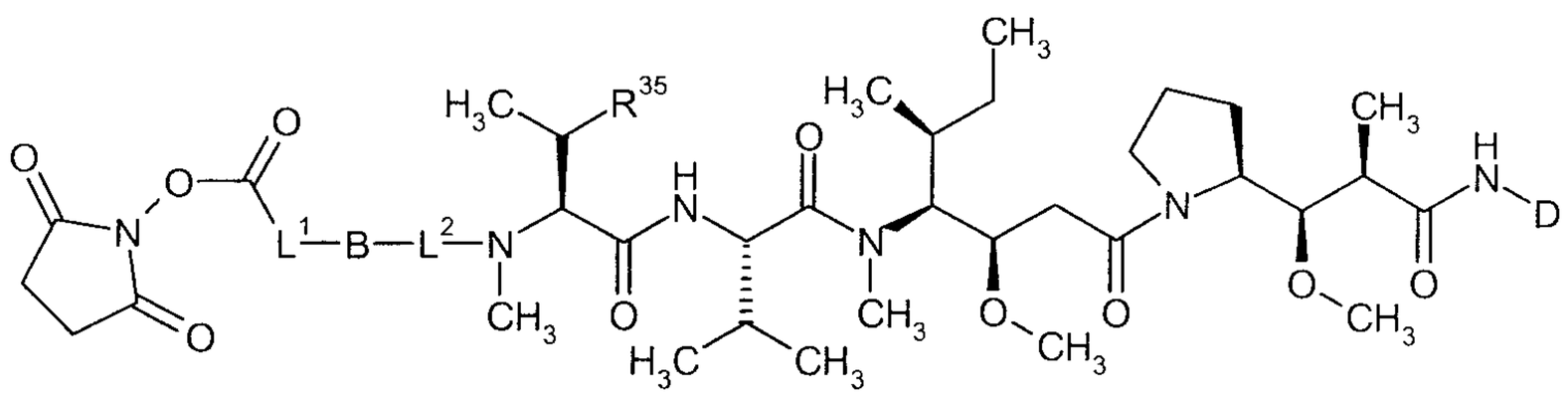


(Ia-A),

in which n, AK_1, D, L^1, B, L^2 and R^{35} each have the meanings given above,

or

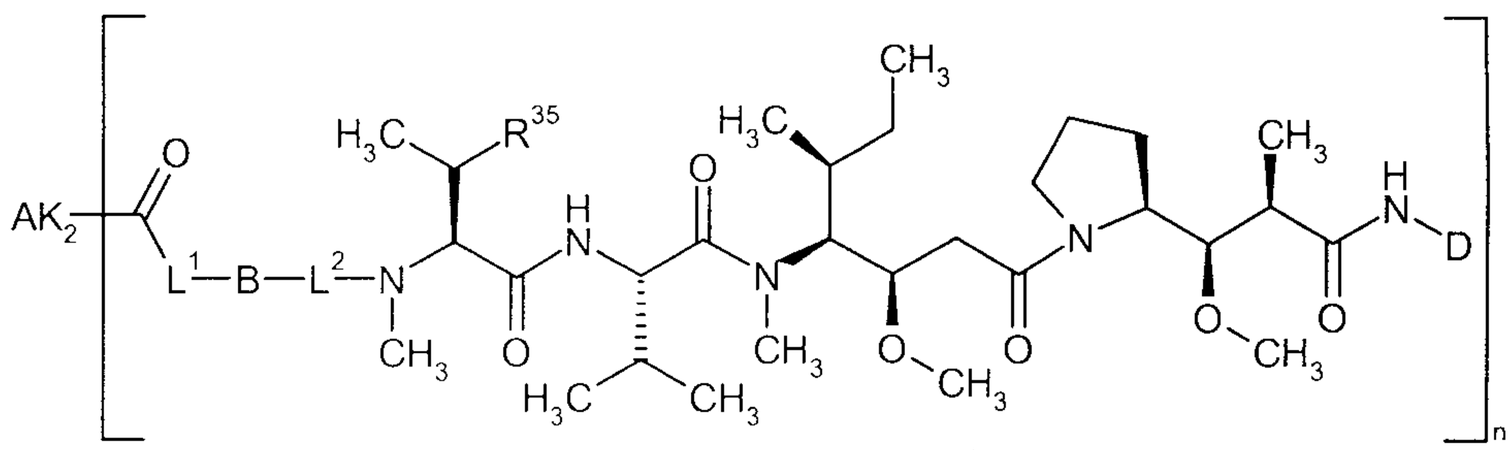
[B] reacting it with a compound of formula (IIIa)



(IIIa),

in which D, L^1, B, L^2 and R^{35} each have the meanings given above,

to form a compound of formula (Ia-B)

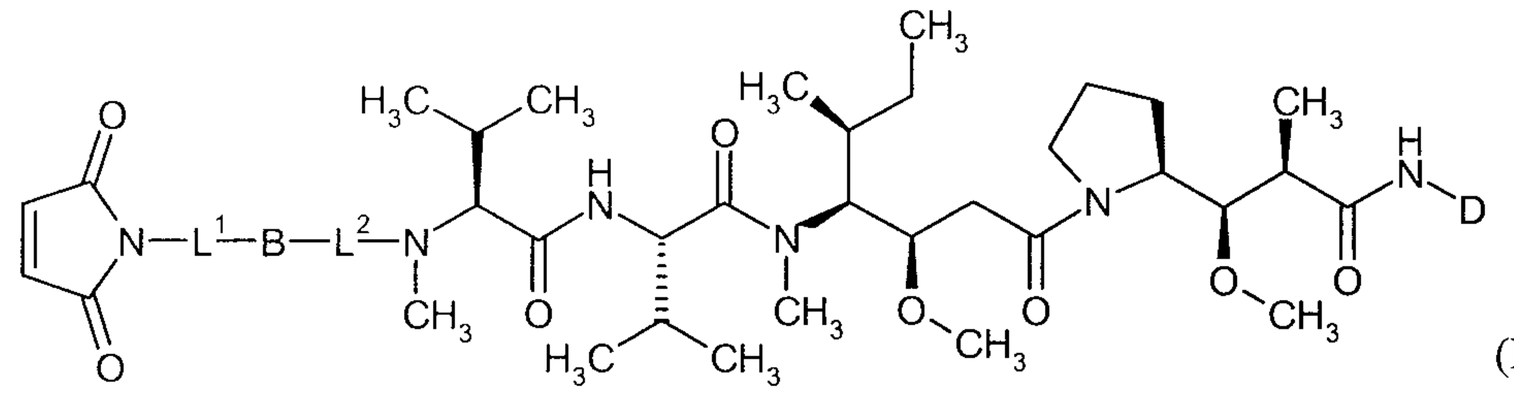


(Ia-B),

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

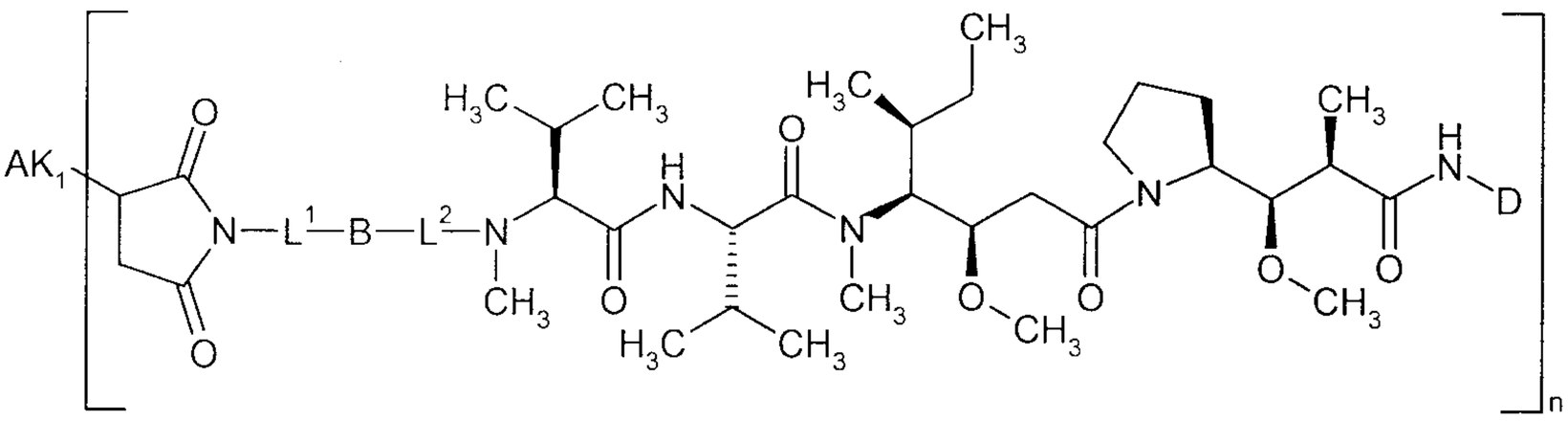
[A] is mixed with a suitable reducing agent such as, for example, dithiothreitol or *tris*-(2-carboxyethyl)phosphine hydrochloride and then is reacted with a compound of formula (II)



(II),

in which D, L^1, B and L^2 each have the meanings given above,

to form a compound of formula (I-A)

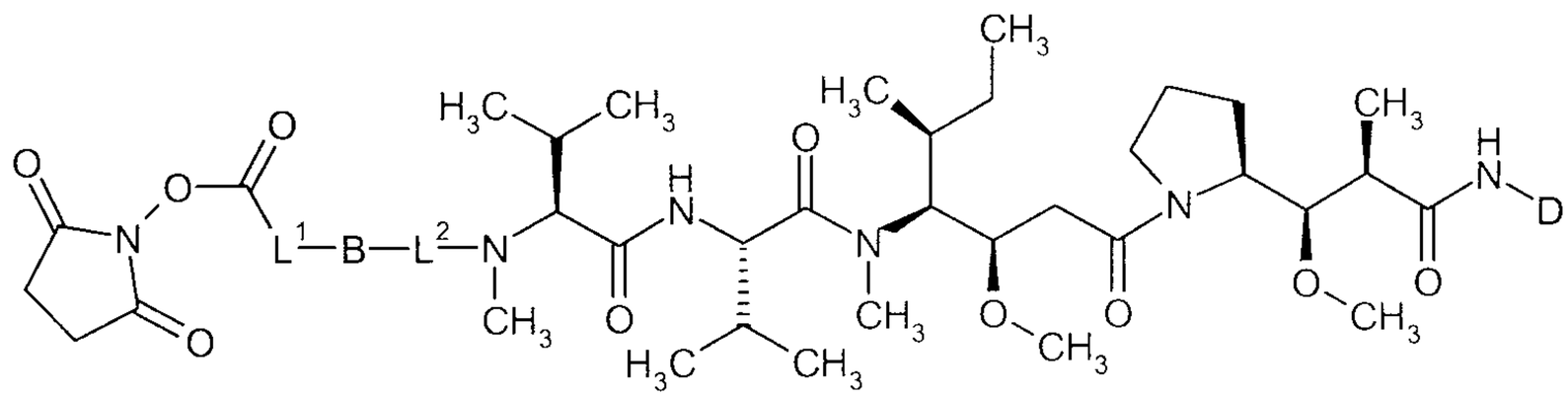


(I-A),

in which n, AK_1, D, L^1, B and L^2 each have the meanings given above,

or

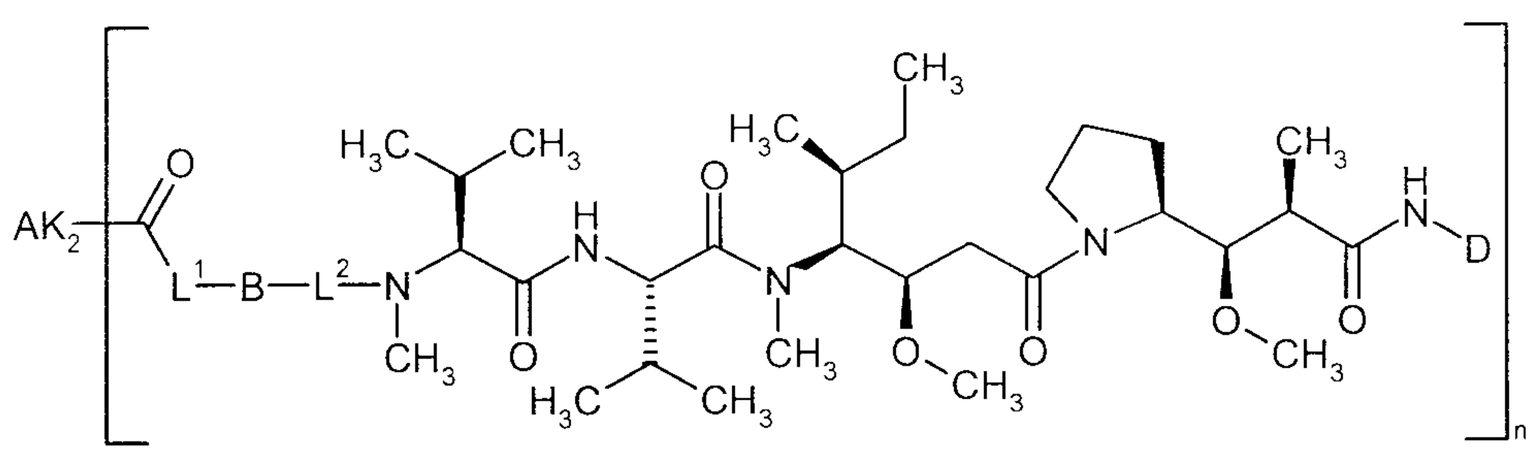
[B] reacting it with a compound of formula (III)



(III),

in which D, L^1, B and L^2 each have the meanings given above,

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 above.

Cysteine coupling:

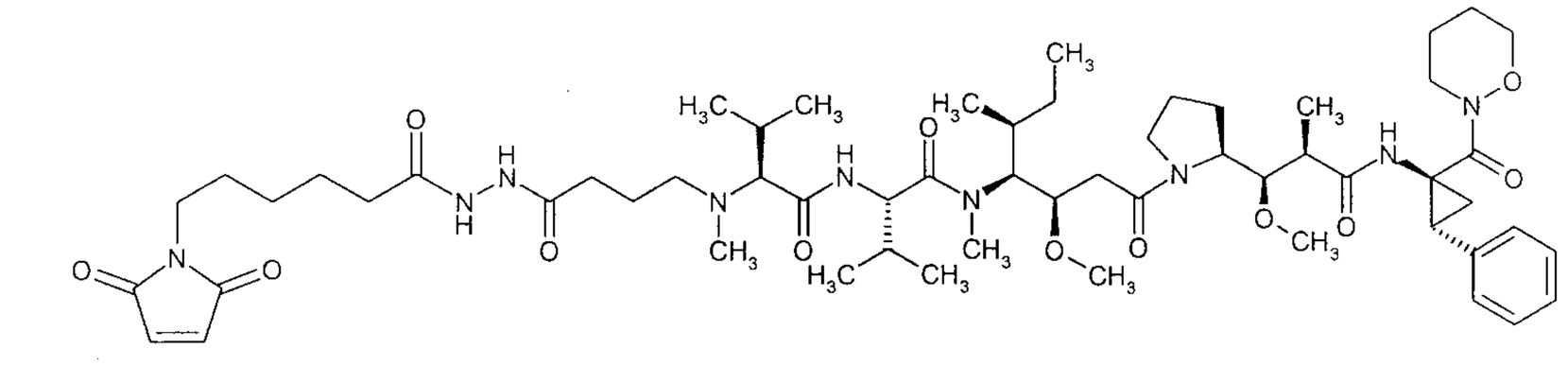
Partial reduction of the antibody and subsequent conjugation of the (partially) reduced antibody with a compound of formula (II) and/or (IIa) takes place according to methods with which those skilled in the art are familiar; see, for example, Ducry et. al., *Bioconj. Chem.* 2010, **21**, 5 and references 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.

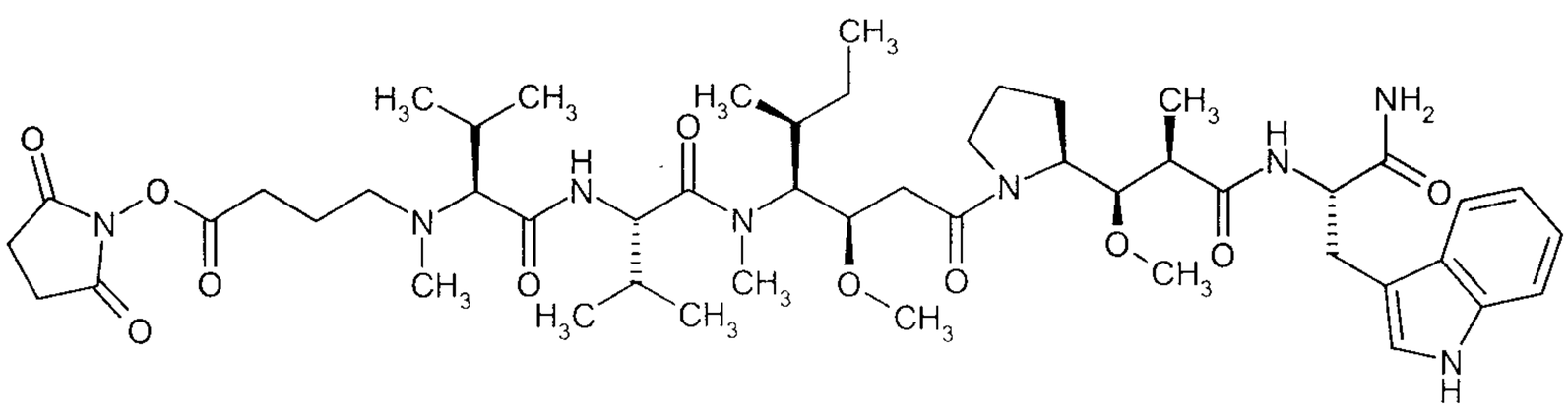
The synthesis processes described above are then illustrated as an example on the basis of the following schemes (schemes 1 and 2):

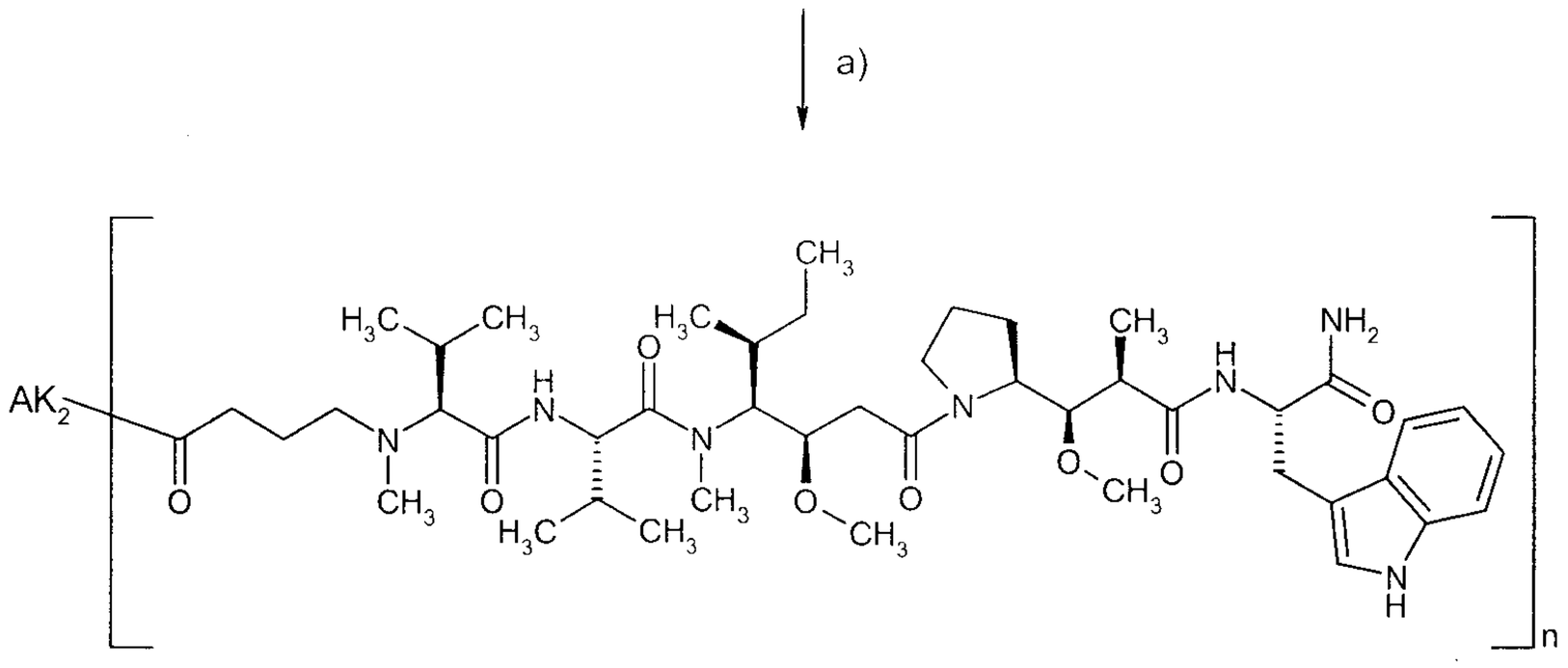
Scheme 1



[a): 1. AK, TCEP, PBS buffer, RT; 2. Addition of maleimide derivative in DMSO, RT].

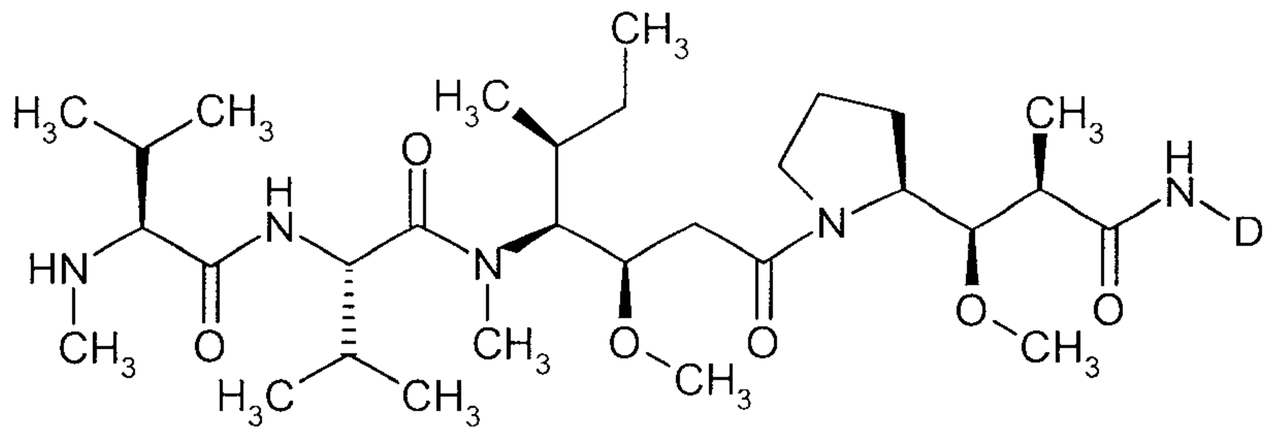
Scheme 2





[a): AK, PBS buffer, RT mixed with activated carboxyl derivative of the linker-drug components].

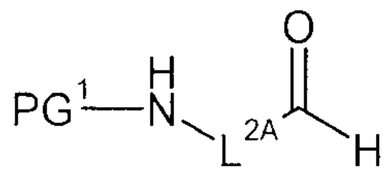
The compounds of formula (II) in which L^1 and B stand for a bond can be synthesized by reductive amination of a compound of formula (IV)



(IV),

in which D has the meaning given above

in an inert solvent with a compound of formula (V)



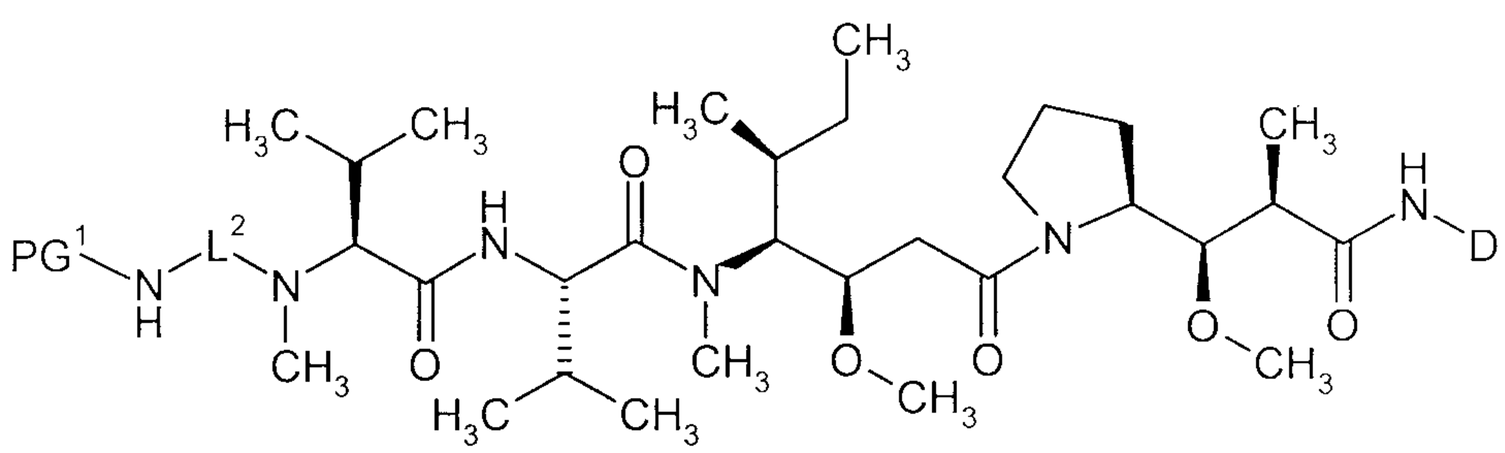
(V),

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,

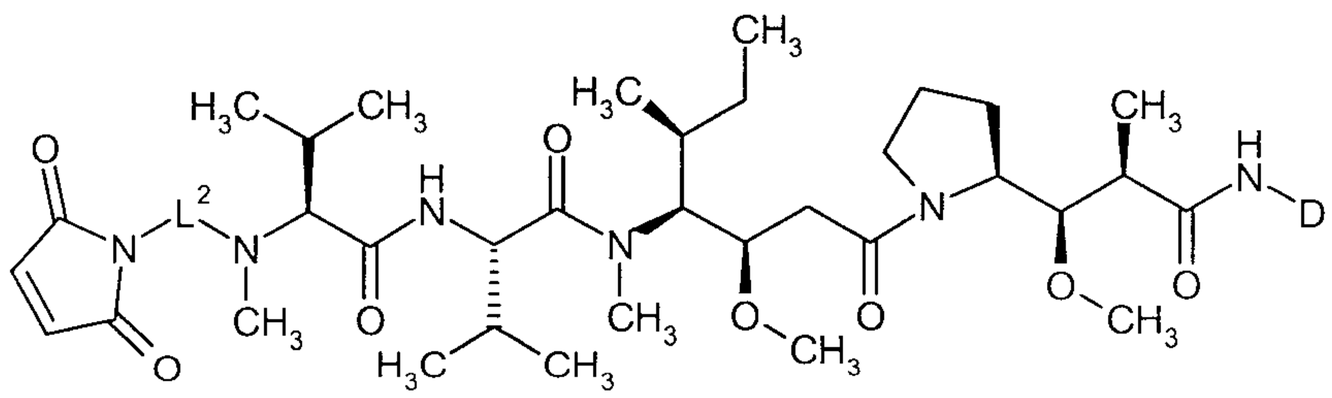
to form a compound of formula (VI)



(VI),

in which D, L^2 and PG^1 have the meanings given above,

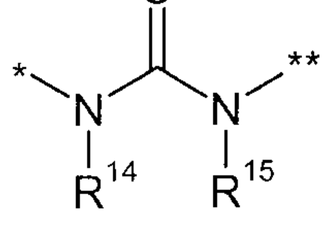
splitting off the protective group PG^1 from this compound by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable base with methyl-2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate to form a compound of formula (II-A)



(II-A),

in which D and L^2 each have the meanings given above.

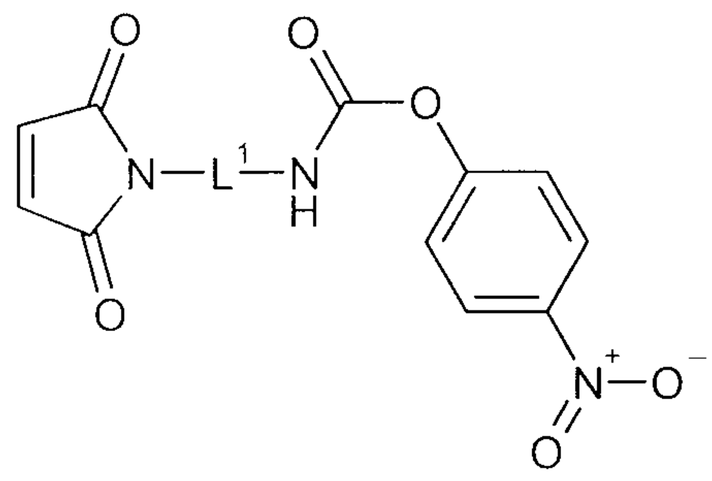
The compounds of formula (II) in which B stands for a group of the formula (B^1)



(B^1),

in which *, **, R^{14} and R^{15} each have the meanings given above,

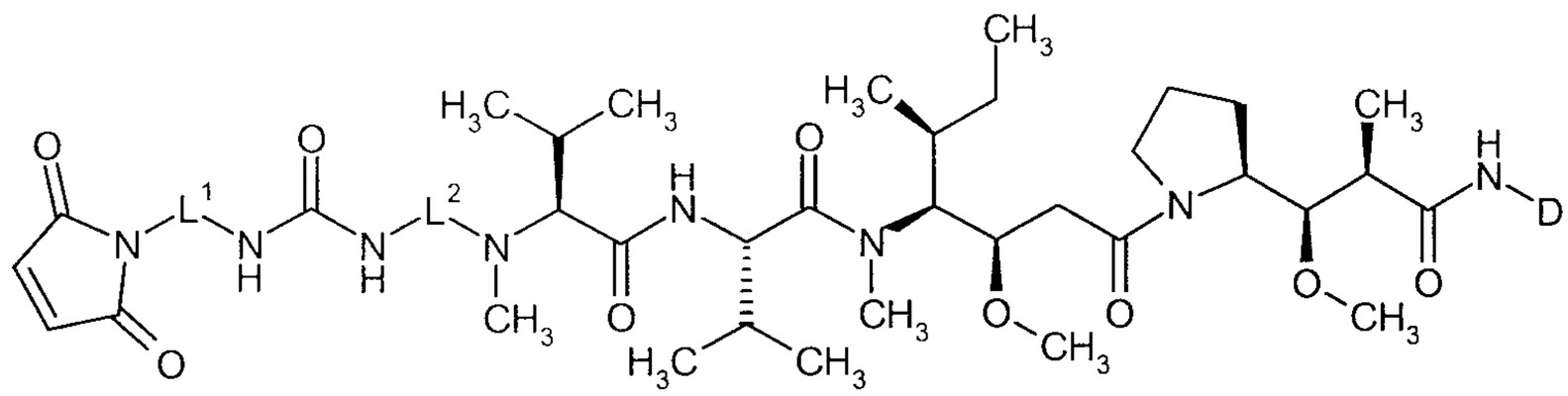
can be synthesized by splitting off the protective group PG^1 from a compound of formula (VI) by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable base with a compound of formula (VII)



(VII),

in which L^1 has the meaning given above

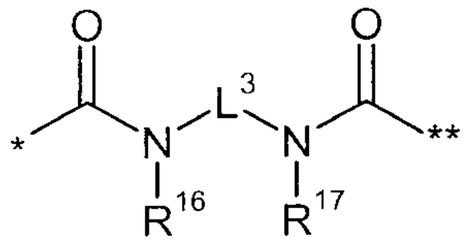
to form a compound of formula (II-B)



(II-B),

in which D, L^1 and L^2 each have the meanings given above.

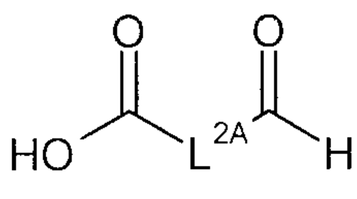
The compounds of formula (II) in which B stands for a group of the formula (B^2)



(B^2),

in which *, **, L^3, R^{16} and R^{17} 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)

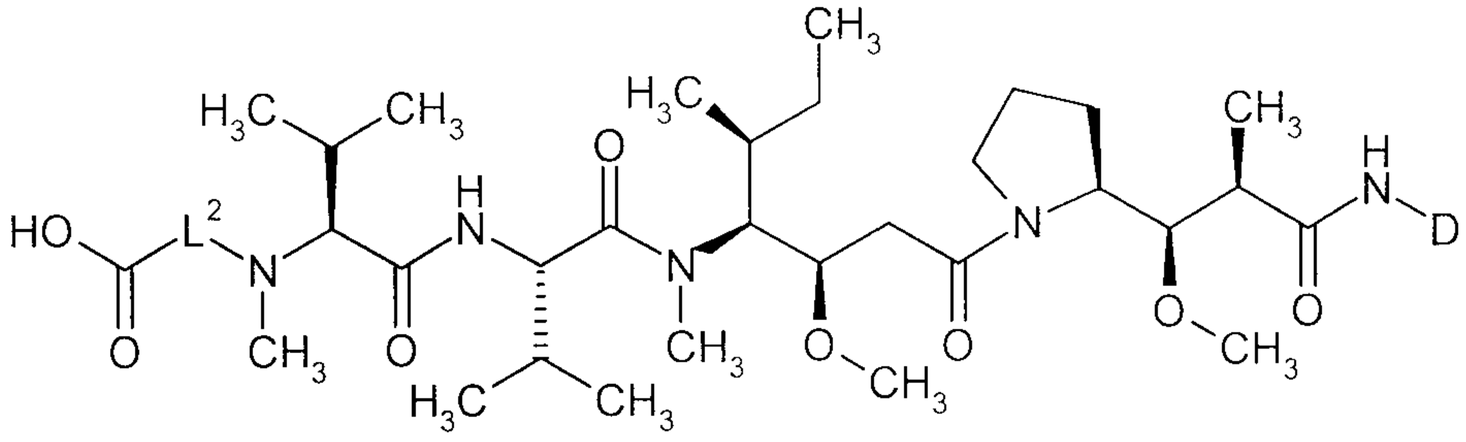


(VIII),

in which

L^{2A} has the meaning given above for L^2 but the alkyl chain length has been shortened by one carbon atom,

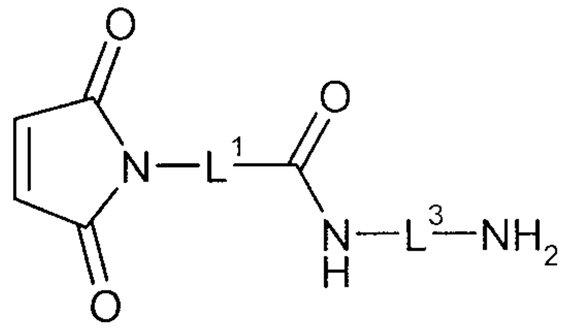
to form a compound of formula (IX)



(IX),

in which D and L^2 have the meanings given above

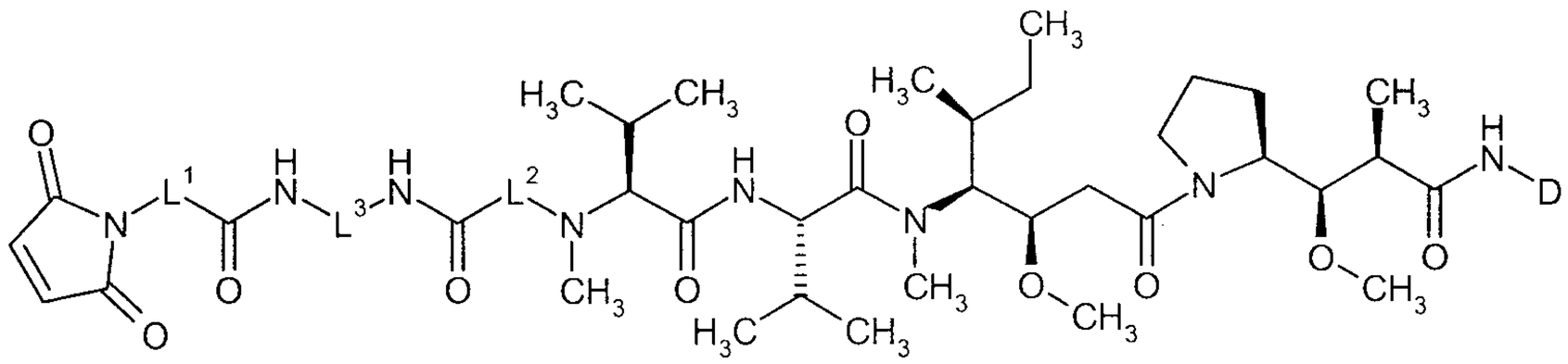
and this compound is reacted in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of formula (X)



(X),

in which L^1 and L^3 each have the meanings given above,

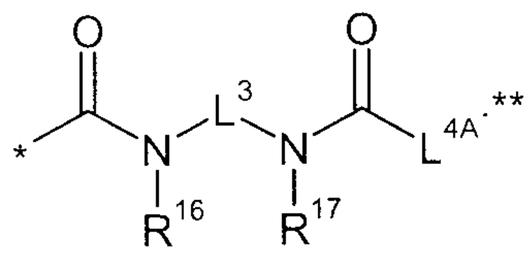
to form a compound of formula (II-C)



(II-C),

in which D, L^1, L^2 and L^3 each have the meanings given above.

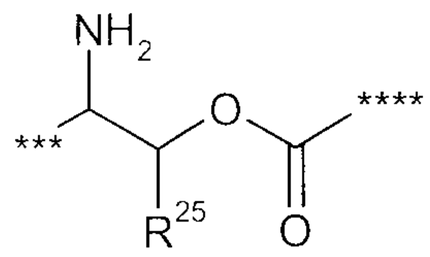
A compound of formula (II), in which B stands for a group of the formula (B^3)



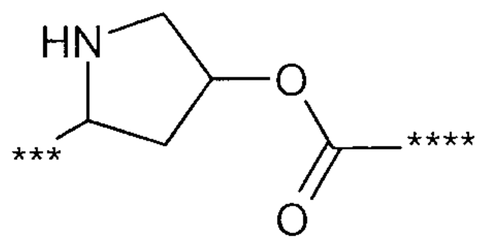
(B^3),

in which *, **, L^3, R^{16} and R^{17} each have the meanings given above and

L^{4A} stands for a group of the formula



or



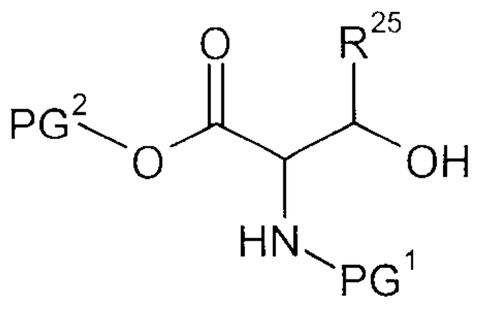
wherein

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

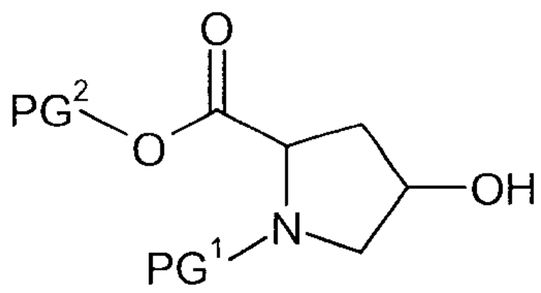
**** denotes the linkage site to L^2,

R^{25} stands for hydrogen or methyl,

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)



(XI-A) or

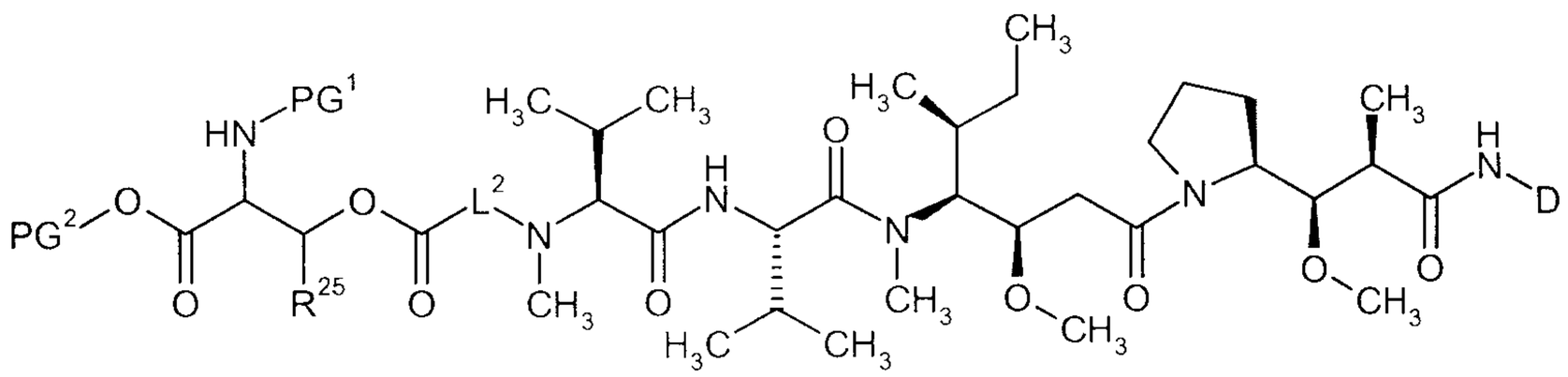


(XI-B),

in which R^{25} and PG^1 each have the meanings given above and

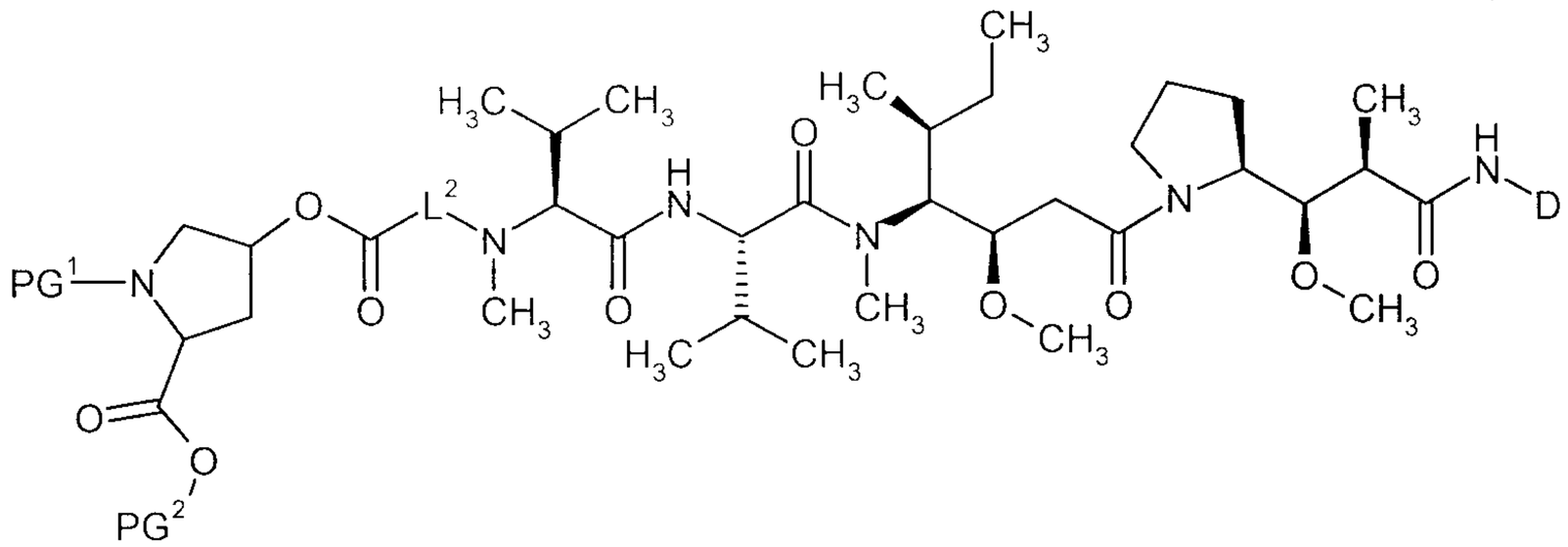
PG^2 stands for a suitable carboxyl protective group, in particular benzyl,

to form a compound (XII-A) and/or (XII-B)



(XII-A)

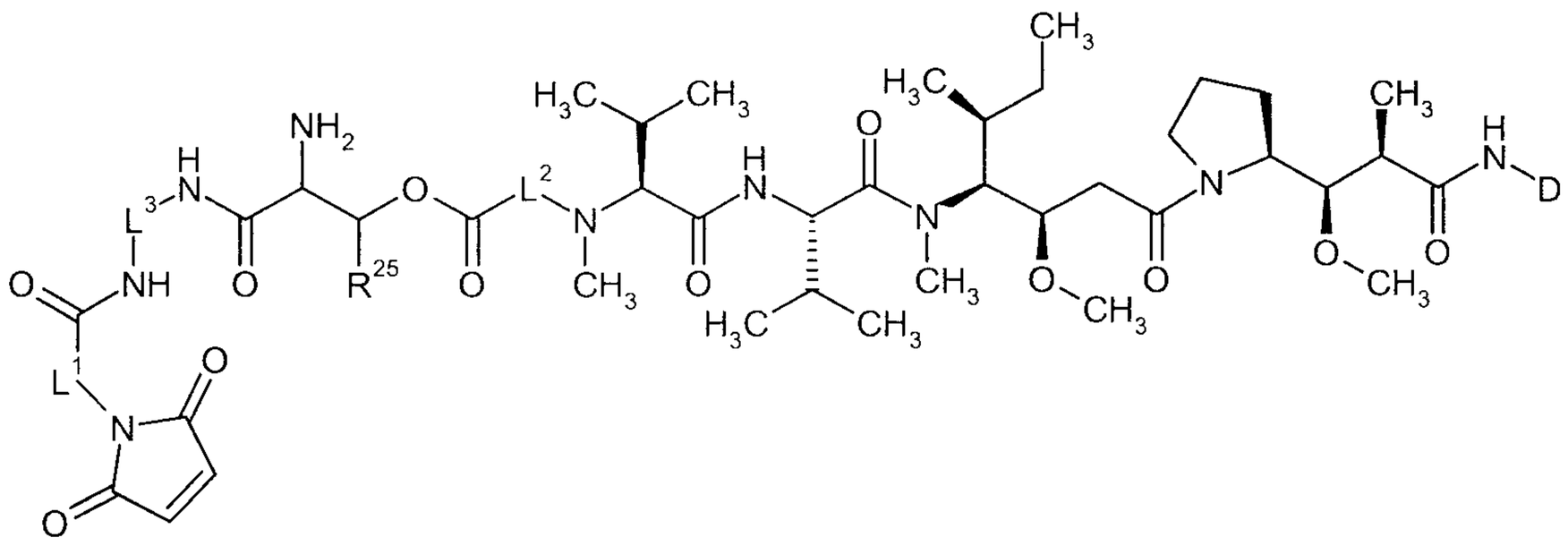
or



(XII-B),

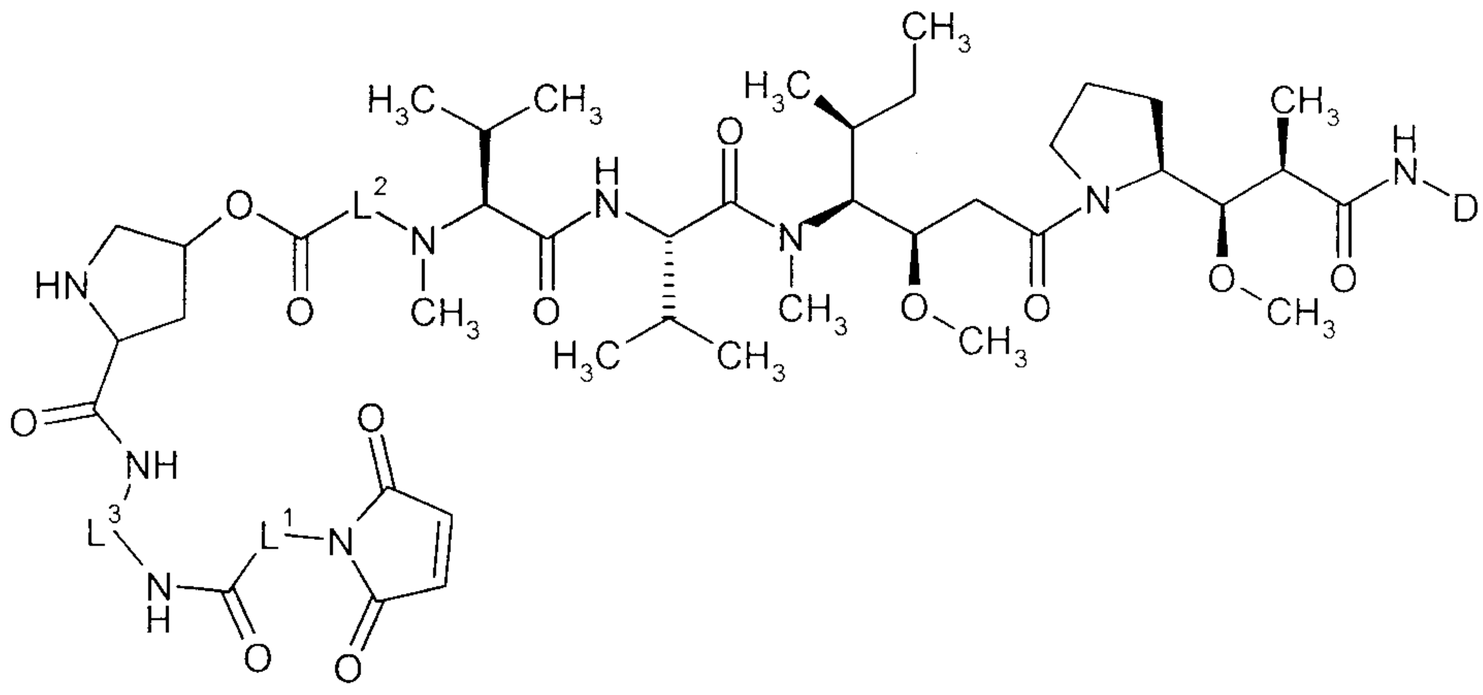
in which D, PG^1, PG^2 and L^2 have the meanings given above,

then splitting off the protective group PG^2 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 off the protective group PG^1 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)

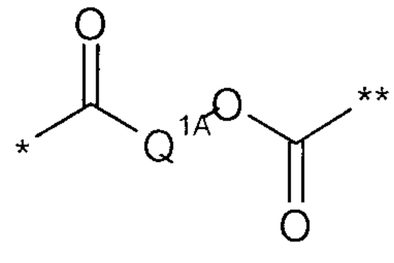
or



(II-D-B),

in which D, L^1, L^2 and L^3 have the meanings given above.

A compound of formula (II) in which B stands for a group of the formula (B^4)

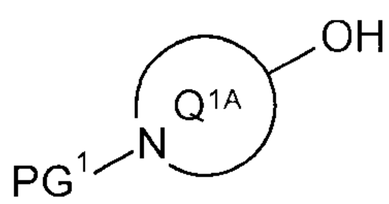


(B^4),

in which *, ** each have the meanings given above and

Q^{1A} stands for an N-linked four- to seven-membered heterocycle,

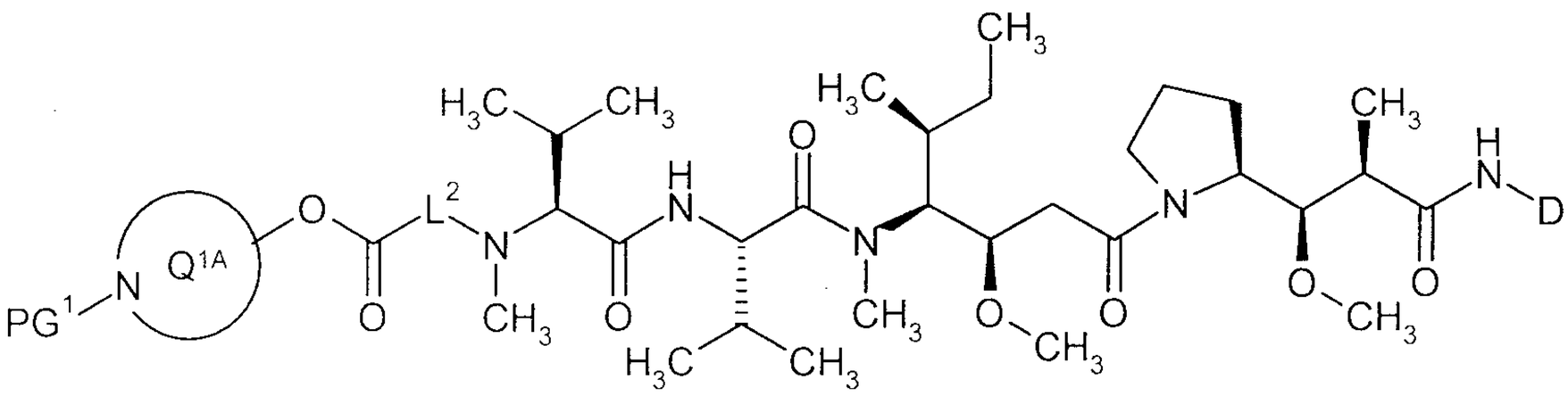
can be synthesized by reacting a compound of formula (IX) in an inert solvent in the presence of a suitable base and a suitable coupling reagent with a compound of formula (XXI)



(XXI),

in which PG^1 and Q^{1A} each have the meanings given above,

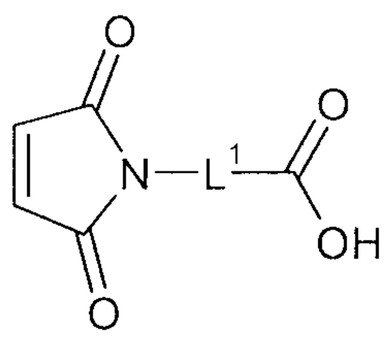
to form a compound of formula (XXII)



(XXII),

in which PG^1, Q^{1A}, D and L^2 have the meanings given above

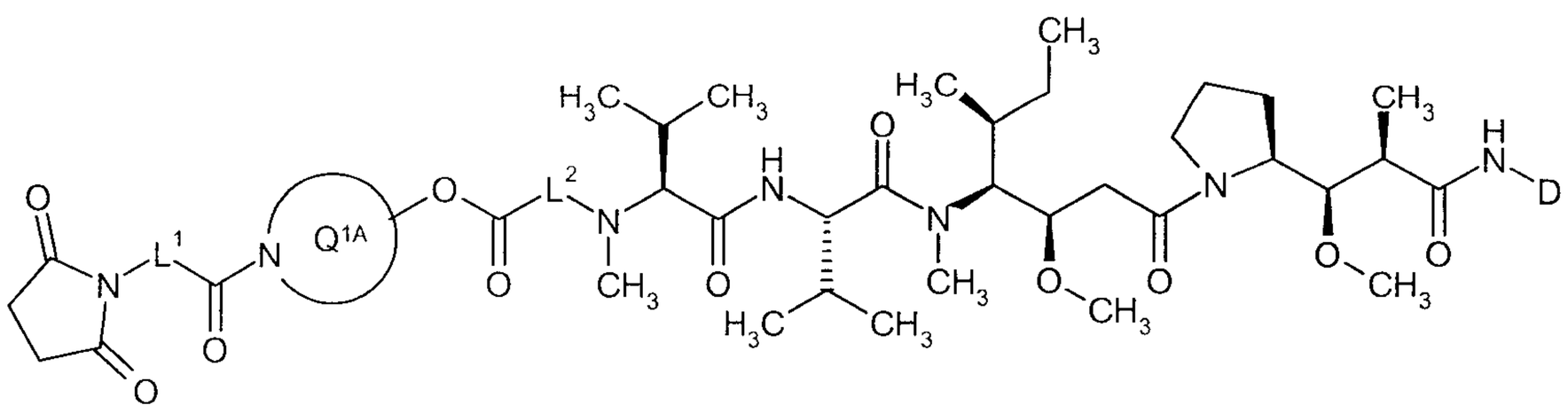
then splitting off the protective group PG^1 from this compound by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of formula (XXIII)



(XXIII),

in which L^1 has the meaning given above

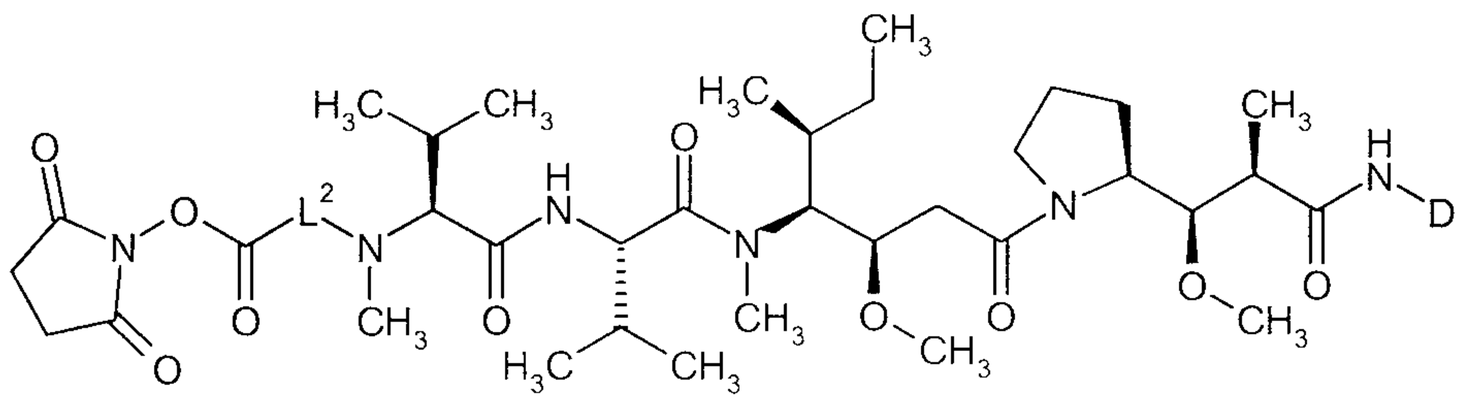
to form a compound of formula (II-D)



(II-D),

in which Q^{1A}, D, L^1 and L^2 have the meanings given above.

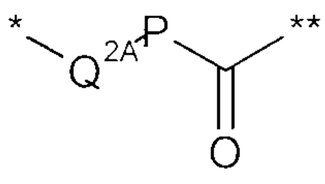
The compounds of formula (III), in which L^1 and B stand for a bond can be synthesized by reacting a compound of formula (IX) with N-hydroxysuccinimide in an inert solvent in the presence of a suitable coupling regent and a suitable base to form a compound of formula (III-A):



(III-A),

in which D and L^2 each have the meanings given above.

The compounds of formula (III), in which L^1 stands for a bond and B stands for a group of the formula (B^{5A})

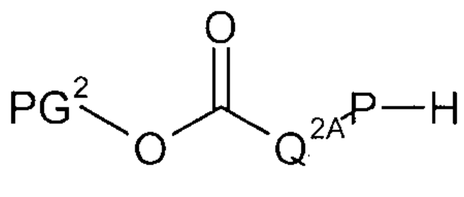


(B^{5A}),

in which *, ** and P each have the meanings given above and

Q^{2A} stands for a three- to seven-membered carbocycle,

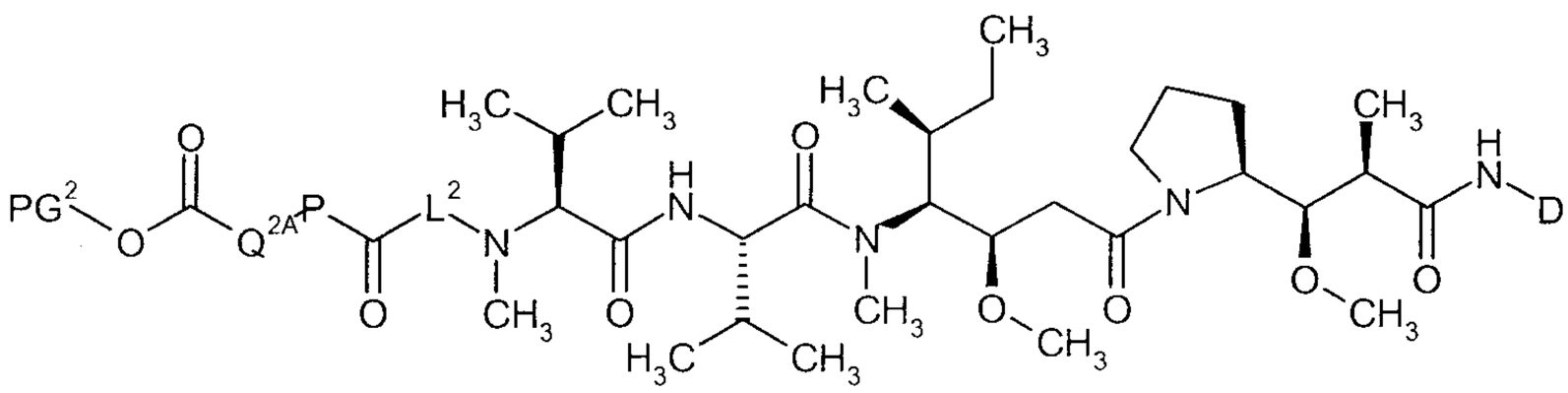
can be synthesized by reacting a compound of formula (IX) in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of formula (XIII)



(XIII),

in which P, Q^{2A} and PG^2 each have the meanings given above,

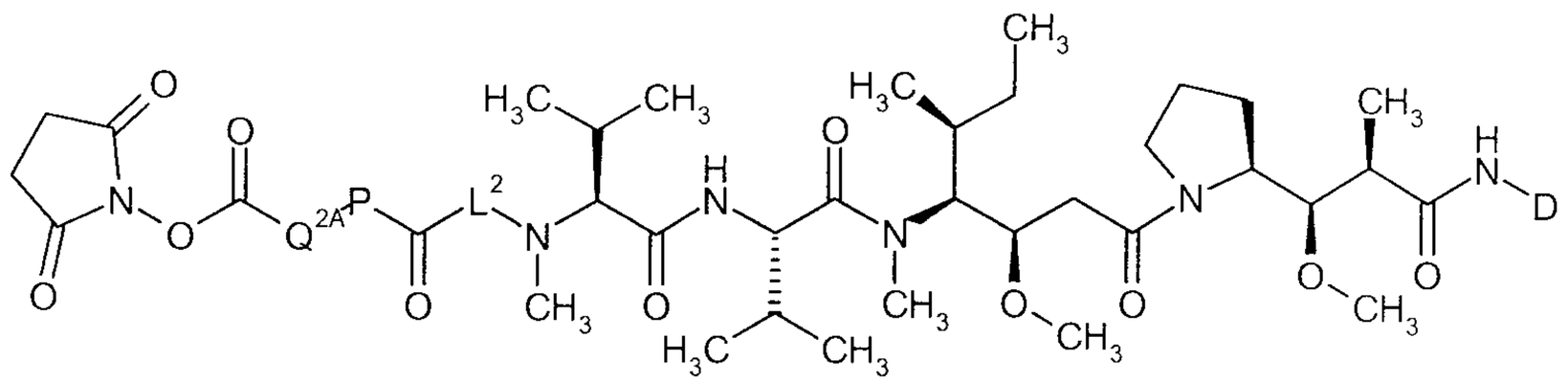
to form a compound of formula (XIV)



(XIV),

in which D, P, Q^{2A}, L^2 and PG^2 each have the meanings given above,

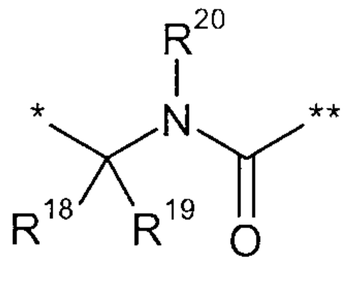
splitting off the protective group PG^2 from this compound by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable base with N-hydroxysuccinimide to form a compound of formula (III-B)



(III-B),

in which D, P, Q^{2A} and L^2 each have the meanings given above.

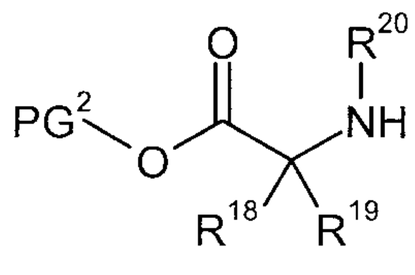
The compounds of formula (III), in which L^1 stands for a bond and B stands for a group of the formula (B^6)



(B^6),

in which *, **, R^{18}, R^{19} and R^{20} each have the meanings given above,

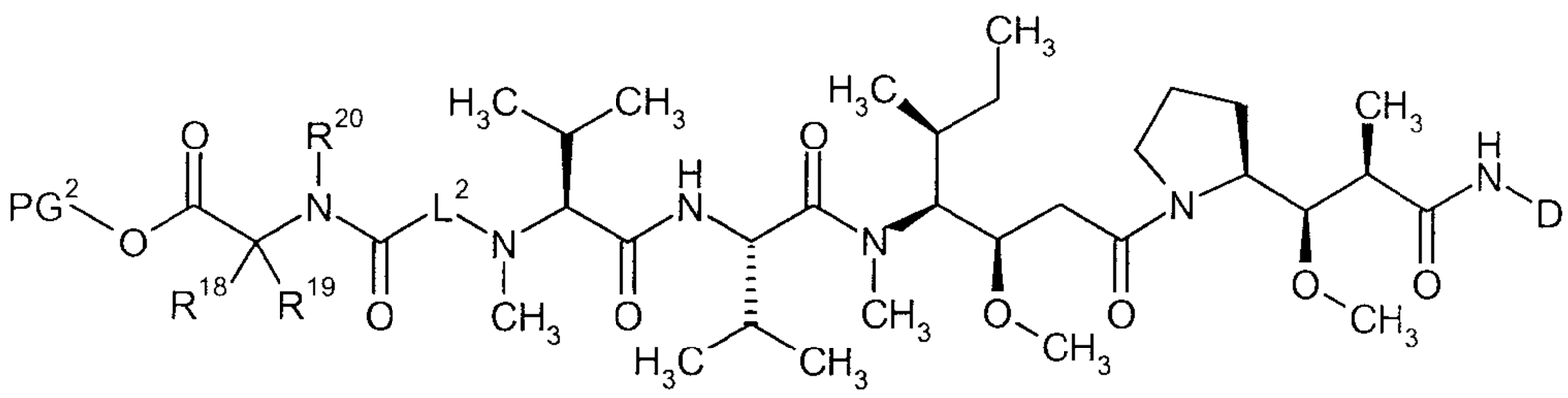
can be synthesized by reacting a compound of formula (IX) in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of formula (XV)



(XV),

in which R^{18}, R^{19}, R^{20} and PG^2 each have the meanings given above,

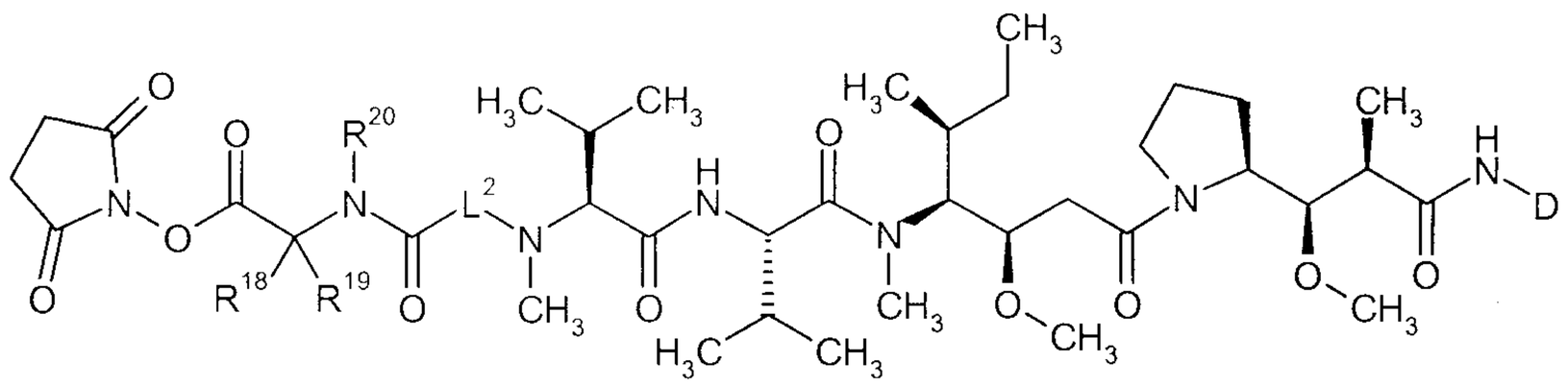
to form a compound of formula (XVI)



(XVI),

in which D, R^{18}, R^{19}, R^{20}, L^2 and PG^2 each have the meanings given above,

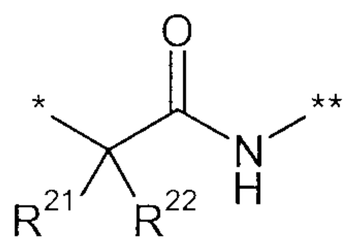
then splitting off the protective group PG^2 from this compound by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with N-hydroxysuccinimide to form a compound of formula (III-C)



(III-C),

in which D, R^{18}, R^{19}, R^{20} and L^2 each have the meanings given above.

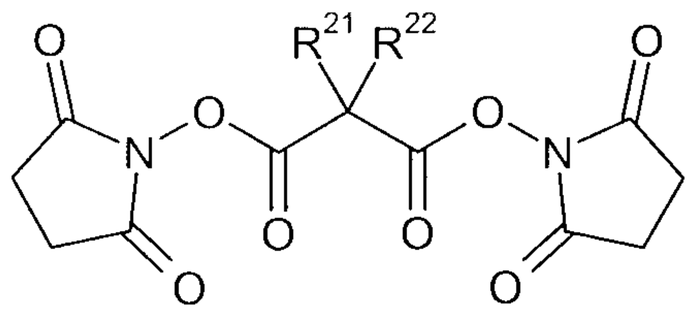
The compounds of formula (III), in which L^1 stands for a bond and B stands for a group of formula (B^7)



(B^7),

in which *, **, R^{21} and R^{22} each have the meanings given above,

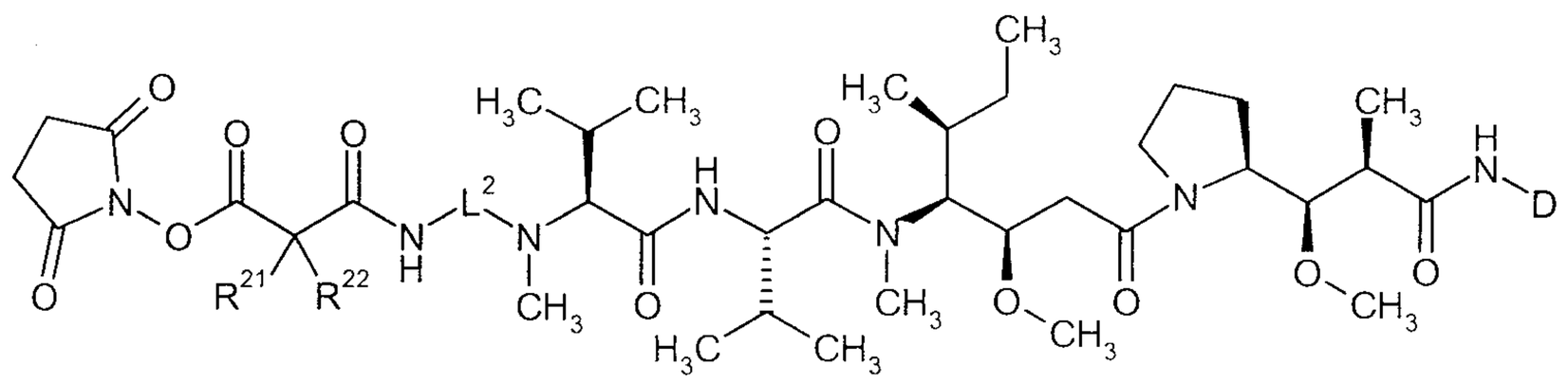
may be synthesized by splitting off the protective group PG^1 from a compound of formula (VI) by methods with which those skilled in the art are familiar, and then reacting the resulting deprotected compound in an inert solvent in the presence of a suitable base with a compound of formula (XVII)



(XVII),

in which R^{21} and R^{22} each have the meanings given above,

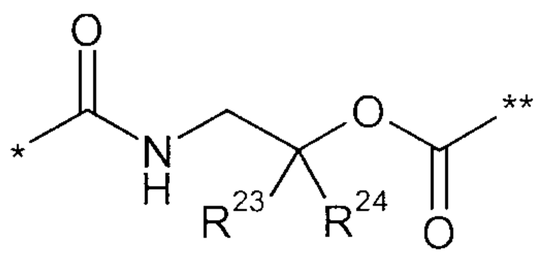
to form a compound of formula (III-D)



(III-D),

in which D, R^{21}, R^{22} and L^2 each have the meanings given above.

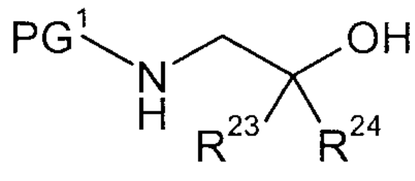
The compounds of formula (III) in which B stands for a group of the formula (B^8)



(B^8),

in which *, **, R^{23} and R^{24} each have the meanings given above,

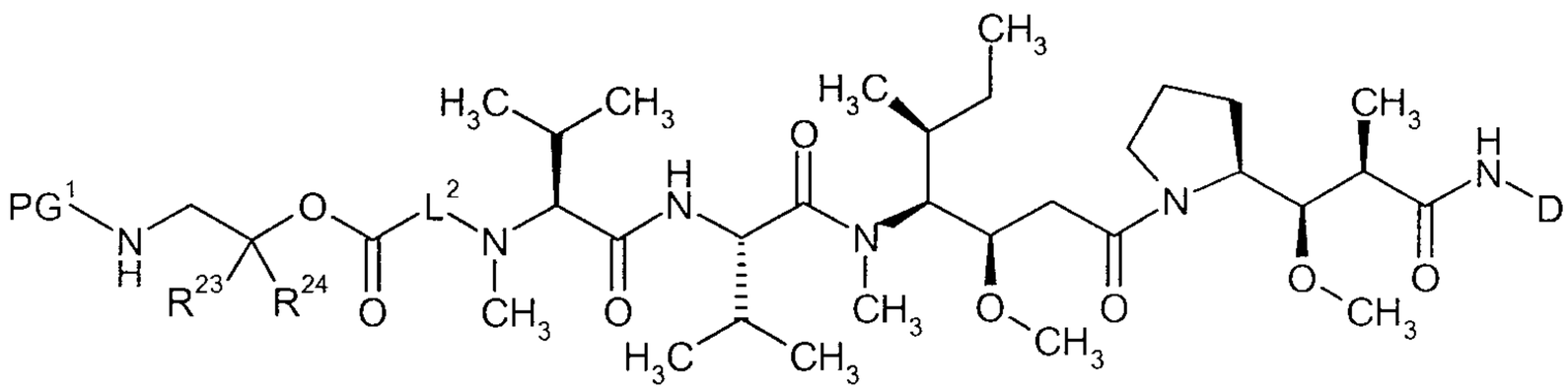
can by synthesized by reacting a compound of formula (IX) in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of formula (XVIII)



(XVIII),

in which R^{23}, R^{24} and PG^1 each have the meanings given above

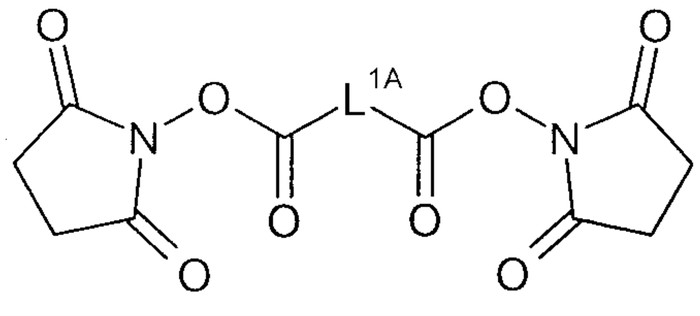
to form a compound of formula (XIX)



(XIX),

in which D, R^{23}, R^{24}, L^2 and PG^1 each have the meanings given above,

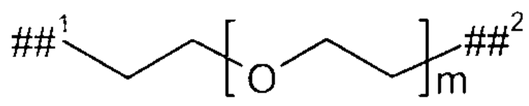
splitting off the protective group PG^1 from this compound by methods with which those skilled in the art are familiar and then reacting the deprotected compound in an inert solvent in the presence of a suitable coupling reagent and a suitable base with a compound of formula (XX)



(XX),

in which

L^{1A} stands for 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,

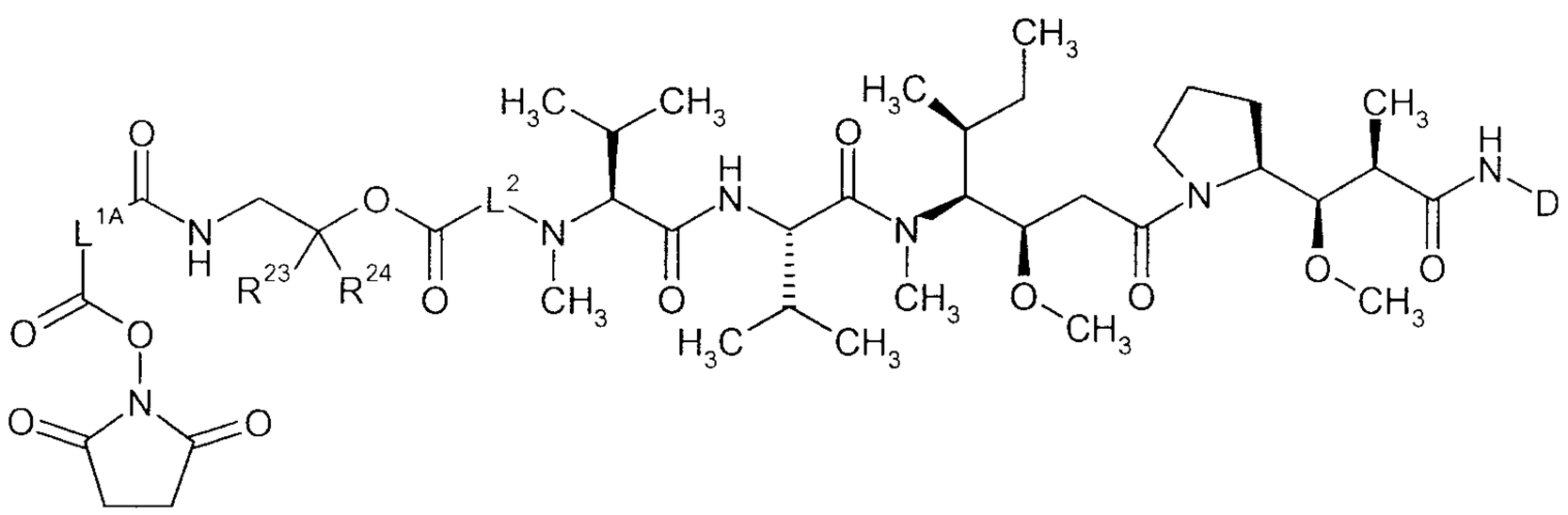
$##^2$ denotes the linkage site to the group B,

wherein (C_1-C_{10})-alkanediyl may be substituted with 1 to 4 methyl substituents,

and

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,

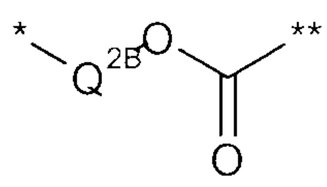
to form a compound of formula (III-E)



(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})

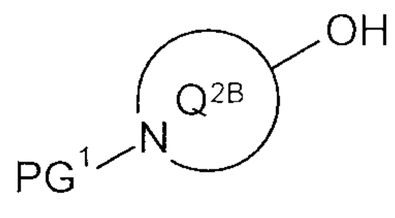


(B^{5B}),

in which * and ** each have the meanings given above and

Q^{2B} stands for a N-linked four- to seven-membered heterocycle,

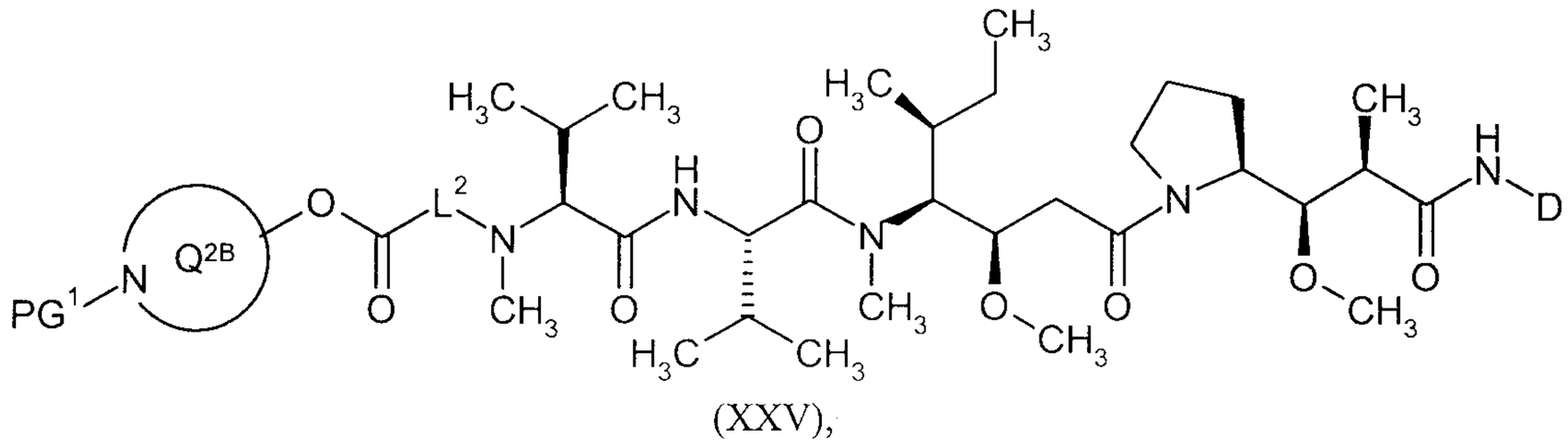
can be synthesized by reacting a compound of formula (IX) in an inert solvent in the presence of a suitable base and a suitable coupling reagent with a compound of formula (XXIV



(XXIV),

in which PG^1 and Q^{2B} each have the meanings given above,

to form a compound of formula (XXV)

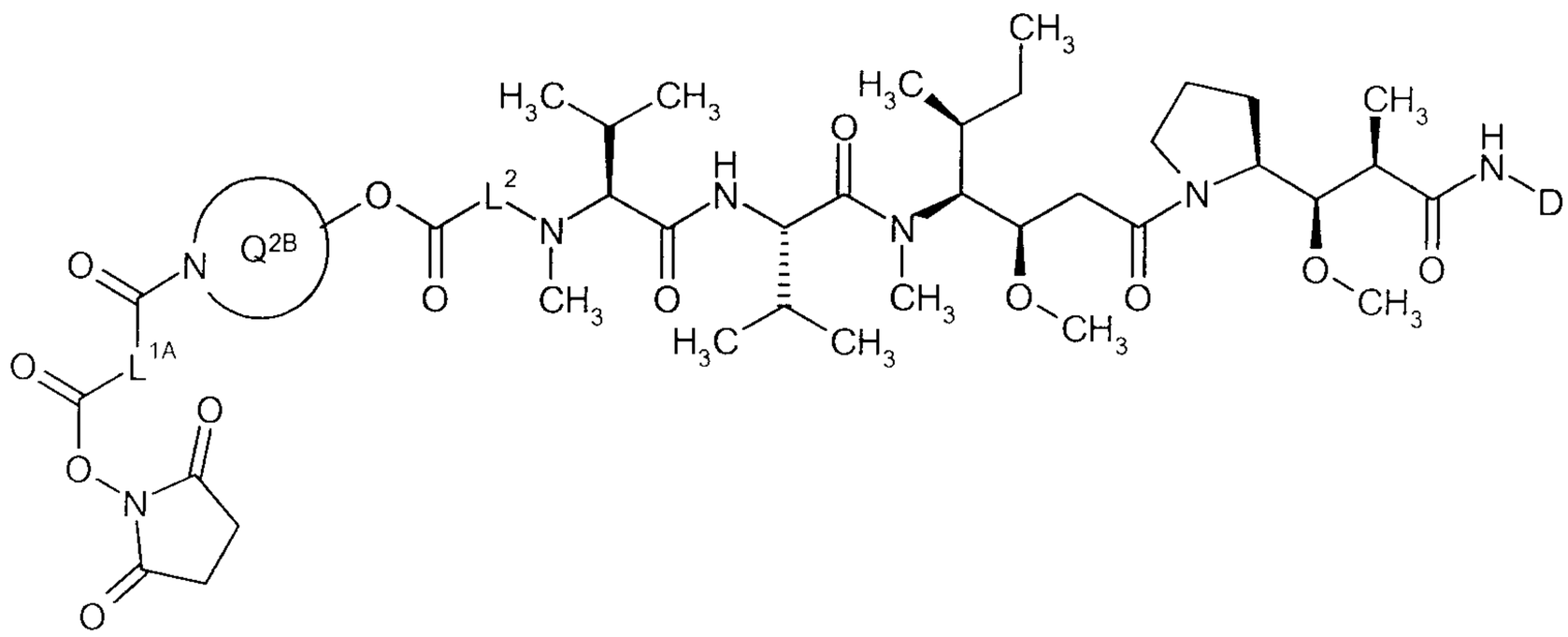


(XXV),

in which PG^1, Q^{2B}, D and L^2 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 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) → (VI) and (IV) + (VIII) → (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 triacetoxyborohydride, tetra-*n*-butylammonium borohydride or borane-pyridine complex. Sodium cyanoborohydride or borane pyridine complex is preferably used.

The reactions (IV) + (V) → (VI) and (IV) + (VIII) → (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) → (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) (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'-dicyclohexyl-carbodiimide (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 benzotriazol-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 PG^1 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 PG^2 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-dimetoxyethane 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*-dimethyl-acetamide (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) → (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) → (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) → (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) → (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) → (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) → (III-A), (XIV) → (III-B) and (XVI) → (III-C) as well as (VI) + (XVII) → (III-D), (XIX) + (XX) → (III-E) and (XXV) + (XX) → (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 to use mixtures of such solvents. *N,N*-Dimethylformamide is preferred.

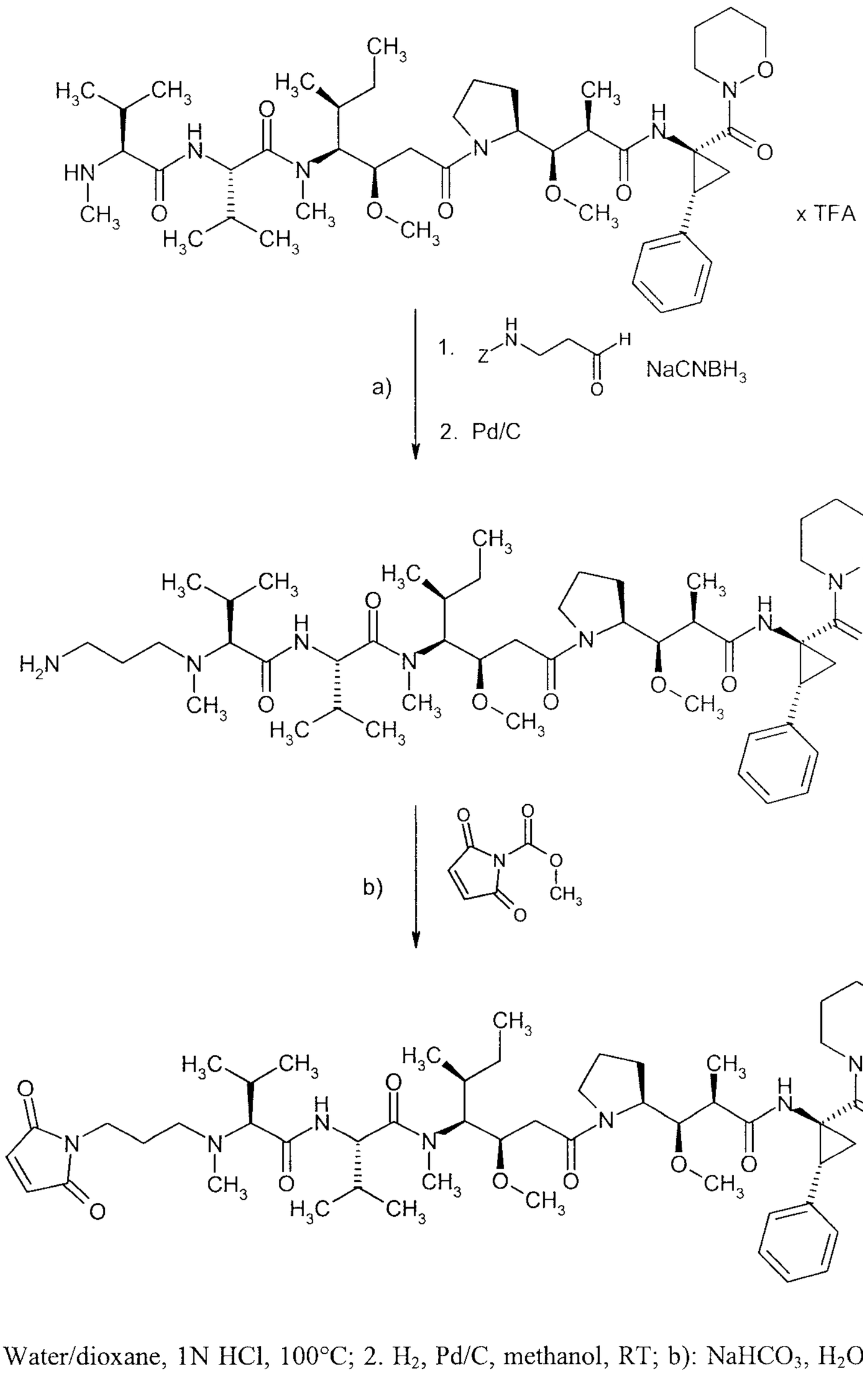
Suitable bases for these reactions include, for example, tertiary amines like triethylamine, *N*-methylmorpholine, *N*-methylpiperidine, *N,N*-diisopropylethylamine, pyridine or 4-*N,N*-dimethylaminopyridine. *N,N*-Diisopropylethylamine is preferred, optionally with the addition of 4-*N,N*-dimethylaminopyridine.

The reactions (IX) → (III-A), (XIV) → (III-B) and (XVI) → (III-C) as well as (VI) + (XVII) → (III-D) and (XIX) + (XX) → (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 formulas (IIa), (IIIa), (Ia-A) and/or (Ia-B), where R^{35} stands for methyl. Compounds (IIa) and (IIIa) are synthesized as in the synthesis of the compound of formula (II) and (III) as described above.

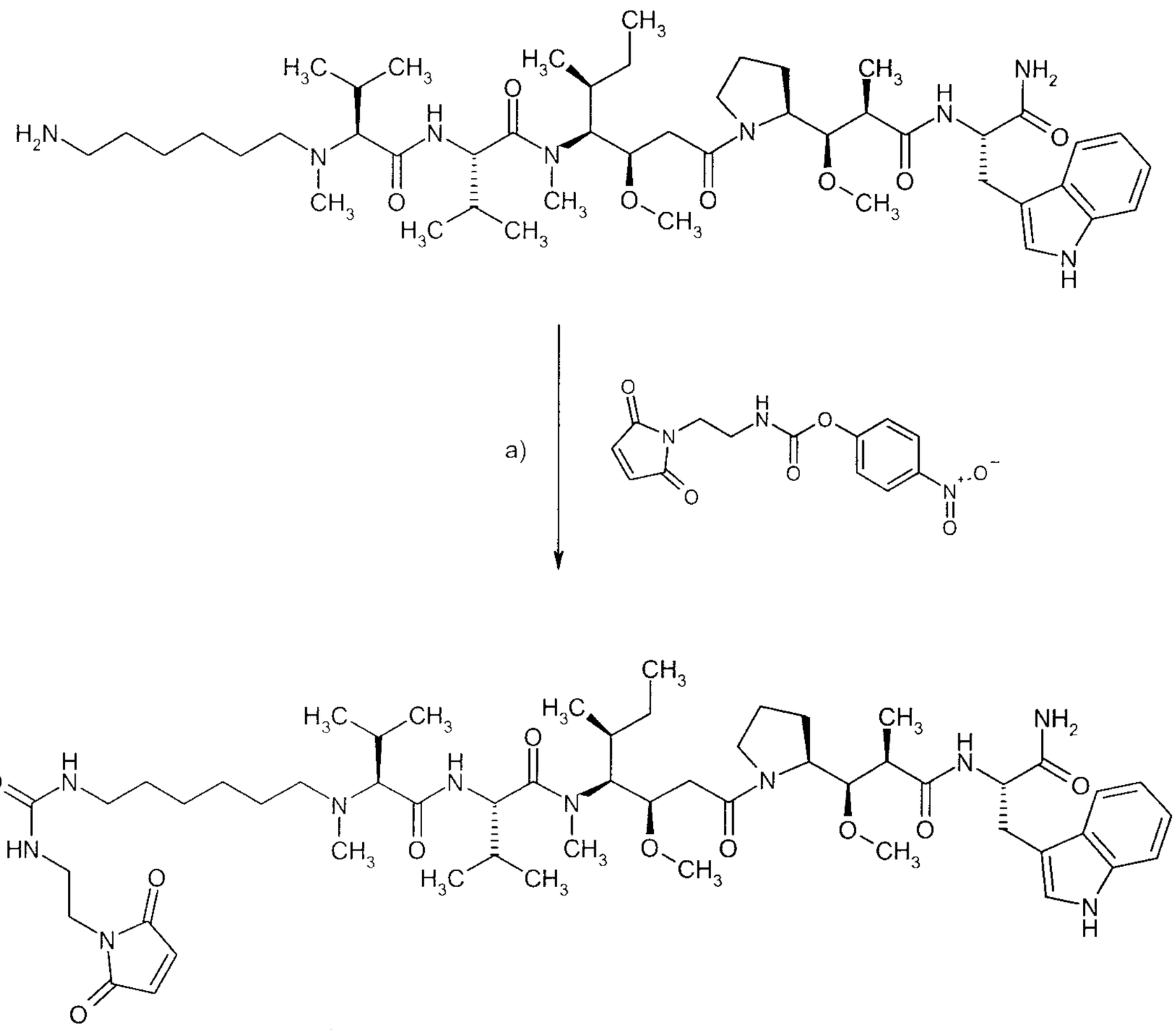
The methods described above are illustrated by the following synthesis schemes (schemes 3 through 13, 18) as examples:

Scheme 3



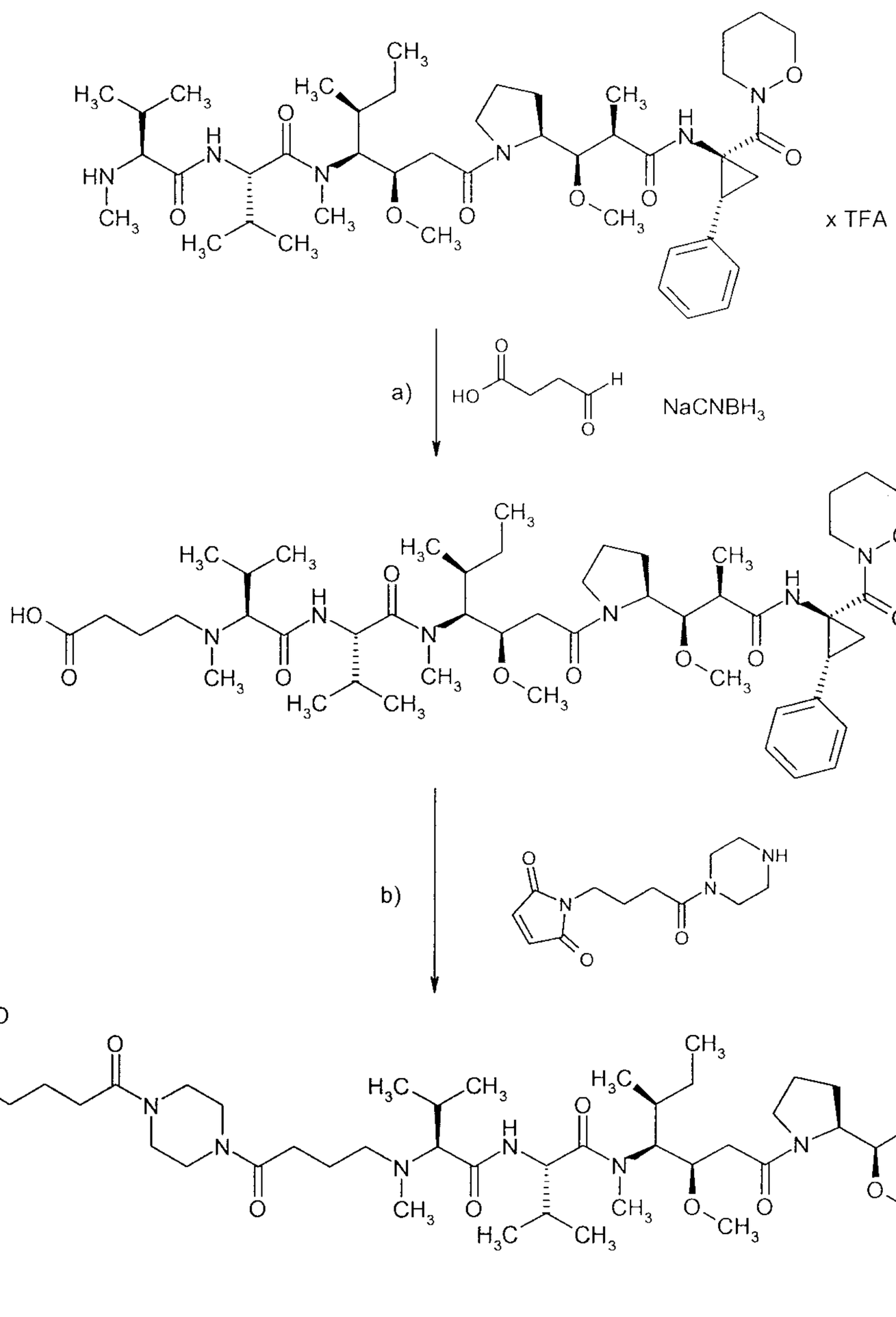
[a): 1. Water/dioxane, 1N HCl, 100°C; 2. H_2, Pd/C, methanol, RT; b): $NaHCO_3$, H_2O, dioxane, RT].

Scheme 4



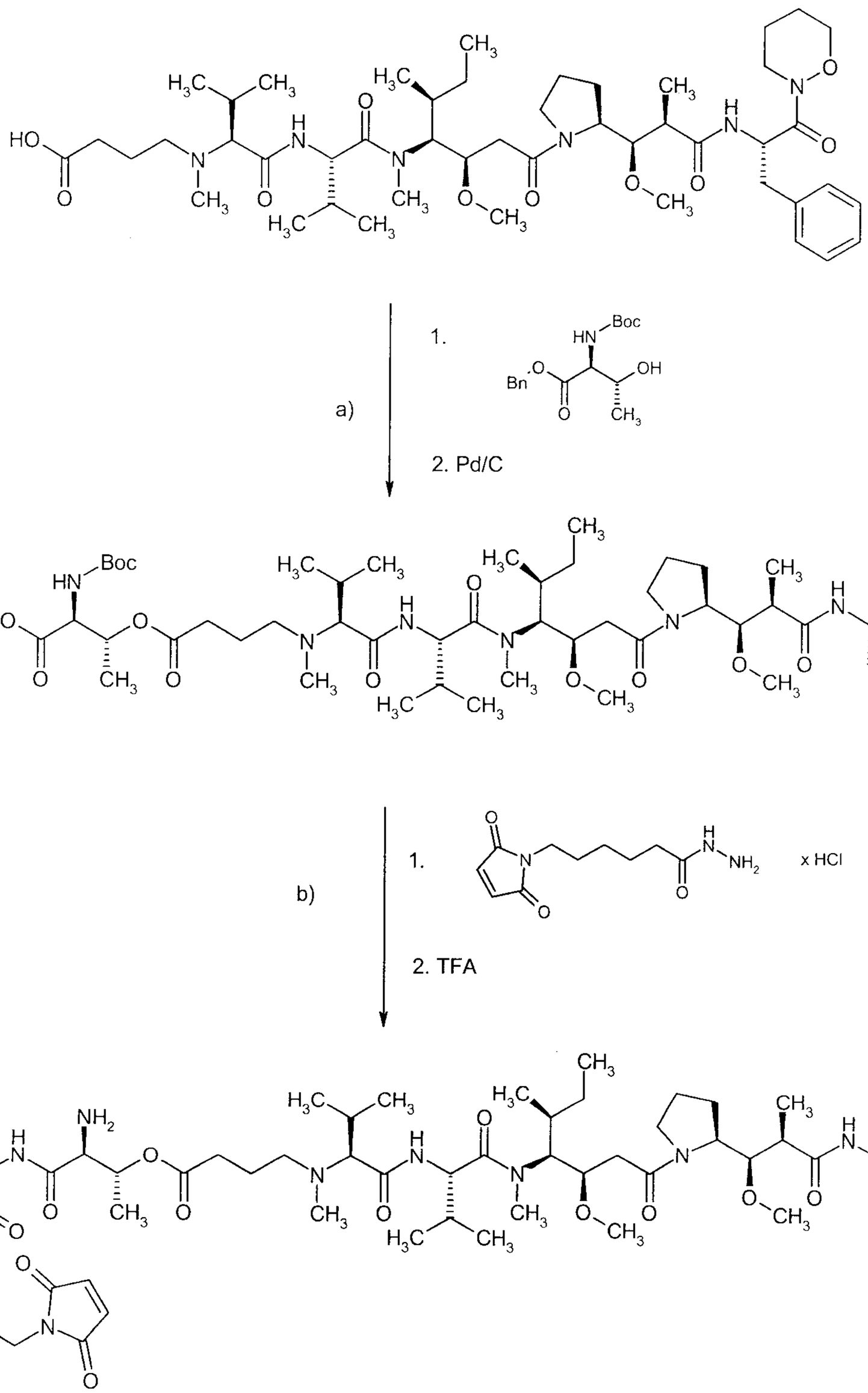
[a): Diisopropylethylamine, DMF, RT].

Scheme 5



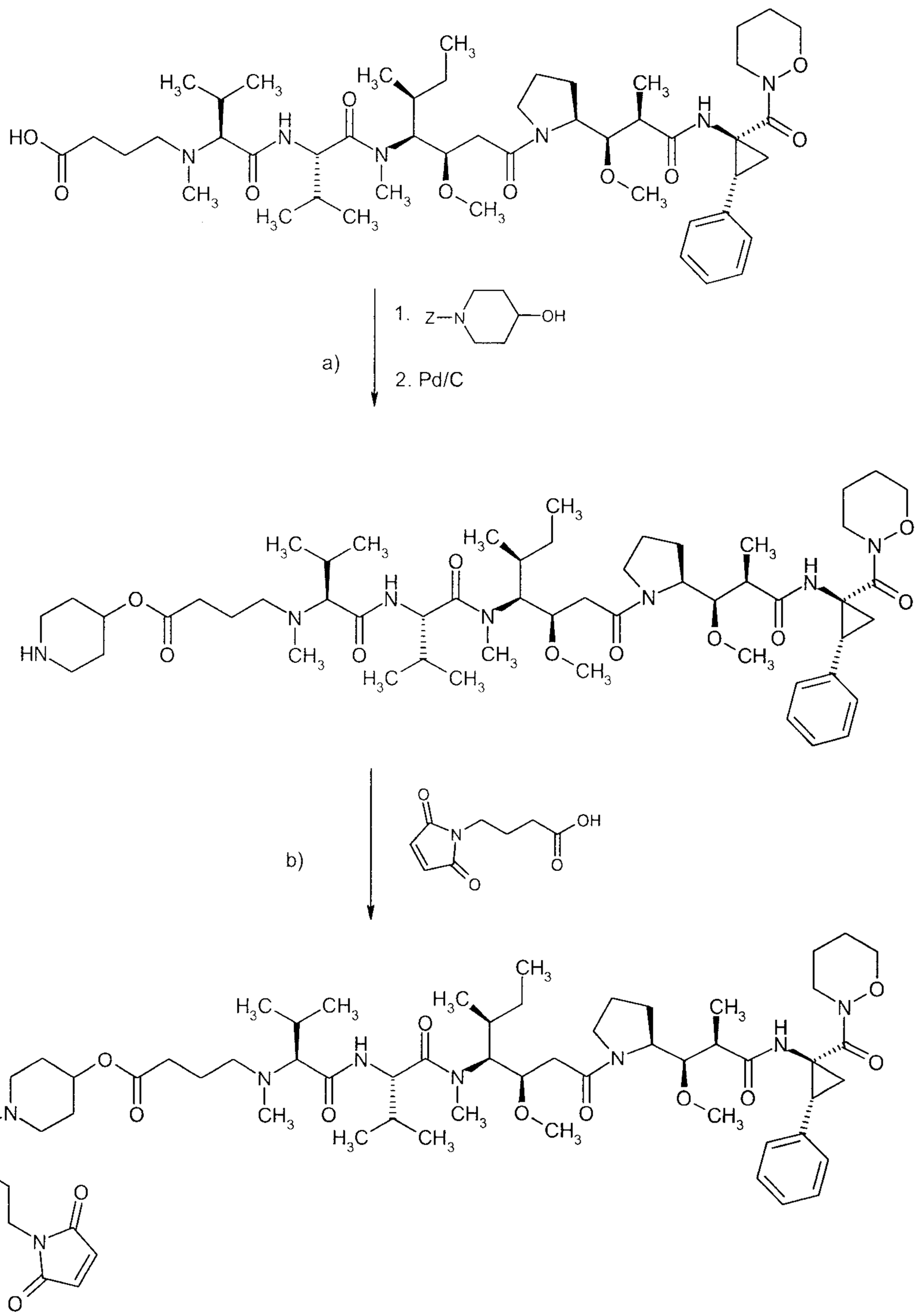
[a): Water/dioxane, 1N HCl, 100°C; b): HATU, diisopropylamine, DMF, RT].

Scheme 6



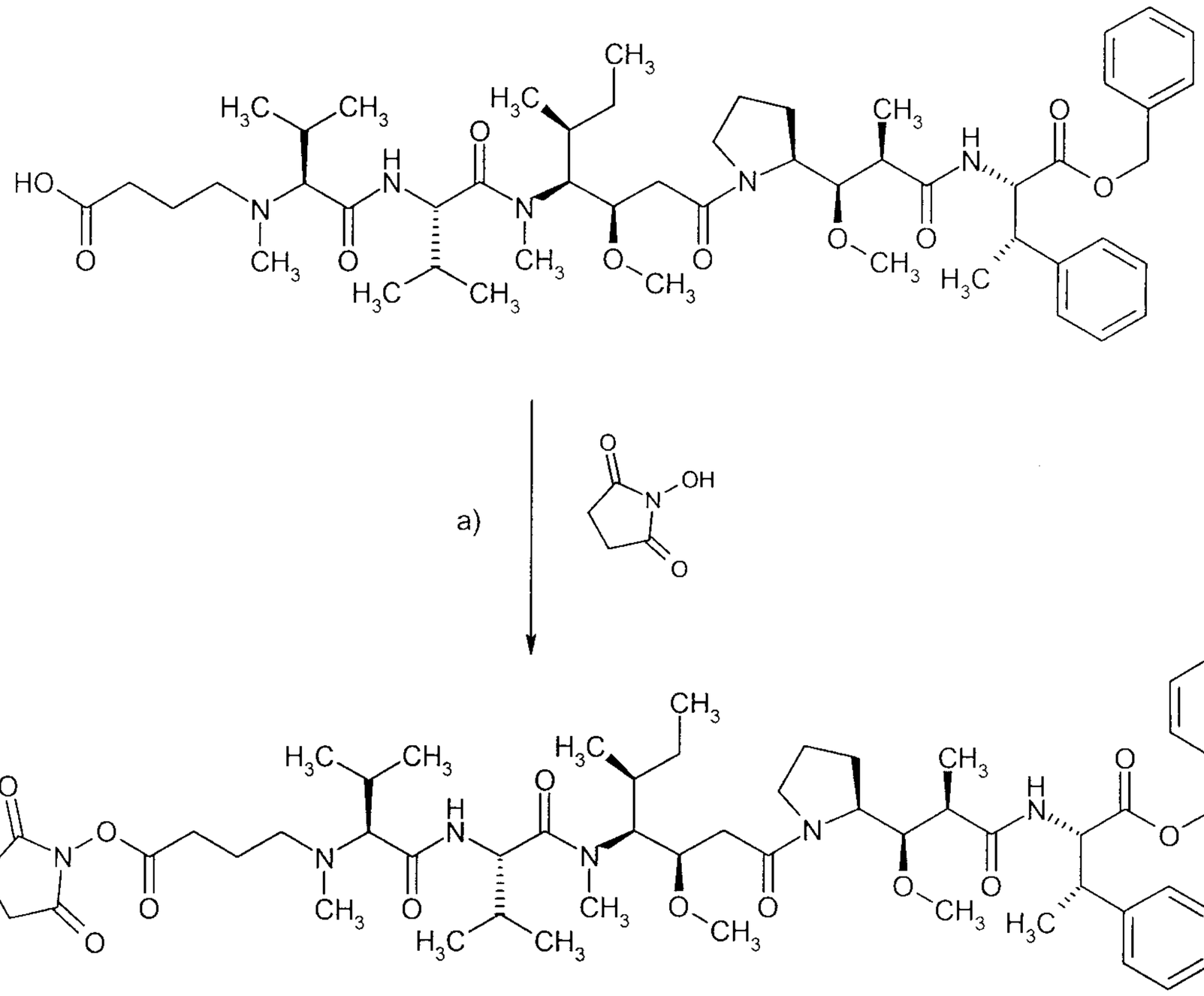
[a): 1. EDCI, DMAP, dichloromethane, RT; 2. H_2, methanol, RT ; b): 1. EDCI, HOBt, diisopropylamine, DMF, RT; 2. dichloromethane, RT].

Scheme 7



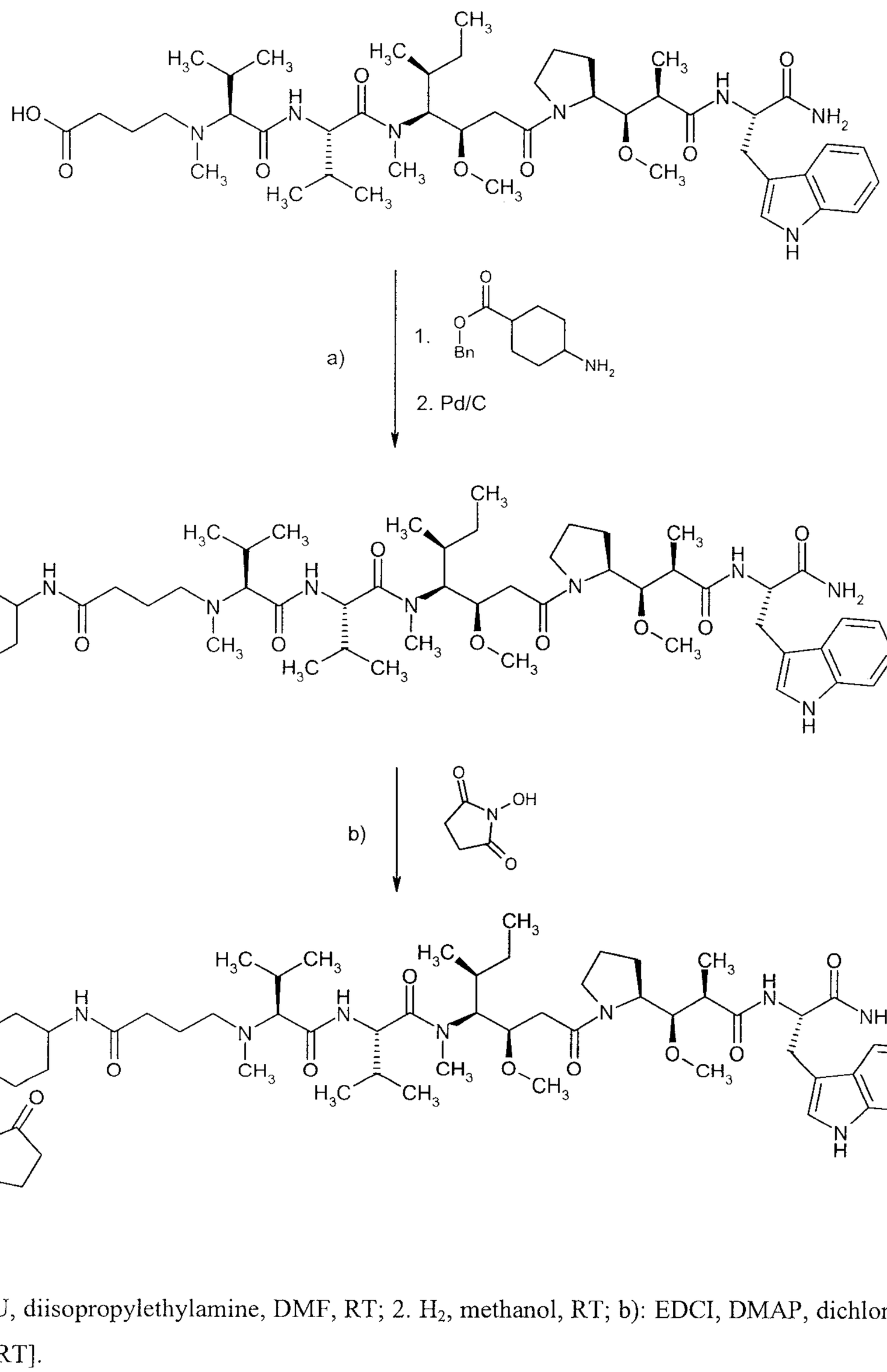
[a): 1. EDCI, DMAP, dichloromethane, RT; 2. H_2, methanol, RT ; b): HATU, diisopropylamine, DMF, RT].

Scheme 8



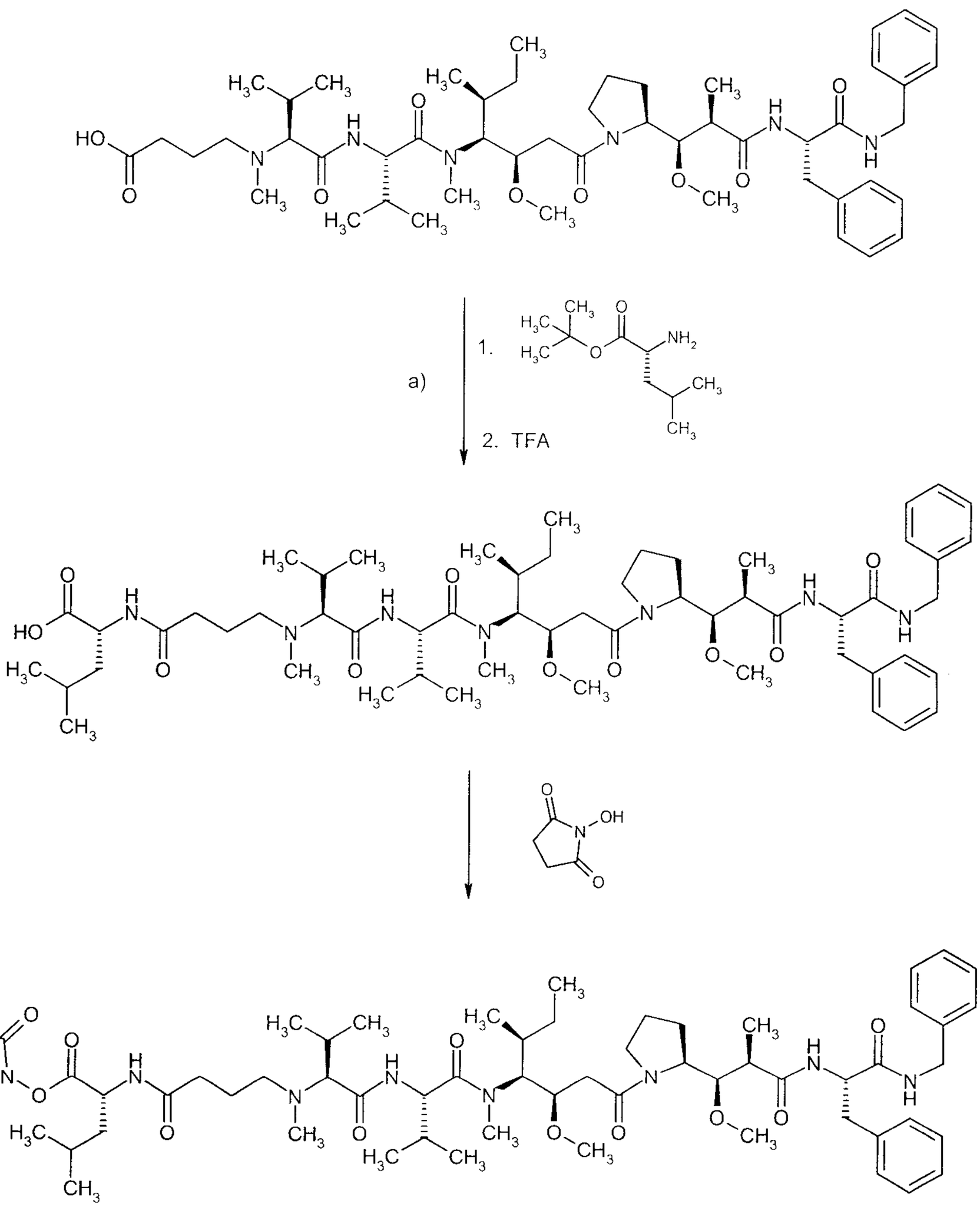
[a): EDCI, dichloromethane, RT].

Scheme 9



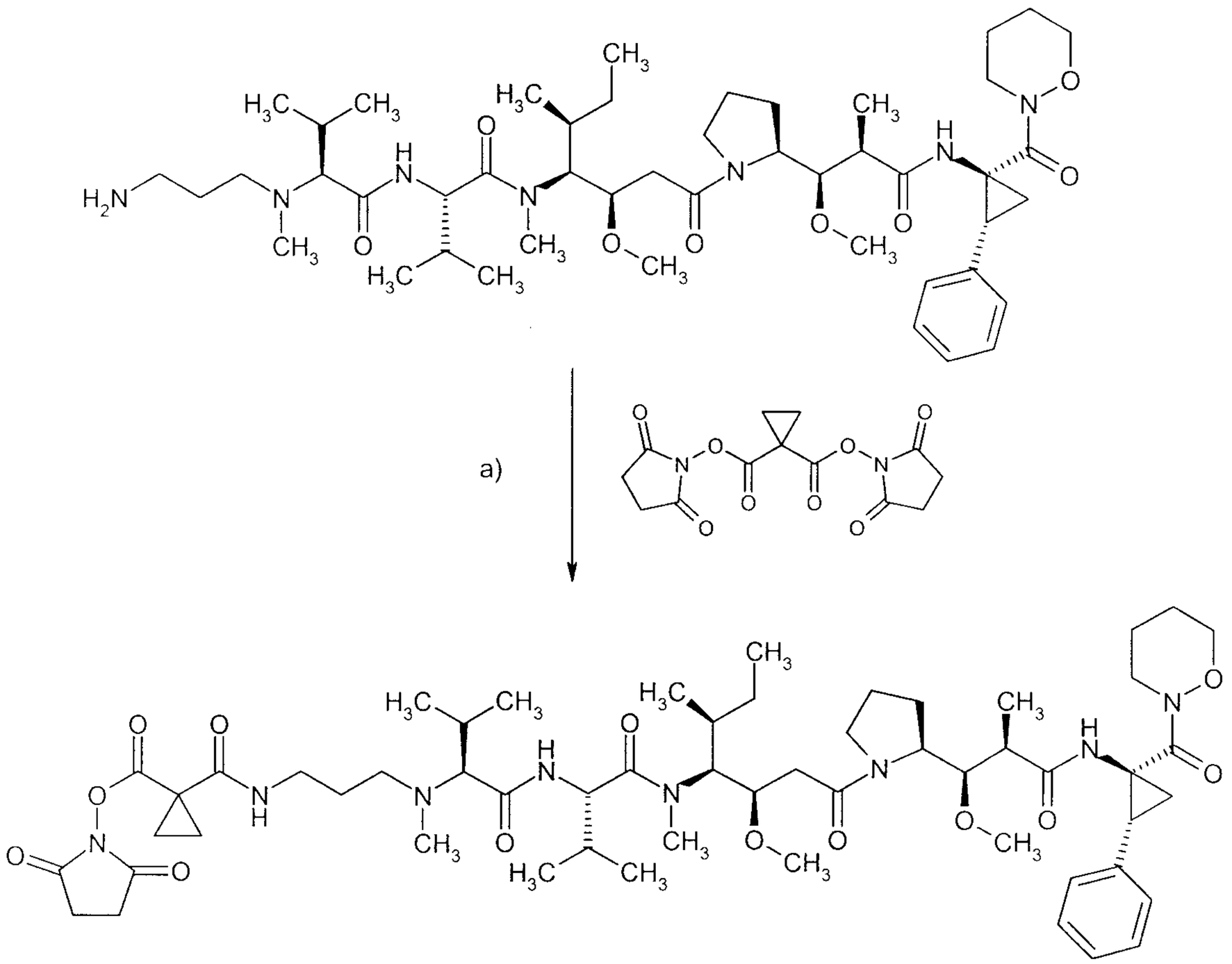
[a): HATU, diisopropylethylamine, DMF, RT; 2. H_2, methanol, RT; b): EDCI, DMAP, dichloro-methane, RT].

Scheme 10



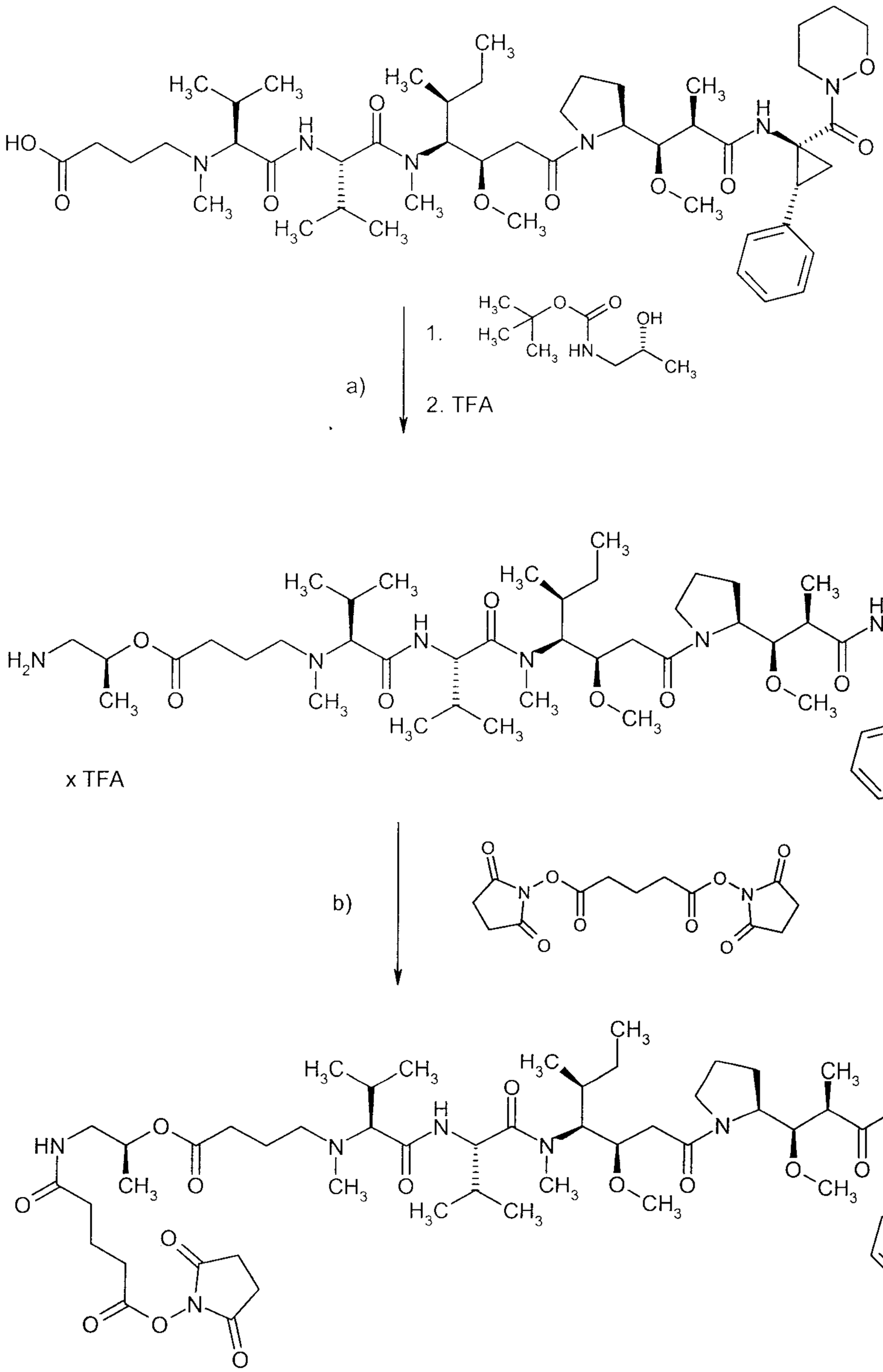
[a): 1. HATU, diisopropylethylamine, DMF, RT; 2. dichloromethane, RT; b): EDCI, DMAP, dichloromethane, RT].

Scheme 11



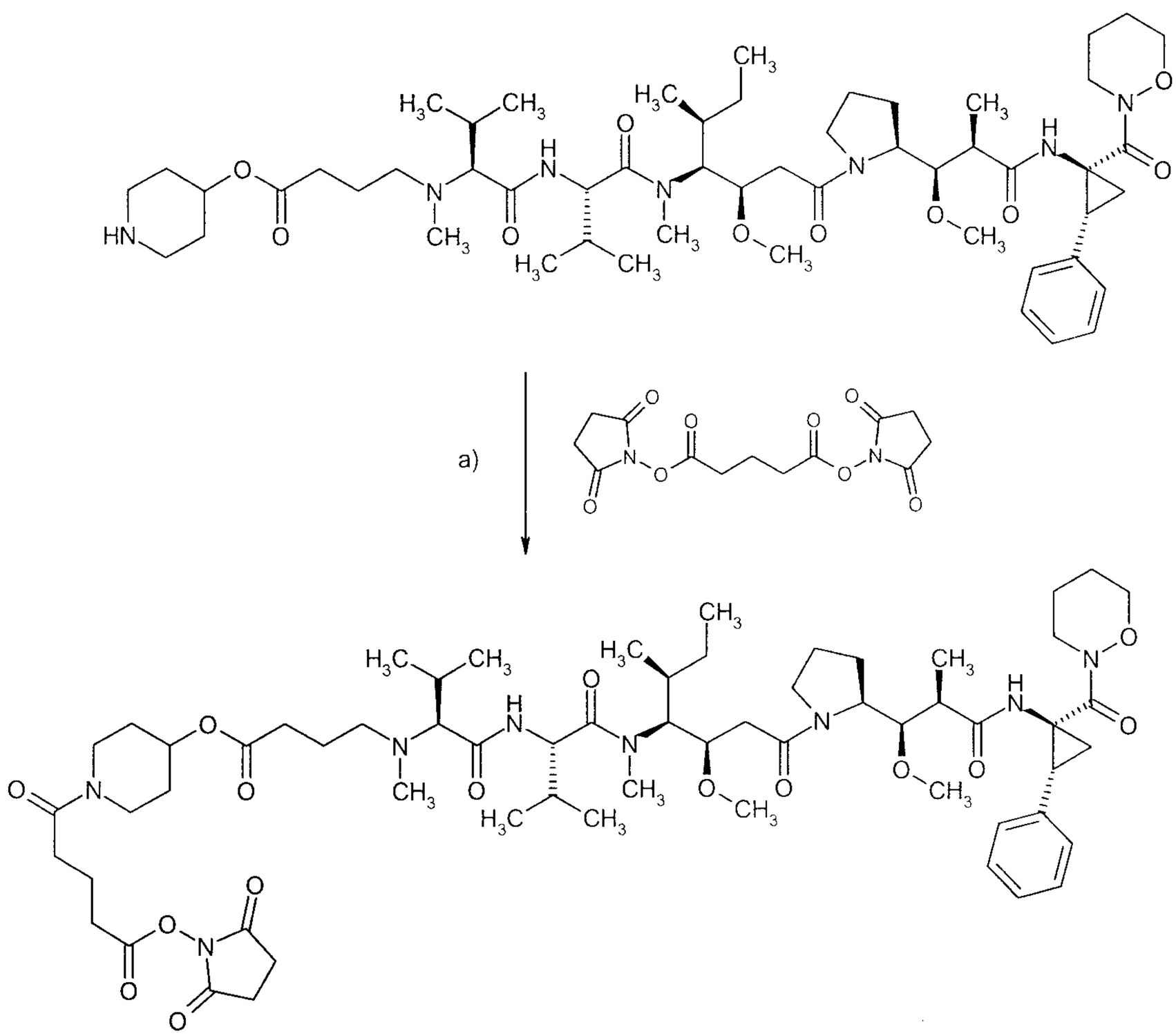
[a): Diisopropylethylamine, DMF, RT].

Scheme 12



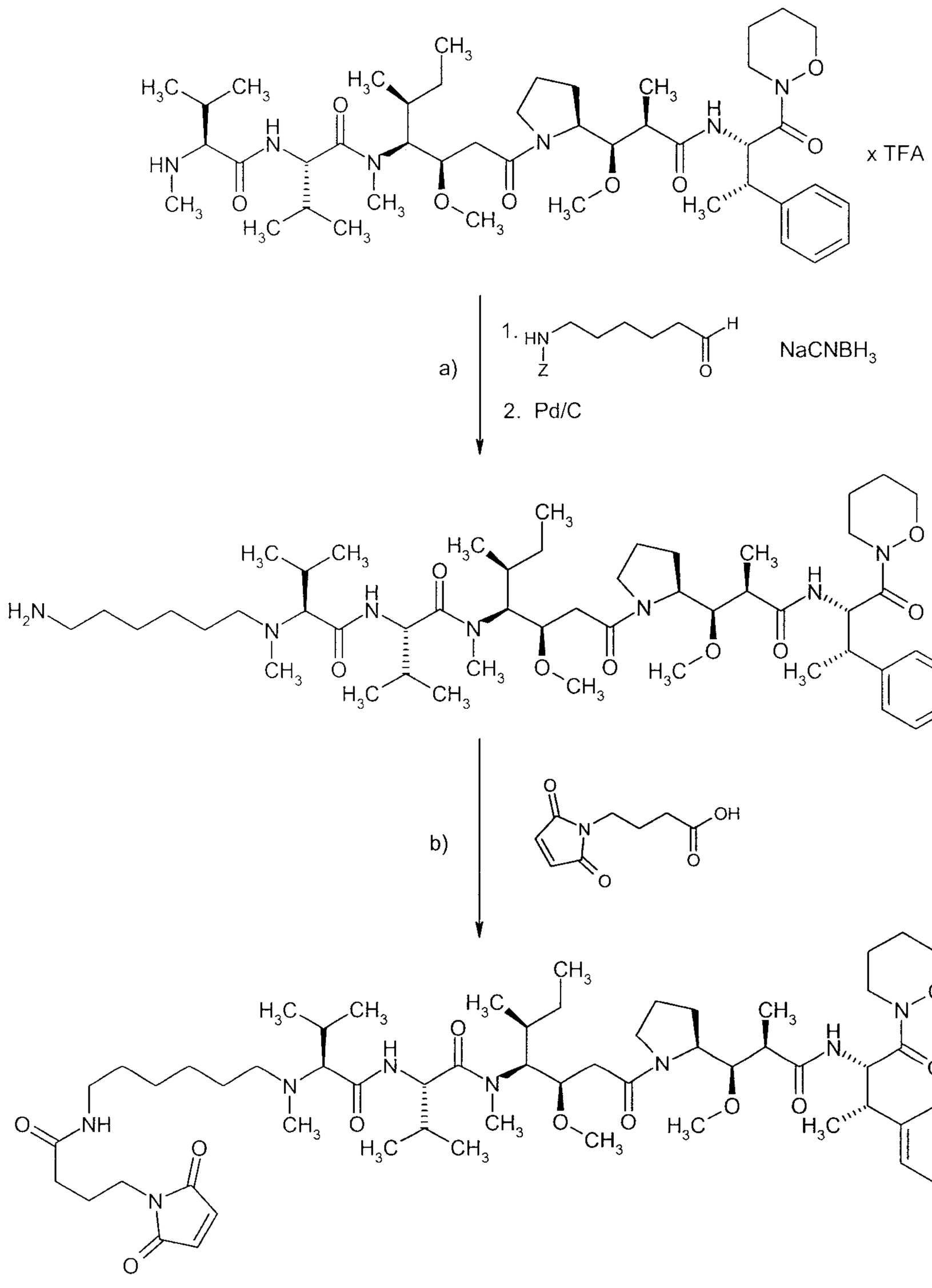
[a): 1. EDCI, DMAP, dichloromethane, RT; 2. Dichloromethane, RT ; b): Diisopropylamine, DMAP, dichloromethane, RT].

Scheme 13



[a): DMAP, diisopropylamine, dichloromethane, RT].

Scheme 18

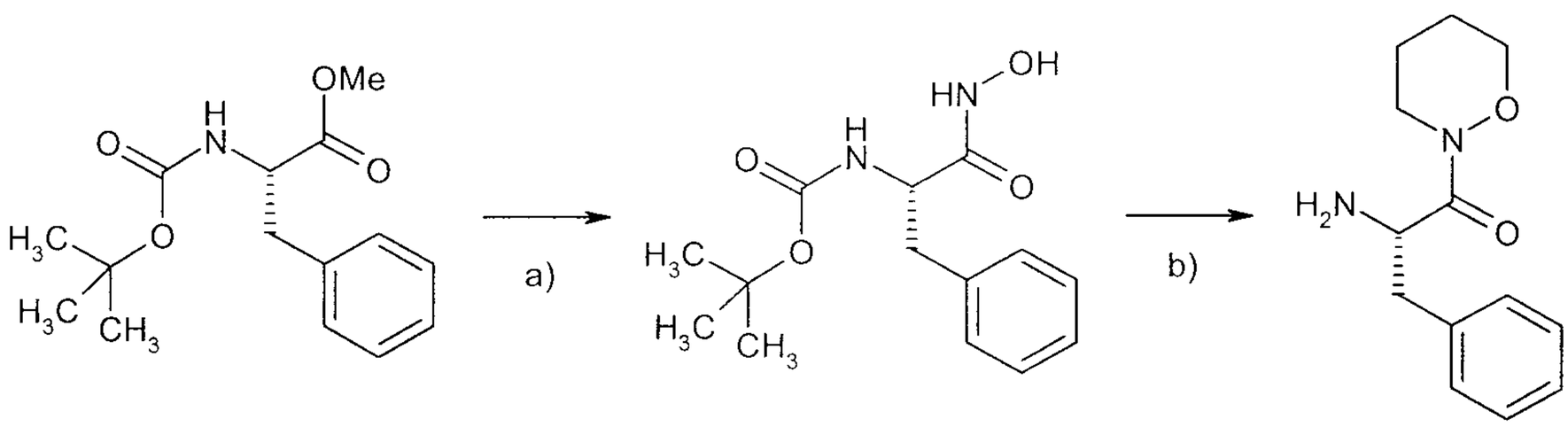


[a): 1. Water/dioxane, 1N HCl, 100°C; 2. H_2, Pd/C, methanol, RT; b): HATU, diisopropylethyl-amine, RT].

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,

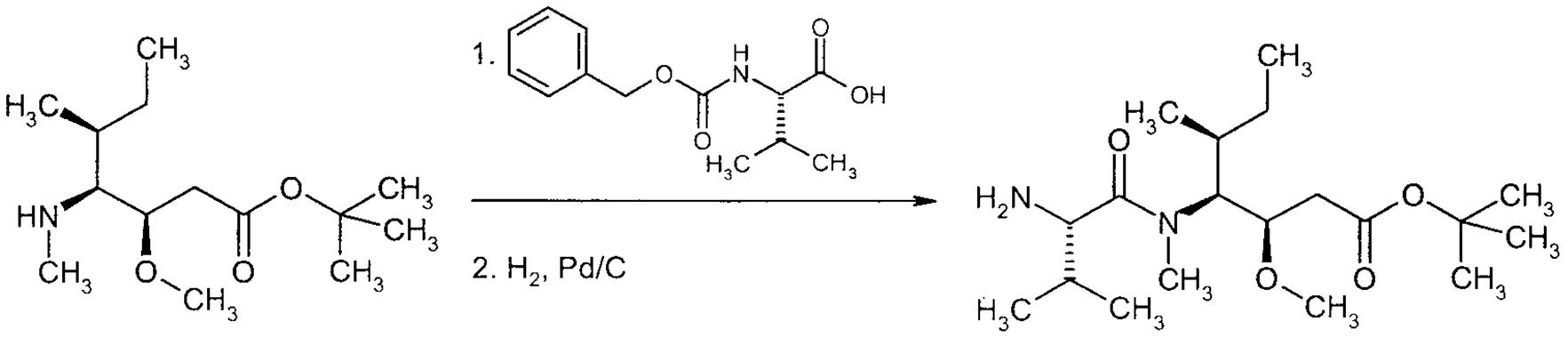
719; Shioiri et al., *Tetrahedron Lett.* 1991, *32*, 931; Shioiri et al., *Tetrahedron* 1993, *49*, 1913; Koga et al., *Tetrahedron Lett.* 1991, *32*, 2395; Vidal et al., *Tetrahedron* 2004, *60*, 9715; Poncet et al., *Tetrahedron* 1994, *50*, 5345. Pettit et al., *J. Org. Chem.* 1994, *59*, 1796) as 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



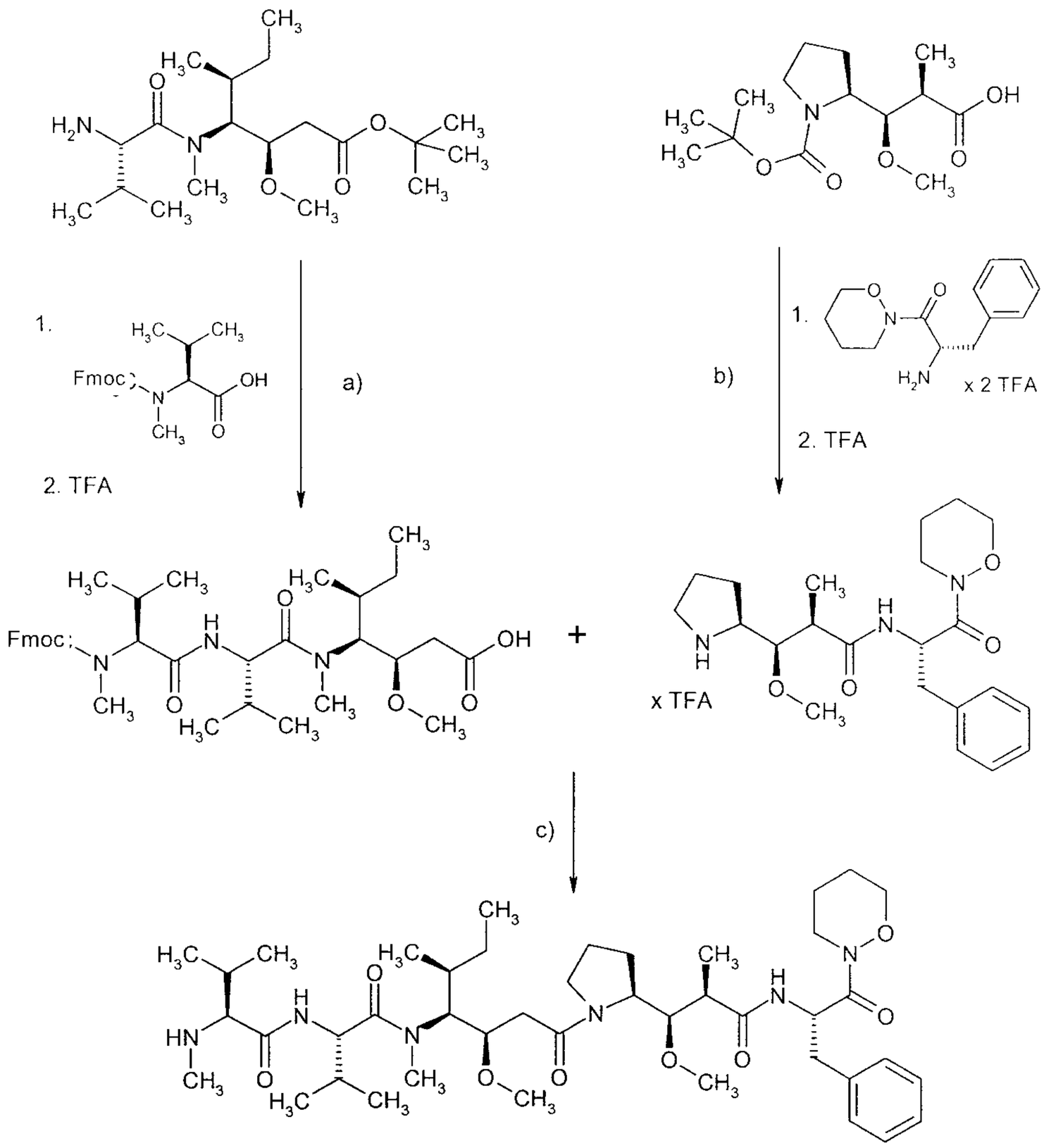
[a): Hydroxylamine hydrochloride, KOH, MeOH, 0°C → RT; b): $BrCH_2(CH_2)_2CH_2Br$, K_2CO_3, acetone, reflux].

Scheme 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].

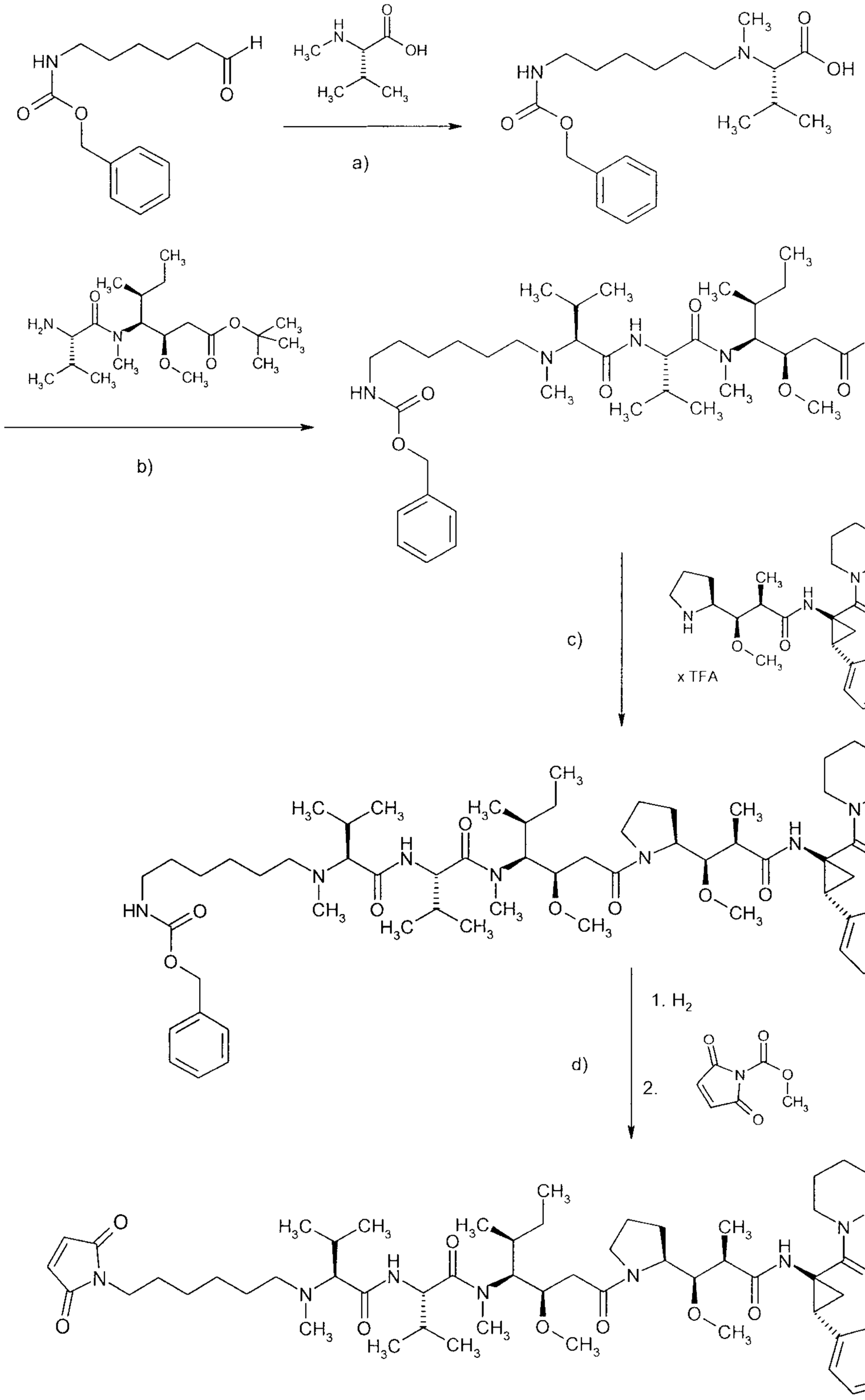
The compounds of formulas (XI), (XIII), (XV), (XVII) and (XXI), including where applicable chiral or diastereomeric forms thereof are commercially available or have been described as such in the literature or can be synthesized by methods like those published in the literature in a manner that would 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.

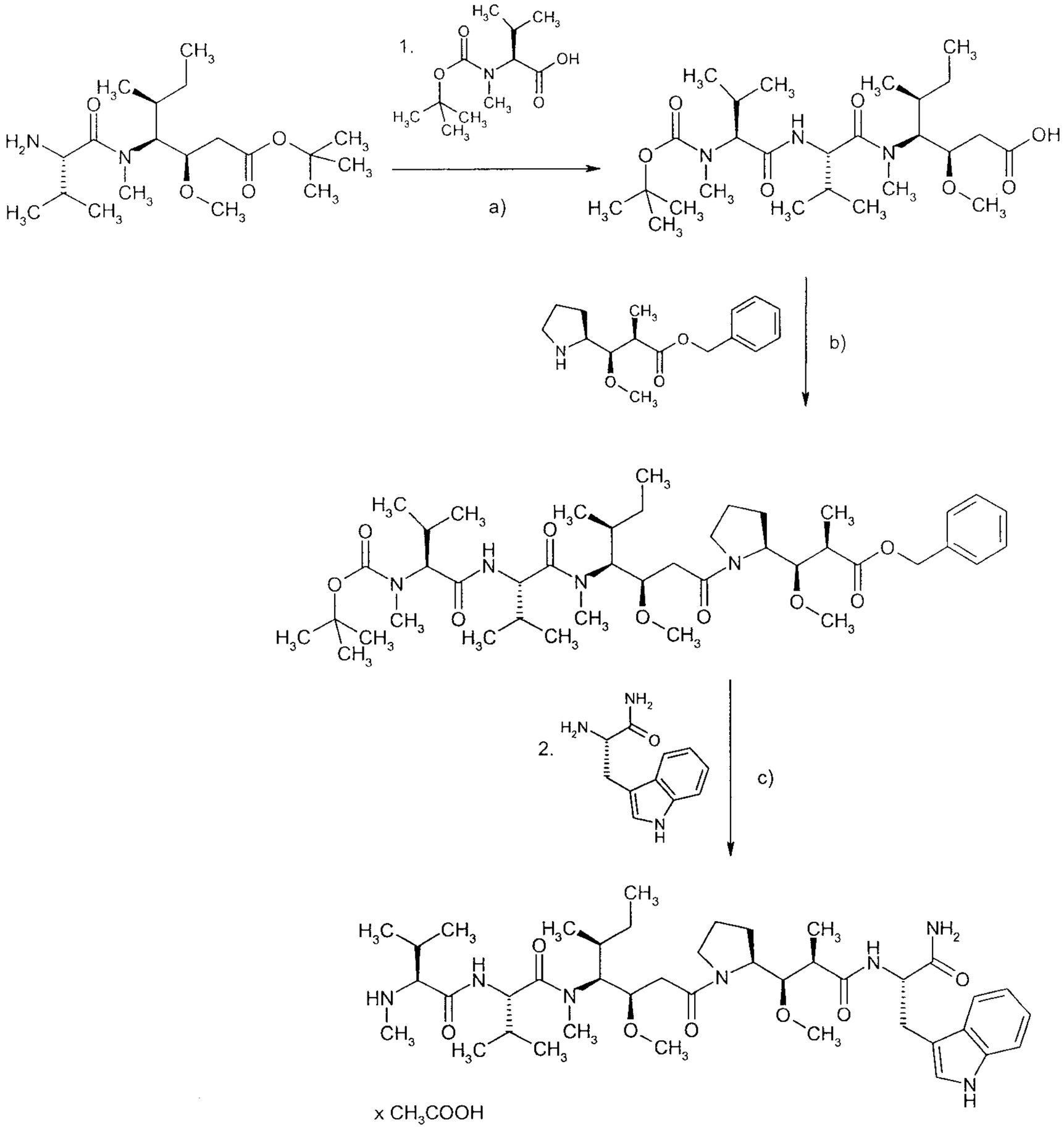
Alternatively individually steps of the synthesis sequence may be performed in a different order. This procedure is illustrated in the following synthesis schemes (schemes 17, 19 and 20) as an example.

Scheme 17



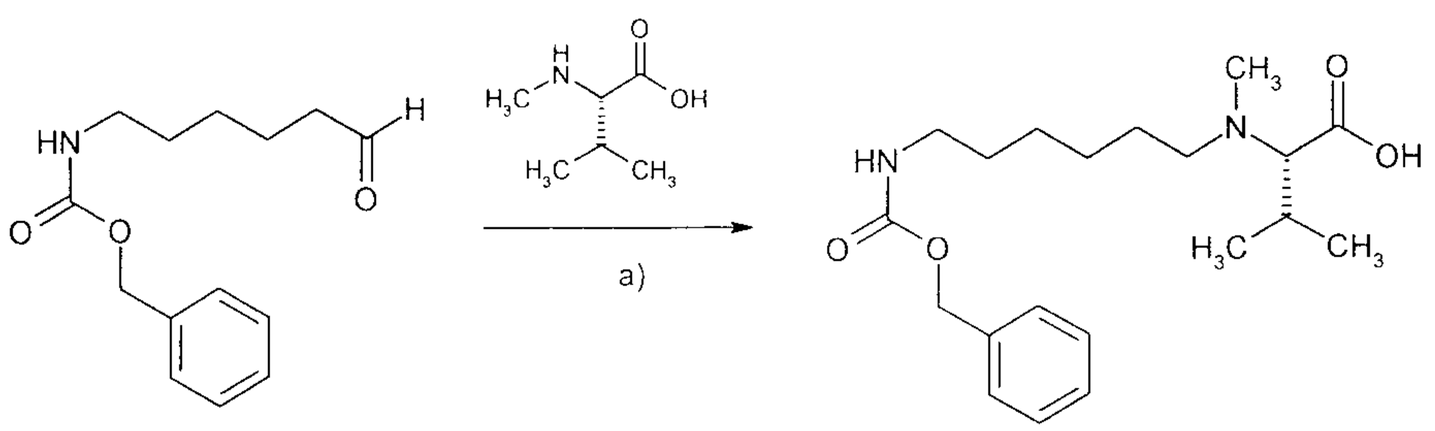
[a): Borane-pyridine complex, acetic acid, MeOH; b): 1. HOBt, EDCI, diisopropylethylamine, DMF, RT; 2. TFA, dichloromethane, RT; c): HATU, diisopropylethylamine, DMF, RT; d) 1. Pd/C, MeOH, RT; 2. $NaHCO_3$, dioxane, water].

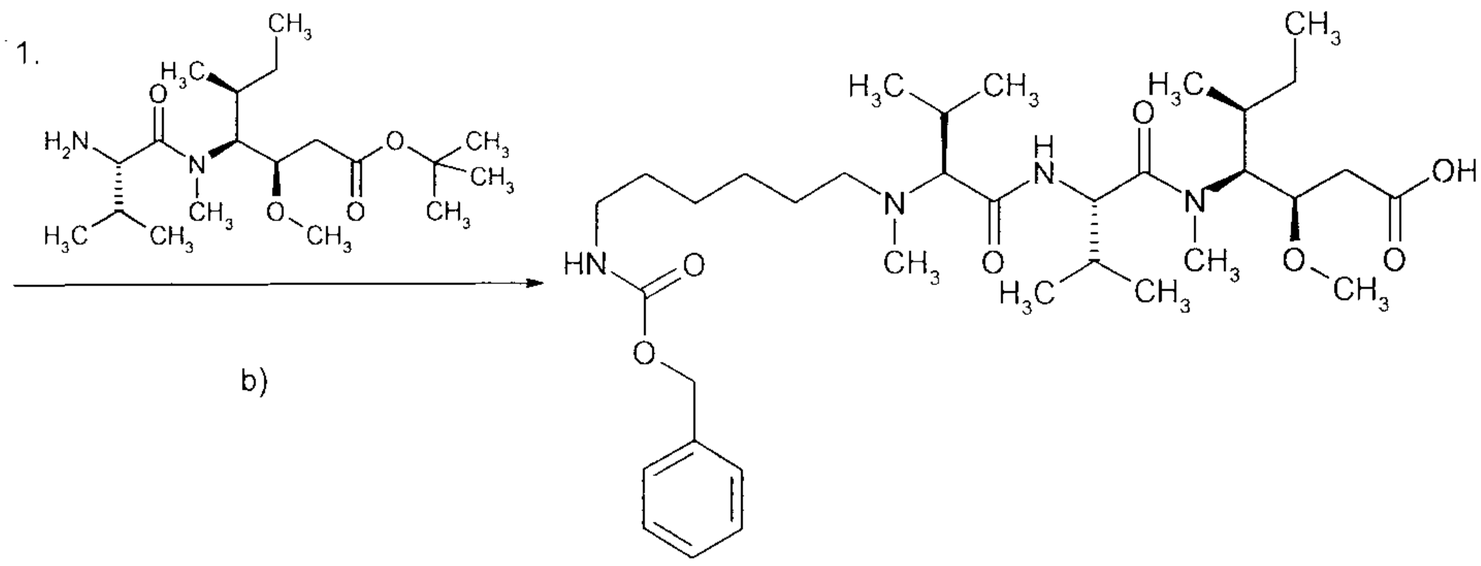
Scheme 19

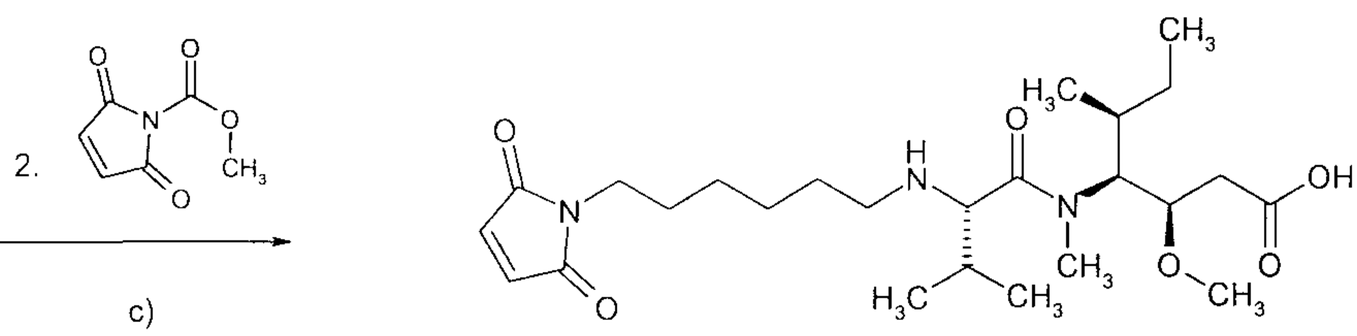


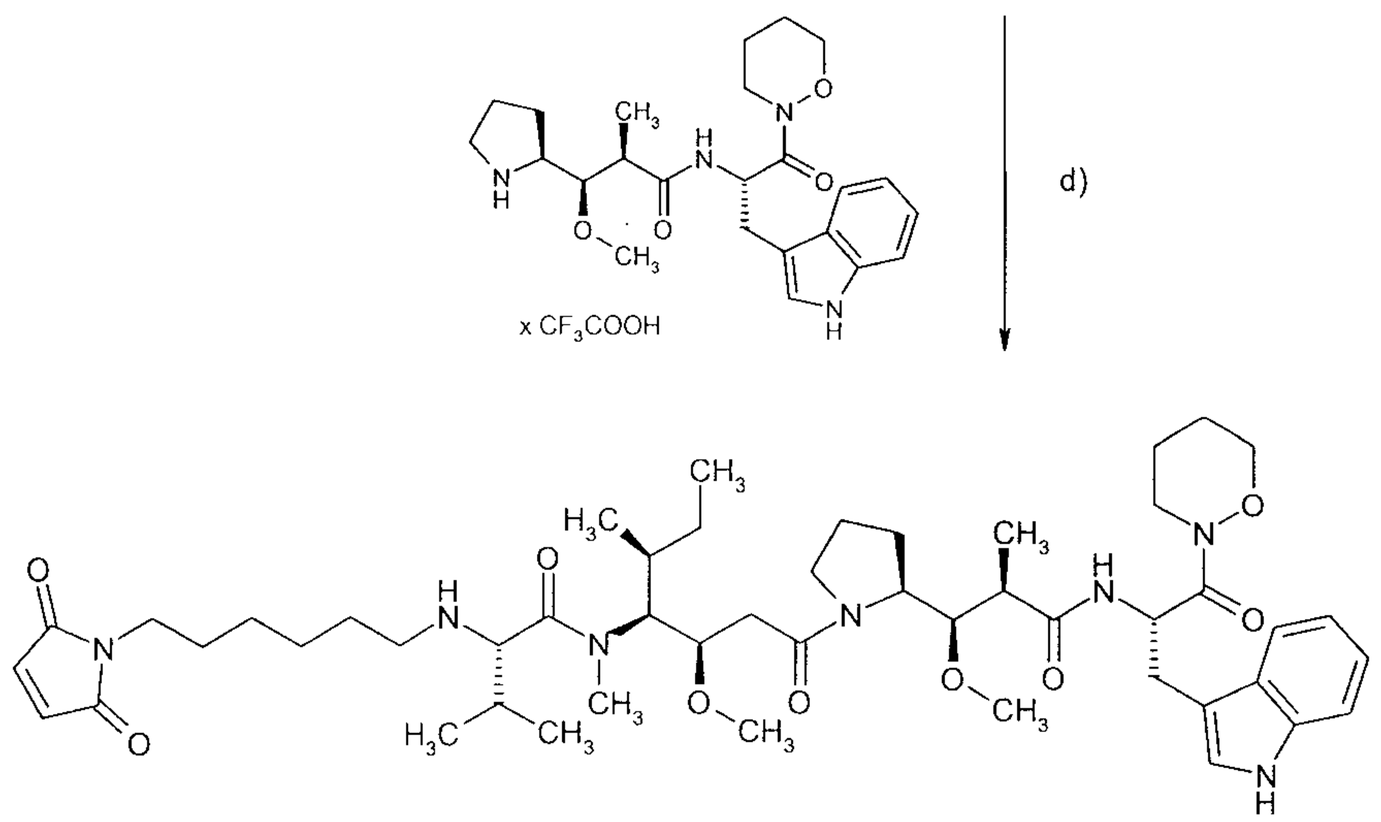
[a): 1. HATU, diisopropylethylamine, DMF, RT; 2. TFA, dichloromethane, RT ; 3. $((H_3C)_3C(C=O))_2O$, DMF, diisopropylethylamine; b): diisopropylethylamine, BEP, DMF, RT; c): 1. H_2, Pd/C (10%). methanol, RT ; 2. HATU, diisopropylethylamine, DMF, RT; 3. TFA, dichloromethane, RT].

Scheme 20









[a): Borane-pyridine complex, acetic acid, MeOH; b): 1. HOBt, EDCI, diisopropylethylamine, DMF, RT; 2. TFA, dichloromethane, RT; c): 1. H_2, Pd/C, MeOH, RT; 2. $NaHCO_3$, 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.

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)

Sequence (1210 amino acids):

>gi|29725609|ref|NP_005219.2| epidermal growth factor receptor isoform a precursor [*Homo sapiens*]

MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLSLQR
MFNNCEVVLGNLEITYVQRNYDLSFLKTIQEVAGYVLIALNTVERIPLENLQIIRG
NMYYENSYALAVLSNYDANKTGLKELPMRNLQEILHGAVRFSNNPALCNVESI
QWRDIVSSDFLSNMSMDFQNHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICA
QQCSGRCRGKSPSDCCHNQCAAGCTGPRESDCLVCRKFRDEATCKDTCPPLML
YNPTTYQMDVNPEGKYSFGATCVKKCPRNYVVTDHGSCVRACGADSYEMEED
GVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFR
GDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQH
GQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTK
IISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLE
GEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAG
VMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMV
GALLLLLVVALGIGLFMRRRHIVRKRTLRRLLQERELVEPLTPSGEAPNQALLRIL
KETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREATSPKANKEILDE
AYVMASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLDYVREHKDNIGSQYLLN
WCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHVKITDFGLAKLLGAEEKE
YHAEGGKVPIKWMALESILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIPASE
ISSILEKGERLPQPPICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYL
VIQGDERMHLPSPTDSNFYRALMDEEDMDDVVDADEYLIPQQGFFSSPSTSRTPL

LSSLSATSNNSTVACIDRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPE
YINQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVGNPEYLNTVQPT
CVNSTFDSPAHWAQKGSHQISLDNPDYQQDFFPKEAKPNGIFKGSTAENAEYLR
VAPQSSEFIGA

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

MALPTARPLLGSCGTPALGSLLFLLFSLGWVQPSRTLAGETGQEAAPLDGVLAN
PPNISS
LSPRQLLGFPCAEVSGLSTERVRELAVALAQKNVKLSTEQLRCLAHRLSEPPEDL
DALPL
DLLLFLNPDAFSGPQACTRFFSRITKANVDLLPRGAPERQRLLPAALACWGVRGS
LLSEA
DVRALGGLACDLPGRFVAESAEVLLPRLVSCPGPLDQDQQEAARAALQGGGPP
YGPPSTW
SVSTMDALRGLLPVLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPERTILRPRFRRE
VEKT
ACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLDVLKH
KLDELY
PQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLEVNKGHEMSPQVATLI
DRFVK
GRGQLDKDTLDTLTAFYPGYLCSLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDV
LYPKA
RLAFQNMNGSEYFVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLP
LTVAEVQ
KLLGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLSM
QEALSGT
PCLLGPGPVLTVLALLLASTLA

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

MAPLCPSPWLPLLIPAPAPGLTVQLLLSLLLLVPVHPQRLPRMQEDSPLGGGSSG
EDDPL
GEEDLPSEEDSPREEDPPGEEDLPGEEDLPGEEDLPEVKPKSEEEGSLKLEDLPTV
EAPG
DPQEPQNNAHRDKEGDDQSHWRYGGDPPWPRVSPACAGRFQSPVDIRPQLAAF
CPALRPL
ELLGFQLPPLPELRLRNNGHSVQLTLPPGLEMALGPGREYRALQLHLHWGAAGR
PGSEHT
VEGHRFPAEIHVVHLSTAFARVDEALGRPGGLAVLAAFLEEGPEENSAYEQLLS
RLEEIA
EEGSETQVPGLDISALLPSDFSRYFQYEGSLTTPPCAQGVIWTVFNQTVMLSAKQ
LHTLS
DTLWGPGDSRLQLNFRATQPLNGRVIEASFPAGVDSSPRAAEPVQLNSCLAAGDI
LALVF
GLLFAVTSVAFLVQMRRQHRRGTKGGVSYRPAEVAETGA

The extracellular domain is underlined for emphasis.

(4) C4.4a (NCBI reference sequence NP_055215.2; synonym LYPD3)

Sequence (346 amino acids):

>gi|93004088|ref|NP_055215.2| ly6/PLAUR domain-containing protein 3 precursor [*Homo sapiens*]

MDPARKAGAQAMIWTAGWLLLLLLRGGAQALECYSCVQKADDGCSPNKMKT
VKCAPGVDVCTEAVGAVETIHGQFSLAVRGCGSGLPGKNDRGLDLHGLLAFIQL
QQCAQDRCNAKLNLTSRALDPAGNESAYPPNGVECYSCVGLSREACQGTSPPV
VSCYNASDHVYKGCFDGNVTLTAANVTVSLPVRGCVQDEFCTRDGVTGPGFTL
SGSCCQGSRCNSDLRNKTYFSPRIPPLVRLPPPEPTTVASTTSVTTSTSAPVRPTST
TKPMPAPTSQTPRQGVEHEASRDEEPRLTGGAAGHQDRSNSGQYPAKGGPQQP
HNKGCVAPTAGLAALLLAVAAGVLL

The matured extracellular domain is underlined for emphasis (SEQ ID NO: 1).

(5) CD52 (NCBI reference sequence NP_001794.2)

>gi|68342030|ref|NP_001794.2| CAMPATH-1 antigen precursor [*Homo sapiens*]

MKRFLFLLLTISLLVMVQIQTGLSGQNDTSQTSSPSASSNISGGIFLFFVANAIIHLF
CFS

(6) HER2 (NCBI reference sequence NP_004439.2)

>gi|54792096|ref|NP_004439.2| receptor tyrosine-protein kinase erbB-2 isoform a [*Homo sapiens*]

MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGC
QVVQGNLELTYLPTNASLSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLF
EDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLC
YQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGESSEDCQSLT
RTVCAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPA
LVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLVCPLHNQEVTA
EDGTQRCEKCSKPCARVCYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLP
ESFDGDPASNTAPLQPEQLQVFETLEEITGYLYISAWPDSLPDLSVFQNLQVIRGR
ILHNGAYSLTLQGLGISWLGLRSLRELGSGLALIHHNTHLCFVHTVPWDQLFRNP
HQALLHTANRPEDECVGEGLACHQLCARGHCWGPGPTQCVNCSQFLRGQECVE
ECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPF
CVARCPSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGCPAEQRAS
PLTSIISAVVGILLVVVLGVVFGILIKRRQQKIRKYTMRRLLQETELVEPLTPSGA
MPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRENT
SPKANKEILDEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLMPYGCLLDHVREN
RGRLGSQDLLNWCMQIAKGMSYLEDVRLVHRDLAARNVLVKSPNHVKITDFGL
ARLLDIDETEYHADGGKVPIKWMALESILRRRFTHQSDVWSYGVTVWELMTFG
AKPYDGIPAREIPDLLEKGERLPQPPICTIDVYMIMVKCWMIDSECRPRFRELVSE
FSRMARDPQRFVVIQNEDLGPASPLDSTFYRSLLEDDDMGDLVDAEEYLVPQQG
FFCPDPAPGAGGMVHHRHRSSSTRSGGGDLTLGLEPSEEEAPRSPLAPSEGAGSD
VFDGDLGMGAAKGLQSLPTHDPSPLQRYSEDPTVPLPSETDGYVAPLTCSPQPE
YVNQPDVRPQPPSPREGPLPAARPAGATLERPKTLSPGKNGVVKDVFAFGGAVE
NPEYLTPQGGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPE
YLGLDVPV

(7) CD20 (NCBI reference sequence NP_068769.2)

>gi|23110987|ref|NP_068769.2| B-lymphocyte antigen CD20 [*Homo sapiens*]

MTTPRNSVNGTFPAEPMKGPIAMQSGPKPLFRRMSSLVGPTQSFFMRESKTLGA
VQIMNGLFHIALGGLLMIPAGIYAPICVTVWYPLWGGIMYIISGSLLAATEKNSR
KCLVKGKMIMNSLSLFAAISGMILSIMDILNIKISHFLKMESLNFIRAHTPYINIYN
CEPANPSEKNSPSTQYCYSIQSLFLGILSVMLIFAFFQELVIAGIVENEWKRTCSRP
KSNIVLLSAEEKKEQTIEIKEEVVGLTETSSQPKNEEDIEIIPIQEEEEEETETNFPEP
PQDQESSPIENDSSP

(8) The lymphocyte activating antigen CD30 (SwissProt ID P28908)

>gi|68348711|ref|NP_001234.2| tumor necrosis factor receptor superfamily member 8 isoform 1 precursor [*Homo sapiens*]

MRVLLAALGLLFLGALRAFPQDRPFEDTCHGNPSHYYDKAVRRCCYRCPMGLF
PTQQCPQRPTDCRKQCEPDYYLDEADRCTACVTCSRDDLVEKTPCAWNSSRVC
ECRPGMFCSTSAVNSCARCFFHSVCPAGMIVKFPGTAQKNTVCEPASPGVSPAC
ASPENCKEPSSGTIPQAKPTPVSPATSSASTMPVRGGTRLAQEAASKLTRAPDSPS
SVGRPSSDPGLSPTQPCPEGSGDCRKQCEPDYYLDEAGRCTACVSCSRDDLVEK
TPCAWNSSRTCECRPGMICATSATNSRARCVPYPICAAETVTKPQDMAEKDTTF
EAPPLGTQPDCNPTPENGEAPASTSPTQSLLVDSQASKTLPIPTSAPVALSSTGKP
VLDAGPVLFWVILVLVVVVGSSAFLLCHRRACRKRIRQKLHLCYPVQTSQPKLE
LVDSRPRRSSTQLRSGASVTEPVAEERGLMSQPLMETCHSVGAAYLESLPLQDA
SPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVGTVKAELPEGRGLAGPA
EPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPTAASGK

(9) The lymphocyte adhesion molecule CD22 (SwissProt ID P20273)

>gi|157168355|ref|NP_001762.2| B-cell receptor CD22 isoform 1 precursor [*Homo sapiens*]

MHLLGPWLLLLVLEYLAFSDSSKWVFEHPETLYAWEGACVWIPCTYRALDGDL
ESFILFHNPEYNKNTSKFDGTRLYESTKDGKVPSEQKRVQFLGDKNKNCTLSIHP
VHLNDSGQLGLRMESKTEKWMERIHLNVSERPFPPHIQLPPEIQESQEVTLTCLL
NFSCYGYPIQLQWLLEGVPMRQAAVTSTSLTIKSVFTRSELKFSPQWSHHGKIVT
CQLQDADGKFLSNDTVQLNVKHTPKLEIKVTPSDAIVREGDSVTMTCEVSSSNP
EYTTVSWLKDGTSLKKQNTFTLNLREVTKDQSGKYCCQVSNDVGPGRSEEVFL

QVQYAPEPSTVQILHSPAVEGSQVEFLCMSLANPLPTNYTWYHNGKEMQGRTE
EKVHIPKILPWHAGTYSCVAENILGTGQRGPGAELDVQYPPKKVTTVIQNPMPIR
EGDTVTLSCNYNSSNPSVTRYEWKPHGAWEEPSLGVLKIQNVGWDNTTIACAA
CNSWCSWASPVALNVQYAPRDVRVRKIKPLSEIHSGNSVSLQCDFSSSHPKEVQ
FFWEKNGRLLGKESQLNFDSISPEDAGSYSCWVNNSIGQTASKAWTLEVLYAPR
RLRVSMSPGDQVMEGKSATLTCESDANPPVSHYTWFDWNNQSLPYHSQKLRLE
PVKVQHSGAYWCQGTNSVGKGRSPLSTLTVYYSPETIGRRVAVGLGSCLAILILA
ICGLKLQRRWKRTQSQQGLQENSSGQSFFVRNKKVRRAPLSEGPHSLGCYNPM
MEDGISYTTLRFPEMNIPRTGDAESSEMQRPPPDCDDTVTYSALHKRQVGDYEN
VIPDFPEDEGIHYSELIQFGVGERPQAQENVDYVILKH

(10) The myeloid cell surface antigen CD33 (SwissProt ID **P20138**)

>gi|130979981|ref|NP_001763.3| myeloid cell surface antigen CD33 isoform 1 precursor [*Homo sapiens*]

MPLLLLLPLLWAGALAMDPNFWLQVQESVTVQEGLCVLVPCTFFHPIPYYDKNS
PVHGYWFREGAIISRDSPVATNKLDQEVQEETQGRFRLLGDPSRNNCSLSIVDAR
RRDNGSYFFRMERGSTKYSYKSPQLSVHVTDLTHRPKILIPGTLEPGHSKNLTCS
VSWACEQGTPPIFSWLSAAPTSLGPRTTHSSVLIITPRPQDHGTNLTCQVKFAGA
GVTTERTIQLNVTYVPQNPTTGIFPGDGSGKQETRAGVVHGAIGGAGVTALLAL
CLCLIFFIVKTHRRKAARTAVGRNDTHPTTGSASPKHQKKSKLHGPTETSSCSGA
APTVEMDEELHYASLNFHGMNPSKDTSTEYSEVRTQ

(11) The transmembrane glycoprotein NMB (SwissProt ID Q14956)

>gi|52694752|ref|NP_001005340.1| transmembrane glycoprotein NMB isoform a precursor [*Homo sapiens*]

MECLYYFLGFLLLAARLPLDAAKRFHDVLGNERPSAYMREHNQLNGWSSDEND
WNEKLYPVWKRGDMRWKNSWKGGRVQAVLTSDSPALVGSNITFAVNLIFPRC
QKEDANGNIVYEKNCRNEAGLSADPYVYNWTAWSEDSDGENGTGQSHHNVFP
DGKPFPHHPGWRRWNFIYVFHTLGQYFQKLGRCSVRVSVNTANVTLGPQLMEV
TVYRRHGRAYVPIAQVKDVYVVTDQIPVFVTMFQKNDRNSSDETFLKDLPIMFD
VLIHDPSHFLNYSTINYKWSFGDNTGLFVSTNHTVNHTYVLNGTFSLNLTVKAA
APGPCPPPPPPPRPSKPTPSLATTLKSYDSNTPGPAGDNPLELSRIPDENCQINRYG
HFQATITIVEGILEVNIIQMTDVLMPVPWPESSLIDFVVTCQGSIPTEVCTIISDPTC
EITQNTVCSPVDVDEMCLLTVRRTFNGSGTYCVNLTLGDDTSLALTSTLISVPDR

DPASPLRMANSALISVGCLAIFVTVISLLVYKKHKEYNPIENSPGNVVRSKGLSVF
LNRAKAVFFPGNQEKDPLLKNQEFKGVS

(12) The adhesion molecule CD56 (SwissProt ID P13591)

>gi|94420689|ref|NP_000606.3| neural cell adhesion molecule 1 isoform 1 [*Homo sapiens*]

MLQTKDLIWTLFFLGTAVSLQVDIVPSQGEISVGESKFFLCQVAGDAKDKDISWF
SPNGEKLTPNQQRISVVWNDDSSSTLTIYNANIDDAGIYKCVVTGEDGSESEATV
NVKIFQKLMFKNAPTPQEFREGEDAVIVCDVVSSLPPTIIWKHKGRDVILKKDVR
FIVLSNNYLQIRGIKKTDEGTYRCEGRILARGEINFKDIQVIVNVPPTIQARQNIVN
ATANLGQSVTLVCDAEGFPEPTMSWTKDGEQIEQEEDDEKYIFSDDSSQLTIKKV
DKNDEAEYICIAENKAGEQDATIHLKVFAKPKITYVENQTAMELEEQVTLTCEA
SGDPIPSITWRTSTRNISSEEKTLDGHMVVRSHARVSSLTLKSIQYTDAGEYICTA
SNTIGQDSQSMYLEVQYAPKLQGPVAVYTWEGN

QVNITCEVFAYPSATISWFRDGQLLPSSNYSNIKIYNTPSASYLEVTPDSENDFGN
YNCTAVNRIGQESLEFILVQADTPSSPSIDQVEPYSSTAQVQFDEPEATGGVPILK
YKAEWRAVGEEVWHSKWYDAKEASMEGIVTIVGLKPETTYAVRLAALNGKGL
GEISAASEFKTQPVQGEPSAPKLEGQMGEDGNSIKVNLIKQDDGGSPIRHYLVRY
RALSSEWKPEIRLPSGSDHVMLKSLDWNAEYEVYVVAENQQGKSKAAHFVFRT
SAQPTAIPANGSPTSGLSTGAIVGILIVIFVLLLVVVDITCYFLNKCGLFMCIAVNL
CGKAGPGAKGKDMEEGKAAFSKDESKEPIVEVRTEEERTPNHDGGKHTEPNETT
PLTEPEKGPVEAKPECQETETKPAPAEVKTVPNDATQTKENESKA

(13) The surface molecule CD70 (SwissProt ID P32970)

>gi|4507605|ref|NP_001243.1| CD70 antigen [*Homo sapiens*]

MPEEGSGCSVRRRPYGCVLRAALVPLVAGLVICLVVCIQRFAQAQQQLPLESLG
WDVAELQLNHTGPQQDPRLYWQGGPALGRSFLHGPELDKGQLRIHRDGIYMVH
IQVTLAICSSTTASRHHPTTLAVGICSPASRSISLLRLSFHQGCTIASQRLTPLARGD
TLCTNLTGTLLPSRNTDETFFGVQWVRP

(14) The surface molecule CD74 (SwissProt ID P04233)

>gi|10835071|ref|NP_004346.1| HLA class II histocompatibility antigen gamma chain isoform b [*Homo sapiens*]

MHRRRSRSCREDQKPVMDDQRDLISNNEQLPMLGRRPGAPESKCSRGALYTGFS
ILVTLLLAGQATTAYFLYQQQGRLDKLTVTSQNLQLENLRMKLPKPPKPVSKMR
MATPLLMQALPMGALPQGPMQNATKYGNMTEDHVMHLLQNADPLKVYPPLK
GSFPENLRHLKNTMETIDWKVFESWMHHWLLFEMSRHSLEQKPTDAPPKESLEL
EDPSSGLGVTKQDLGPVPM

(15) The B-lymphocyte antigen CD19 (SwissProt ID P15391)

>gi|296010921|ref|NP_001171569.1| B-lymphocyte antigen CD19 isoform 1 precursor [*Homo sapiens*]

MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSR
ESPLKPFLKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQP
GWTVNVEGSGELFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAK
DRPEIWEGEPPCLPPRDSLNQSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTH
VHPKGPKSLLSLELKDDRPARDMWVMETGLLLPRATAQDAGKYYCHRGNLTM
SFHLEITARPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRRK
RKRMTDPTRRFFKVTPPPGSGPQNQYGNVLSLPTPTSGLGRAQRWAAGLGGTAP
SYGNPSSDVQADGALGSRSPPGVGPEEEEGEGYEEPDSEEDSEFYENDSNLGQD
QLSQDGSGYENPEDEPLGPEDEDSFSNAESYENEDEELTQPVARTMDFLSPHGSA
WDPSREATSLAGSQSYEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENMDNPD
GPDPAWGGGGRMGTWSTR

(16) The surface protein mucin 1 (SwissProt ID P15941)

>gi|65301117|ref|NP_002447.4| mucin-1 isoform 1 precursor [*Homo sapiens*]

MTPGTQSPFFLLLLLTVLTVVTGSGHASSTPGGEKETSATQRSSVPSSTEKNALST
GVSFFFLSFHISNLQFNSSLEDPSTDYYQELQRDISEMFLQIYKQGGFLGLSNIKFR
PGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISDVSVSDVPFPFSA
QSGAGVPGWGIALLVLVCVLVALAIVYLIALAVCQCRRKNYGQLDIFPARDTYH
PMSEYPTYHTHGRYVPPSSTDRSPYEKVSAGNGGSSLSYTNPAVAATSANL

(17) The surface protein CD138 (SwissProt ID P18827)

>gi|29568086|ref|NP_002988.3| syndecan-1 precursor [*Homo sapiens*]

MRRAALWLWLCALALSLQPALPQIVATNLPPEDQDGSGDDSDNFSGSGAGALQ
DITLSQQTPSTWKDTQLLTAIPTSPEPTGLEATAASTSTLPAGEGPKEGEAVVLPE
VEPGLTAREQEATPRPRETTQLPTTHQASTTTATTAQEPATSHPHRDMQPGHHE

TSTPAGPSQADLHTPHTEDGGPSATERAAEDGASSQLPAAEGSGEQDFTFETSGE
NTAVVAVEPDRRNQSPVDQGATGASQGLLDRKEVLGGVIAGGLVGLIFAVCLV
GFMLYRMKKKDEGSYSLEEPKQANGGAYQKPTKQEEFYA

(18) Integrin alphaV (GenBank Accession No. NP_002201.1)

>gi|4504763|ref|NP_002201.1| integrin alpha-V isoform 1 precursor [*Homo sapiens*]

MAFPPRRRLRLGPRGLPLLLSGLLLPLCRAFNLDVDSPAEYSGPEGSYFGFAVDF
FVPSASSRMFLLVGAPKANTTQPGIVEGGQVLKCDWSSTRRCQPIEFDATGNRD
YAKDDPLEFKSHQWFGASVRSKQDKILACAPLYHWRTEMKQEREPVGTCFLQD
GTKTVEYAPCRSQDIDADGQGFCQGGFSIDFTKADRVLLGGPGSFYWQGQLISD
QVAEIVSKYDPNVYSIKYNNQLATRTAQAIFDDSYLGYSVAVGDFNGDGIDDFV
SGVPRAARTLGMVYIYDGKNMSSLYNFTGEQMAAYFGFSVAATDINGDDYAD
VFIGAPLFMDRGSDGKLQEVGQVSVSLQRASGDFQTTKLNGFEVFARFGSAIAPL
GDLDQDGFNDIAIAAPYGGEDKKGIVYIFNGRSTGLNAVPSQILEGQWAARSMP
PSFGYSMKGATDIDKNGYPDLIVGAFGVDRAILYRARPVITVNAGLEVYPSILNQ
DNKTCSLPGTALKVSCFNVRFCLKADGKGVLPRKLNFQVELLLDKLKQKGAIRR
ALFLYSRSPSHSKNMTISRGGLMQCEELIAYLRDESEFRDKLTPITIFMEYRLDYR
TAADTTGLQPILNQFTPANISRQAHILLDCGEDNVCKPKLEVSVDSDQKKIYIGD
DNPLTLIVKAQNQGEGAYEAELIVSIPLQADFIGVVRNNEALARLSCAFKTENQT
RQVVCDLGNPMKAGTQLLAGLRFSVHQQSEMDTSVKFDLQIQSSNLFDKVSPV
VSHKVDLAVLAAVEIRGVSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRN
NGPSSFSKAMLHLQWPYKYNNNTLLYILHYDIDGPMNCTSDMEINPLRIKISSLQ
TTEKNDTVAGQGERDHLITKRDLALSEGDIHTLGCGVAQCLKIVCQVGRLDRGK
SAILYVKSLLWTETFMNKENQNHSYSLKSSASFNVIEFPYKNLPIEDITNSTLVTT
NVTWGIQPAPMPVPVWVIILAVLAGLLLLAVLVFVMYRMGFFKRVRPPQEEQE
REQLQPHENGEGNSET

(19) The teratocarcinoma-derived growth factor 1 protein TDGF1 (GenBank Accession No.: NP_003203.1)

>gi|4507425|ref|NP_003203.1| teratocarcinoma-derived growth factor 1 isoform 1 precursor [*Homo sapiens*]

MDCRKMARFSYSVIWIMAISKVFELGLVAGLGHQEFARPSRGYLAFRDDSIWPQ
EEPAIRPRSSQRVPPMGIQHSKELNRTCCLNGGTCMLGSFCACPPSFYGRNCEHD

VRKENCGSVPHDTWLPKKCSLCKCWHGQLRCFPQAFLPGCDGLVMDEHLVAS
RTPELPPSARTTTFMLVGICLSIQSYY

(20) The prostate-specific membrane antigen PSMA (SwissProt ID: Q04609)

>gi|4758398|ref|NP_004467.1| glutamate carboxypeptidase 2 isoform 1 [*Homo sapiens*]

MWNLLHETDSAVATARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEATNITP
KHNMKAFLDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKEFGLDSV
ELAHYDVLLSYPNKTHPNYISIINEDGNEIFNTSLFEPPPPGYENVSDIVPPFSAFSP
QGMPEGDLVYVNYARTEDFFKLERDMKINCSGKIVIARYGKVFRGNKVKNAQL
AGAKGVILYSDPADYFAPGVKSYPDGWNLPGGGVQRGNILNLNGAGDPLTPGY
PANEYAYRRGIAEAVGLPSIPVHPIGYYDAQKLLEKMGGSAPPDSSWRGSLKVP
YNVGPGFTGNFSTQKVKMHIHSTNEVTRIYNVIGTLRGAVEPDRYVILGGHRDS
WVFGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTILFASWDAEEFGLLGSTEW
AEENSRLLQERGVAYINADSSIEGNYTLRVDCTPLMYSLVHNLTKELKSPDEGFE
GKSLYESWTKKSPSPEFSGMPRISKLGSGNDFEVFFQRLGIASGRARYTKNWETN
KFSGYPLYHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVFELANSIVLPFDCR
DYAVVLRKYADKIYSISMKHPQEMKTYSVSFDSLFSAVKNFTEIASKFSERLQDF
DKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGESFPGI
YDALFDIESKVDPSKAWGEVKRQIYVAAFTVQAAAETLSEVA

(21) Tyrosine protein kinase EPHA2 (SwissProt ID: P29317)

>gi|32967311|ref|NP_004422.2| ephrin type-A receptor 2 precursor [*Homo sapiens*]

MELQAARACFALLWGCALAAAAAAQGKEVVLLDFAAAGGELGWLTHPYGKG
WDLMQNIMNDMPIYMYSVCNVMSGDQDNWLRTNWVYRGEAERIFIELKFTVR
DCNSFPGGASSCKETFNLYYAESDLDYGTNFQKRLFTKIDTIAPDEITVSSDFEAR
HVKLNVEERSVGPLTRKGFYLAFQDIGACVALLSVRVYYKKCPELLQGLAHFPE
TIAGSDAPSLATVAGTCVDHAVVPPGGEEPRMHCAVDGEWLVPIGQCLCQAGY
EKVEDACQACSPGFFKFEASESPCLECPEHTLPSPEGATSCECEEGFFRAPQDPAS
MPCTRPPSAPHYLTAVGMGAKVELRWTPPQDSGGREDIVYSVTCEQCWPESGE
CGPCEASVRYSEPPHGLTRTSVTVSDLEPHMNYTFTVEARNGVSGLVTSRSFRTA
SVSINQTEPPKVRLEGRSTTSLSVSWSIPPPQQSRVWKYEVTYRKKGDSNSYNVR
RTEGFSVTLDDLAPDTTYLVQVQALTQEGQGAGSKVHEFQTLSPEGSGNLAVIG
GVAVGVVLLLVLAGVGFFIHRRRKNQRARQSPEDVYFSKSEQLKPLKTYVDPHT
YEDPNQAVLKFTTEIHPSCVTRQKVIGAGEFGEVYKGMLKTSSGKKEVPVAIKT

LKAGYTEKQRVDFLGEAGIMGQFSHHNIIRLEGVISKYKPMMIITEYMENGALD
KFLREKDGEFSVLQLVGMLRGIAAGMKYLANMNYVHRDLAARNILVNSNLVC
KVSDFGLSRVLEDDPEATYTTSGGKIPIRWTAPEAISYRKFTSASDVWSFGIVMW
EVMTYGERPYWELSNHEVMKAINDGFRLPTPMDCPSAIYQLMMQCWQQERAR
RPKFADIVSILDKLIRAPDSLKTLADFDPRVSIRLPSTSGSEGVPFRTVSEWLESIK
MQQYTEHFMAAGYTAIEKVVQMTNDDIKRIGVRLPGHQKRIAYSLLGLKDQVN
TVGIPI

(22) The surface protein SLC44A4 (GenBank Accession No. NP_001171515)

>gi|295849282|ref|NP_001171515.1| choline transporter-like protein 4 isoform 2 [*Homo sapiens*]

MGGKQRDEDDEAYGKPVKYDPSFRGPIKNRSCTDVICCVLFLLFILGYIVVGIVA
WLYGDPRQVLYPRNSTGAYCGMGENKDKPYLLYFNIFSCILSSNIISVAENGLQC
PTPQTVITSLQQELCPSFLLPSAPALGRCFPWTNVTPPALPGITNDTTIQQGISGLID
SLNARDISVKIFEDFAQSWYWILVALGVALVLSLLFILLLRLVAGPLVLVLILGVL
GVLAYGIYYCWEEYRVLRDKGASISQLGFTTNLSAYQSVQETWLAALIVLAVLE
AILLLMLIFLRQRIRIAIALLKEASKAVGQMMSTMFYPLVTFVLLLICIAYWAMT
ALYLATSGQPQYVLWASNISSPGCEKVPINTSCNPTAHLVNSSCPGLMCVFQGYS
SKGLIQRSVFNLQIYGVLGLFWTLNWVLALGQCVLAGAFASFYWAFHKPQDIPT
FPLISAFIRTLRYHTGSLAFGALILTLVQIARVILEYIDHKLRGVQNPVARCIMCCF
KCCLWCLEKFIKFLNRNAYIMIAIYGKNFCVSAKNAFMLLMRNIVRVVVLDKVT
DLLLFFGKLLVVGGVGVLSFFFFSGRIPGLGKDFKSPHLNYYWLPIMTSILGAYVI
ASGFFSVFGMCVDTLFLCFLEDLERNNGSLDRPYYMSKSLLKILGKKNEAPPDN
KKRKK

(23) The surface protein BMPR1B (SwissProt: O00238)

(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)

(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)

(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)

(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)

(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)

(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

>sp|Q9NZQ7|PD1L1_HUMAN Programmed cell death 1 ligand 1 OS=*Homo sapiens* GN=CD274 PE=1 SV=1

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALI
VYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQ
DAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYP
KAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPE
ENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKC
GIQDTNSKKQSDTHLEET

(53) Single-pass type I membrane protein "ICOSLG" (synonyms:B7H2, B7RP1, ICOSL, KIAA0653, CD275) – (SwissProt: O75144), both are isoforms

>sp|O75144|ICOSL_HUMAN ICOS ligand OS=*Homo sapiens* GN=ICOSLG PE=1 SV=2

MRLGSPGLLFLLFSSLRADTQEKEVRAMVGSDVELSCACPEGSRFDLNDVYVY
WQTSESKTVVTYHIPQNSSLENVDSRYRNRALMSPAGMLRGDFSLRLFNVTPQD
EQKFHCLVLSQSLGFQEVLSVEVTLHVAANFSVPVVSAPHSPSQDELTFTCTSIN
GYPRPNVYWINKTDNSLLDQALQNDTVFLNMRGLYDVVSVLRIARTPSVNIGCC
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)

>sp|P22607|FGFR3_HUMAN Fibroblast growth factor receptor 3 OS=*Homo sapiens* GN=FGFR3 PE=1 SV=1

MGAPACALALCVAVAIVAGASSESLGTEQRVVGRAAEVPGPEPGQQEQLVFGS
GDAVELSCPPPGGGPMGPTVWVKDGTGLVPSERVLVGPQRLQVLNASHEDSGA
YSCRQRLTQRVLCHFSVRVTDAPSSGDDEDGEDEAEDTGVDTGAPYWTRPERM
DKKLLAVPAANTVRFRCPAAGNPTPSISWLKNGREFRGEHRIGGIKLRHQQWSL
VMESVVPSDRGNYTCVVENKFGSIRQTYTLDVLERSPHRPILQAGLPANQTAVL
GSDVEFHCKVYSDAQPHIQWLKHVEVNGSKVGPDGTPYVTVLKTAGANTTDKE

LEVLSLHNVTFEDAGEYTCLAGNSIGFSHHSAWLVVLPAEEELVEADEAGSVYA
GILSYGVGFFLFILVVAAVTLCRLRSPPKKGLGSPTVHKISRFPLKRQVSLESNAS
MSSNTPLVRIARLSSGEGPTLANVSELELPADPKWELSRARLTLGKPLGEGCFGQ
VVMAEAIGIDKDRAAKPVTVAVKMLKDDATDKDLSDLVSEMEMMKMIGKHK
NIINLLGACTQGGPLYVLVEYAAKGNLREFLRARRPPGLDYSFDTCKPPEEQLTF
KDLVSCAYQVARGMEYLASQKCIHRDLAARNVLVTEDNVMKIADFGLARDVH
NLDYYKKTTNGRLPVKWMAPEALFDRVYTHQSDVWSFGVLLWEIFTLGGSPYP
GIPVEELFKLLKEGHRMDKPANCTHDLYMIMRECWHAAPSQRPTFKQLVEDLD
RVLTVTSTDEYLDLSAPFEQYSPGGQDTPSSSSSGDDSVFAHDLLPPAPPSSGGSR
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 OS=*Homo sapiens* GN=TYRP1 PE=1 SV=2

MSAPKLLSLGCIFFPLLLFQQARAQFPRQCATVEALRSGMCCPDLSPVSGPGTDR
CGSSSGRGRCEAVTADSRPHSPQYPHDGRDDREVWPLRFFNRTCHCNGNFSGH
NCGTCRPGWRGAACDQRVLIVRRNLLDLSKEEKNHFVRALDMAKRTTHPLFVI
ATRRSEEILGPDGNTPQFENISIYNYFVWTHYYSVKKTFLGVGQESFGEVDFSHE
GPAFLTWHRYHLLRLEKDMQEMLQEPSFSLPYWNFATGKNVCDICTDDLMGSR
SNFDSTLISPNSVFSQWRVVCDSLEDYDTLGTLCNSTEDGPIRRNPAGNVARPMV
QRLPEPQDVAQCLEVGLFDTPPFYSNSTNSFRNTVEGYSDPTGKYDPAVRSLHN
LAHLFLNGTGGQTHLSPNDPIFVLLHTFTDAVFDEWLRRYNADISTFPLENAPIG
HNRQYNMVPFWPPVTNTEMFVTAPDNLGYTYEIQWPSREFSVPEIIAIAVVGALL
LVALIFGTASYLIRARRSMDEANQPLLTDQYQCYAEEYEKLQNPNQSVV

(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

MAGTVRTACLVVAMLLSLDFPGQAQPPPPPPDATCHQVRSFFQRLQPGLKWVP
ETPVPGSDLQVCLPKGPTCCSRKMEEKYQLTARLNMEQLLQSASMELKFLIIQN
AAVFQEAFEIVVRHAKNYTNAMFKNNYPSLTPQAFEFVGEFFTDVSLYILGSDIN
VDDMVNELFDSLFPVIYTQLMNPGLPDSALDINECLRGARRDLKVFGNFPKLIM

TQVSKSLQVTRIFLQALNLGIEVINTTDHLKFSKDCGRMLTRMWYCSYCQGLM
MVKPCGGYCNVVMQGCMAGVVEIDKYWREYILSLEELVNGMYRIYDMENVLL
GLFSTIHDSIQYVQKNAGKLTTTIGKLCAHSQQRQYRSAYYPEDLFIDKKVLKVA
HVEHEETLSSRRRELIQKLKSFISFYSALPGYICSHSPVAENDTLCWNGQELVERY
SQKAARNGMKNQFNLHELKMKGPEPVVSQIIDKLKHINQLLRTMSMPKGRVLD
KNLDEEGFESGDCGDDEDECIGGSGDGMIKVKNQLRFLAELAYDLDVDDAPGN
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) though (56).

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 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 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-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 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.

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 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 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., 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 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, 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):

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGS
GSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC

Cetuximab heavy chain:

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYN
TPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSAA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
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):

DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPS
RFSGSGSGTDFTFTISSLQPEDIATYFCQHFDHLPLAFGGGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Panitumumab heavy chain:

QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKGLEWIGHIYYSGNTN
YNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTMVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSV
LTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSC
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 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:

- 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)

- 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)

- 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)

- 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)

- 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)

- 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, 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 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 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

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 particular human or humanized anti-CAIX antibodies. The antibodies preferably have an affinity of at least 10^{-7} M (as the Kd value, i.e., preferably those with Kd 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 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. The 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 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 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 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 rate. 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 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 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 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

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.

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,

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,

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,

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 he 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: GISSLGSTTYYADSVKG; 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 TYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTGSPGTFMHHGDHWGQ 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 GVPSRFSGSGSGTDFTLTISLQPEDFAVYYCQQYYGRPTTFGQGTKVEIKRT, 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:

QVELVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVSGISSLGSTTYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTGSPGTFMHGDHWGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

and the amino acid sequence of the light chain comprises the following sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDINNYLSWYQQKPGKAPKLLIYGASNLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFAVYYCQQYYGRPTTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA
DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

anti-carboanhydrase IX antibody 3ee9-IgG1:

Another aspect of the present invention is supplying the anti-carboanhydrase IX antibody 3ee9-IgG1.

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 Kd value, i.e., preferably those with Kd 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 Kd values can be 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 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 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, 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 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.

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 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).

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 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.

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 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 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 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).

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 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 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: 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),

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 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),

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),

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),

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).

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), 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 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 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 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 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 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 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.

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),

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

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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.

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 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 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 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).

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 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.

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.

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.

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).

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,

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),

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

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

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),

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),

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:

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 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 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).

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 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).

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).

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).

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.

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.

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.

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.

Examples of antibodies that bind the cancer target molecule TDGF1 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-TDGF1 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.

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.

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.

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 antibody comprises the following sequences;

Light chain:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLVWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQL
KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYGFSWVRQAPGQGLEWMGWITAYNGNT
NYAQKLQGRVTMTTDTSTSTVYMELRSLRSDDTAVYYCARDYFYGMDVWGQGTTVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPG

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:

DIQMTQSPSSLSASVGDRVTITCRASQGISNWLAWYQQKPEKAPKSLIYAASSLQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYDSYPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQL
KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

EVQLVESGGGLVQPGGSLRLSCAGSGFTFSSYWMSWVRQAPGKGLEWVAYIKQDGNEK
YYVDSVKGRFTISRDNAKKSLYLQMNSLRAEDTAVYYCAREGILWFGDLPTFWGQGILVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPG

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:

DIQLTQSPSSLSASVGDRVTITCSASSSVSYMYWFQQKPGKAPKPLIYLTSYLASGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSYPLTFGGGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYNMYWVRQMPGKGLEWMGYIDPYNGGTS
YNQKFKGKATLTVDKSISTAYLQWSSLKASDTAMYYCAREGGNYEAWFAYWGQGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPG

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:

Light chain

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWLMYTFGQGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

QVQLVQSGSELKKPGASVKISCKASGYTFTSYAMNWVRQAPGQGLESMGWINTNTGNPT
YAQGFTGRFVFSMDTSVSTAYLQISSLKAEDTAIYYCAPRYSSSWYLDYWGQGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPG

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:

DVVMTQSPLSLPVTPGEPASISCRSSQSLVHSNGNTYLHWYLQKPGQSPQLLIYKVSNRFS
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQNTHVPPTFGQGTKLEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

QVQLVESGAEVKKPGASVKVSCKASGYTFTDYEMHWVRQAPGQGLEWMGALDPKTGD
TAYSQKFKGRVTITADESTSTAYMELSSLRSEDTAVYYCARFYSYTYWGQGTLVTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
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 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 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 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 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 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 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 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., 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 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 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.

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 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 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 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 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.

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 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.

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.

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 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 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.

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, 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, 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, ethyol, etidronic acid, etopophos, etoposide, fadrozole, farstone, filgrastim, finasteride, fligrastim, floxuridine, fluconazole, fludarabin, 5-fluorodeoxyuridine monophosphate, 5-fluorouracil (5-FU), fluoxymesterone, flutamide, formestane, fosteabine, fotemustine, fulvestrant, gammagard, gemcitabine, gemtuzumab, gleevec, gliadel, gosereline, granisetrone hydrochloride, histreline, hycamtine, hydrocortone, erythrohydroxynonyladenine, hydroxyurea, ibritumomab tiuxetan, idarubicin, ifosfamide, interferon-alpha, interferon-alpha-2, interferon-alpha-2α, interferon-alpha-2β, interferon-alpha-n1, interferon-alpha-n3, interferon-beta, interferon-gamma-1α, 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, melphalane, menest, 6-mercaptopurine, mesna, methotrexate, metvix, miltefosine, minocycline, mitomycin C, mitotane, mitoxantrone, modrenal, myocet, nedaplatin, neulasta, neumega, neupogen, nilutamide, nolvadex, NSC-631570, OCT-43, octreotide, ondansetrone hydrochloride, orapred, oxaliplatin, paclitaxel, pediapred, pegaspargase, pegasys, pentostatin, picibanil, pilocarpine hydrochloride, pirarubicin, plicamycin, porfimer sodium, prednimustin, prednisolone, prednisone, premarin, procarbazine, procrit, raltitrexed, rebif, rhenium 186 etidronate, rituximab, roferone A, 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, triptoreline pamoate, uft, uridine, valrubicin, vesnarinone, vinblastine, vincristine, vindesine, vinorelbine, virulizine, zinecard, zinostatin stimalamer, zofran; ABI-007, acolbifene, actimmune, affinitak, aminopterine, 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, 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, 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 with antihyperproliferative agents, which may include the following, for example, although this list is not conclusive:

aminoglutethimide, L-asparaginase, azathioprine, 5-azacytidine, bleomycin, busulfan, carboplatin, carmustin, chlorambucil, cisplatin, colaspase, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, diethylstilbestrol, 2',2'-difluorodeoxycytidine, docetaxel, doxorubicin (adriamycin), epirubicin, epothilone and seine derivate, erythrohydroxynonyladenine, ethinyl estradiol, etoposide, fludarabine phosphate, 5-fluorodeoxyuridine, 5-fluordeoxyuridine monophosphate, 5-fluoruracil, fluoxymesterone, flutamide, hexamethyl melamine, hydroxyurea, hydroxyprogesterone caproate, idarubicin, ifosfamide, interferon, irinotecan, leucovorin, lomustine, mechlorethamine, medroxyprogesterone acetate, megestrol acetate, melphalan, 6-mercaptopurine, 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, 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:

- 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 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 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 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 intralumbarly) or with the inclusion 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:

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 and related substances (glucose, saccharose, mannitol, trehalose, sucrose, mannose, lactose, sorbitol), glycerol, sodium, potassium, ammonium and calcium salts (e.g., NaCl, KCl or Na_2HPO_4 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, 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 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 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 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	*tert*-butoxycarbonyl
br.	broad (in NMR)
Ex.	example
ca.	*circa*, 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-*N,N*-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMEM	Dulbecco's modified eagle medium (standardized nutrient medium for the cell culture)
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulphoxide

DPBS, D-PBS, PBS Dulbecco's phosphate-buffered saline solution

PBS = DPBS = D-PBS, pH 7.4, from Sigma, No. D8537

Composition:

0.2 g KCl

0.2 g KH_2PO_4 (anhydrous)

8.0 g NaCl

1.15 g Na_2HPO_4 (anhydrous)

make up to 1 l with H_2O

dt	doublet of a triplet (in NMR)
DTT	DL-dithiothreitol
EDC	*N'*-(3-dimethylaminopropyl)-*N*-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	ESI- MicroTofq (name of the mass spectrometer, with Tof = time of flight and q = quadrupole)
FCS	foetal calf serum
Fmoc	(9*H*-fluoren-9-ylmethoxy)carbonyl
sat.	Saturated
GTP	guanosine 5'-triphosphate
h	hour(s)
HATU	*O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HCT-116	human tumour cell line
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulphonic acid
HOAc	acetic acid
HOBt	1-hydroxy-1*H*-benzotriazole hydrate

HOSu	*N*-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 resistence protein 1
min	minute(s)
MS	mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide
NCI-H292	human tumour cell line
NCI-H520	human tumour cell line
NMM	*N*-methylmorpholine
NMP	*N*-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)
tert	tertiary
TFA	trifluoroacetic acid
THF	tetrahydrofuran
UV	ultraviolet spectrometry
v/v	volume to volume ratio (of a solution)
Z	benzyloxycarbonyl

HPLC and LC-MS methods:

Method 1 (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% strength formic acid, eluent B: 1 l acetonitrile + 0.25 ml 99% strength formic 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 GOLD 1.9µ 50 mm x 1 mm; eluent A: 1 l water + 0.5 ml 50% strength formic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strength formic 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 Hypersil GOLD 3µ 20 mm x 4 mm; eluent A: 1 l water + 0.5 ml 50% strength formic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strength formic 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 3µ 30 mm x 3.00 mm; eluent A: 1 l water + 0.5 ml 50% strength formic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strength formic acid; gradient: 0.0 min 90% A → 2.5 min 30% A → 3.0 min 5% A → 4.5 min 5% A; flow rate: 0.0 min 1 ml/min → 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 Speed ROD 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 µl; eluent A: 70% $HClO_4$ in water (4 ml/litre), eluent B: acetonitrile; gradient: 0.00 min 20% B → 0.50 min 20% B → 3.00 min 90% B → 3.50 min 90% B → 3.51 min 20% B → 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% $HClO_4$ 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.

Method 7 (LC-MS):

MS instrument: Waters ZQ; HPLC instrument: Agilent 1100 Series; UV DAD; column: Thermo Hypersil GOLD 3µ 20 mm x 4 mm; eluent A: 1 l water + 0.5 ml 50% strength formic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strength formic acid; gradient: 0.0 min 100% A → 3.0 min 10% A → 4.0 min 10% A → 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 3µ 20 mm x 4 mm; eluent A: 1 l water + 0.5 ml 50% strength formic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% strength formic acid; gradient: 0.0 min 100% A → 2.0 min 60% A → 2.3 min 40% A → 3.0 min 20% A → 4.0 min 10% A → 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% strength formic acid, eluent B: 1 l acetonitrile + 0.25 ml 99% strength formic acid; gradient: 0.0 min 95% A → 6.0 min 5% A → 7.5 min 5% A; 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 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 reacents whose preparation is likewise not described below, and which were not available commercially 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 + 0.25 ml 99% strength formic 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 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% $HClO_4$ 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.

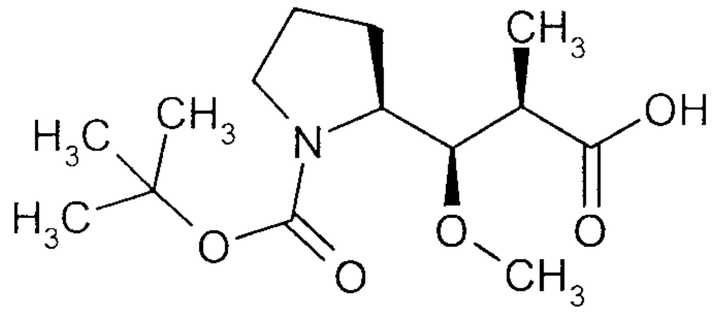
Method 13 (LC-MS):

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 ammonium carbonate, eluent B: 1 l acetonitrile; gradient: 0.0 min 100% A → 2.75 min 5% A → 4.5 min 5% A; oven: 40°C; flow rate: 1.25 ml/min; UV detection: 210 nm.

Starting Compounds and Intermediates:

Starting Compound 1

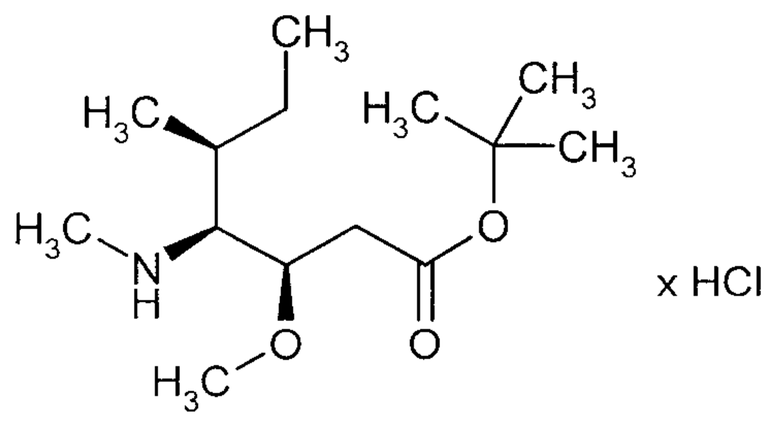
(2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Boc-dolaproine)



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 the free acid or as a 1:1 salt with dicyclohexylamine.

Starting Compound 2a

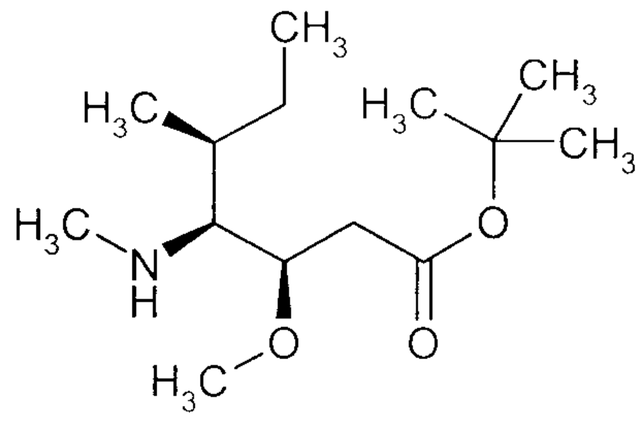
tert-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-(methylamino)heptanoate hydrochloride (dolaisoleucine-OtBu x HCl)



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

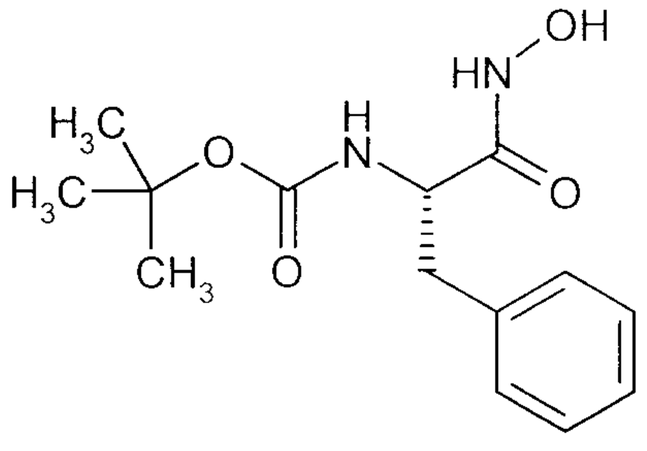
tert-butyl-(3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-(methylamino)heptanoate (dolaisoleucine-O^{t}Bu)



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

N^{α}-(*tert*-butoxycarbonyl)-*N*-hydroxy-L-phenylalaninamide



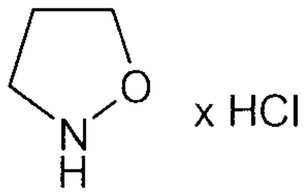
The title compound was prepared by the literature method (A. Ritter *et al.*, *J. Org. Chem.* 1994, *59*, 4602).

Yield: 750 mg (75% of theory)

LC-MS (Method 3): R_t = 1.67 min; MS (ESIpos): m/z = 281 $(M+H)^+$.

Starting Compound 4

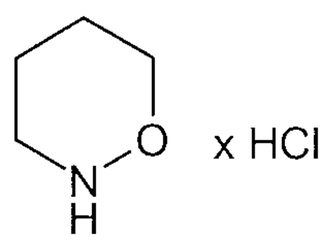
1,2-oxazolidine hydrochloride



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

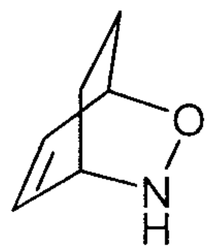
1,2-oxazinane hydrochloride



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

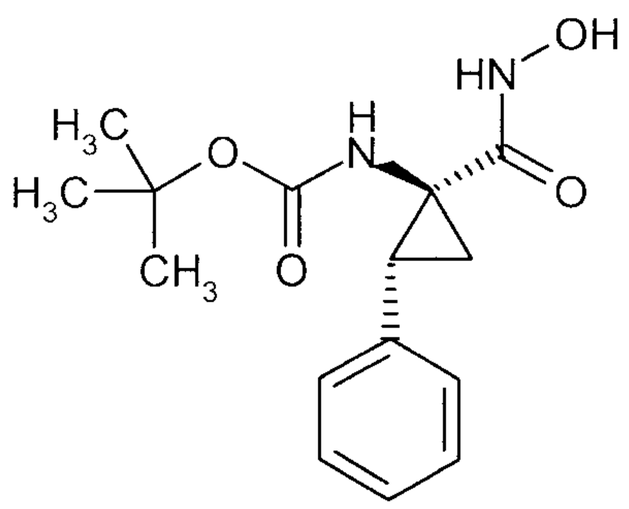


The title compound can be prepared in Boc-protected form by the literature method (see, for example, C. Johnson *et al.*, *Tetrahedron Lett.* 1998, *39*, 2059); the deprotection was effected in a customary manner by treatment with trifluoroacetic acid and subsequent neutralization.

Yield: 149 mg (89% of theory)

Starting Compound 7

tert-butyl (1*S*,2*R*)-1-(hydroxycarbamoyl)-2-phenylcyclopropyl carbamate



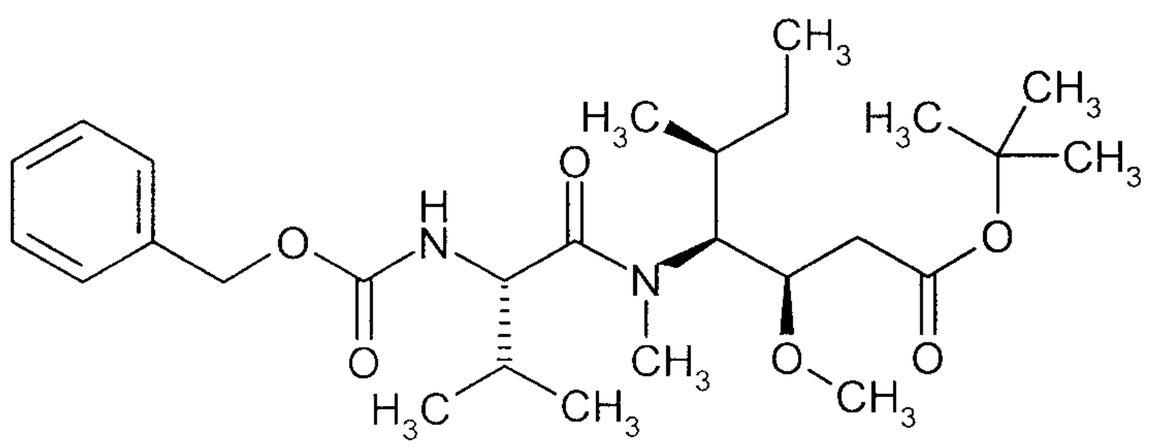
The title compound was prepared by a literature method (A. Ritter *et al.*, *J. Org. Chem.* 1994, *59*, 4602) proceeding from commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid (C. Cativiela *et al.*, *Chirality* 1999, 11, 583).

Yield: 339 mg (59% of theory)

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}(methyl)amino]-3-methoxy-5-methyl heptanoate



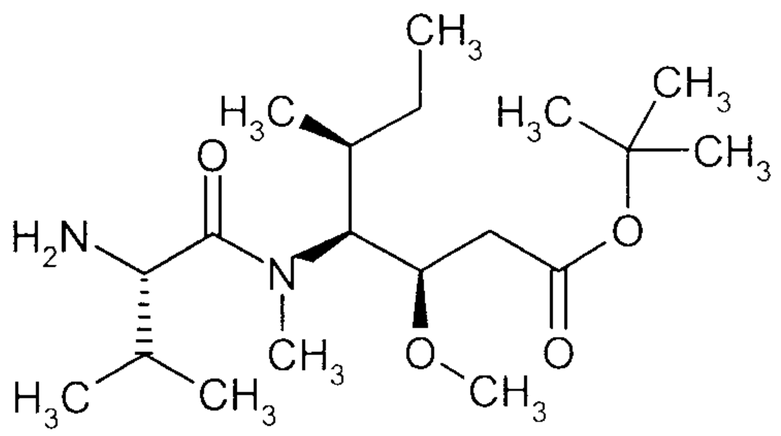
10.65 g (41.058 mmol) of *tert*-butyl (3*R*,4*S*,5*S*)-3-methoxy-5-methyl-4-(methylamino)-heptanoate (starting compound 2b) were taken up in 250 ml of dichloromethane, and the solution was cooled to -10°C. Then, while stirring, 10.317 g (41.058 mmol) of N-[(benzyloxy)carbonyl]-L-valine, 16.866 g (61.586 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 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 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



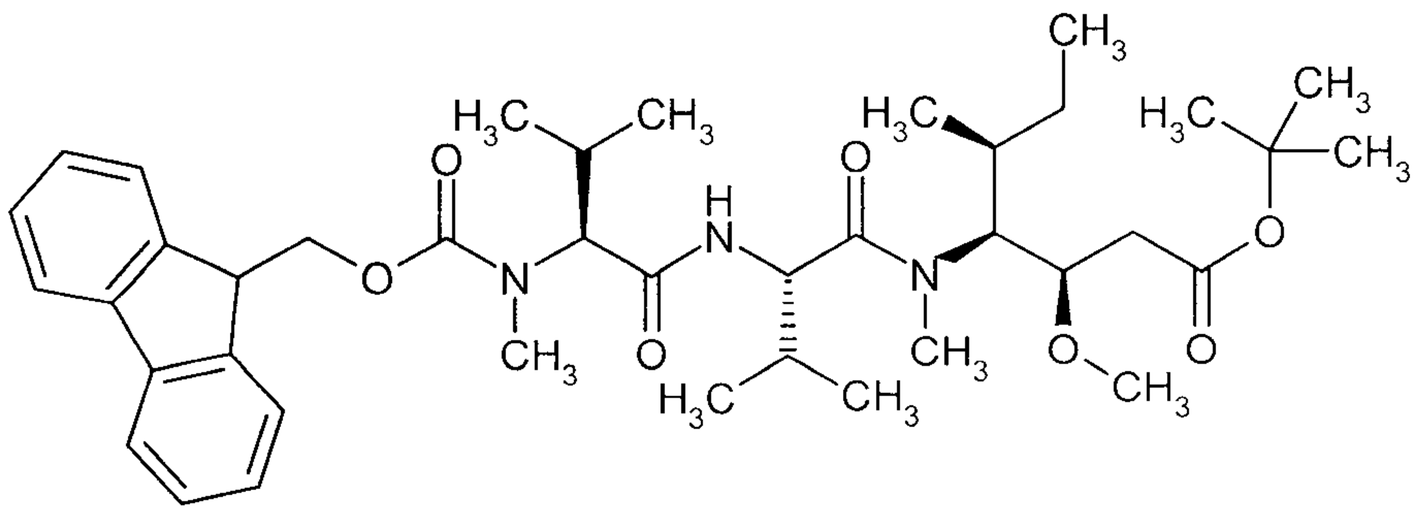
500 mg (1 mmol) of *tert*-butyl (3*R*,4*S*,5*S*)-4-[{*N*-[(benzyloxy)carbonyl]-L-valyl}(methyl)amino]-3-methoxy-5-methylheptanoate (intermediate 1) were dissolved in 50 ml of methanol and, after addition of 100 mg of 10% palladium on activated carbon, hydrogenated under standard hydrogen pressure at 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 $(M+H)^+$.

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.

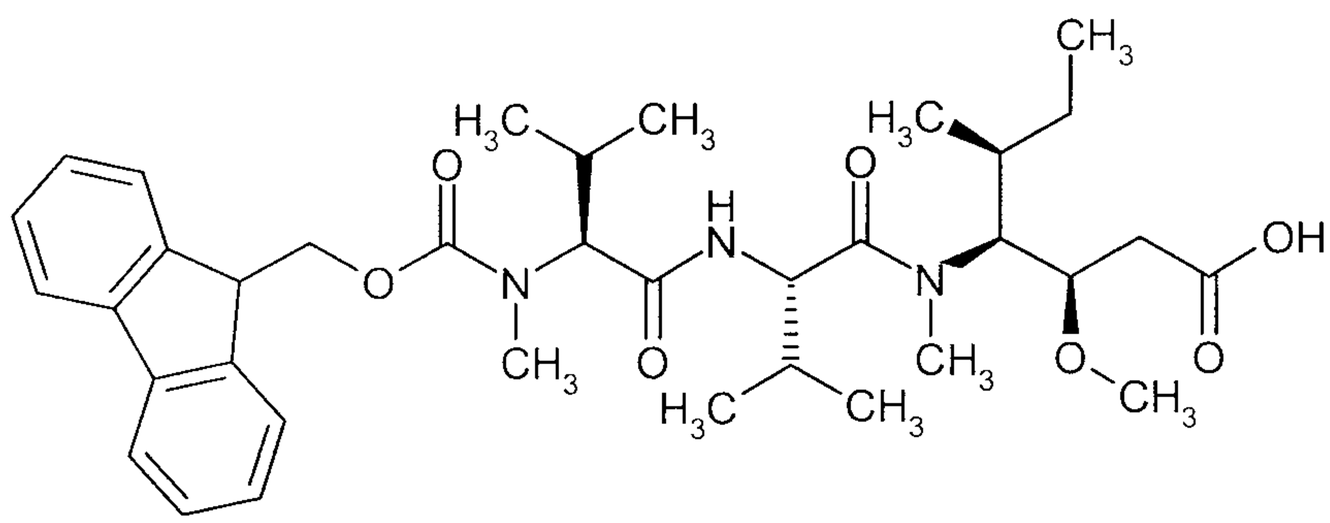
Yield: 9.1 g (quant., 60% purity)

HPLC (Method 5): R_t = 2.7 min;

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



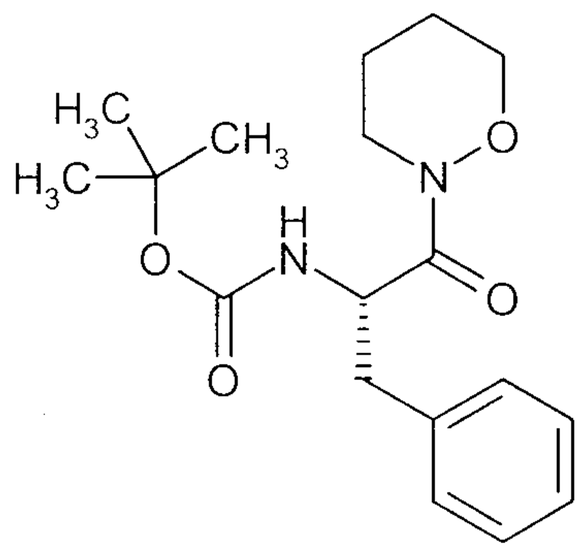
9.1 g of the crude product *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-*tert*-butoxy-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 3) were taken up in 56.6 ml of dichloromethane, 56.6 ml of trifluoroacetic acid were added, and the mixture 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 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 $(M+H)^+$.

Intermediate 5

tert-butyl (2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl carbamate



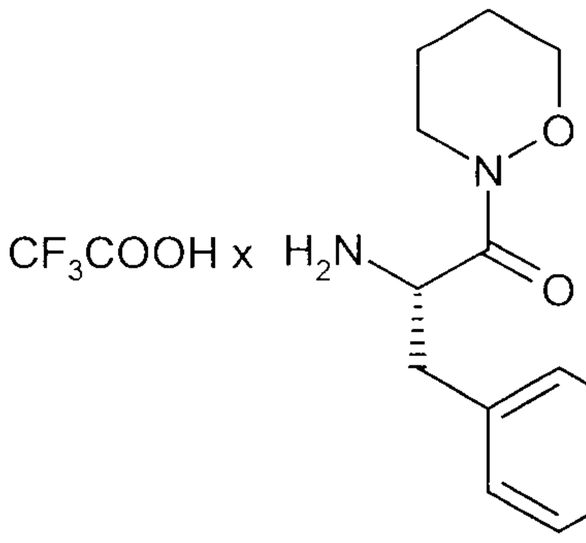
500 mg (1.9 mmol) of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine were dissolved in 10 ml of DMF and admixed successively with 466 mg (3.8 mmol) of 1,2-oxazinane hydrochloride (Starting Compound 5), 433 mg (2.3 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 382 mg (2.8 mmol) of 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



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

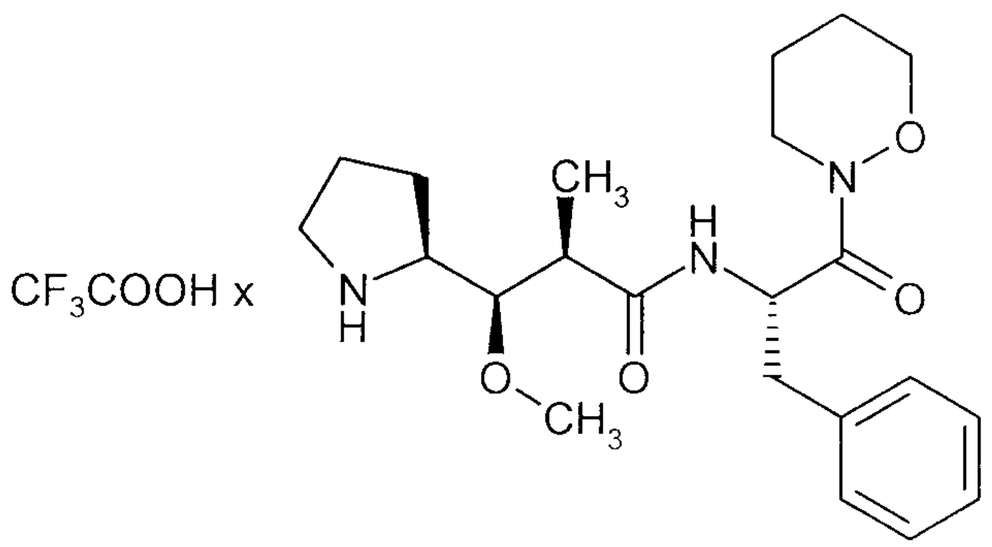
was concentrated *in vacuo*, and the remaining residue was lyophilized from water/acetonitrile. In this way, 779 mg (91% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 5): R_t = 0.45 min;

LC-MS (Method 3): R_t = 1.09 min; MS (ESIpos): m/z = 235 $(M+H)^+$.

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 µl 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 $(M+H)^+$.

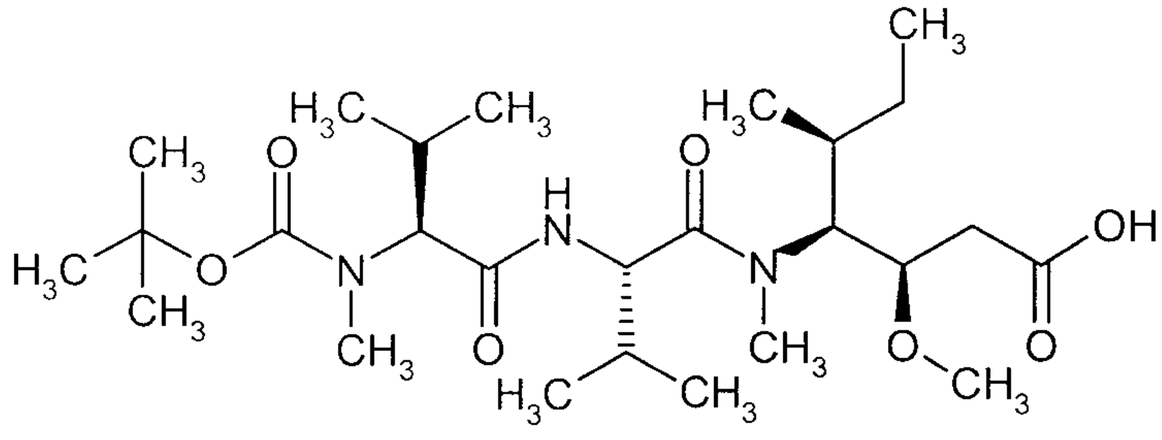
503 mg (1 mmol) of this intermediate were taken up in 20 ml of dichloromethane, 10 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated *in vacuo* and redistilled with dichloromethane. The remaining residue was precipitated 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;

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



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

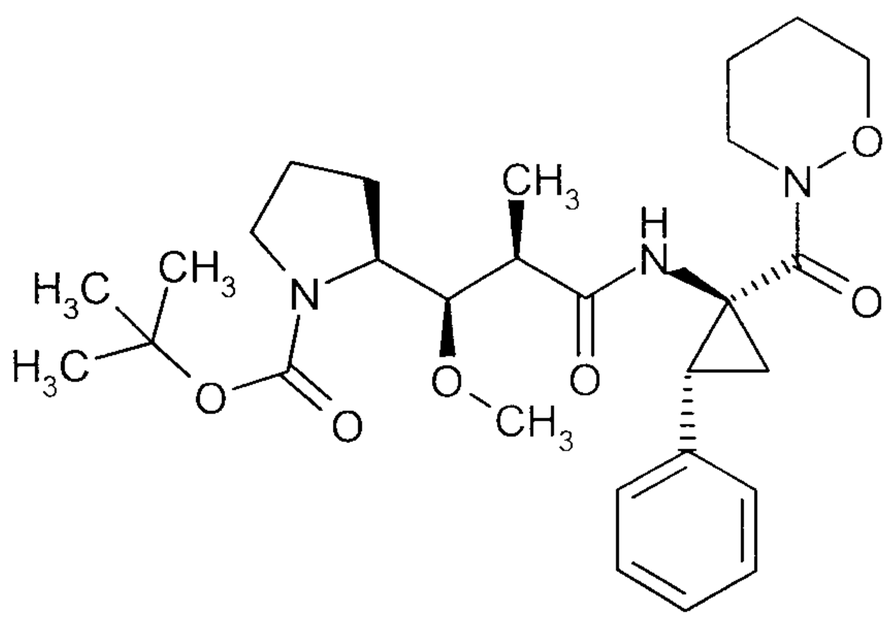
decantation. The residue was digested several times more with pentane and finally dried under high vacuum. 40 mg (97% of theory) of the title compound were thus obtained.

HPLC (Method 6): R_t = 2.2 min;

LC-MS (Method 2): R_t = 1.32 min; MS (ESIpos): m/z = 516 $(M+H)^+$.

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



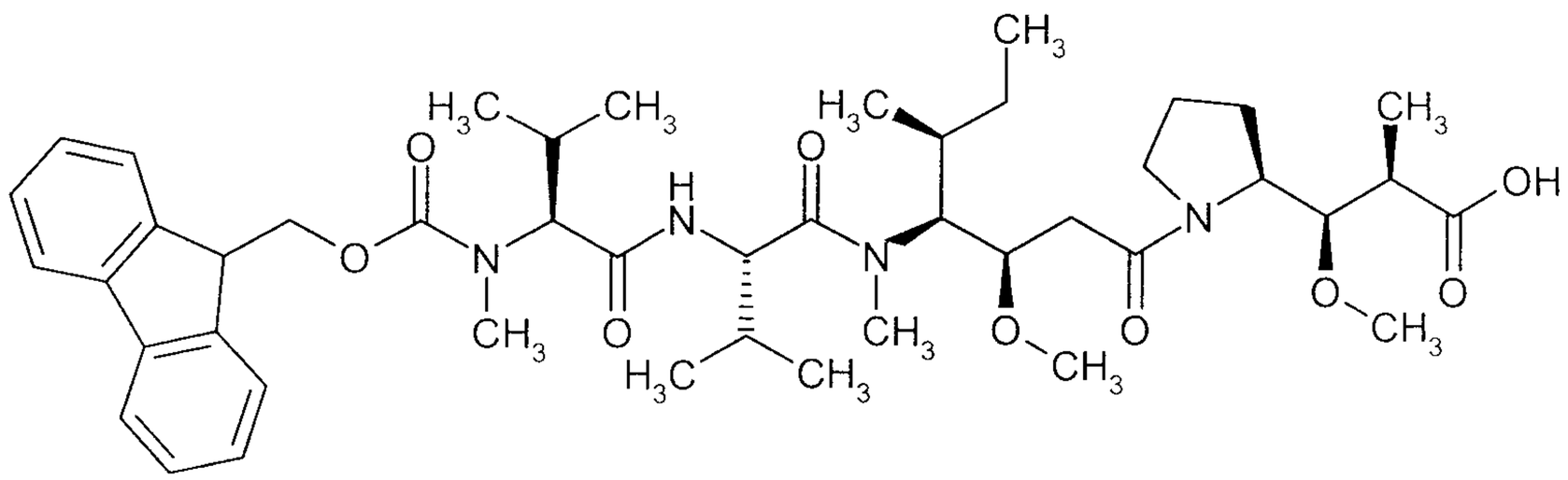
The title compound was prepared in analogy to the synthesis of Intermediates 5, 6 and 7 over three stages by coupling of commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with 1,2-oxazinane hydrochloride (Starting Compound 5), subsequent deprotection 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;

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



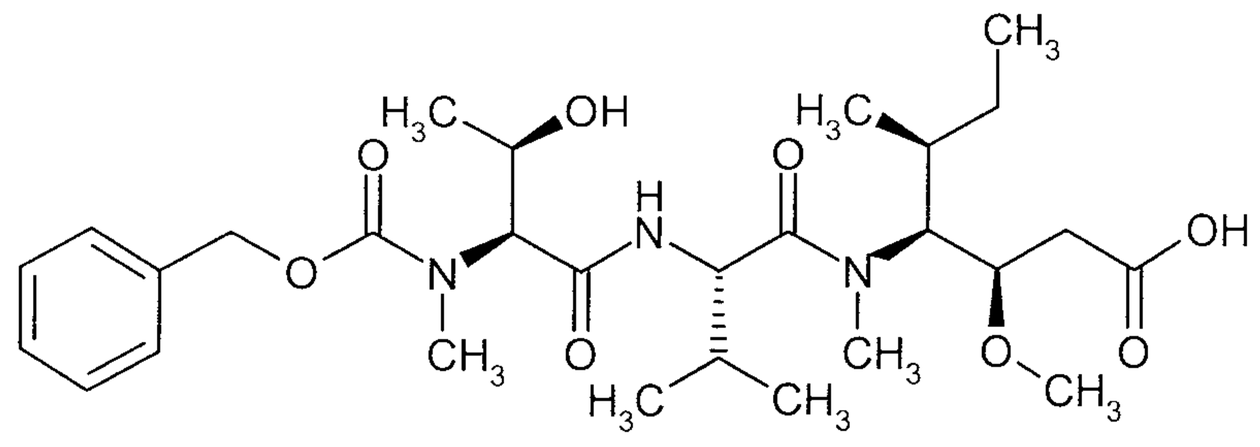
315 mg (0.494 mmol) of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) were dissolved in 12 ml of DMF and admixed with 104 mg (0.543 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide 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 µl 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 $(M+H)^+$.

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.

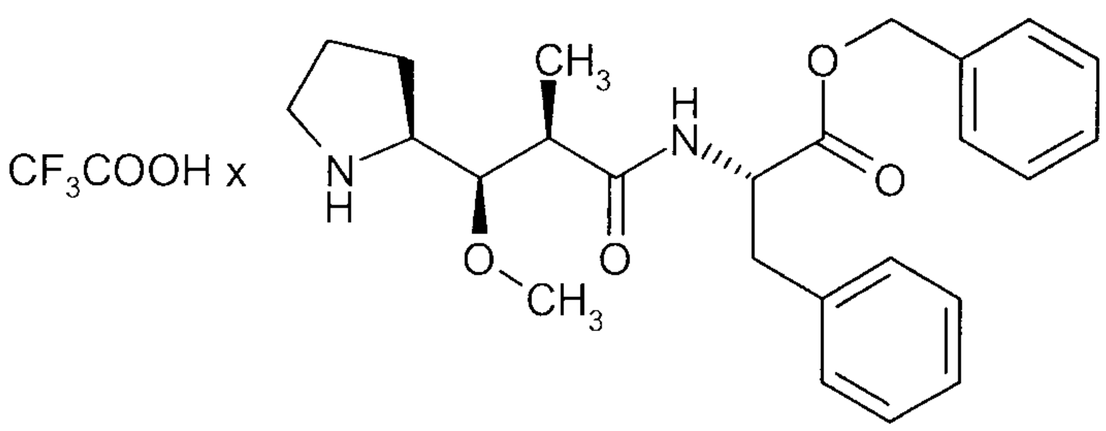
283 mg (0.466 mmol) of this intermediate were taken up in 5 ml of dichloromethane, 5 ml of anhydrous trifluoroacetic acid were added, and the mixture was stirred at RT for 2 h. Subsequently, the reaction mixture was concentrated under high vacuum and the remaining residue was purified by means of preparative 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 $(M+H)^+$.

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.

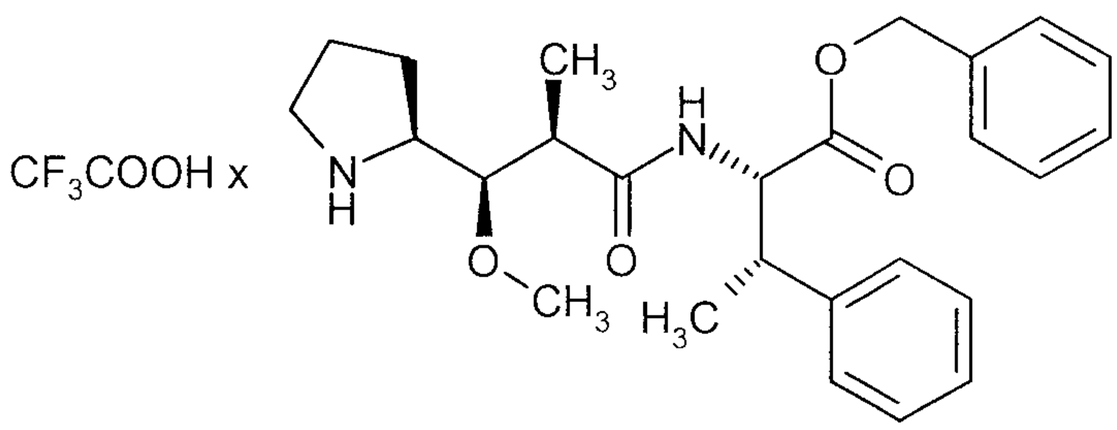
Yield: 650 mg (94% over 2 stages)

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-(β*S*)-*N*-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-β-methyl-L-phenylalaninate trifluoroacetate



First, (2*R*,3*R*)-3-[(2*S*)-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-(β*S*)-β-methyl-L-phenylalaninate trifluoroacetate [prepared from commercially available (β*S*)-*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

intermediate benzyl-(β*S*)-*N*-{(2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoyl}-β-methyl-L-phenylalaninate.

HPLC (Method 6): R_t = 2.3 min;

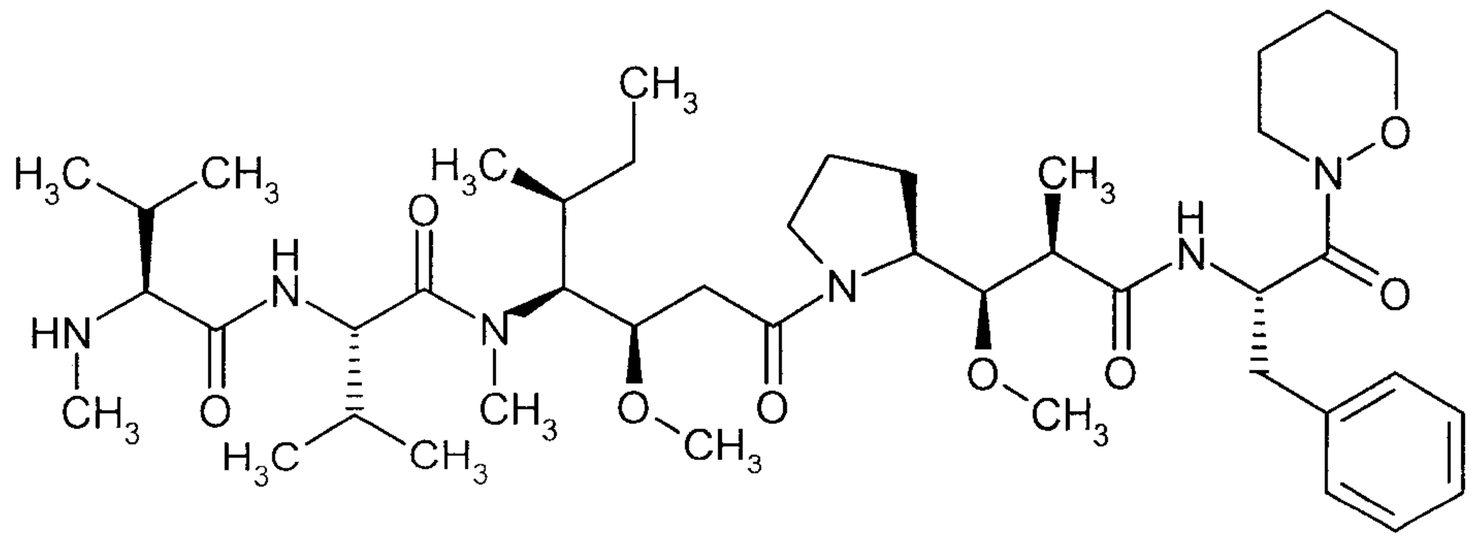
LC-MS (Method 1): R_t = 1.36 min; MS (ESIpos): m/z = 539 $(M+H)^+$.

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.

HPLC (Method 6): R_t = 1.6 min.

Intermediate 14

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



143 mg (0.223 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 15 ml of DMF and admixed successively with 141 mg (0.22 mmol) of (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 (Intermediate 7), 102 mg (0.27 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and 128 µl (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

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.

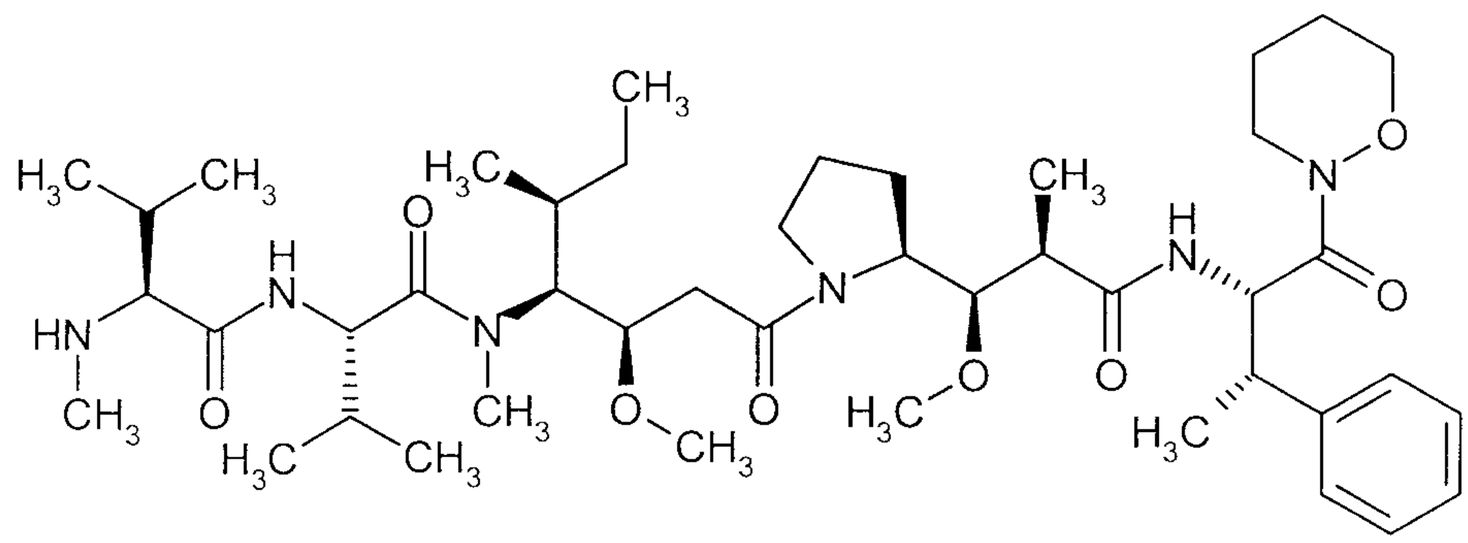
HPLC (Method 5): R_t = 1.68 min;

LC-MS (Method 2): R_t = 1.03 min; MS (ESIpos): m/z = 801 $(M+H)^+$

^{1}H NMR (600 MHz, DMSO-d_6): δ = 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 H_2O 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



x CF_3COOH

126 mg (0.198 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 105 mg (0.198 mmol) of (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 µl (0.454 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. This gave 220 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*,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.

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.

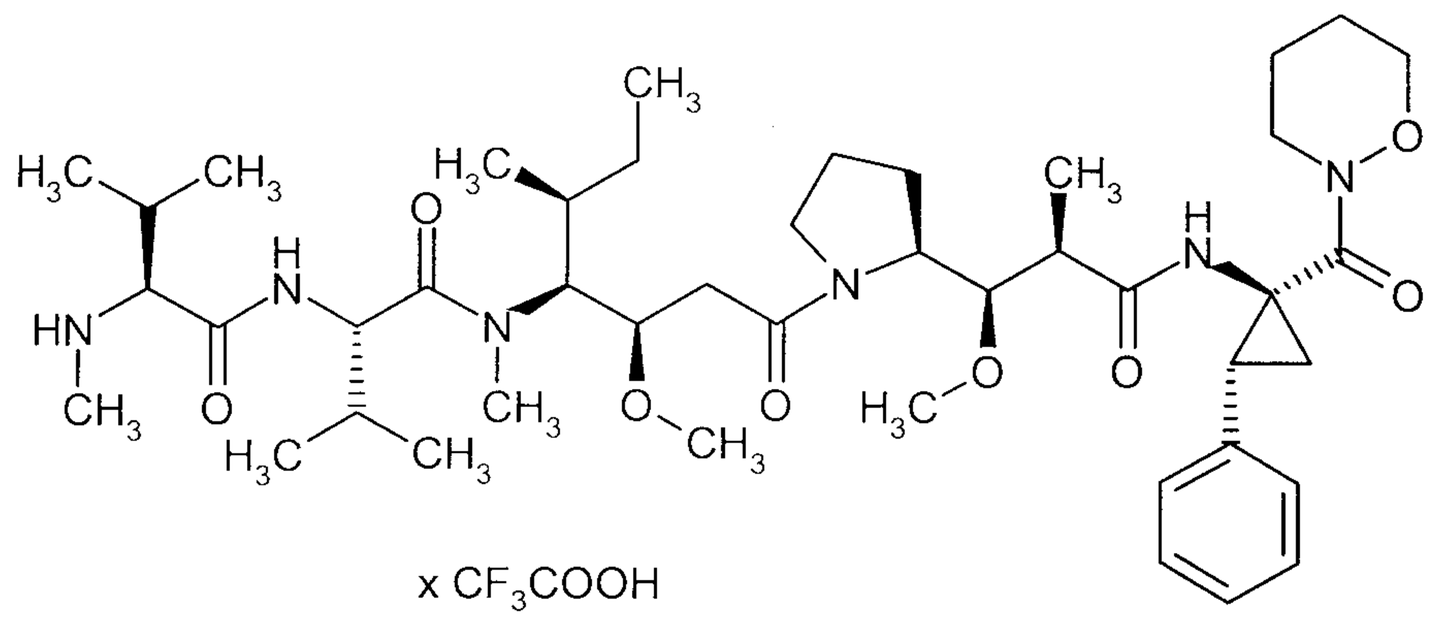
HPLC (Method 5): R_t = 1.71 min;

LC-MS (Method 1): R_t = 0.9 min; MS (ESIpos): m/z = 815 $(M+H)^+$

^{1}H NMR (600 MHz, DMSO-d_6): 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 H_2O 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



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

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.

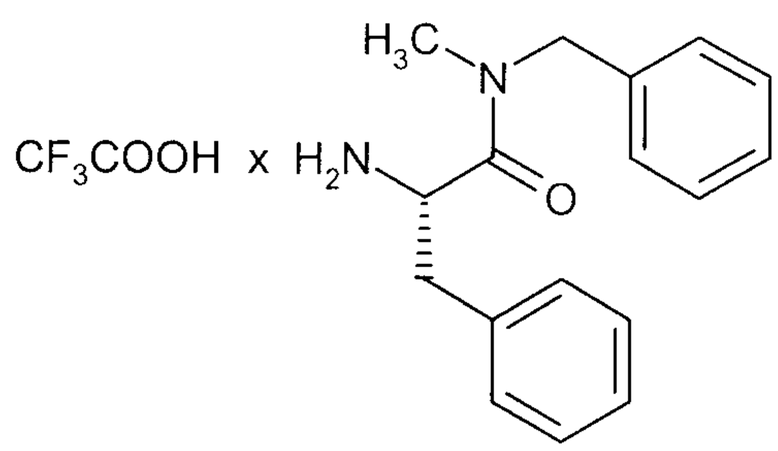
HPLC (Method 5): R_t = 1.64 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 813 $(M+H)^+$

^{1}H NMR (400 MHz, DMSO-d_6): δ = 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 H_2O 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 µl 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 $(M+H)^+$.

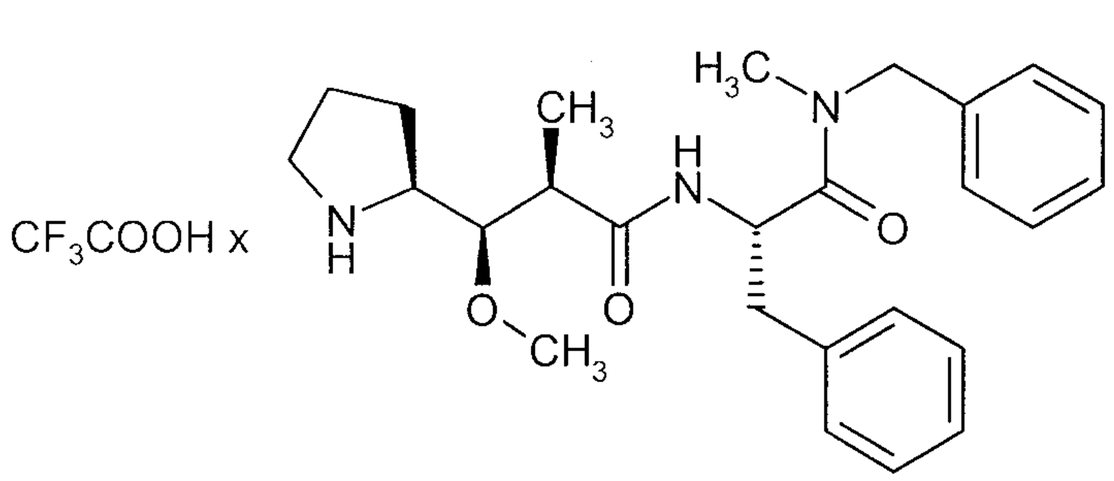
1108 mg (3,007 mmol) of this intermediate were taken up in 30 ml of dichloromethane, 10 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated *in vacuo*, the remaining residue was stirred with dichloromethane, and the 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 $(M+H)^+$.

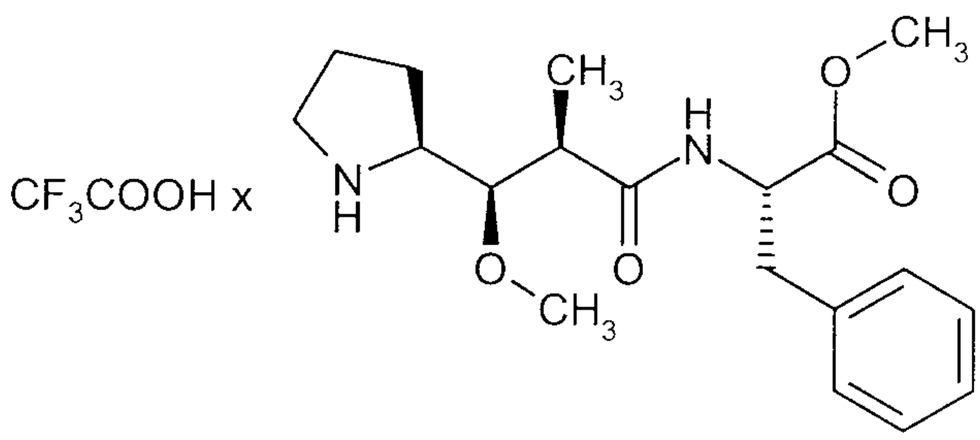
168 mg (0.312 mmol) of this intermediate were taken up in 15 ml of dichloromethane, 3 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated *in vacuo*. The remaining residue was stirred first with dichloromethane, then 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



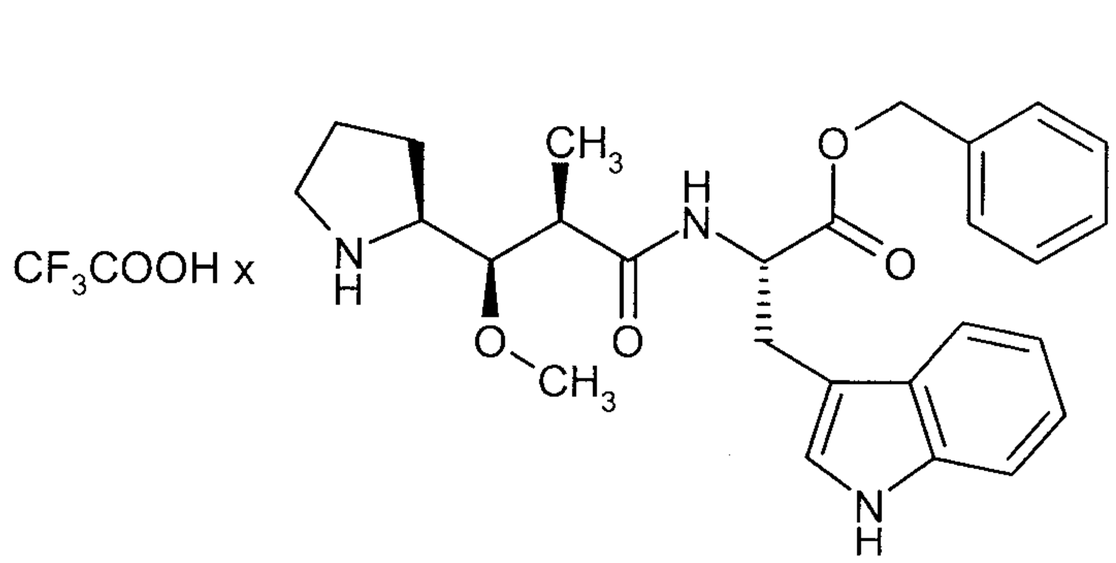
The title compound was prepared in analogy to the synthesis of Intermediate 18, proceeding from (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1), which was released from the dicyclohexylamine salt, and methyl L-phenylalaninate hydrochloride.

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



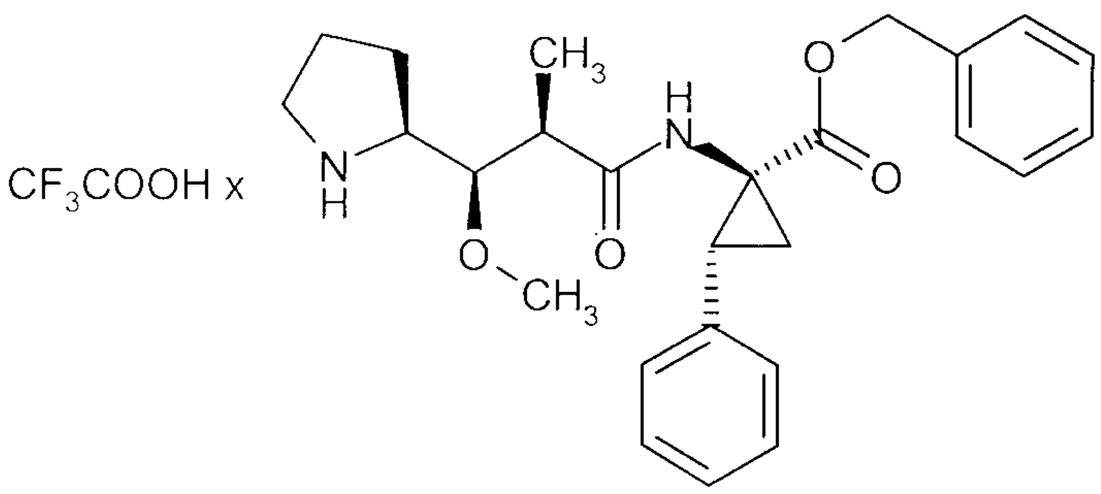
The title compound was prepared in analogy to the synthesis of Intermediate 18, proceeding from (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1), which was released from the dicyclohexylamine salt, and benzyl L-tryptophanate.

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



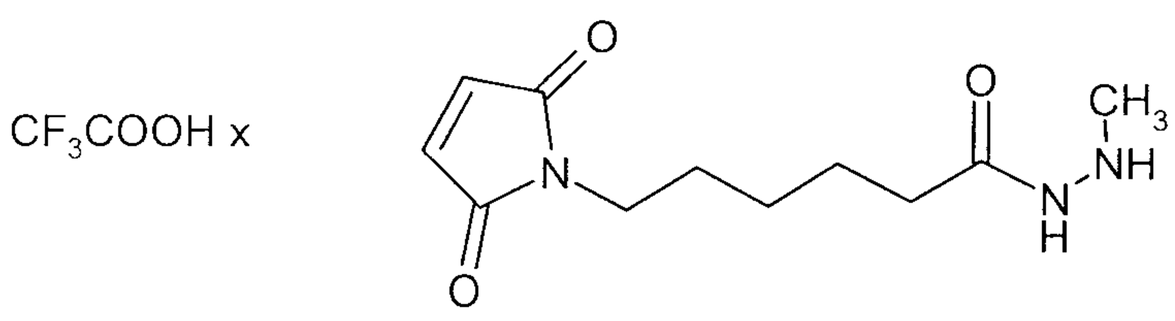
The title compound was prepared in analogy to the synthesis of Intermediate 18, proceeding from (2*R*,3*R*)-3-[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-3-methoxy-2-methylpropanoic acid (Starting Compound 1), which was released from the dicyclohexylamine salt, and benzyl (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate. 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 $(M+H)^+$.

Intermediate 22

6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-N'-methylhexanehydrazide trifluoroacetate



100 mg (473 µmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoic acid were dissolved in 71 µl of DMF and then admixed with 139 mg (947 µmol) of tert-butyl-1-methylhydrazinecarboxylate, 182 mg (947 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 145 mg (947 µmol) of 1-hydroxy-1*H*-benzotriazole

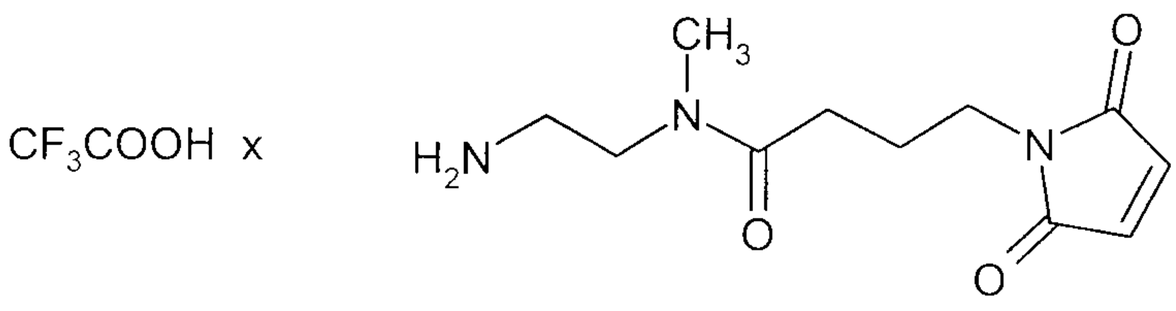
hydrate. The mixture was stirred at RT overnight and then concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization from dioxane/water, 129 mg (80% of theory) of the protected intermediate were obtained as a colourless foam.

Subsequently, the 129 mg (380 µmol) 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

N-(2-aminoethyl)-4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-N-methylbutanamide trifluoroacetate



First, 35 mg (164 µmol) of tert-butyl-[2-(methylamino)ethyl]carbamate-hydrochloride-trifluoroacetate, 30 mg (164 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid, 75 mg (197 µmol) of O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium-hexafluorophosphate and 57 µl 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;

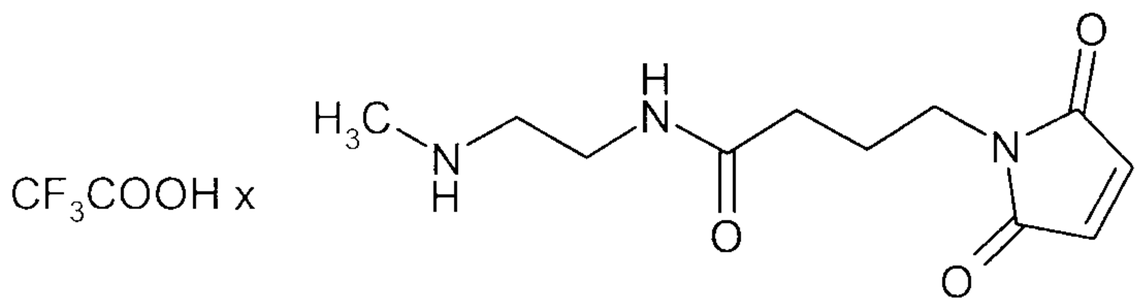
LC-MS (Method 1): R_t = 0.71 min; MS (ESIpos): m/z = 340 $(M+H)^+$.

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 µmol) of tert-butyl-(2-aminoethyl)methylcarbamate hydrochloride trifluoroacetate, 30 mg (164 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid, 75 mg (197 µmol) of O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 57 µl 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 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;

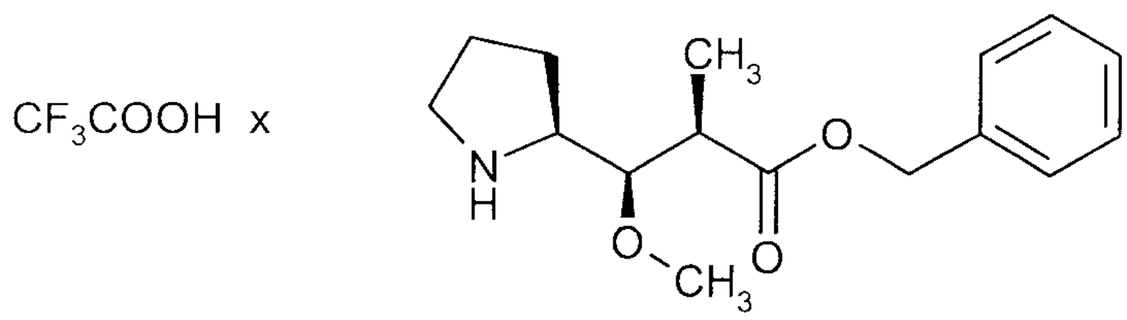
LC-MS (Method 1): R_t = 0.77 min; MS (ESIpos): m/z = 340 $(M+H)^+$.

Subsequently, the entire amount was deprotected with 1 ml of trifluoroacetic acid in 5 ml of 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



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 1990 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.

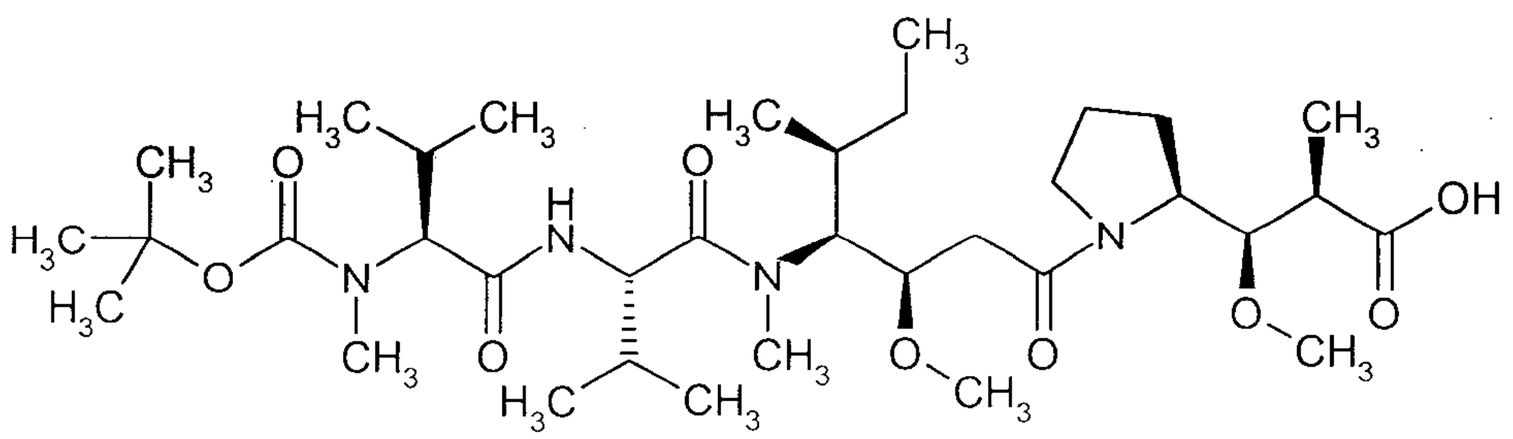
Subsequently, these 1170 mg were deprotected immediately with 5 ml of trifluoroacetic acid in 15 ml of dichloromethane. After stirring at RT for 15 min, the reaction mixture was concentrated *in vacuo*. The residue was lyophilized from dioxane. After drying under high vacuum, there remained 1333 mg (84% 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 µl 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 $(M+H)^+$.

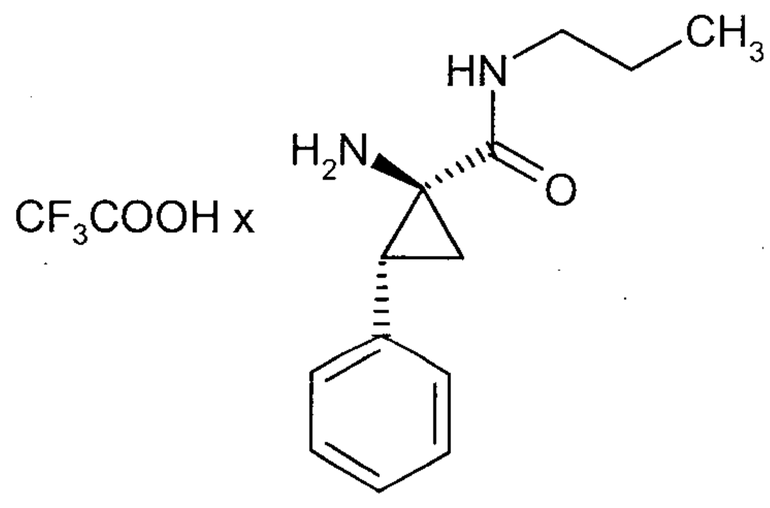
The entire amount of this intermediate obtained was taken up in 25 ml of a mixture of methanol and dichloromethane (20:1), and the benzyl ester group was removed by hydrogenation under standard hydrogen pressure with 10% palladium on activated carbon as a catalyst. After stirring at RT for 30 min, the 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 $(M+H)^+$.

Intermediate 27

(1*S*,2*R*)-1-amino-2-phenyl-*N*-propylcyclopropanecarboxamide trifluoroacetate



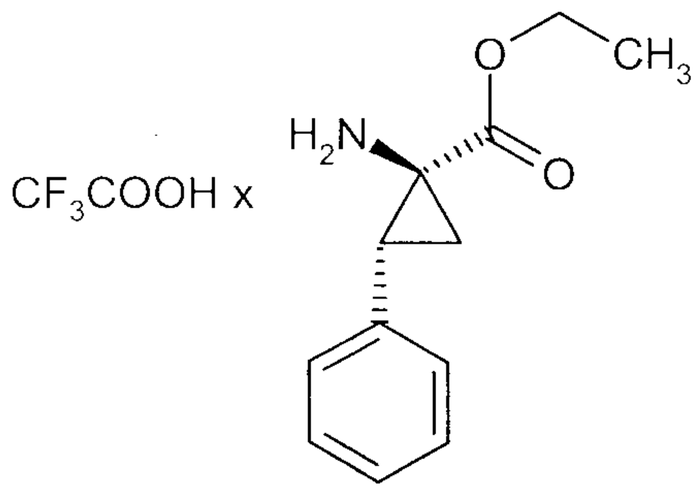
The title compound was prepared by coupling commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with *n*-propylamine in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and subsequent Boc cleaving 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

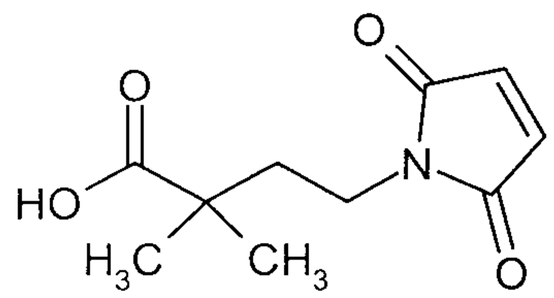


The title compound was prepared according to standard methods by esterifying commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with ethanol and subsequent Boc cleaving with trifluoroacetic acid.

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

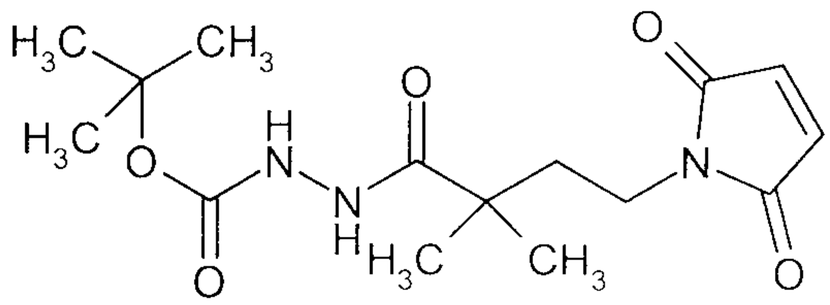


To a solution of 1.39 g (8.95 mmol) of N-methoxycarbonylmaleimide in 44 ml of saturated sodium hydrogencarbonate solution were added, at 0°C, 1.5 g (8.95 mmol) of 4-amino-2,2-dimethylbutyric acid, and the mixture was stirred for 40 min. Subsequently, the cooling bath was removed, and the reaction mixture 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.

LC-MS (Method 1): R_t = 0.64 min; m/z = 212 $(M+H)^+$.

Intermediate 30

tert-butyl 2-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoyl]hydrazinecarboxylate

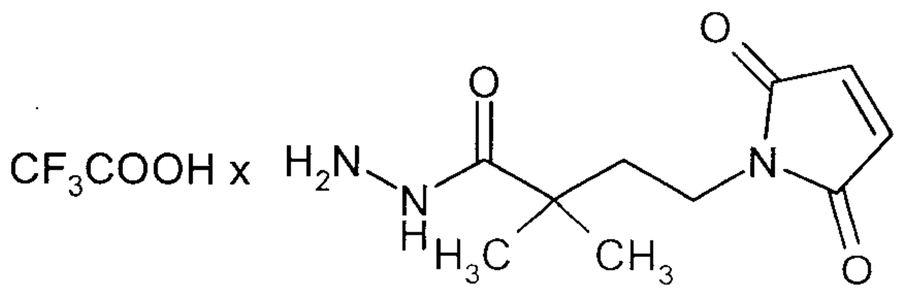


To a solution of 50 mg (237 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoic acid in 2 ml of THF were added, at 0°C, first 26 µl (237 µmol) of 4-methylmorpholine and then 31 µl (237 µmol) of isobutyl chloroformate. After removing the cooling bath and stirring at RT for a further 15 min, 31.3 mg (237 µmol) of tert-butyloxycarbonyl hydrazide were added. The reaction mixture was stirred overnight and then concentrated. The 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

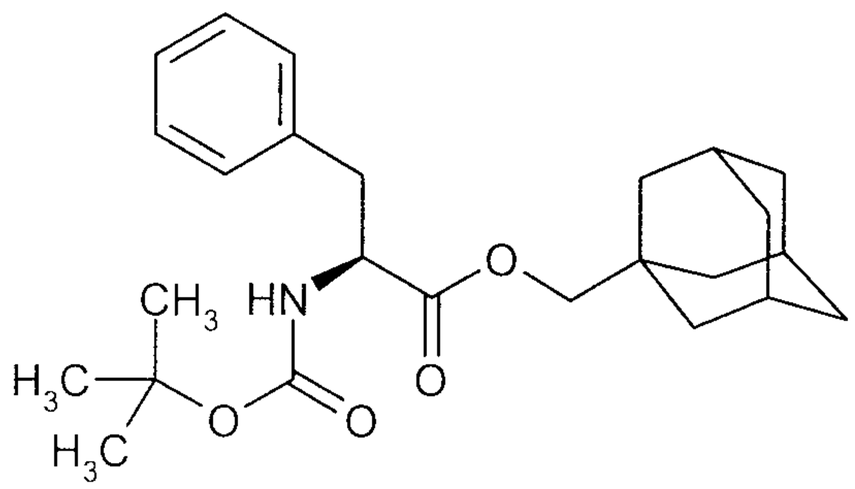


50 mg (154 mmol) of *tert*-butyl 2-[4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-2,2-dimethylbutanoyl]hydrazinecarboxylate were dissolved in 2 ml of dichloromethane, and 0.4 ml of trifluoroacetic acid was added. The reaction mixture was stirred at RT for 30 min and then concentrated. 55.2 mg (93% purity, 99% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.36 min; m/z = 226 $(M+H)^+$.

Intermediate 32

Adamantan-1-ylmethyl *N*-(*tert*-butoxycarbonyl)-L-phenylalaninate

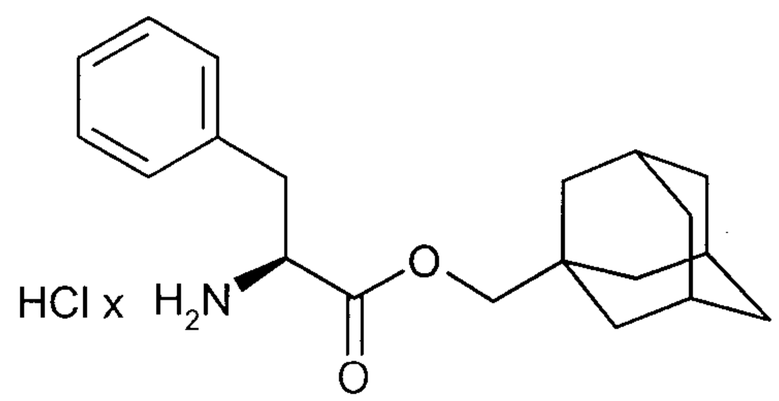


To a solution of 500 mg (1.89 mmol) of *N*-Boc-L-phenylalanine in 25 ml of dichloromethane were added, at RT, 1192 mg (6.2 mmol) of EDC, 578 µl (4.1 mmol) of triethylamine, 345 mg (2.8 mmol) of DMAP and 345 mg (2.1 mmol) of 1-adamantylmethanol. The reaction mixture was stirred overnight, then diluted 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 $(M+H)^+$.

Intermediate 33

Adamantan-1-ylmethyl *L*-phenylalaninate hydrochloride

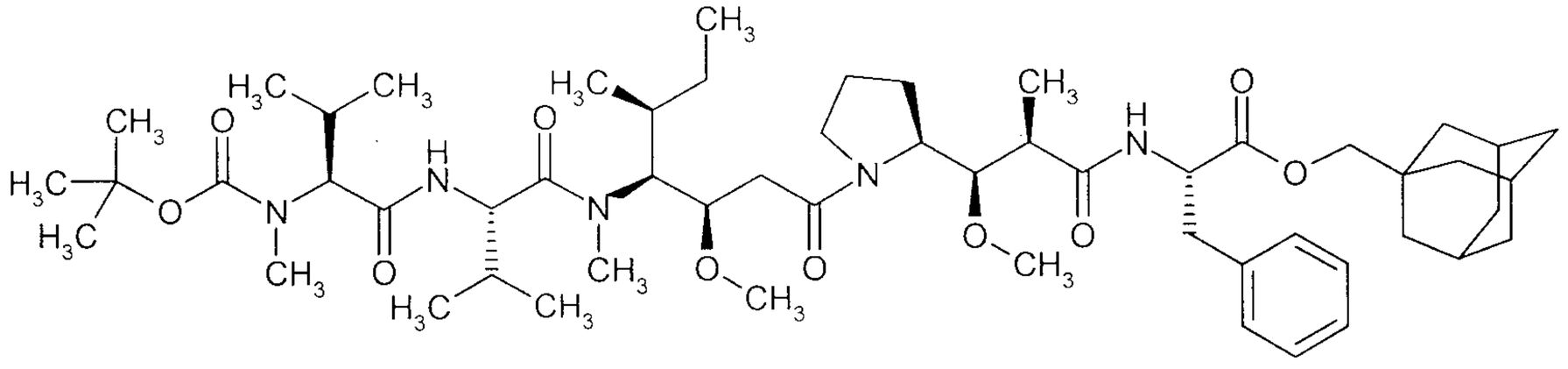


769 mg (1.86 mmol) of adamantan-1-ylmethyl N-(*tert*-butoxycarbonyl)-*L*-phenylalaninate (Intermediate 13) were dissolved in 25 ml of a 4 N solution of hydrogen chloride in dioxane and stirred at RT for 1 h. Subsequently, the reaction mixture was concentrated, and the residue was dried *in vacuo*. 619 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

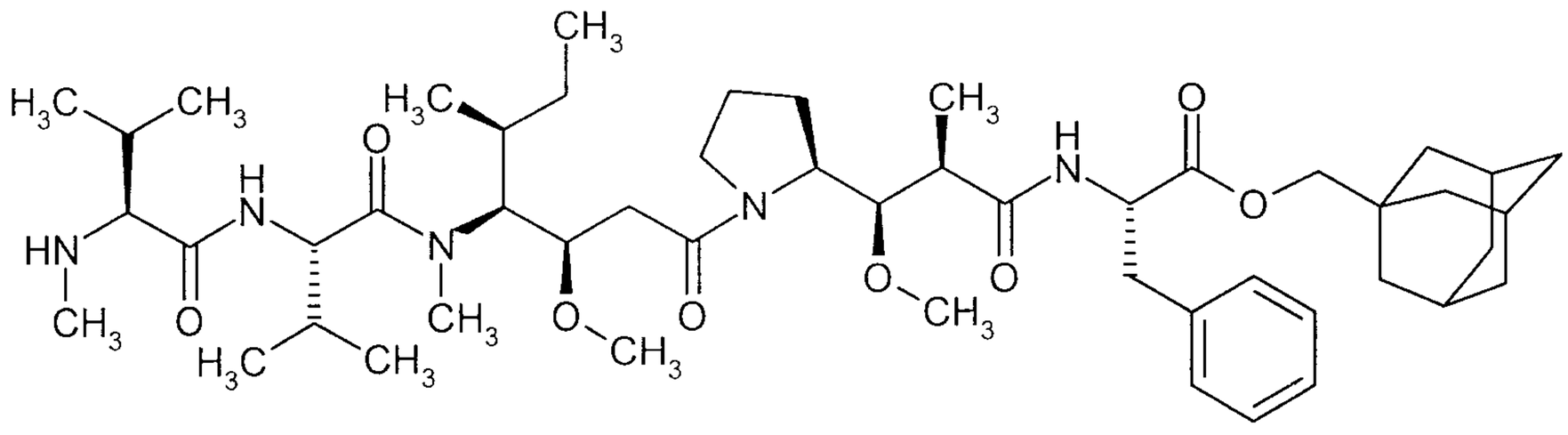


To a solution of 20 mg (29 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide in 1 ml of DMF were added, at RT, 15.3 µl (88 µmol) of *N,N*-diisopropylethylamine, 6.7 mg (44 µmol) of HOBt and 6.7 mg (35 µmol) of EDC, and the mixture was stirred for 30 min. Subsequently, 10.1 mg (32 µmol) 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.

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



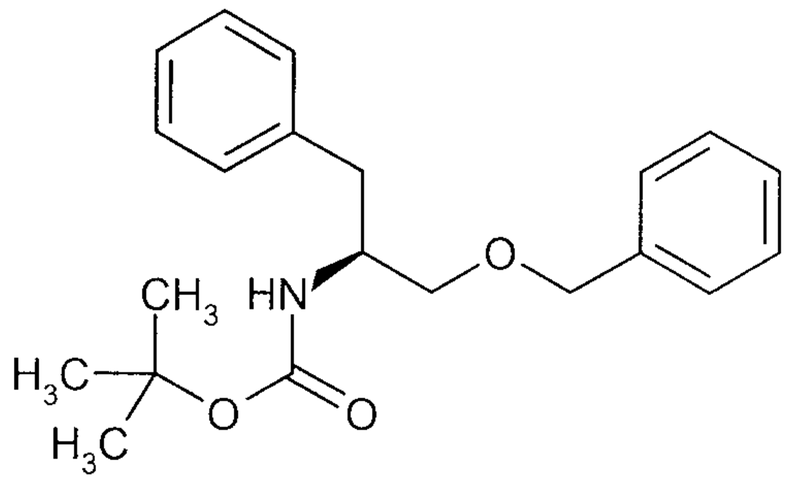
x CF_3COOH

27.5 mg (28 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-(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 µl 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

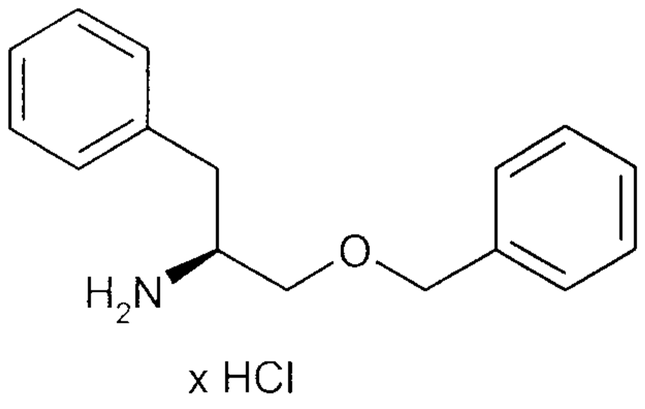


Under an argon atmosphere, 500 mg (1.99 mmol) of *N*-Boc-*L*-phenylalaninol were dissolved in 5 ml of DMF and cooled to 0°C. Subsequently, 159 mg (3.98 mmol) of a 60% suspension of sodium hydride in paraffin oil were added. The reaction mixture was stirred until the evolution of gas had ended, and then 260 µl (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.

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



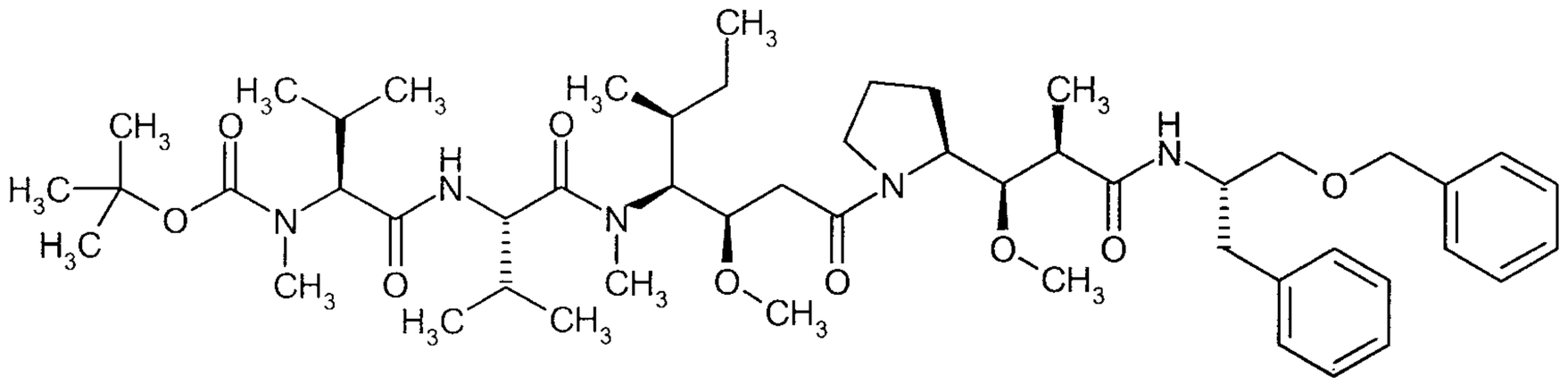
x HCl

220 mg (644 µmol) 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

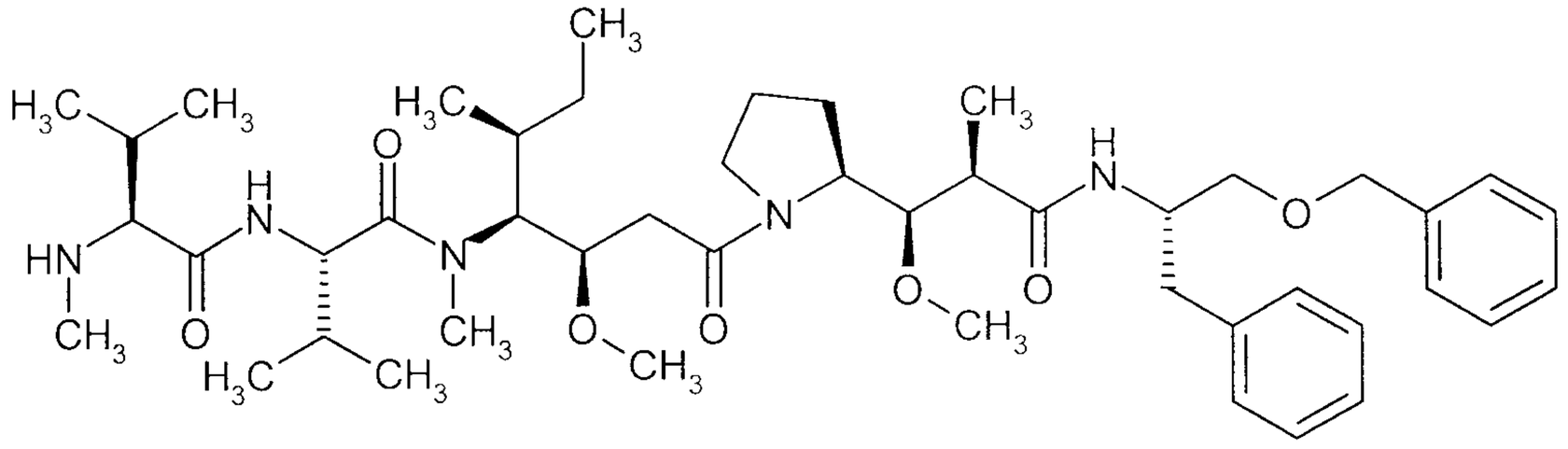


To a solution of 20 mg (29 µmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide in 1 ml of DMF were added, at RT, 15.3 µl (88 µmol) of *N,N*-diisopropylethylamine, 6.7 mg (44 µmol) of HOBt and 6.7 mg (35 µmol) of EDC, and the mixture was stirred for 30 min. Subsequently, 7.8 mg (32 µmol) 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): Rt = 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



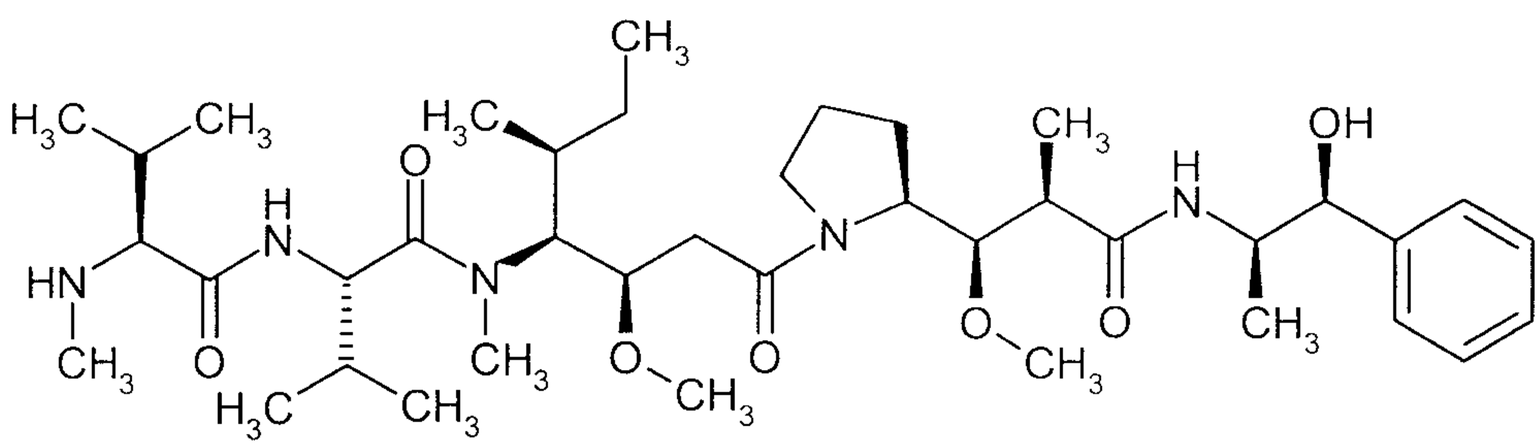
x CF_3COOH

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-[(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



50 mg (70 µmol) of Intermediate 26 and 11 mg (70 µmol) of (1S, 2R)-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,

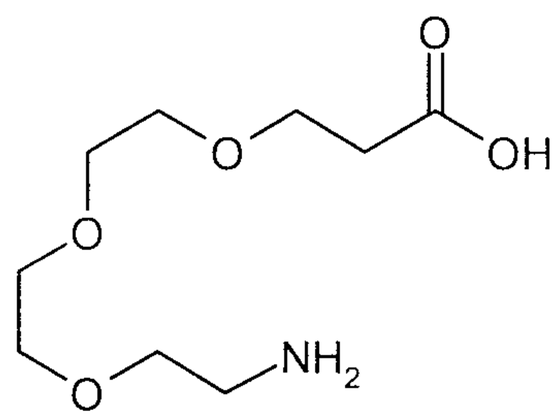
and the reaction mixture was stirred at RT for 5 min. This was followed by concentration and purification of the residue by means of preparative HPLC. After combining the corresponding fractions, concentrating and drying under high vacuum, 49 mg (81%) of the protected intermediate were obtained. Subsequently, 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

3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoic acid trifluoroacetate



x CF_3COOH

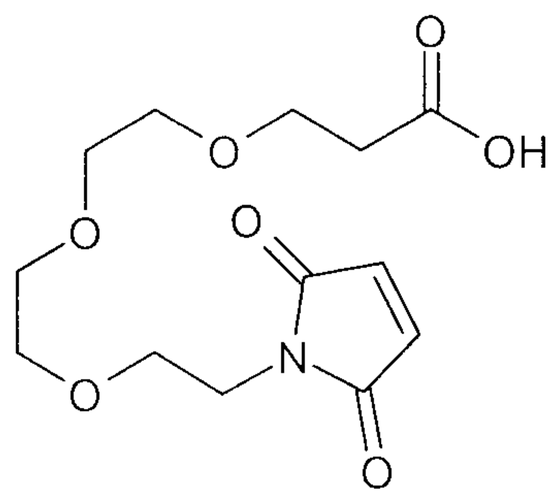
150 mg (541 µmol) of *tert*-butyl 3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoate were dissolved in 3 ml of dichloromethane, 1.5 ml of trifluoroacetic acid were added, and the reaction mixture was stirred at RT for 1 h, then concentrated.

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



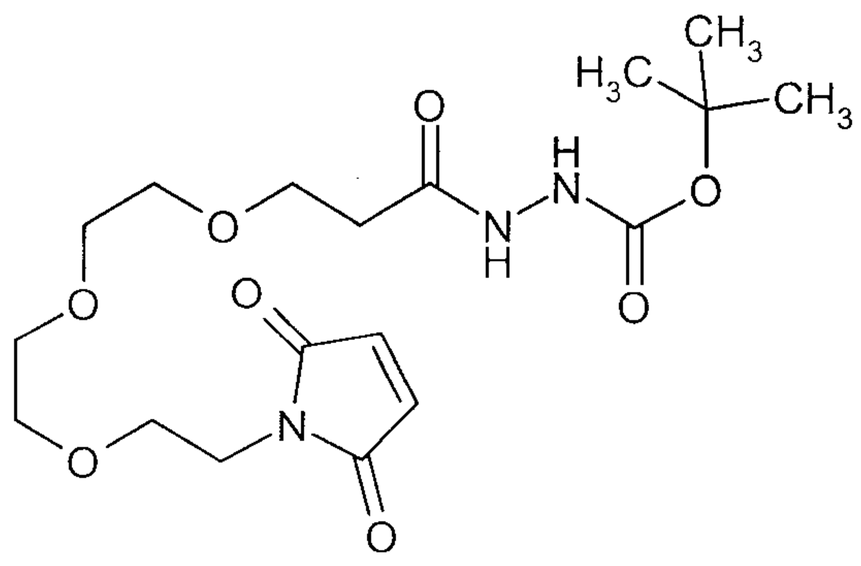
186 mg (555 µmol) of 3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propanoic acid trifluoroacetate were dissolved in 2.6 ml of saturated sodium hydrogencarbonate solution and admixed at 0°C with 86 mg (555 µmol) of N-methoxycarbonylmaleimide. The reaction mixture was stirred at 0°C for 40 min and at RT for 1 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 $(M+H)^+$.

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



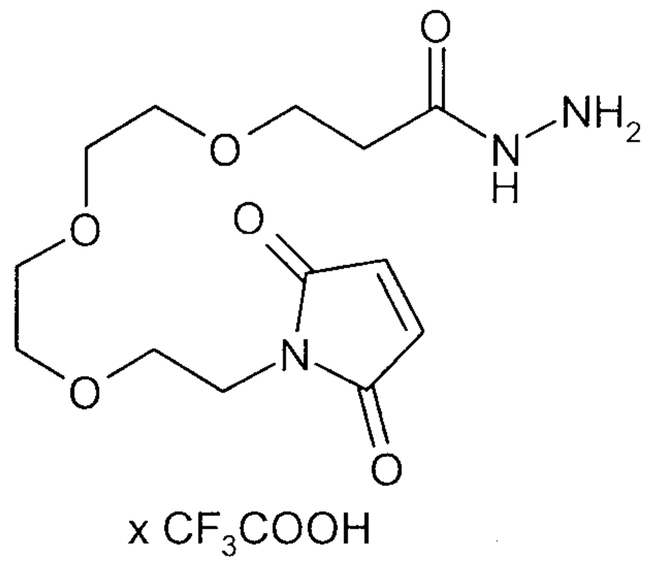
125 mg (417 µmol) of 3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy]ethoxy}ethoxy) propanoic acid were dissolved at 0°C in 2.1 ml of THF and admixed with 46 µl (417 mmol) of 4-methylmorpholine and 54.5 µl (417 µmol) of isobutyl chloroformate. The ice bath was removed, and the reaction mixture was stirred at RT for 30 min. Subsequently, at 0°C, 55 mg (417 µmol) 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 trifluoroacetate



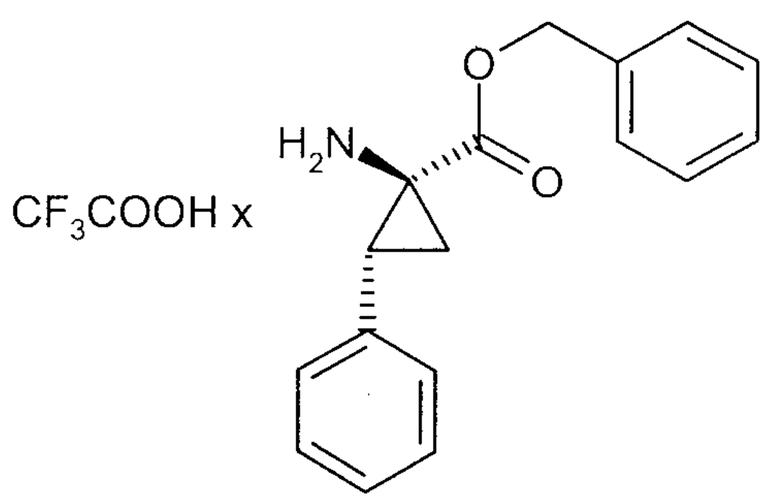
60 mg (145 µmol) of *tert*-butyl-15-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-4-oxo-7,10,13-trioxa-2,3-diazapentadecan-1-oate were dissolved in 1 ml of dichloromethane, and 0.2 ml of trifluoroacetic acid was added. The reaction mixture was stirred at RT for 30 min and then concentrated.

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

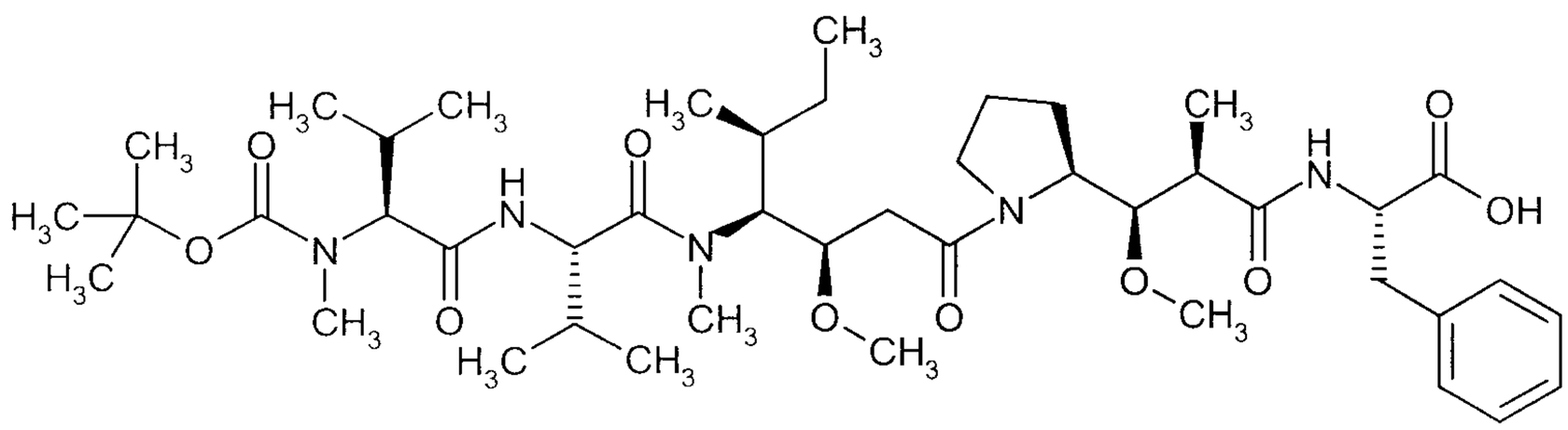


The title compound was prepared according to standard methods by esterifying commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with benzyl alcohol and subsequent Boc cleaving with trifluoroacetic acid.

LC-MS (Method 1): R_t = 0.72 min; MS (ESIpos): m/z = 268 $(M+H)^+$.

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



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 µl of *N*,*N*-diisopropylethylamine in 15 ml of DMF, and the mixture was stirred at RT for 10 min. 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 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 activated carbon as a catalyst. After stirring at RT for 1 h, the catalyst was filtered off and the

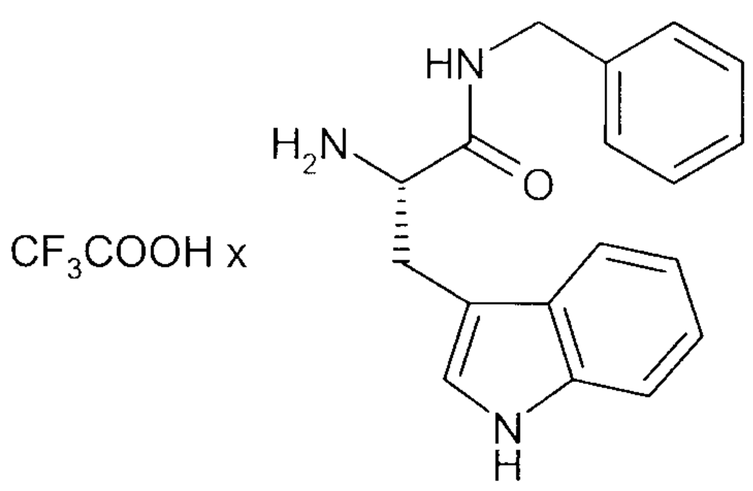
filtrate was concentrated *in vacuo*. After lyophilization from dioxane, 85 mg (94% of theory) of the title compound were obtained as a solid.

HPLC (Method 12): R_t = 2.4 min;

LC-MS (Method 1): R_t = 1.24 min; MS (ESIpos): m/z = 832 $(M+H)^+$.

Intermediate 47

N-benzyl-L-tryptophanamide trifluoroacetate



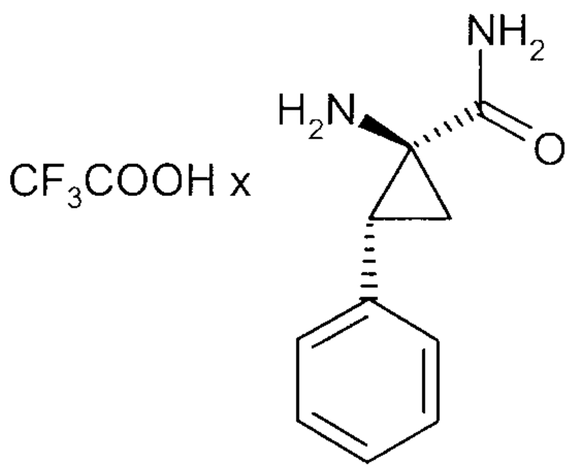
202 mg (0.5 mmol) of 2,5-dioxopyrrolidin-1-yl *N*-(tert-butoxycarbonyl)-L-tryptophanate and 45 mg (0.42 mmol) of benzylamine were dissolved in 10 ml of DMF, and 110 µl (630 µmol) of *N,N*-diisopropylethylamine were added. The reaction mixture was stirred at RT for 3 h. Subsequently, the reaction mixture 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;

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



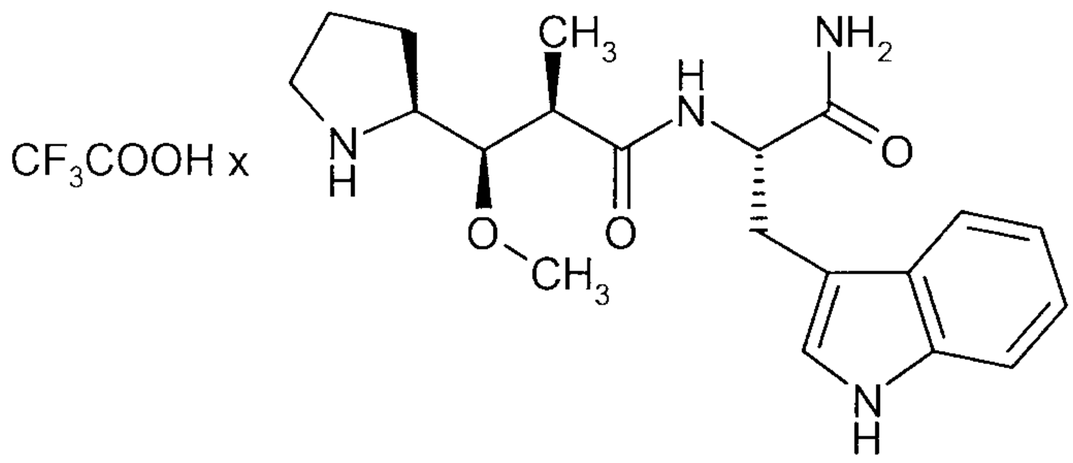
50 mg (180 µmol) of commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid were dissolved in 5 ml of DMF, 94 µl (541 µmol) of *N*,*N*-diisopropylethylamine, 31 mg (270 µmol) of *N*-hydroxysuccinimide and 41.5 mg (216 µmol) of EDC were added, and then the mixture was stirred at RT overnight. The reaction mixture was then concentrated, the residue was taken up in dioxane, 71 mg (901 µmol) 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;

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



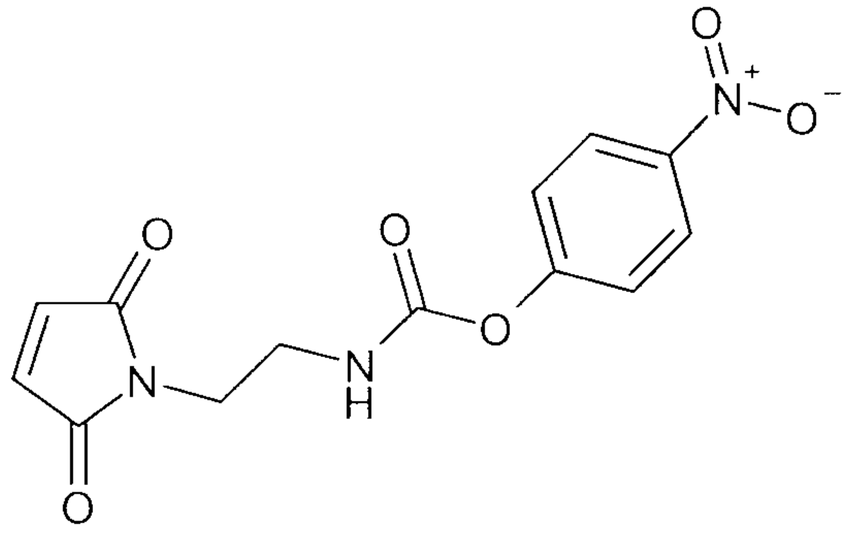
The title compound was prepared in analogy to the synthesis of Intermediate 13 from Starting Compound 1 and L-tryptophanamide hydrochloride.

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



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.

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.

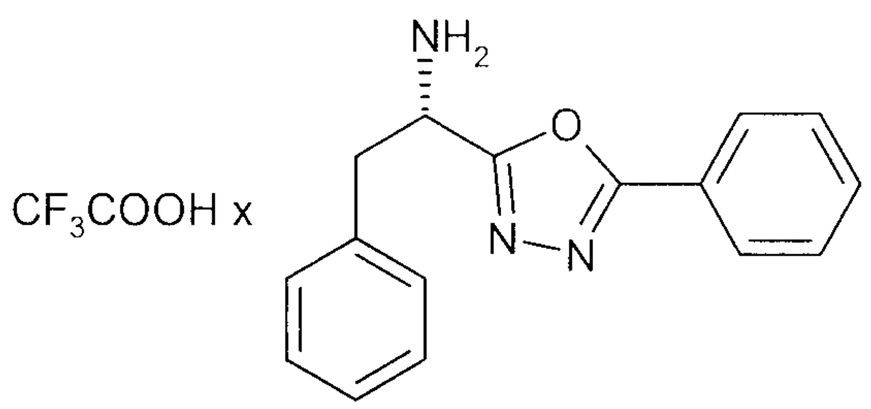
112.9 mg (543 µmol) of nitrophenyl chloroformate were dissolved in 30 ml of THF and, after the addition of 100 mg (271.6 µmol) of 1-(2-aminoethyl)-1*H*-pyrrole-2,5-dione trifluoroacetate, the mixture was stirred at RT for 30 min. The mixture was filtered, and the filtrate was concentrated to dryness and then slurried with diethyl ether. After suctioning off and drying, 60 mg (95% of theory) of the title compound were obtained.

HPLC (Method 5): Rt = 0.65 min;

LC-MS (Method 1): Rt = 0.74 min; MS (ESIpos): m/z = 306 (M+H)+.

Intermediate 51

(*1S*)-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 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 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.

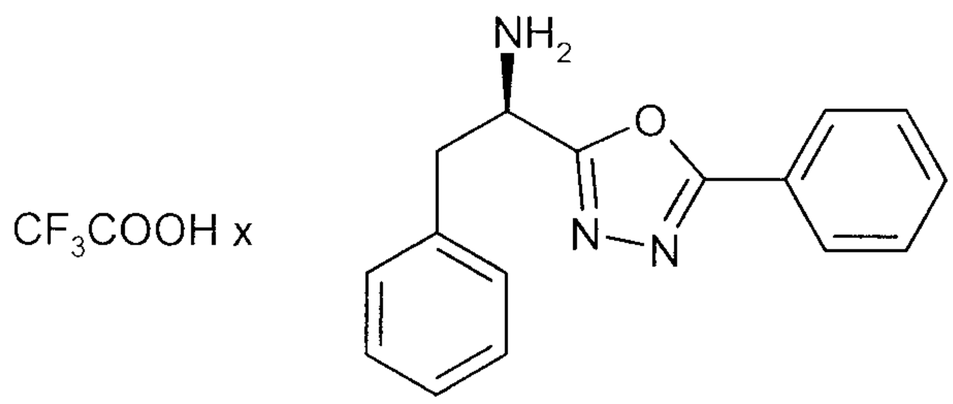
LC-MS (Method 12): R_t = 1.15 min; MS (ESIpos): m/z = 366 $(M+H)^+$

217 mg (0.59 mmol) of this intermediate were taken up in 3 ml of dichloromethane, 0.6 ml of 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 $(M+H)^+$

Intermediate 52

(*1R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethanamine trifluoroacetate



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 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.

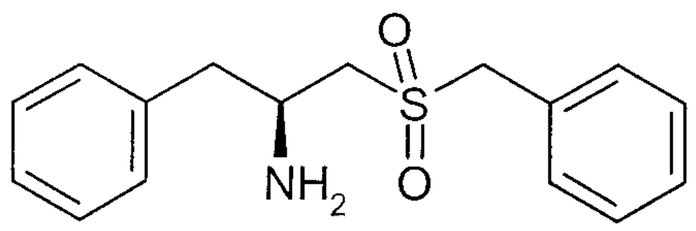
LC-MS (Method 2): R_t = 1.36 min; MS (ESIpos): m/z = 366 $(M+H)^+$

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 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

(*2S*)-1-(benzylsulphonyl)-3-phenylpropan-2-amine



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 $(M+H)^+$

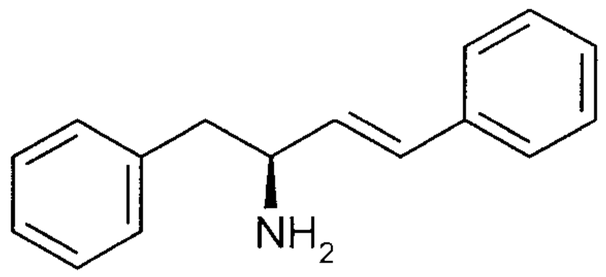
The crude intermediate obtained above was dissolved in a solution of 2 ml of 30% hydrogen peroxide and 5 ml of formic acid, and the mixture was stirred at RT for 12 h. Then the reaction mixture was added to saturated sodium sulphate solution and extracted three times with ethyl acetate. The organic phase 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.

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

(*2S,3E*)-1,4-diphenylbut-3-en-2-amine



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 (*2S*)-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.

HPLC (Method 10): R_t = 2.46 min;

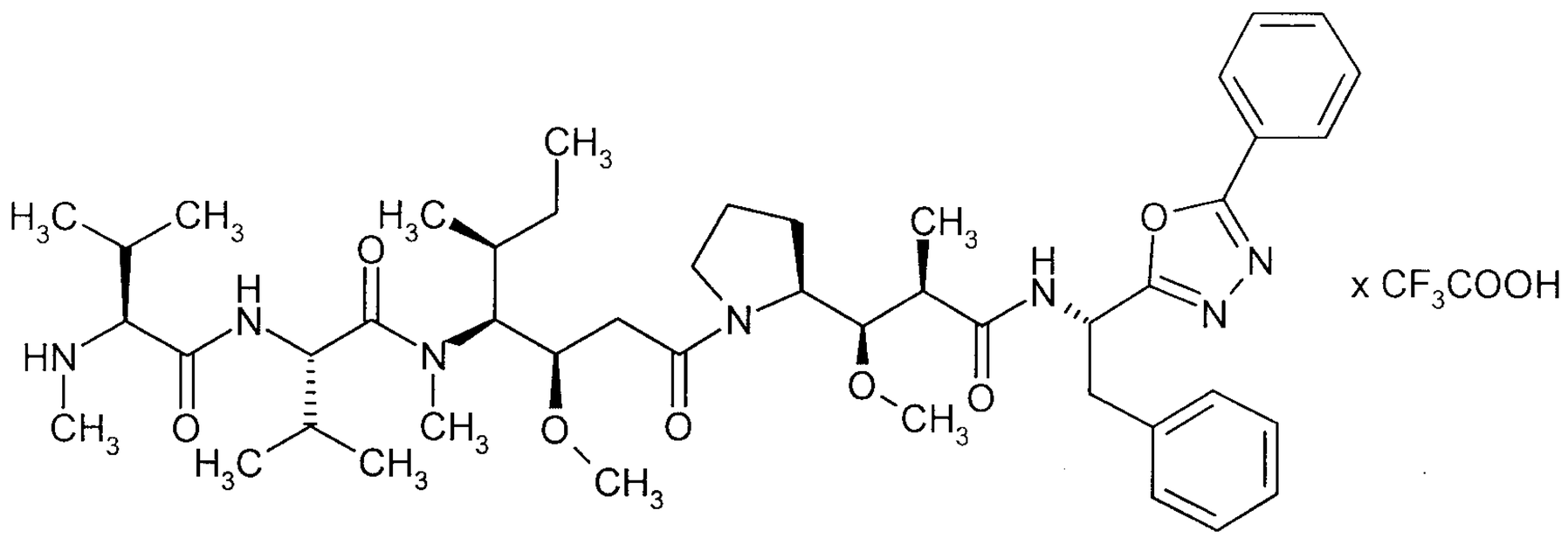
LC-MS (Method 12): R_t = 0.75 min; MS (ESIpos): m/z = 224 $(M+H)^+$

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 µm, 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.

^{1}H 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].

Intermediate 55

N-methyl-*L*-valyl-N-[(*3R,4S,5S*)-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



20 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*)-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 µmol) of HATU and 15.3 µl (88 µmol) of *N,N*-diisopropylethylamine were added, and the mixture was stirred at RT for 30 min. Subsequently, 12.2 mg (32 µmol) 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.

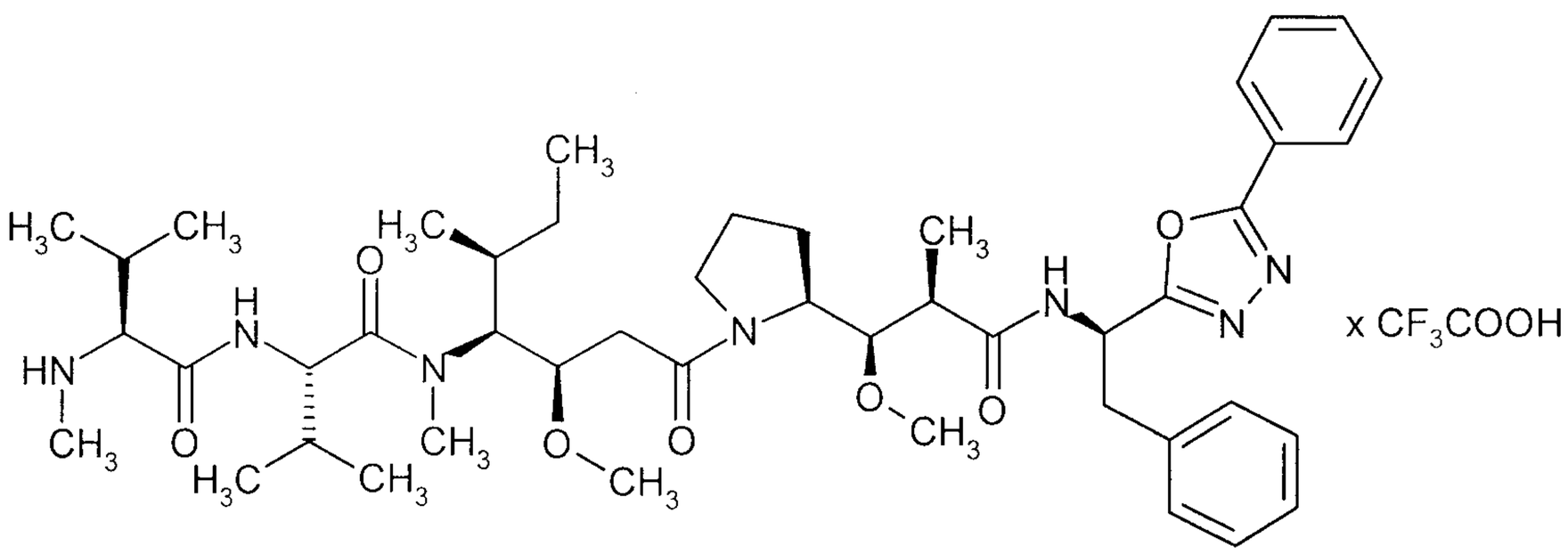
LC-MS (Method 12): R_t = 1.45 min; MS (ESIpos): m/z = 933 $(M+H)^+$

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 $(M+H)^+$

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



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 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 with 12.2 mg (32 µmol) of (1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethanamine trifluoroacetate.

Yield: 17 mg (64% of theory)

HPLC (Method 10): R_t = 3.74 min;

LC-MS (Method 1): R_t = 1.45 min; MS (ESIpos): m/z = 933 $(M+H)^+$

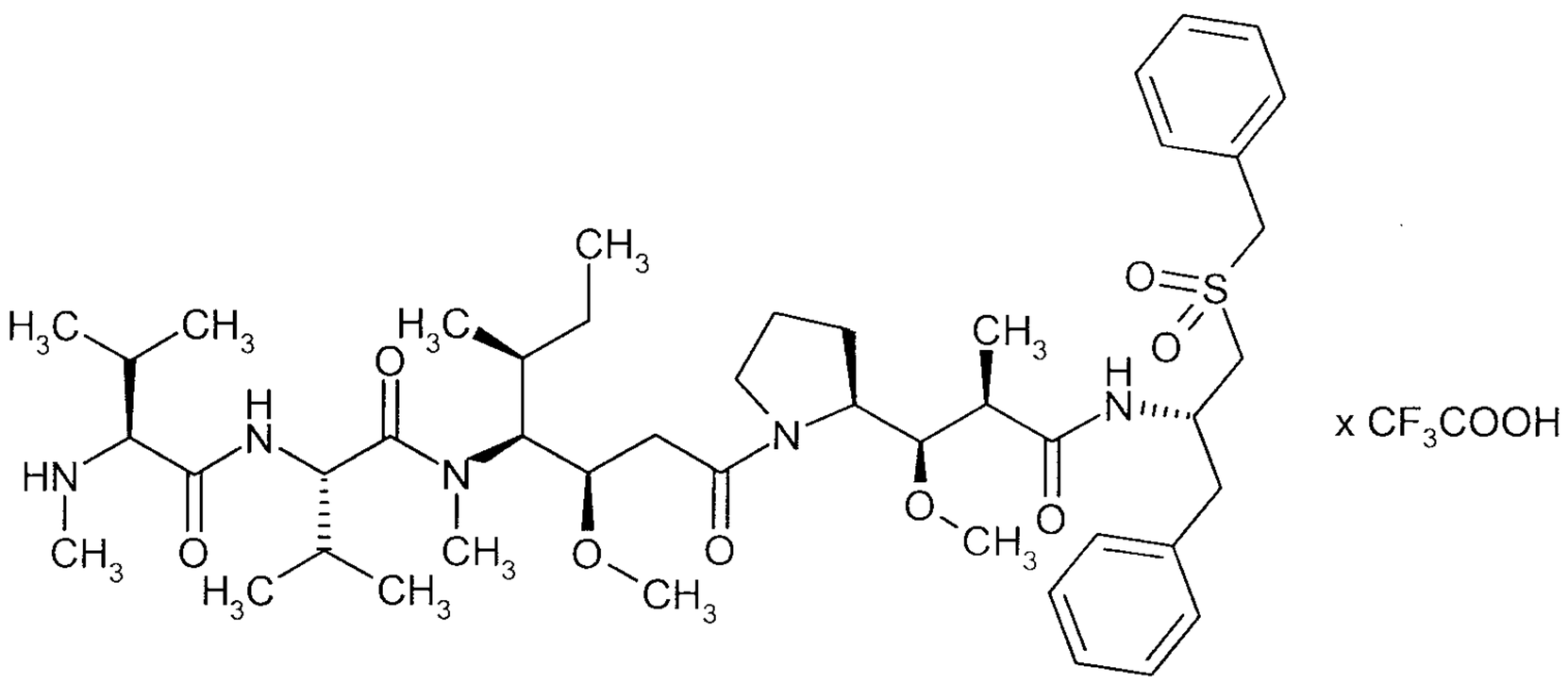
By subsequently cleaving the BOC protecting group with trifluoroacetic acid, 17.1 mg (99% of theory) of the title compound were thus obtained.

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

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



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 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 with 9.3 mg (20 µmol) of (2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-amine.

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 $(M+H)^+$

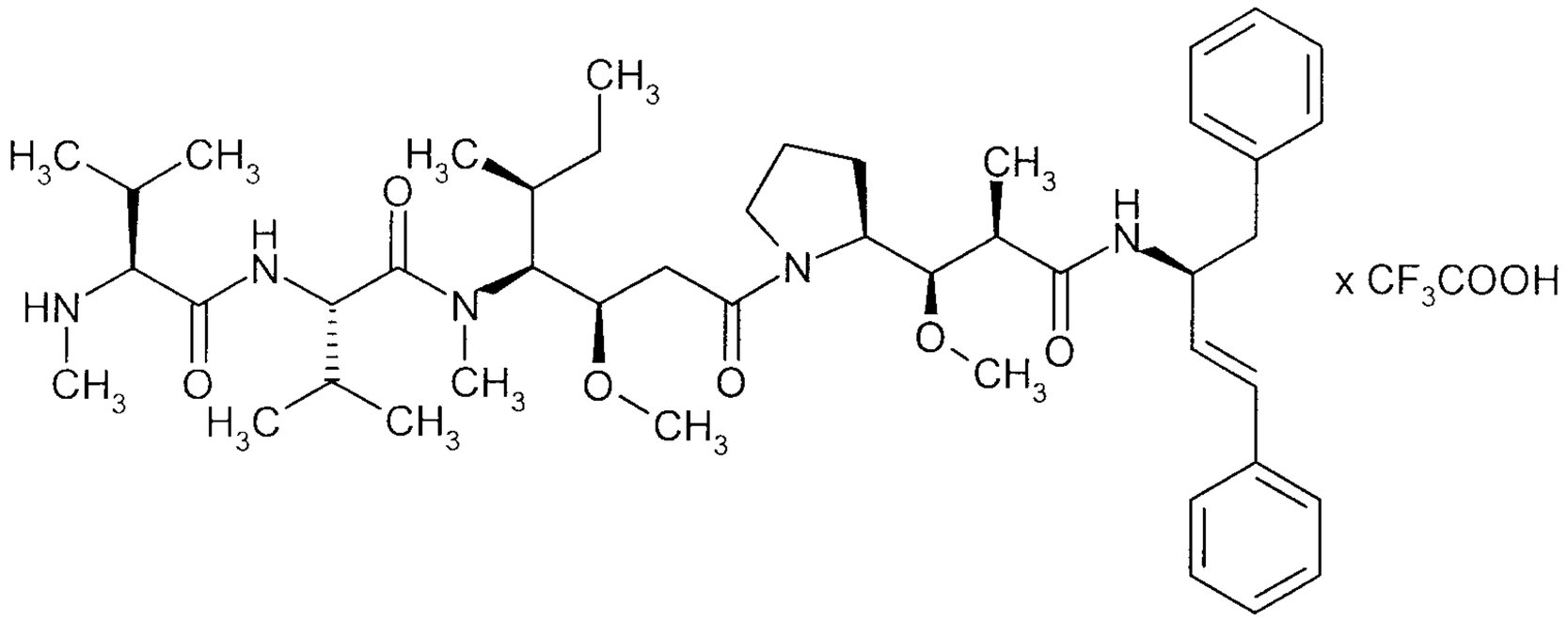
By subsequently cleaving the BOC protecting group with trifluoroacetic acid, 19.3 mg (99% of theory) of the title compound were thus obtained.

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 µmol) *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 with 7.1 mg (32 µmol) 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 $(M+H)^+$

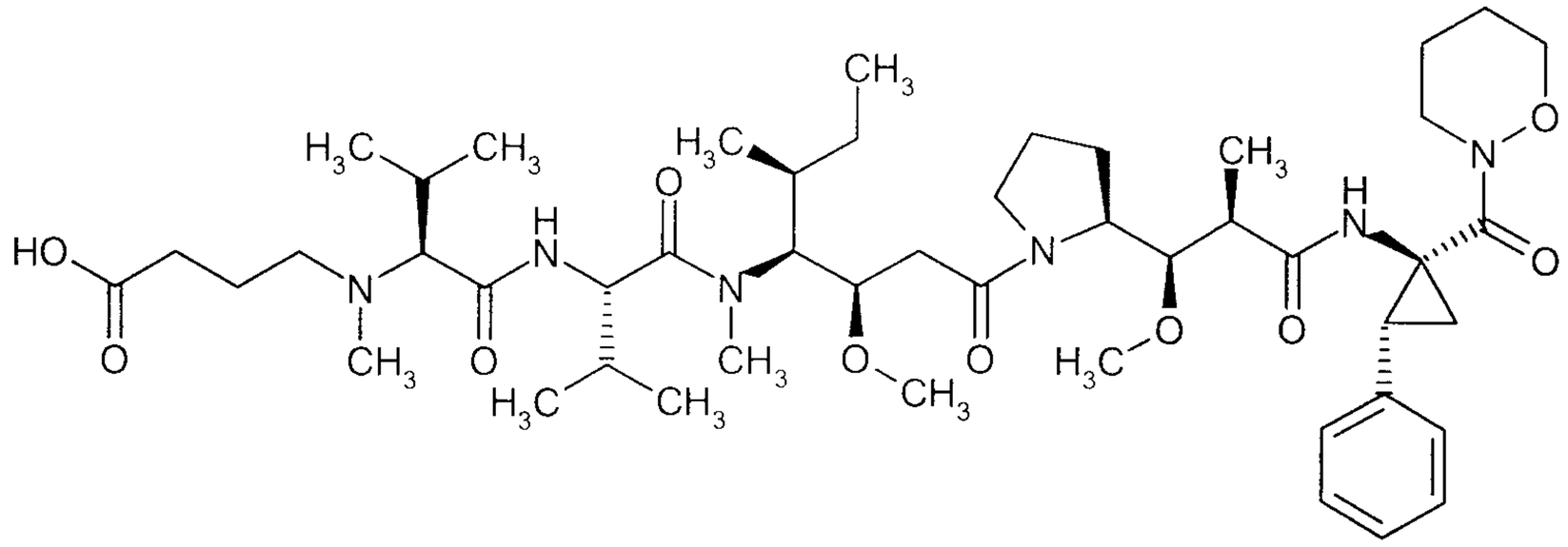
By subsequently cleaving the BOC protecting group with trifluoroacetic acid, 15.7 mg (99% of theory) of the title compound were then obtained.

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



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-{[(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 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;

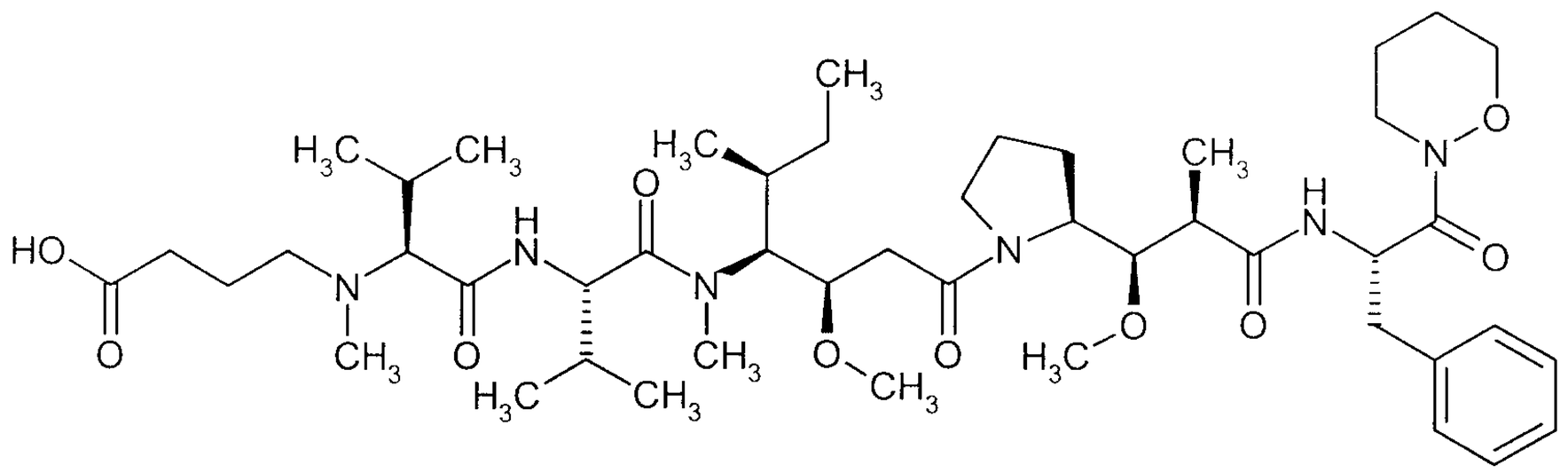
LC-MS (Method 9): R_t = 4.76 min; MS (ESIpos): m/z = 899 $(M+H)^+$

^{1}H NMR (500 MHz, DMSO-d_6): 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 H_2O 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.

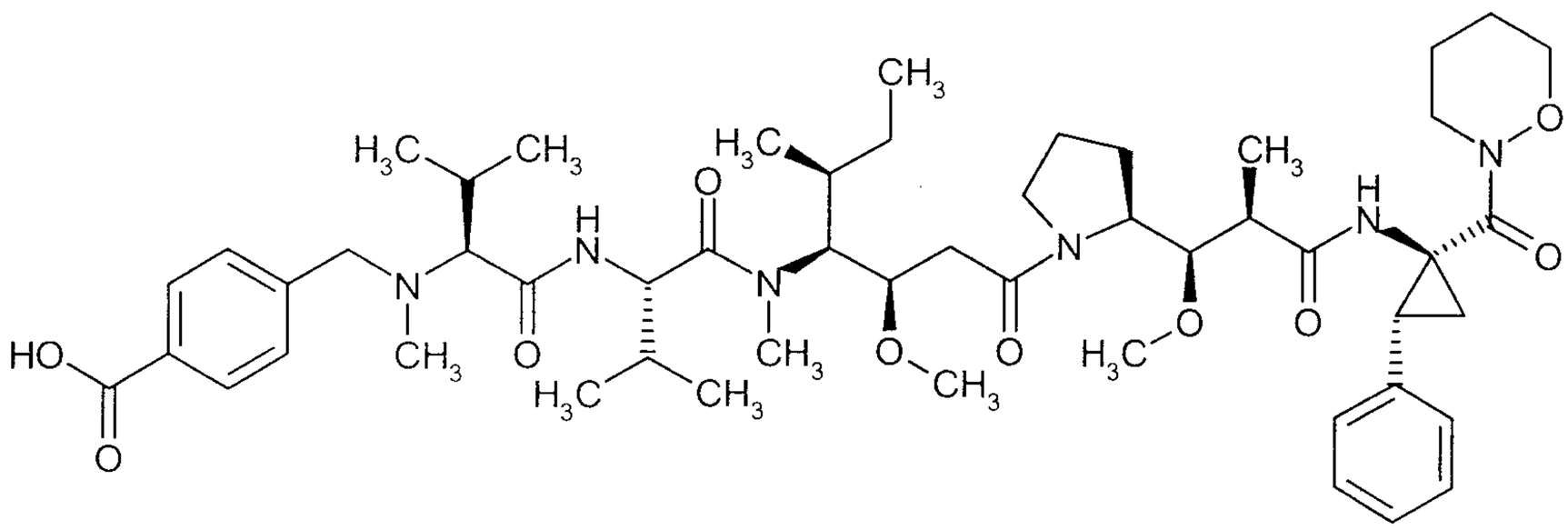
Yield: 34 mg (70% of theory)

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.

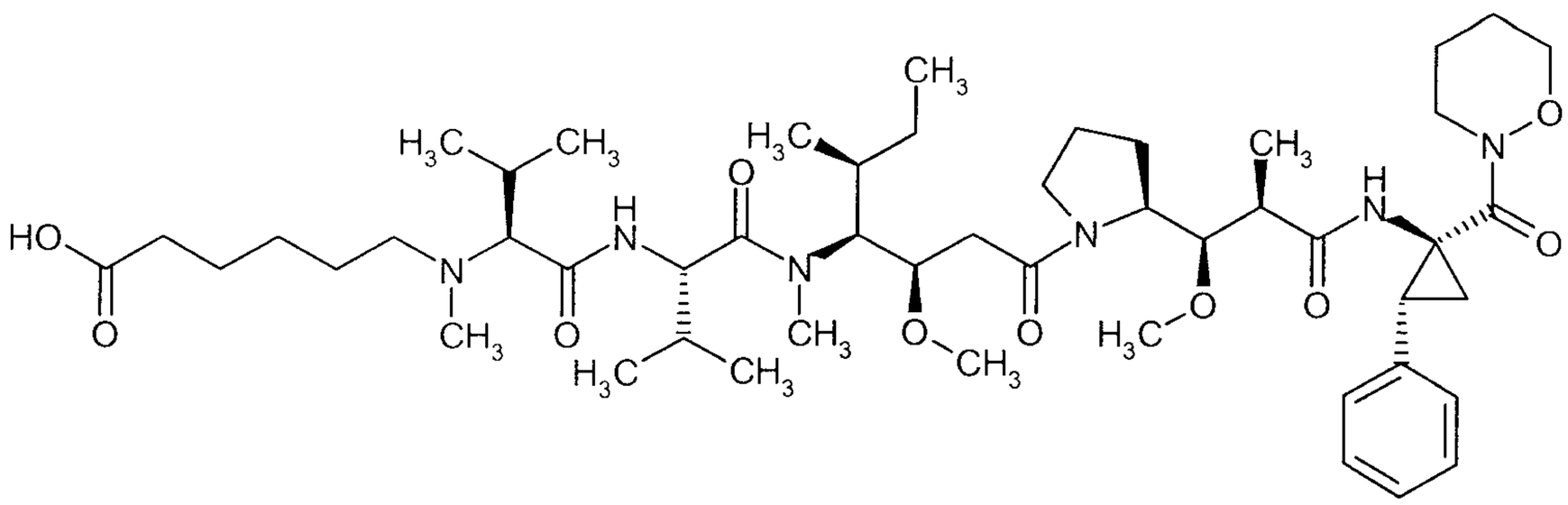
Yield: 7.5 mg (48% of theory)

HPLC (Method 5): R_t = 1.75 min;

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 947 $(M+H)^+$.

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

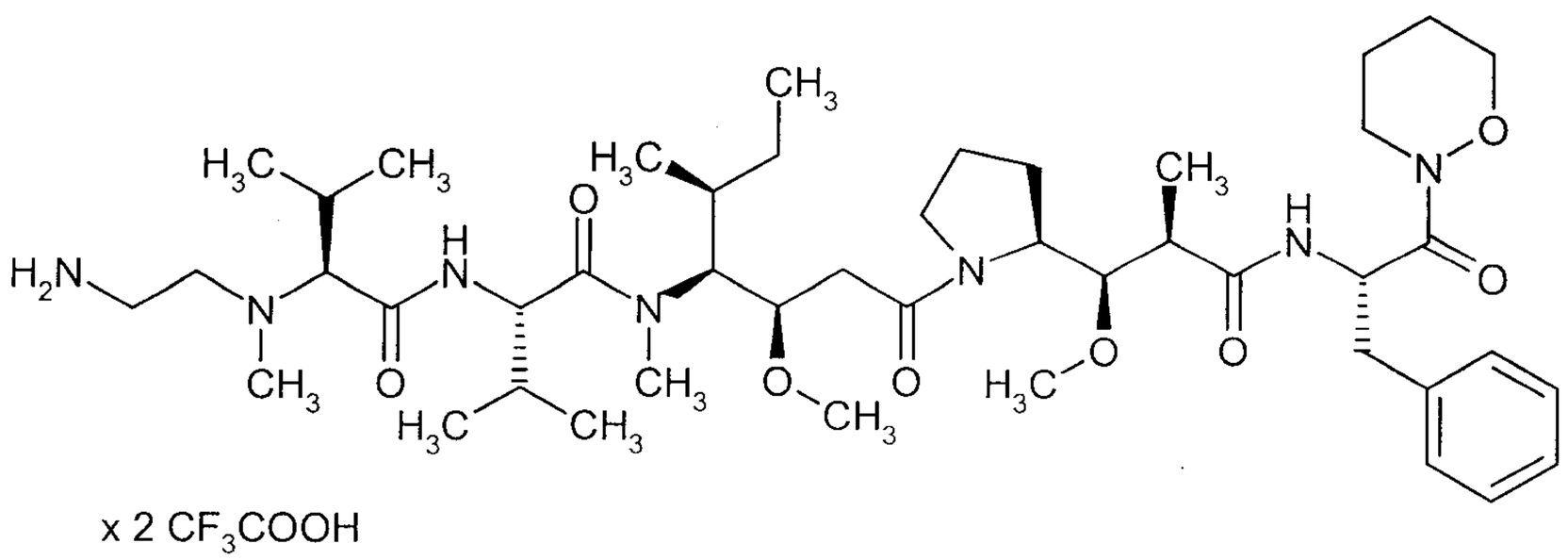
(Intermediate 16) were dissolved in 2 ml of dioxane/water, and 2.8 mg (0.022 mmol) of 6-oxohexanoic acid were added. The reaction mixture was subsequently stirred at 100°C for 1 h. After cooling to RT, 0.75 mg (0.012 mmol) of sodium cyanoborohydride were added, and the mixture was adjusted to a pH of 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)

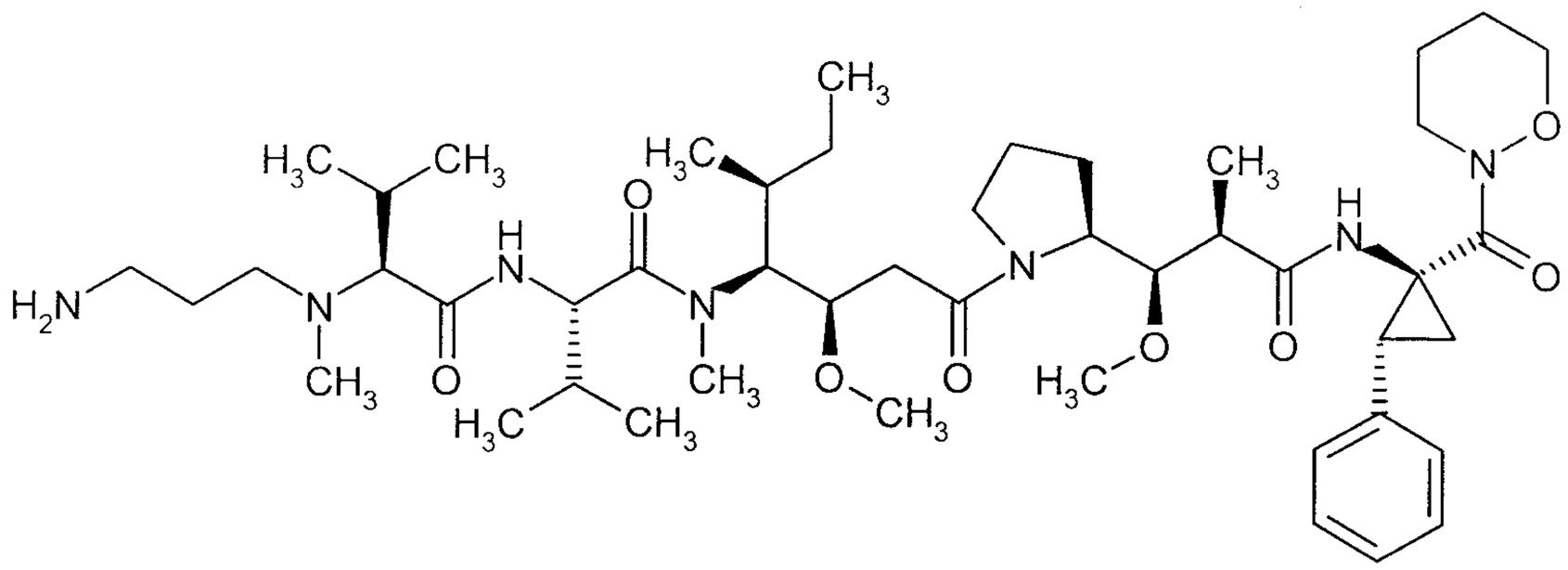
HPLC (Method 5): R_t = 1.58 min;

LC-MS (Method 2): R_t = 1.05 min; MS (ESIpos): m/z = 844 $(M+H)^+$

^{1}H NMR (600 MHz, DMSO-d_6): δ = 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 H_2O 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).

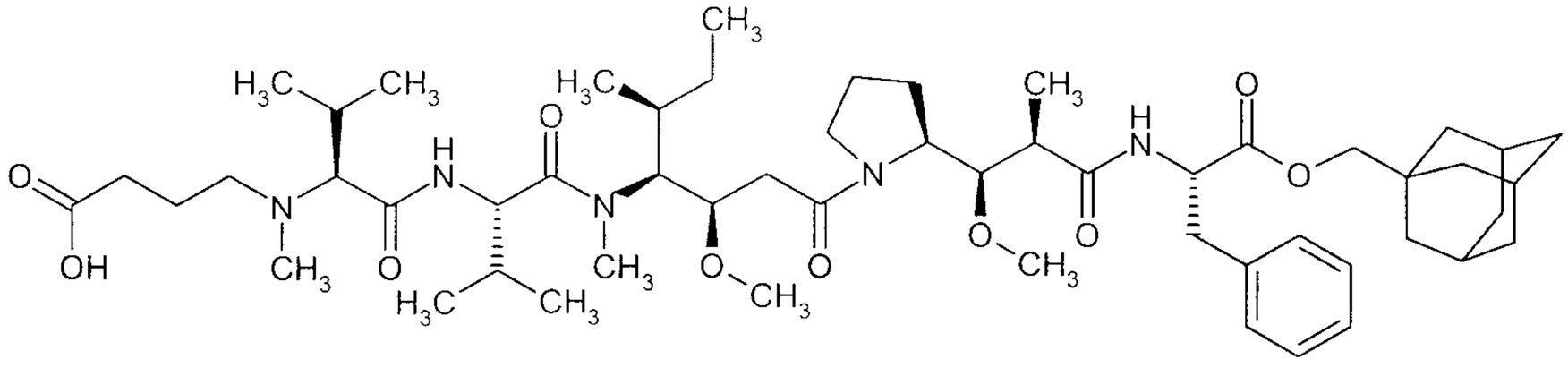
Yield: 11 mg (41% of theory over two stages)

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

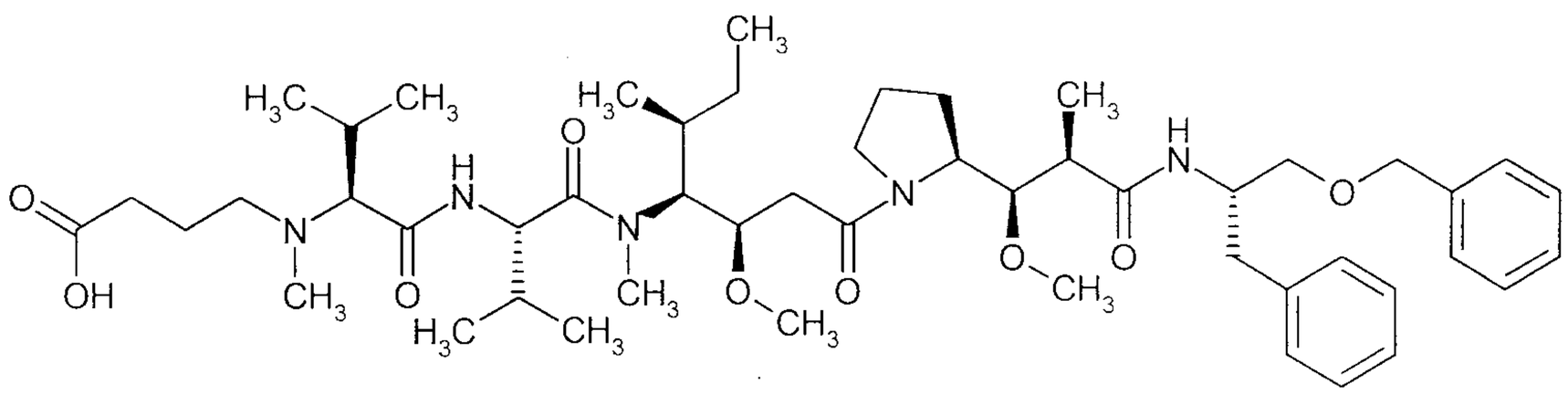


26 mg (26 µmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(adamantan-1-ylmethoxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate and 33.9 µl of a 15% aqueous succinaldehydic acid solution (53 µmol) were dissolved in 957 µl of a 1:1-dioxane/water mixture and heated to 100°C for 1 h. After brief cooling, 1.81 mg (29 µmol) 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, 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

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

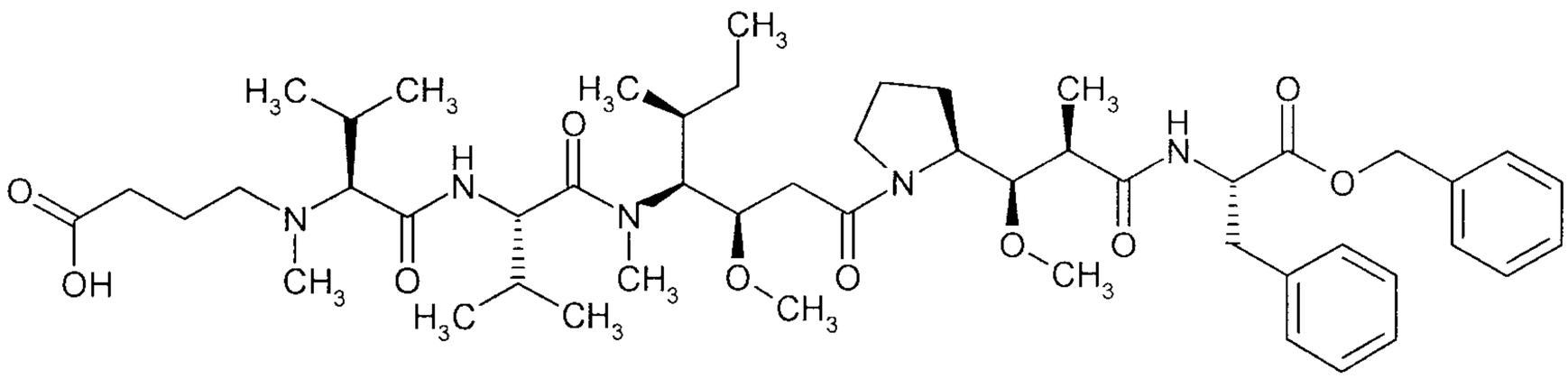


24 mg (26 µmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate and 33.7 µl of a 15% aqueous succinaldehydic acid solution (52 µmol) were dissolved in 953 µl of a 1:1-dioxane/water mixture and heated to 100°C for 1 h. After brief cooling, 1.80 mg (29 µmol) 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 $(M+H)^+$

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



53 mg (84 µmol) 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 µmol) 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 $(M+H)^+$.

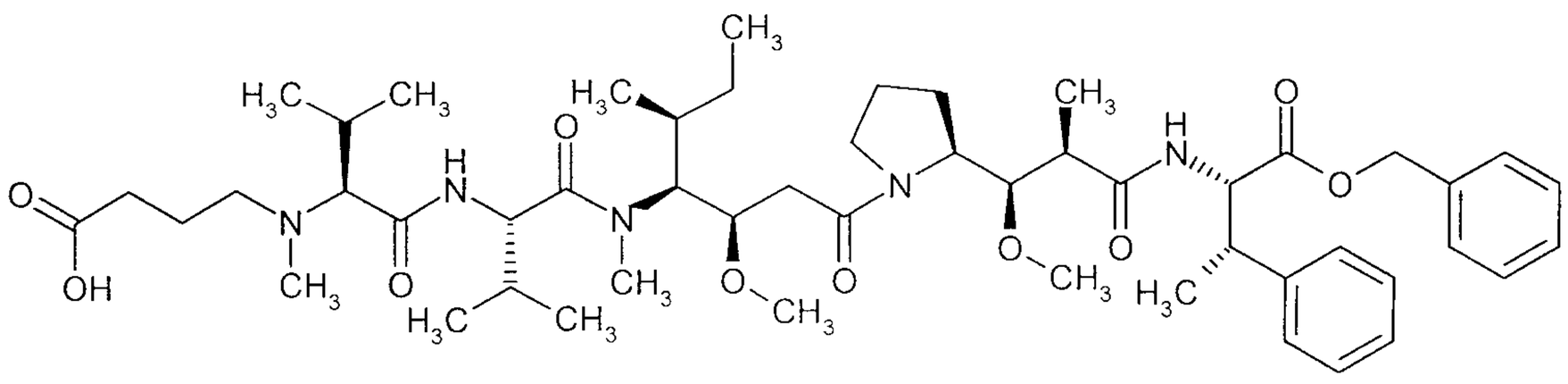
37 mg (0.045 mmol) of this intermediate were dissolved in 5 ml of dioxane/water and, in analogy to the preparation of the compound in Intermediate 66, reacted with a 15% aqueous solution of 4-oxobutanoic acid in the presence of sodium cyanoborohydride. 16 mg (39% of theory) of the title compound were 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.

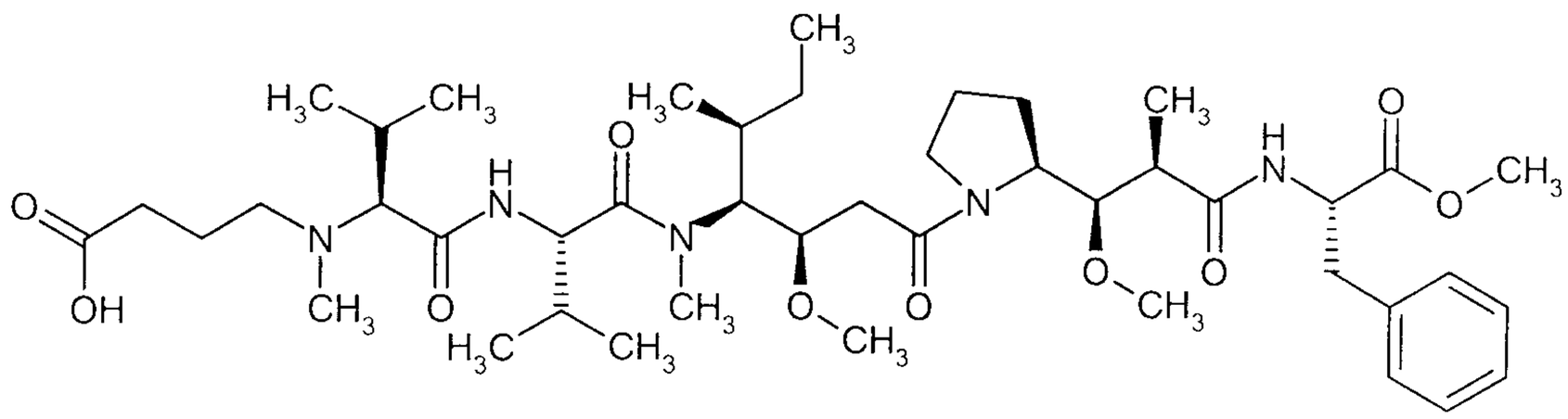
30 mg (0.032 mmol) of this compound were dissolved in 6 ml of dioxane/water, and 41 µl (0.063 mmol) of a 15% aqueous solution of 4-oxobutanoic acid were added. The reaction mixture was subsequently stirred at 100°C for 1 h. After cooling to RT, 2.2 mg (0.035 mmol) of sodium cyanoborohydride were added, and the mixture was adjusted to a pH of 3 by adding about 300 µl of 0.1 N hydrochloric acid. The reaction mixture was then stirred at 100°C for another 2 h. After cooling, another 41 µl (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 µl 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



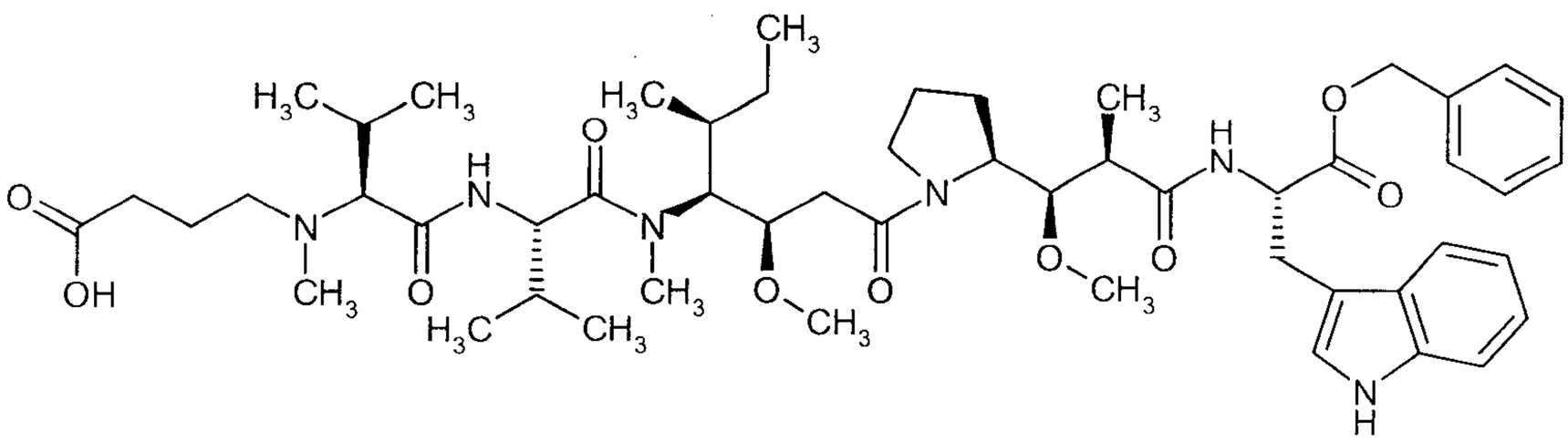
First, in analogy to the synthesis described in Intermediate 14, proceeding from Intermediates 4 and 39, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-3-{[(2*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino}-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide 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;

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



212 mg (411 µmol) 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 µmol) 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 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 215 µl *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 µmol) 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 $(M+H)^+$.

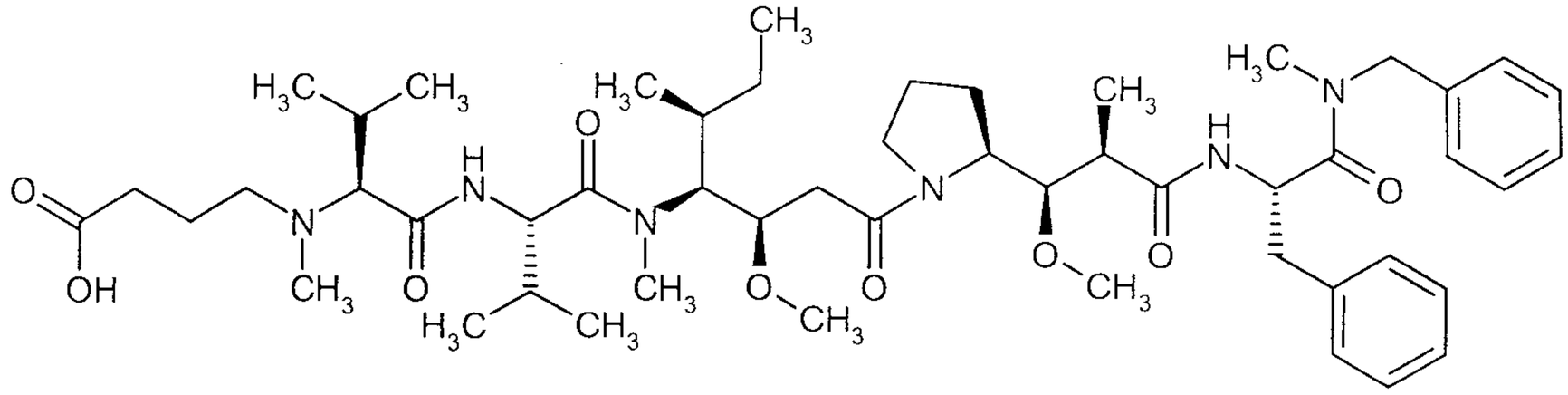
29 mg (0.03 mmol) of this intermediate were dissolved in 6 ml of dioxane/water, and 39 µl (0.059 mmol) of a 15% aqueous solution of 4-oxobutanoic acid were added. The reaction mixture was subsequently stirred at 100°C for 1 h. After cooling to RT, 2 mg (0.033 mmol) of sodium cyanoborohydride were added, and the mixture was adjusted to a pH of 3 by adding about 300 µl of 0.1 N hydrochloric acid. The reaction mixture was then stirred at 100°C for a further 2 h. After cooling, another 39 µl (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 µl 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



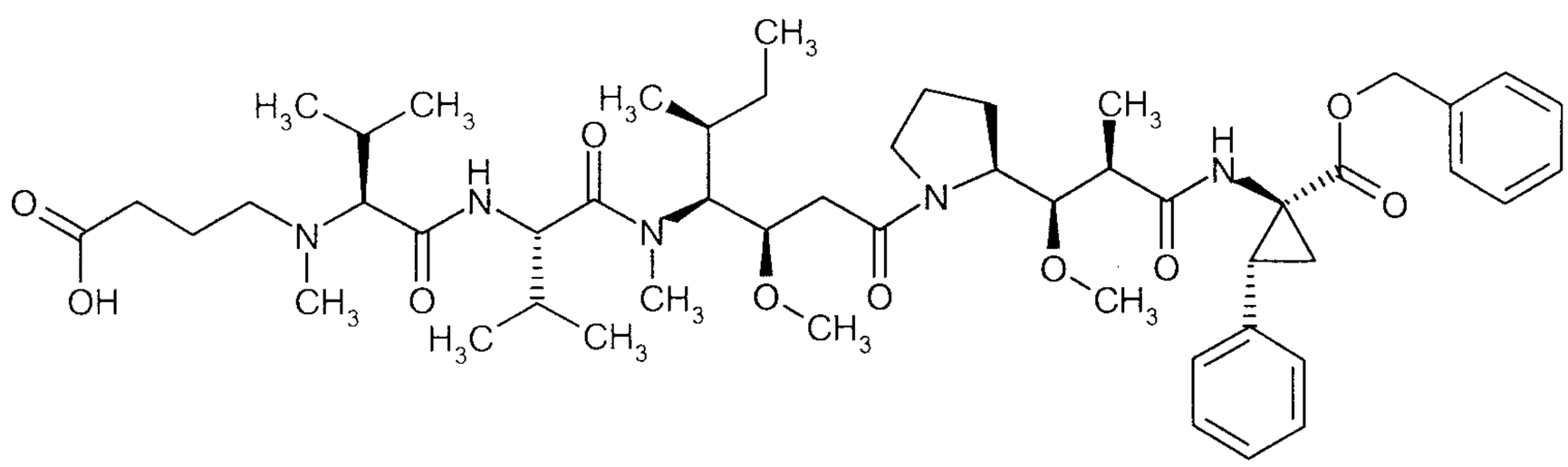
First, in analogy to the synthesis described in Intermediate 14, proceeding from Intermediates 4 and 38, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-({(2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl}amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide 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.

HPLC (Method 12): R_t = 2.2 min;

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 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 28 mg (73 µmol) 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 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 38 µl 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 $(M+H)^+$.

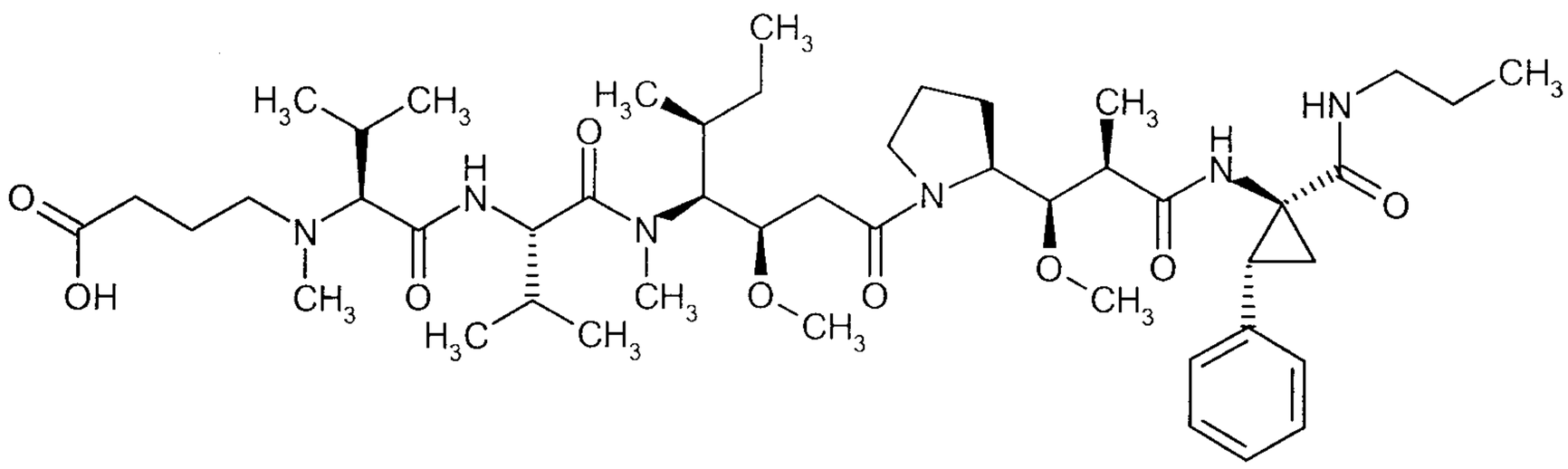
11 mg (0.011 mmol) of this intermediate were then used, in analogy to the preparation of Intermediate 66, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 2.5 mg (24% of theory) of the title compound in the form of a colourless foam.

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



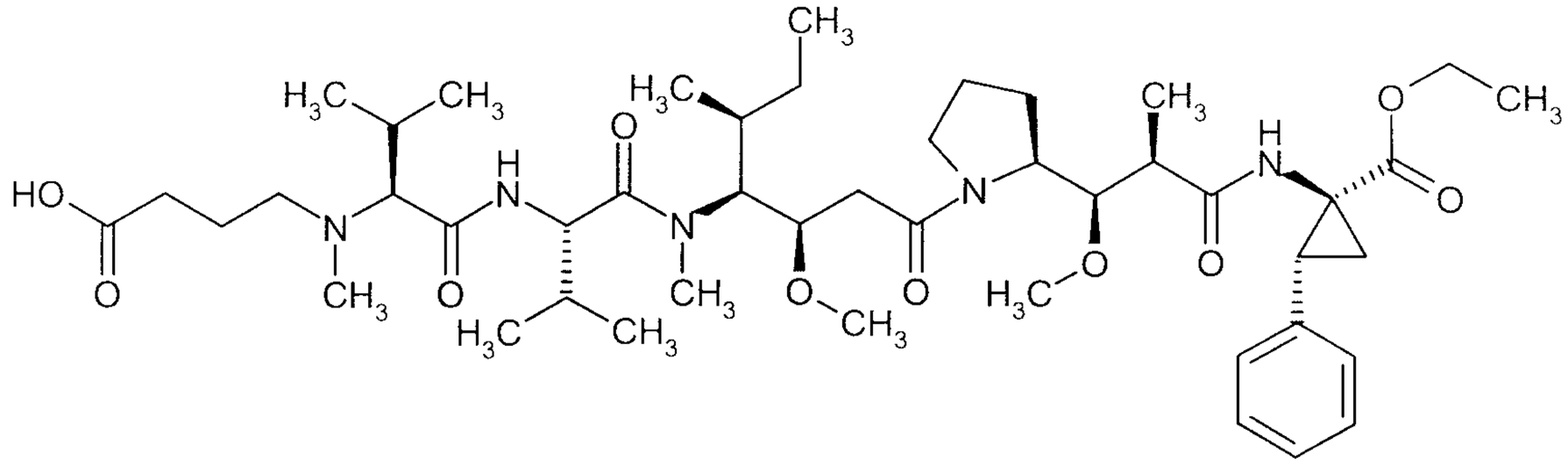
First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and (1*S*,2*R*)-1-amino-2-phenyl-*N*-propylcyclopropanecarboxamide 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



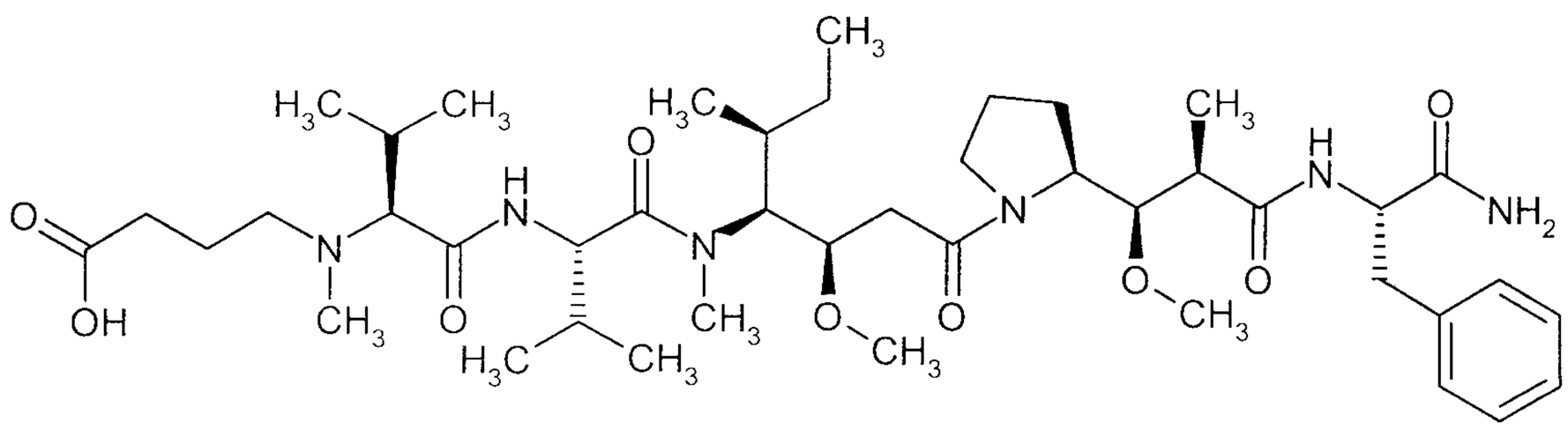
First, by coupling of Intermediate 46 (*N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide) with Intermediate 48 (ethyl (1*S*,2*R*)-1-amino-2-phenylcyclopropanecarboxylate 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

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.

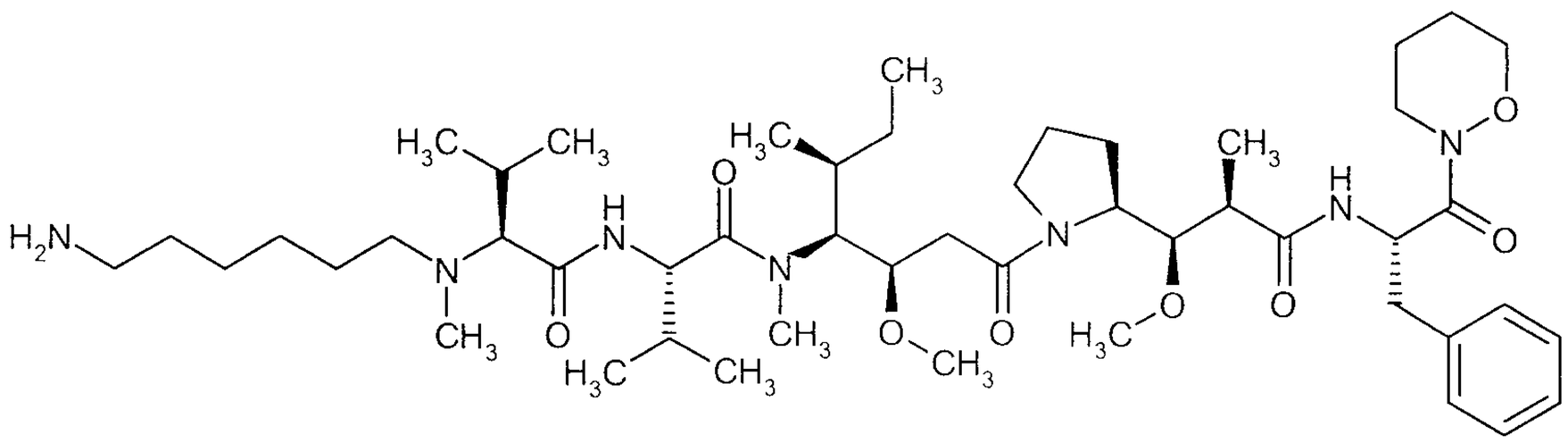
HPLC (Method 6): R_t = 1.7 min;

LC-MS (Method 9): R_t = 4.44 min; MS (ESIpos): m/z = 817 $(M+H)^+$

^{1}H NMR (500 MHz, DMSO-d_6): δ = 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*,5S)-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 µmol) of the compound from Intermediate 14 and benzyl-(6-oxohexyl)carbamate, and the hydrogenation was performed in methanol as the solvent.

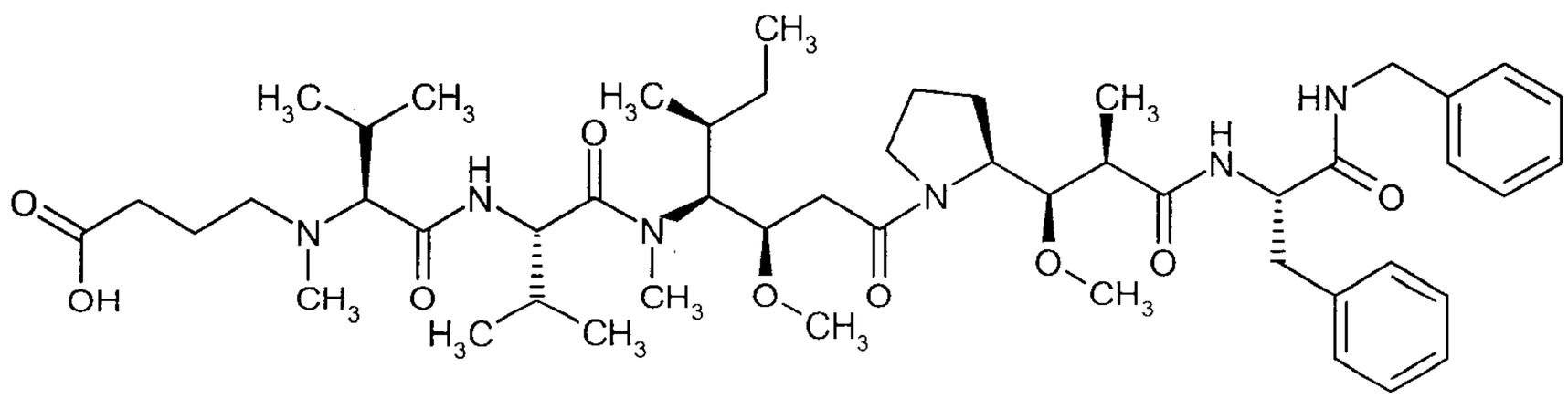
Yield: 7.6 mg (55% of theory over 2 stages)

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

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 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-{[(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 µmol) of benzylamine were taken up in 5 ml of DMF, 7.5 µl (88 µmol) of *N,N*-diisopropylethylamine, 10 mg (65 µmol) of HOBt and 10 mg (52 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. 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-

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-{(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 were obtained as the trifluoroacetate.

LC-MS (Method 1): R_t = 0.95 min; m/z = 821 $(M+H)^+$.

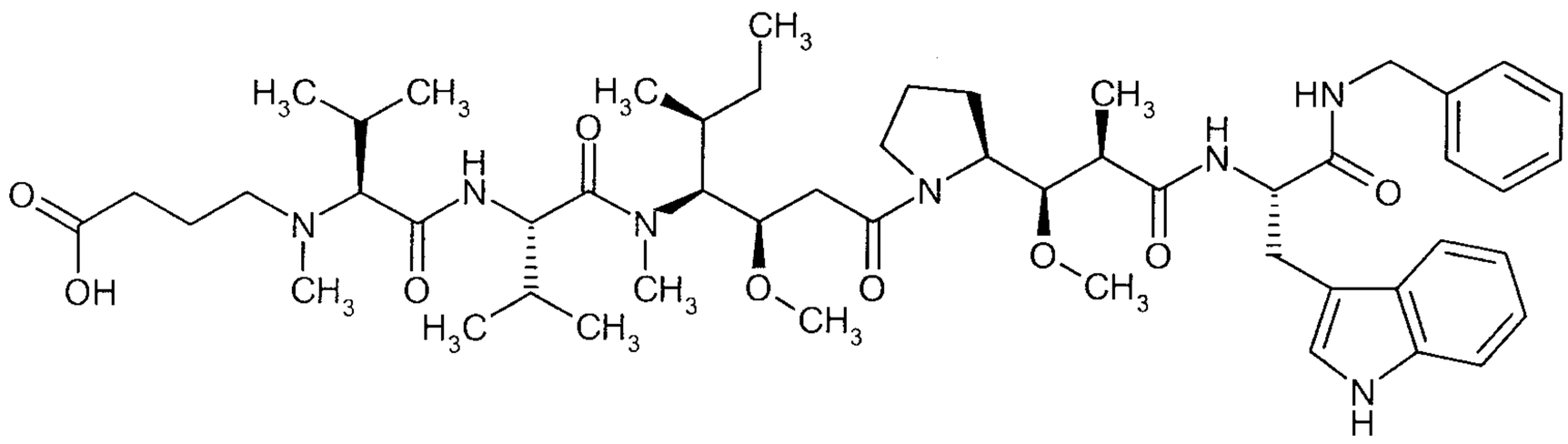
17 mg (0.018 mmol) of this intermediate were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 13 mg (80% of theory) of the title compound in the form of a colourless foam.

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-{(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



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

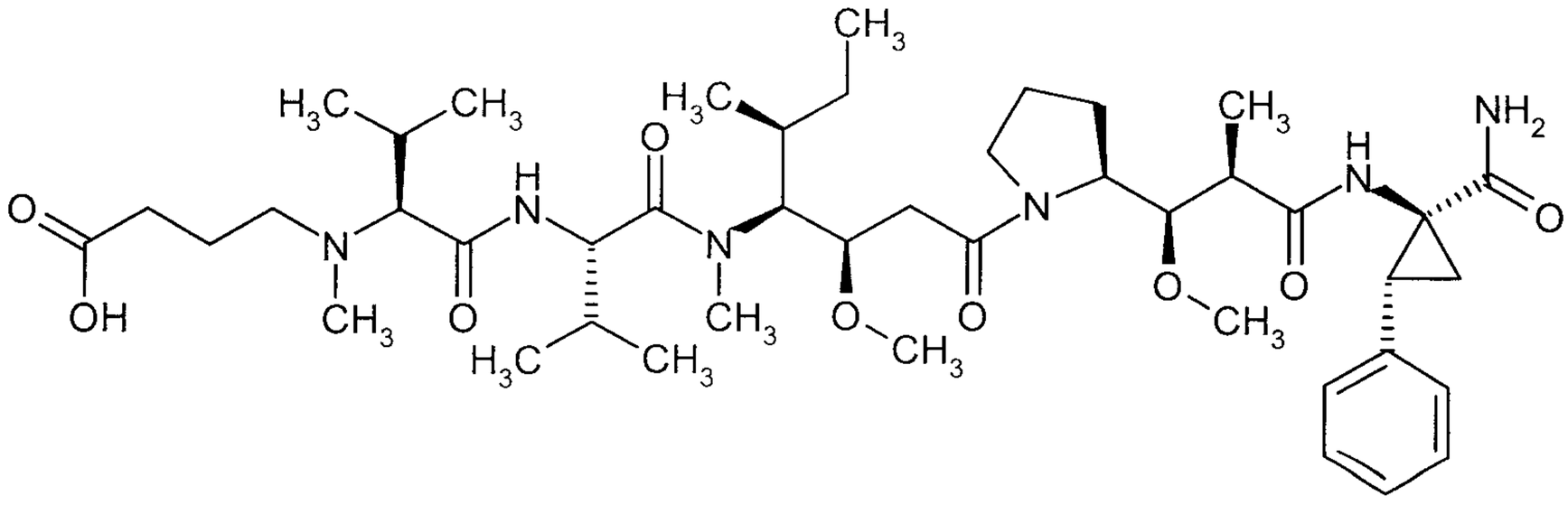
(Intermediate 26) and *N*-benzyl-L-tryptophanamide trifluoroacetate (Intermediate 47) in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleaving of the Boc protecting group by means of trifluoroacetic acid, the amine compound *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-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.

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

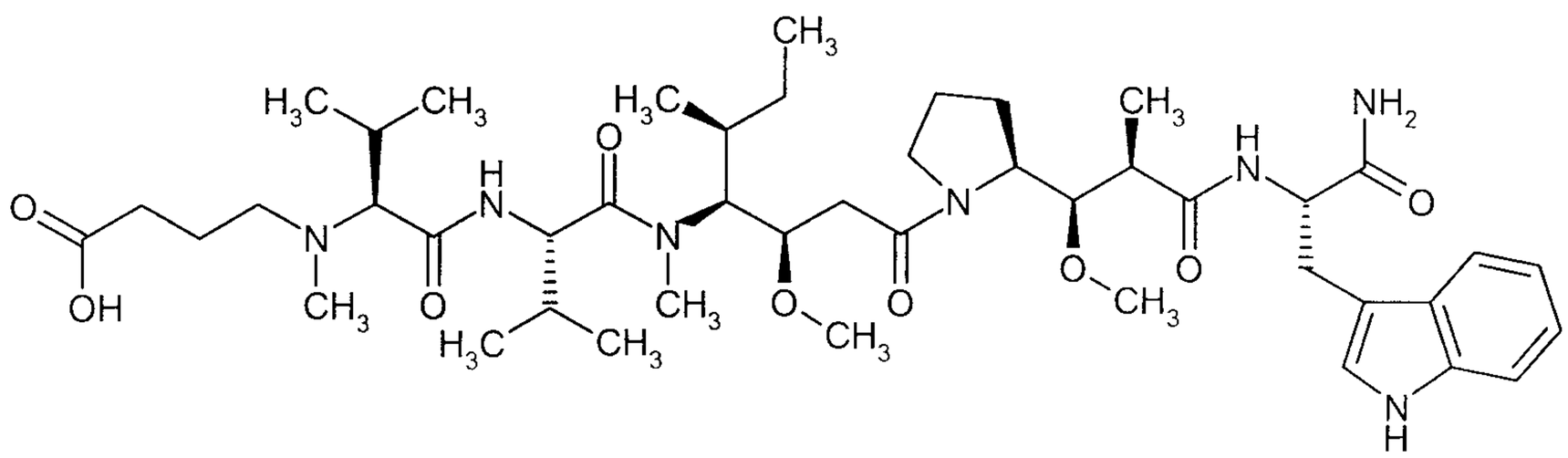
analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 8 mg (57% of theory) of the title compound.

HPLC (Method 5): R_t = 1.6 min;

LC-MS (Method 9): R_t = 4.64 min; MS (ESIpos): m/z = 829 $(M+H)^+$.

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



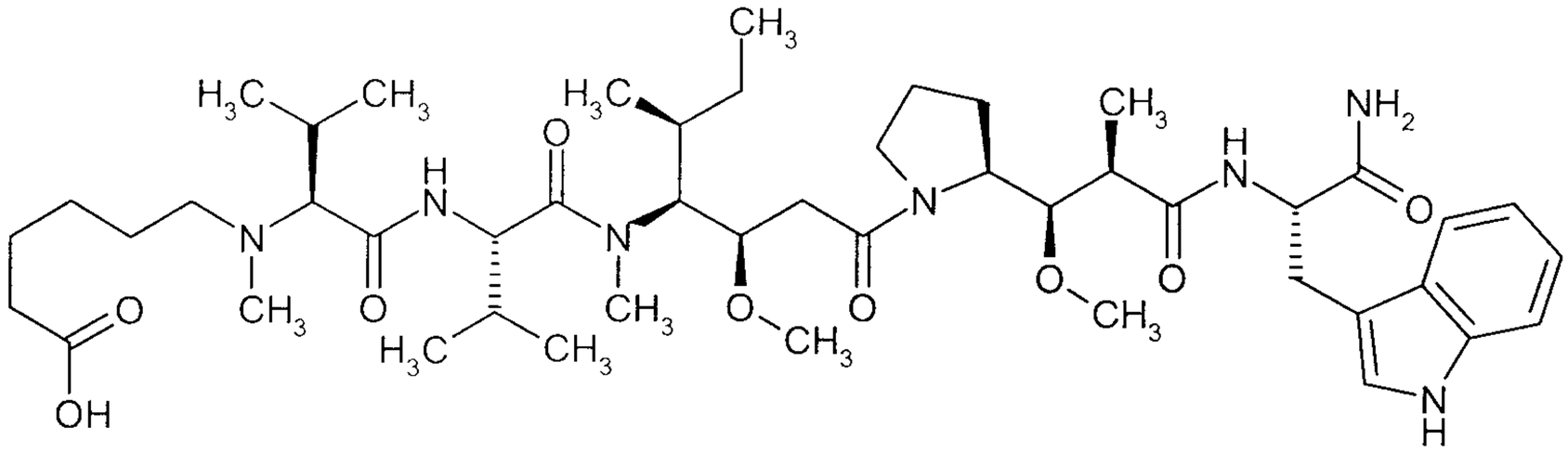
First, in analogy to the synthesis described in Intermediate 69, by coupling of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-L-valyl-*N*-[(2*R*,3*S*,4*S*)-1-carboxy-2-methoxy-4-methylhexan-3-yl]-*N*-methyl-L-valinamide (Intermediate 4) and N^{α}-{(2*R*,3*R*)-3-methoxy-2-methyl-3-[(2*S*)-pyrrolidin-2-yl]propanoyl}-L-tryptophanamide 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 *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, 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



This compound was prepared in analogy to the compound in Intermediate 82, proceeding from 20 mg (26 µmol) 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.

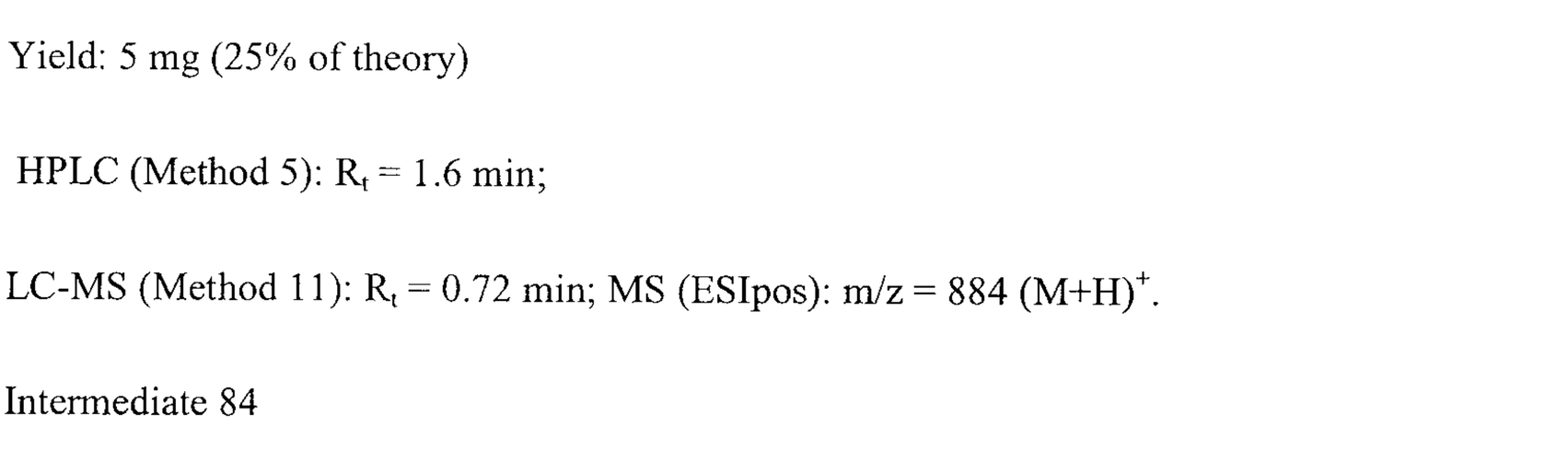
Yield: 5 mg (25% of theory)

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



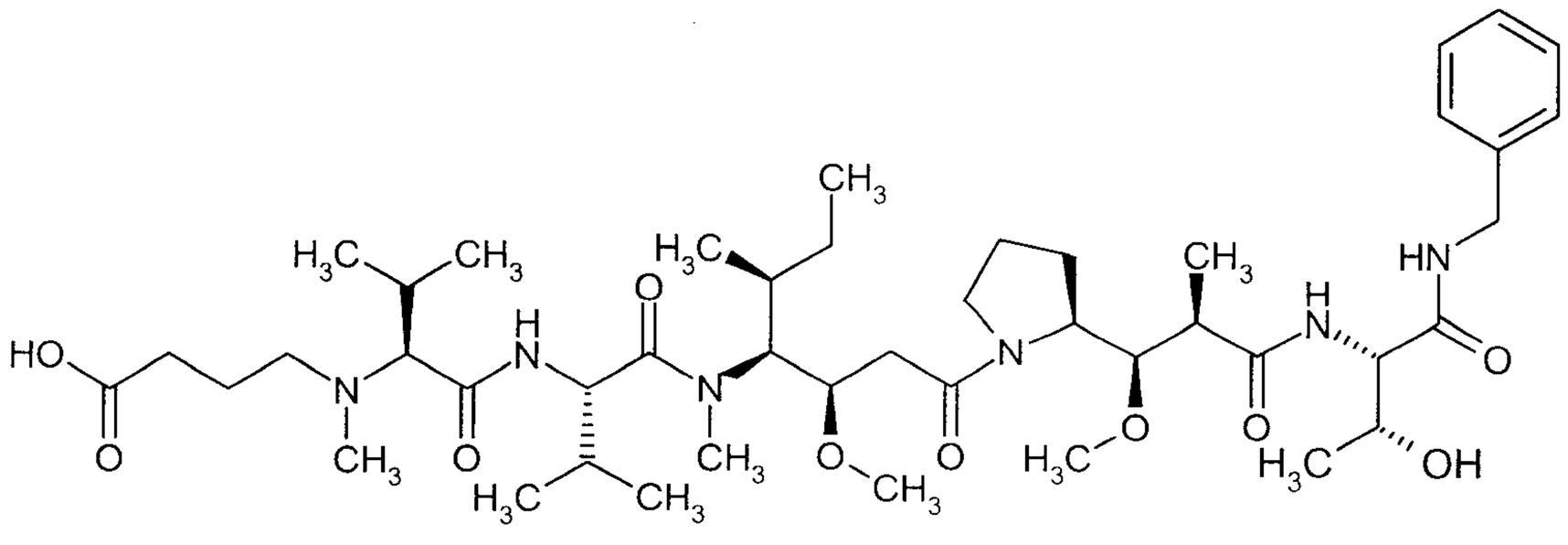
First, in analogy to the synthesis described in Intermediate 79, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(1*S*)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (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-

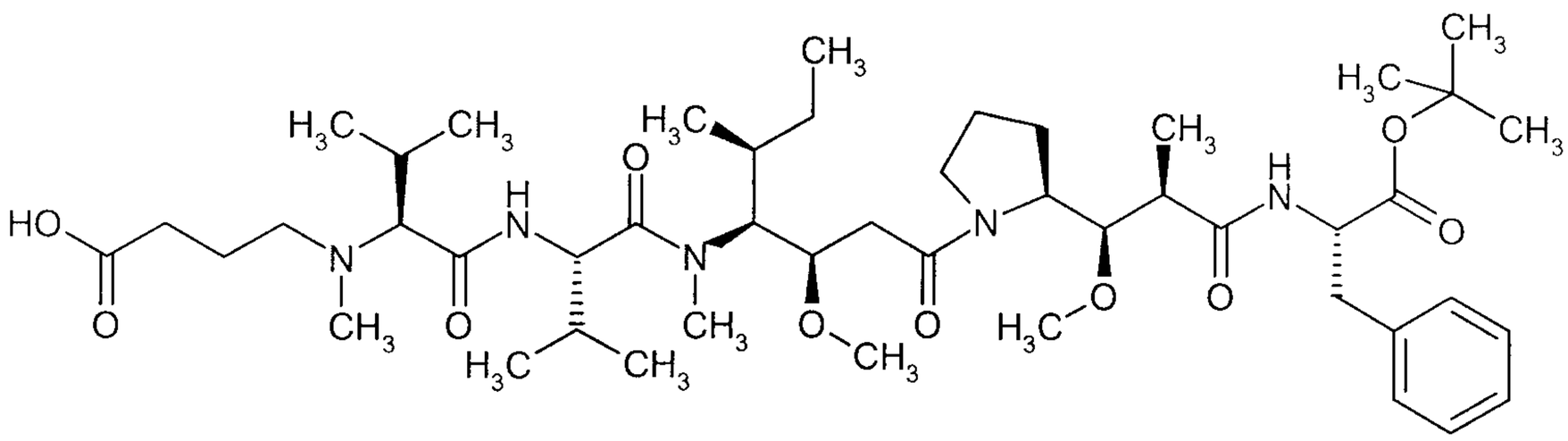
3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide was prepared as the trifluoroacetate. 21 mg (0.024 mmol) of this compound were then used, in analogy to the preparation of Intermediate 61, by reaction with 4-oxobutanoic acid in the presence of sodium cyanoborohydride, to obtain 20 mg (97% of theory) of the title compound.

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-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-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



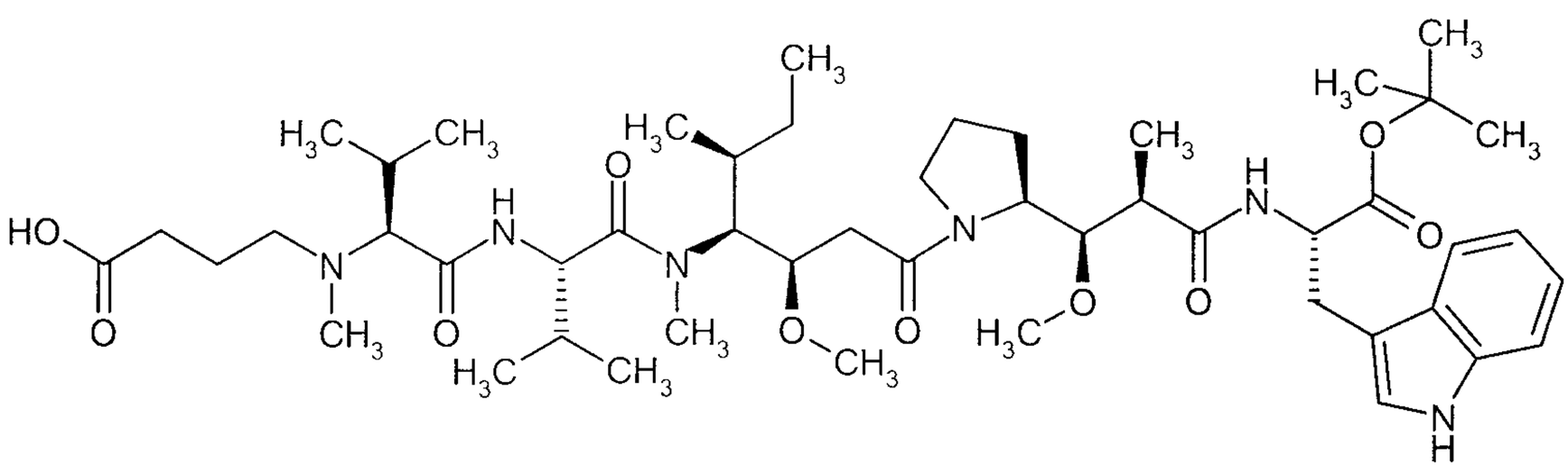
First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and *tert*-butyl-L-phenylalaninate hydrochloride in the presence of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and subsequent cleavingt 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 *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 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-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-1-tert-butoxy-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 in analogy to the synthesis described in Intermediate 86, proceeding from 230 mg (336 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 *tert*-butyl-L-tryptophanate hydrochloride over 3 stages.

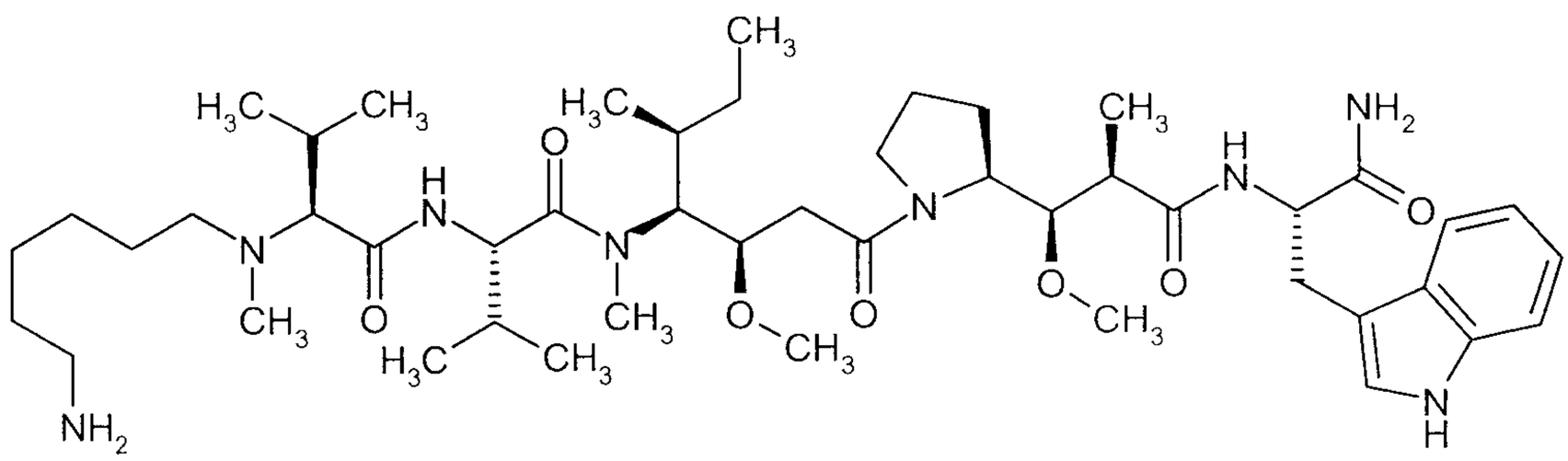
Yield: 95 mg (31% of theory over 3 stages)

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.

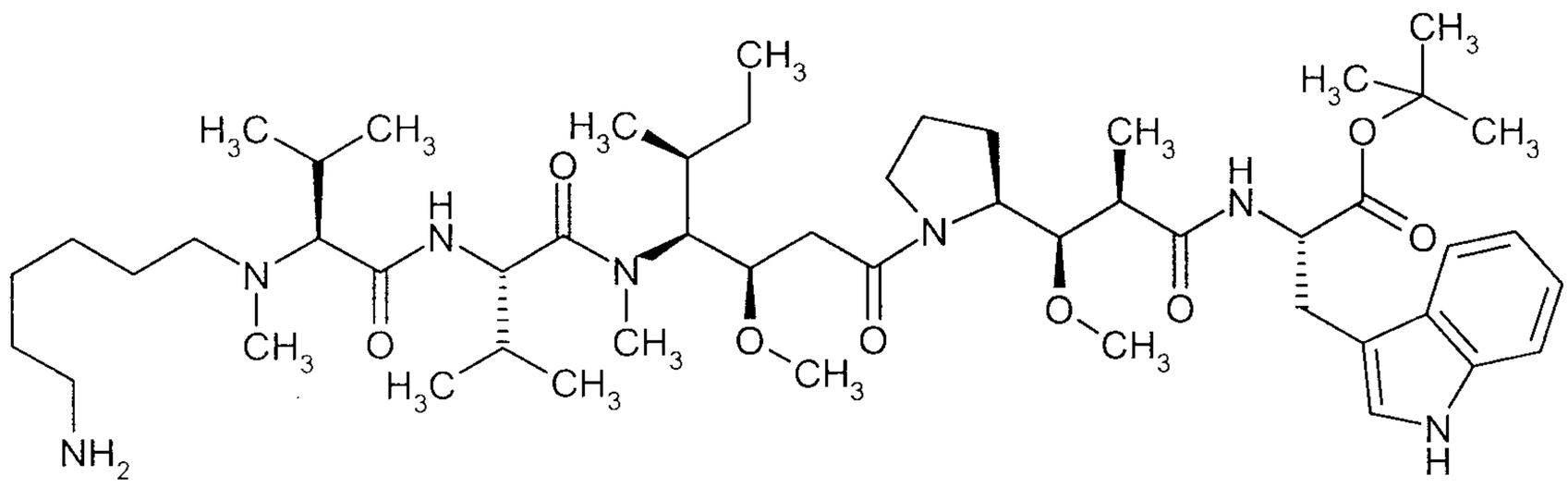
Yield: 14 mg (95% of theory)

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*-*but*oxy-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.

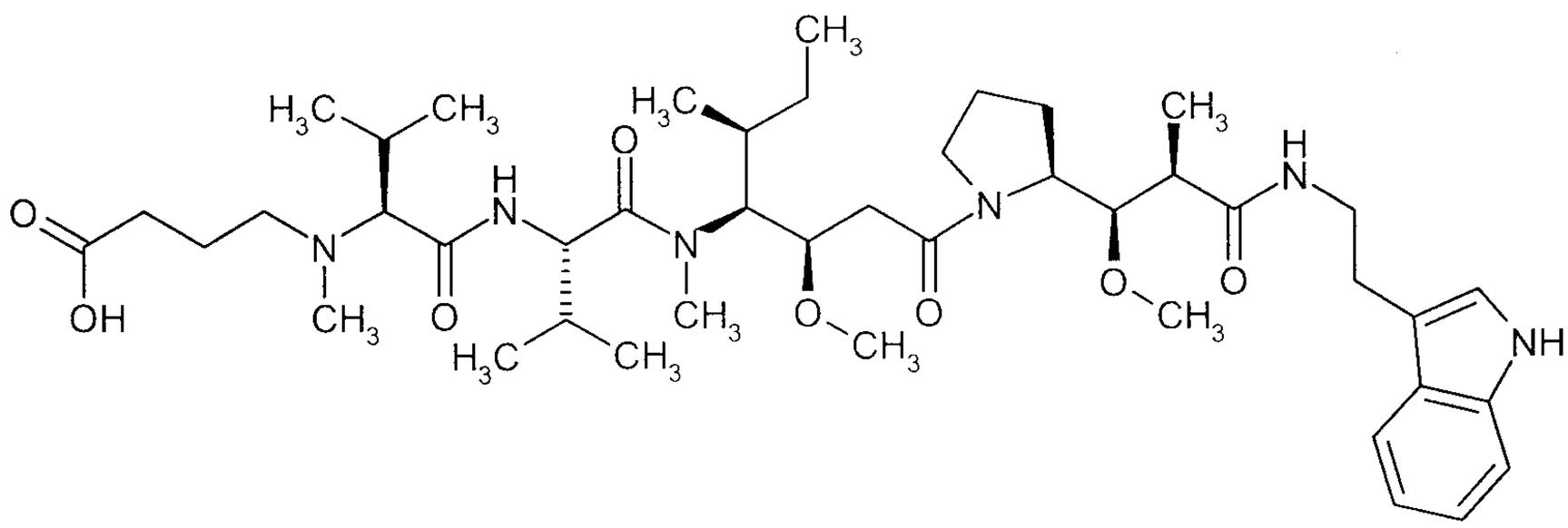
Yield: 30 mg (98% of theory)

HPLC (Method 5): Rt = 1.9 min;

LC-MS (Method 1): Rt = 0.77 min; MS (ESIpos): m/z = 926 (M+H)+.

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



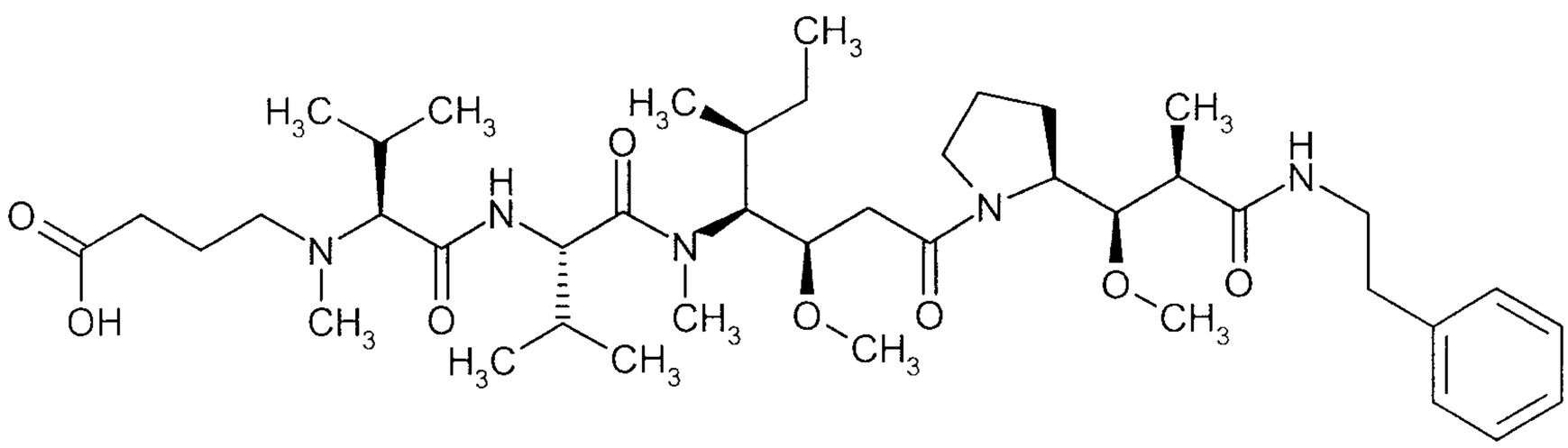
First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and 2-(1*H*-indol-3-yl)ethanamine 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 15/2/02 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



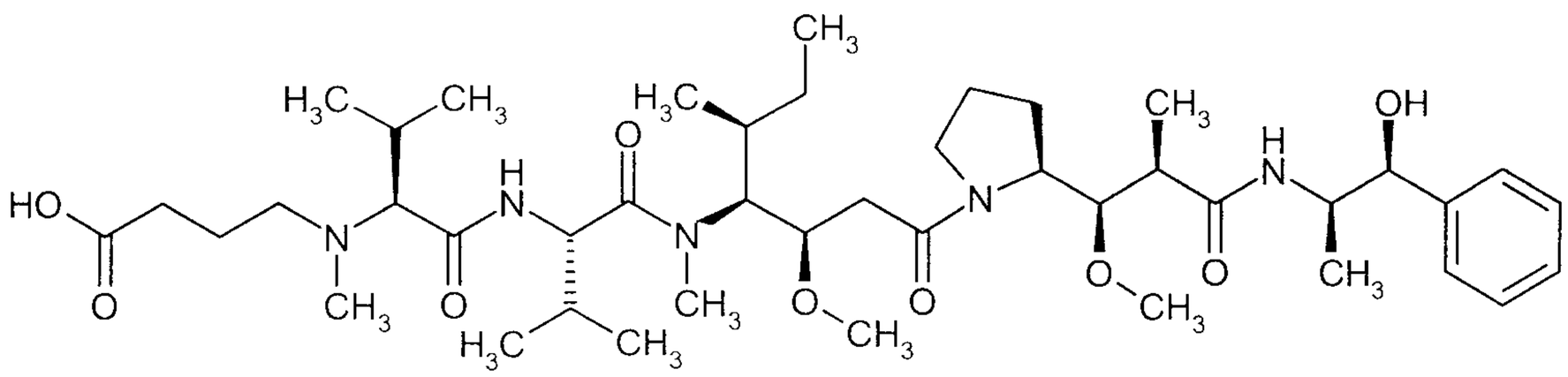
First, in analogy to the synthesis described in Intermediate 74, by coupling of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) and phenylethylamine 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



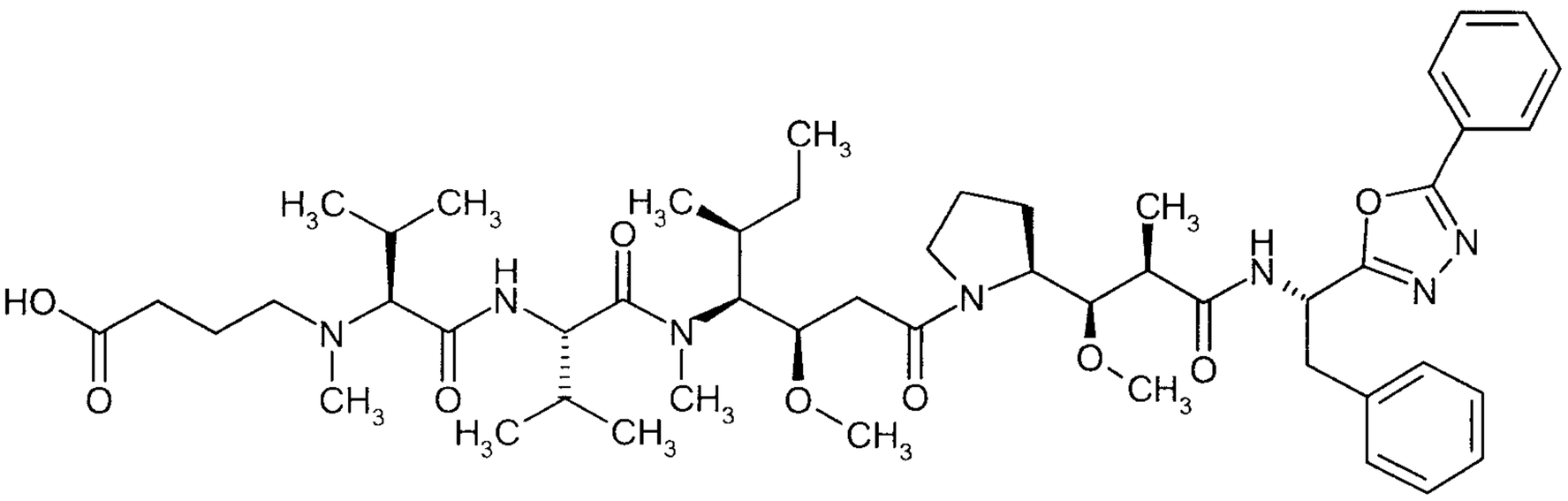
100 mg (0.139 mmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(*1R*,2*R*)-3-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 40) were used, in analogy to the preparation 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;

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



22.4 mg (24 µmol) 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

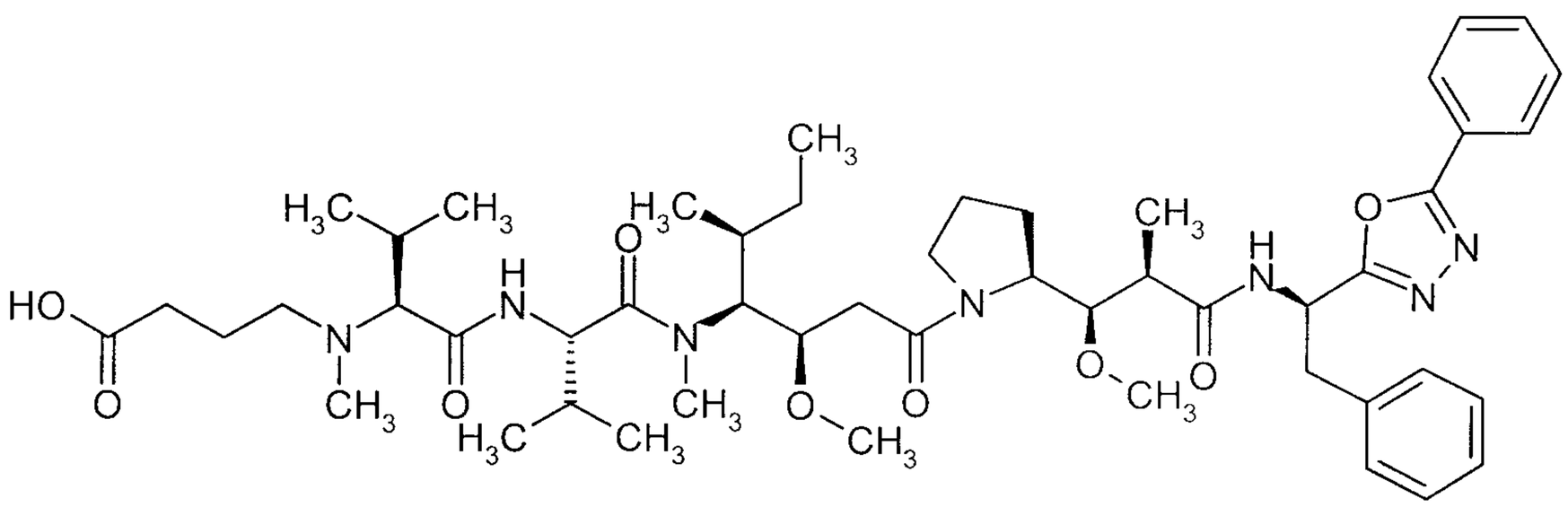
sodium cyanoborohydride. After lyophilization from dioxane, 8.2 mg (38% of theory) of the title compound were obtained in the form of a white solid.

HPLC (Method 10): R_t = 2.54 min

LC-MS (Method 12): R_t = 0.94 min; MS (ESIpos): m/z = 919 $(M+H)^+$

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



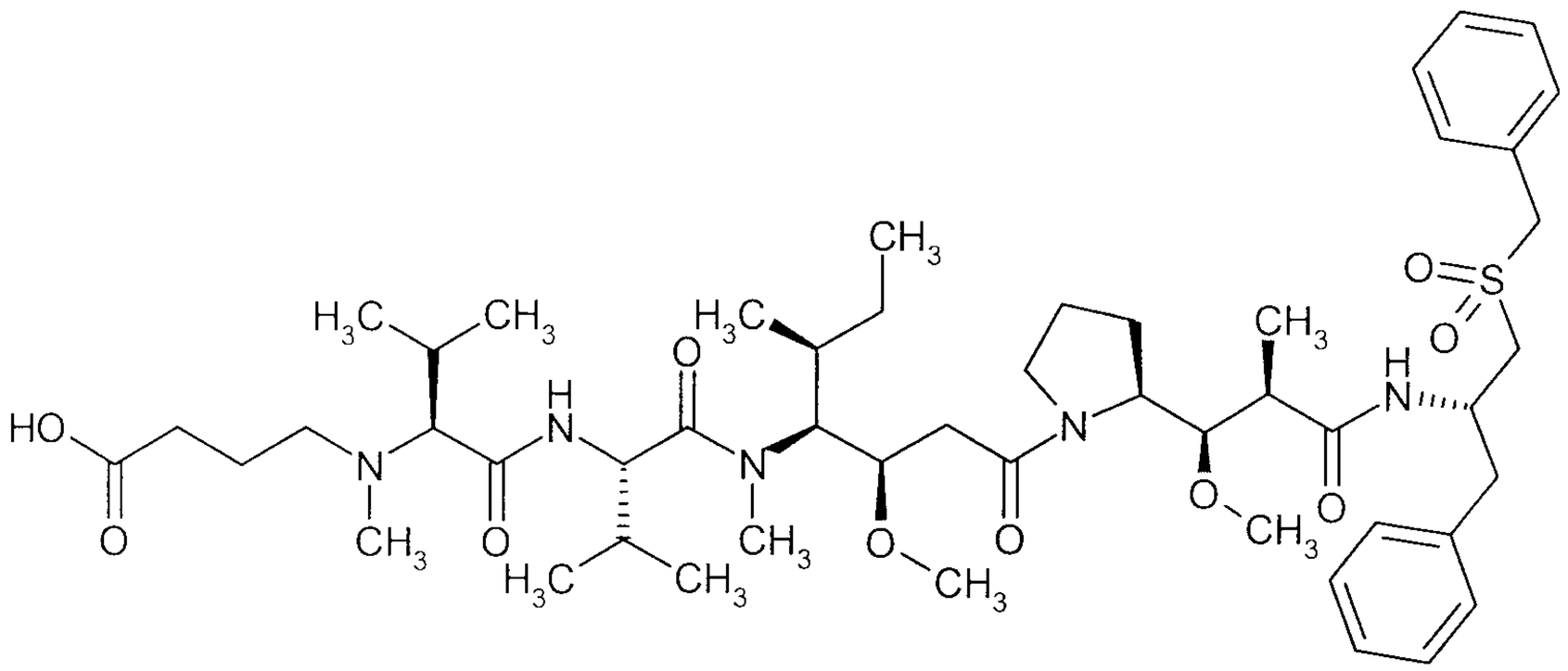
17.1 mg (18 µmol) of *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate were dissolved in 1.1 ml of dioxane/water and, analogously to the preparation of 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 $(M+H)^+$

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

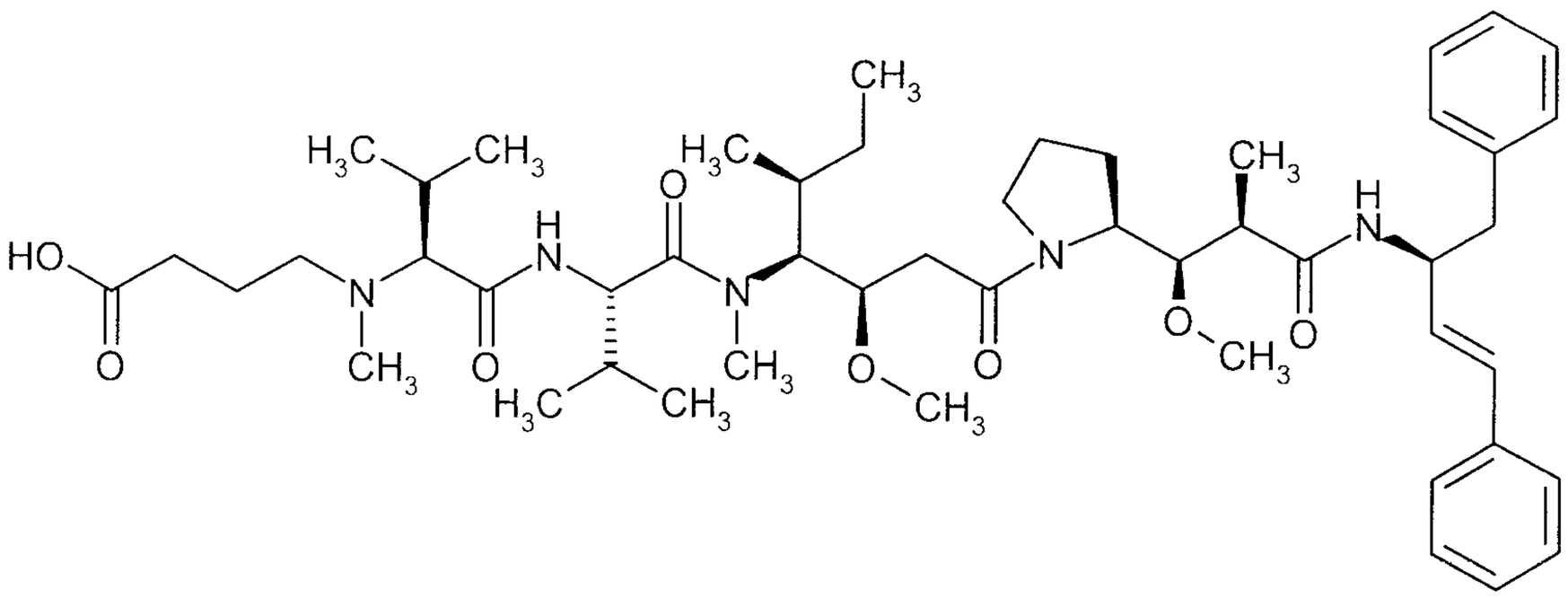


19.3 mg (20 µmol) *N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate were dissolved in 1.2 ml of dioxane/water 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 $(M+H)^+$

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 µmol) 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-

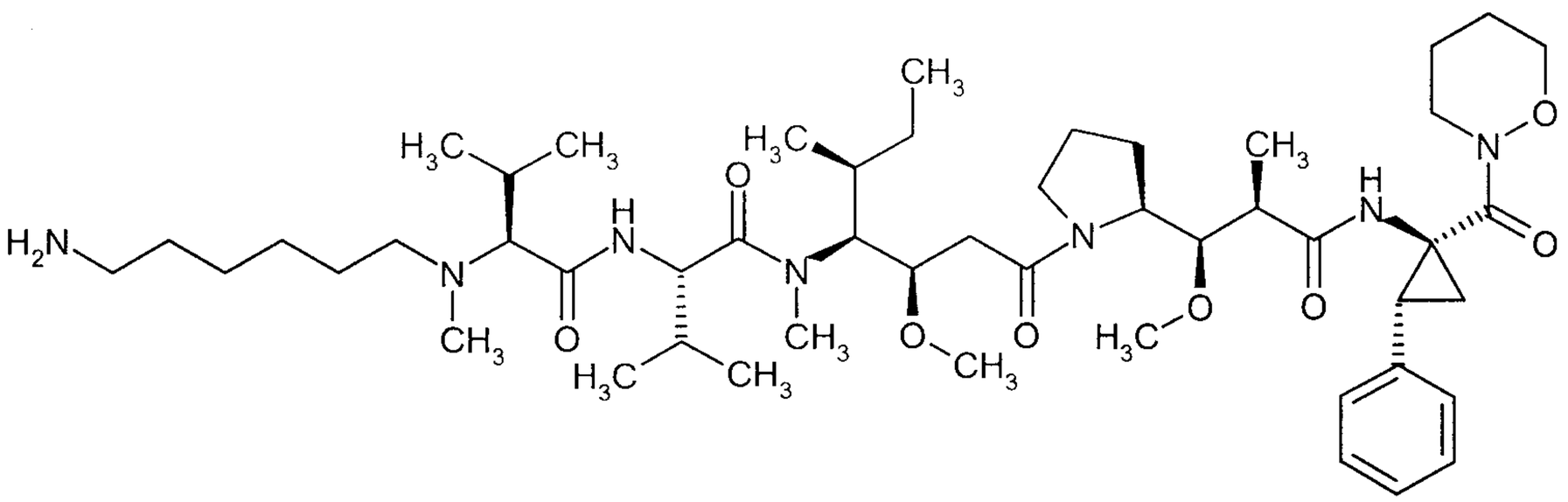
methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide trifluoroacetate were dissolved in 1.0 ml of dioxane/water and, analogously to the preparation of Intermediate 61, reacted with 15% aqueous solution of 4-oxobutanoic acid in the presence of sodium cyanoborohydride. After lyophilization from dioxane, 10.3 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).

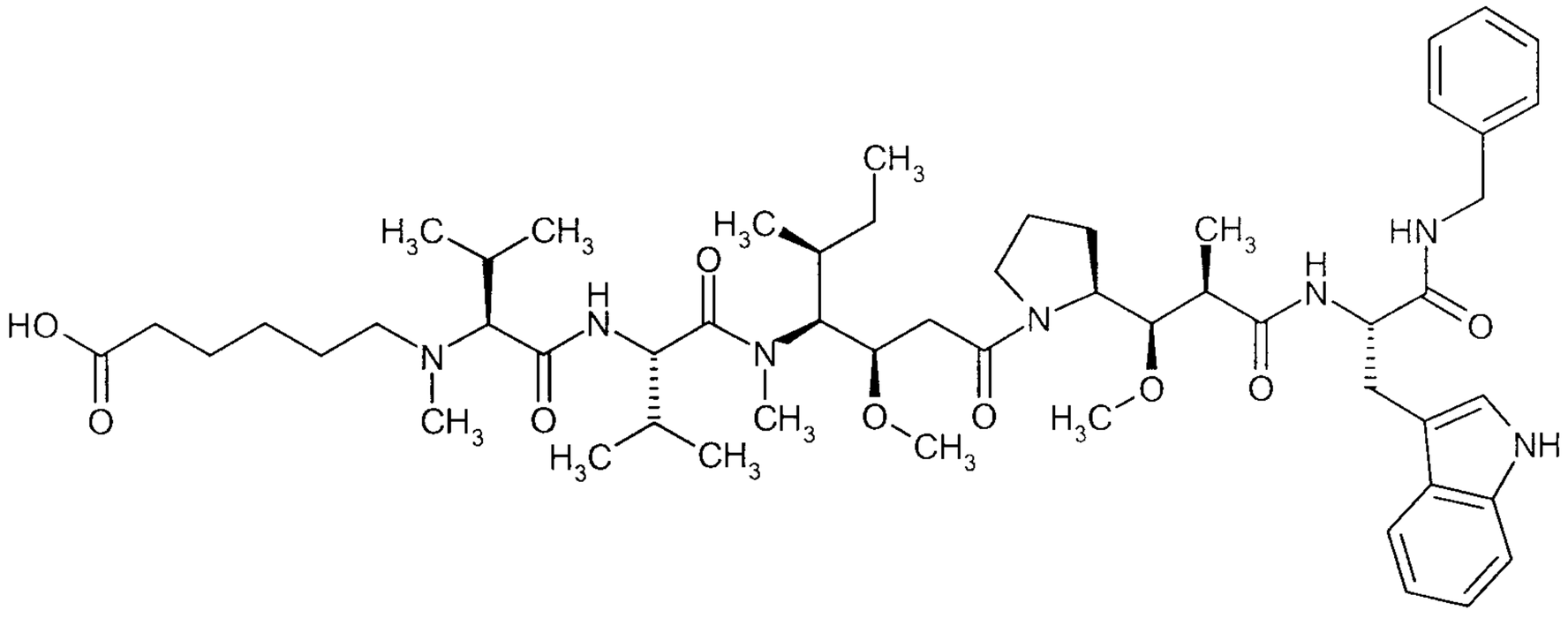
Yield: 69 mg (65% of theory over two stages)

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



This compound was prepared in analogy to the synthesis described in Intermediate 80. The purification was effected by preparative HPLC.

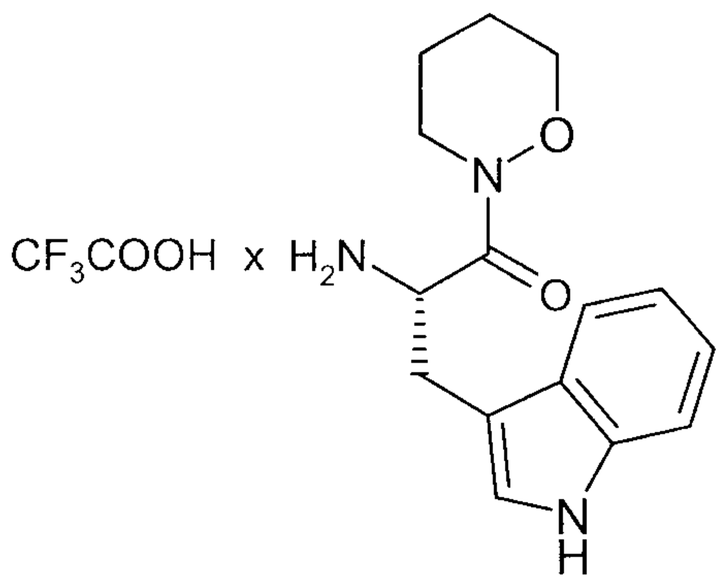
Yield: 40 mg (29% of theory over 3 stages)

HPLC (Method 5): R_t = 1.9 min;

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 dissolved in 20 ml of DMF, and 200 mg (1.62 mmol) of 1,2-oxazinane 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 $(M+H)^+$.

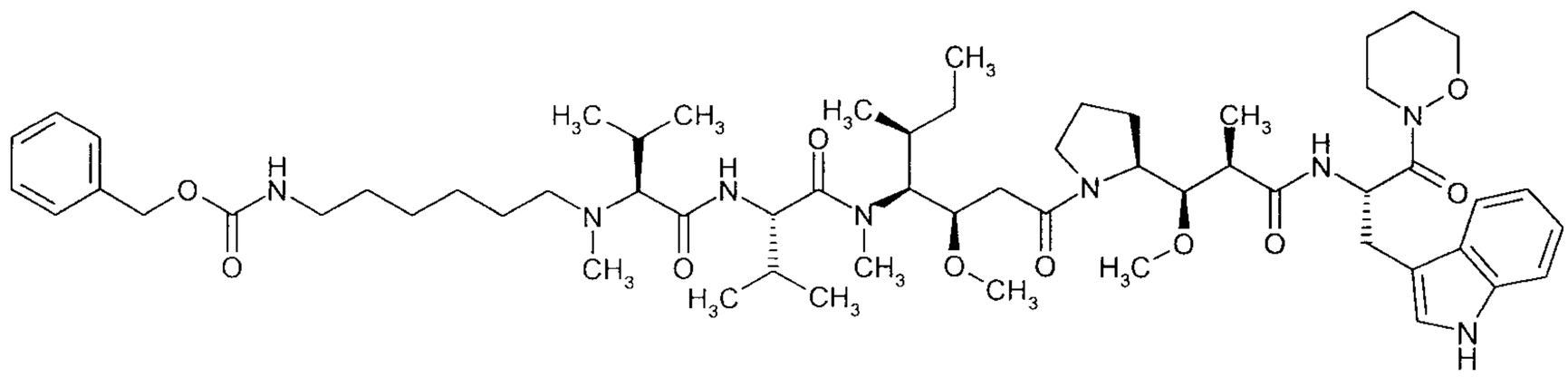
Using 166 mg (444.5 µmol) of this intermediate, under standard conditions with 3 ml of trifluoroacetic acid in 20 ml of dichloromethane, the Boc protecting group was cleaved and, after HPLC purification, 155 mg (86% of theory) of the title compound were obtained.

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 $(M+H)^+$.

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.

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.

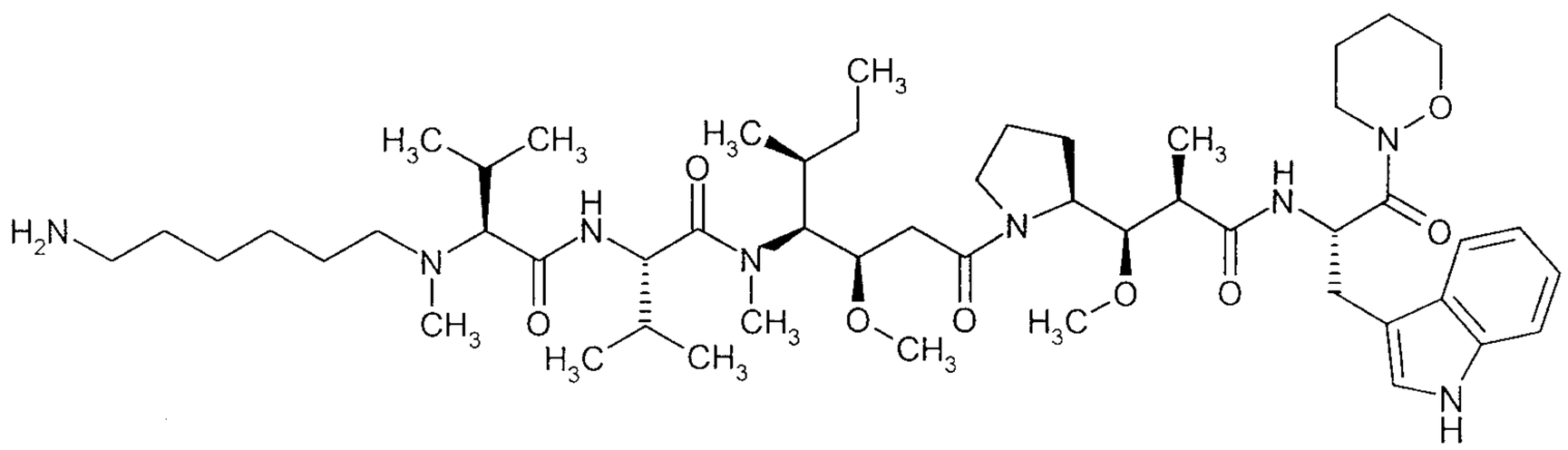
Yield: 21 mg (37% of theory)

HPLC (Method 12): R_t = 2.1 min;

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*-[(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



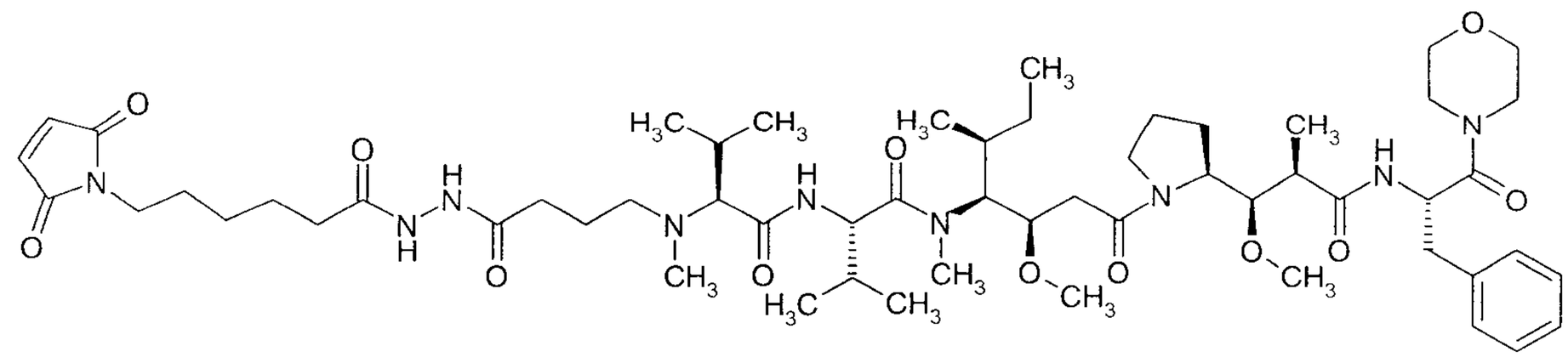
26.7 mg (24.87 µmol) of Intermediate 100 were dissolved in 10 ml of methanol and hydrogenated over palladium/activated carbon (5%) under standard hydrogen pressure for 30 min. The catalyst was filtered off and the solvent was evaporated off *in vacuo*. After the residue had been dried under high vacuum, 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.

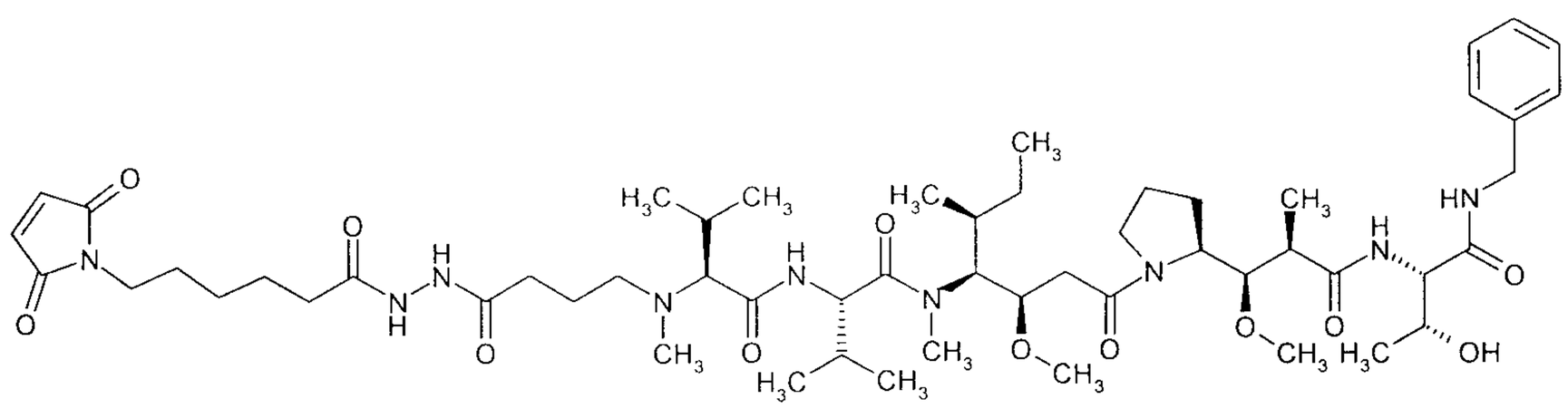
Yield: 8 mg (71% of theory)

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.

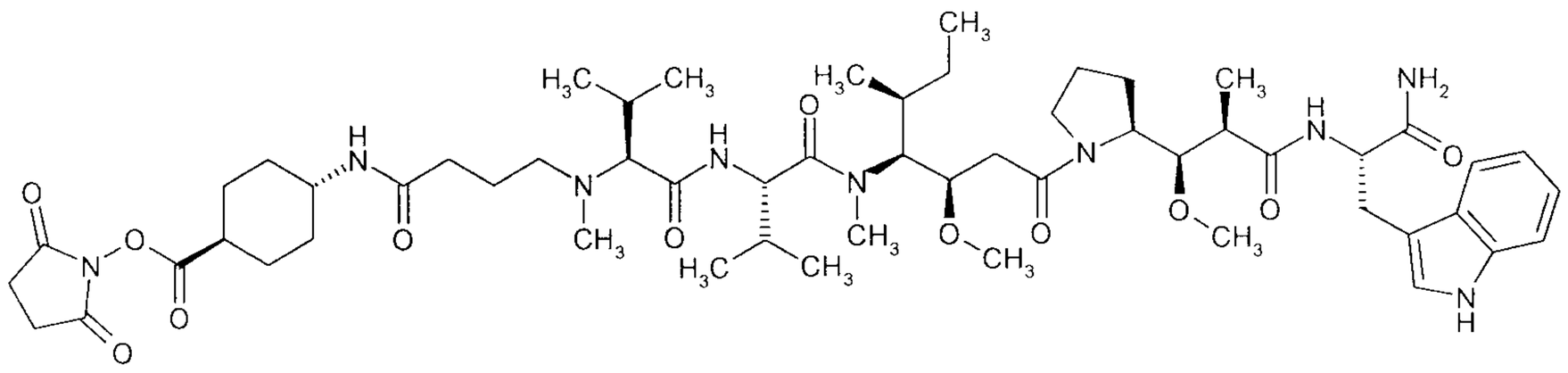
Yield: 3 mg (22% of theory)

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 conventional peptide chemistry methods.

15 mg (18 µmol) 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 dissolved in 5 ml of dimethylformamide and subsequently admixed with 13 mg (35 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 9 µl of *N,N*-diisopropylethylamine and with 15 mg (44 µmol) 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 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, and the free carboxyl component was obtained in quantitative yield. 14 mg (14 µmol; 1 equiv.) of the deprotected compound were taken up in 5 ml of DMF and admixed with 3.3 mg (29 µmol; 2.1 equiv.) of *N*-hydroxysuccinimide in the presence of 4.1 mg (21 µmol; 1.5 equiv.) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 7.5 µl (44 µmol; 3.1 equiv.) of *N,N*-diisopropylethylamine and 0.9 mg (7 µmol; 0.5 equiv.) of 4-dimethylaminopyridine, and the 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

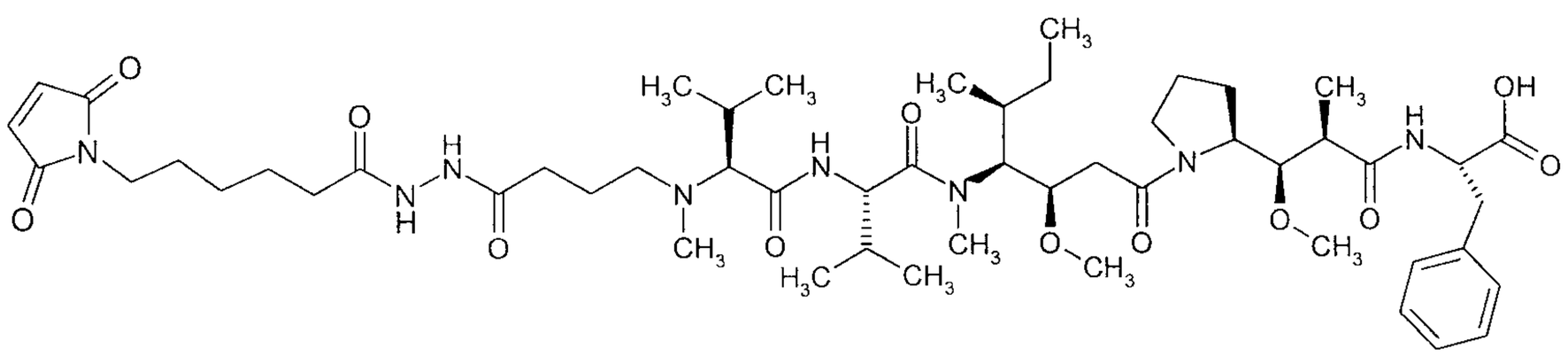
combined and concentrated. After lyophilization of the residue from dioxane, 9.7 mg (62% of theory) of the title compound were obtained as a colourless foam.

HPLC (Method 6): R_t = 1.8 min;

LC-MS (Method 11): R_t = 0.77 min; MS (ESIpos): m/z = 1078 $(M+H)^+$.

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



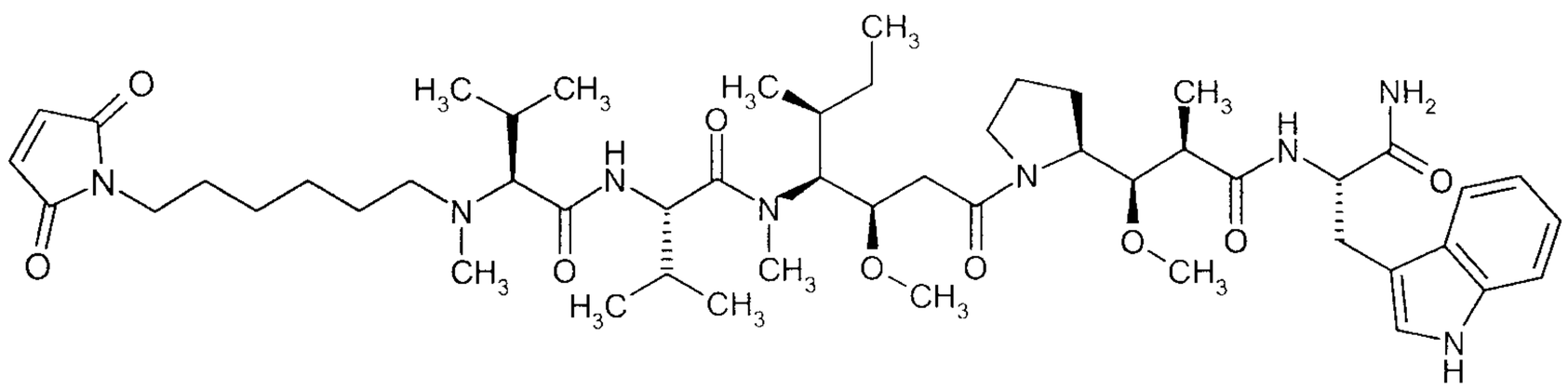
This compound was prepared in analogy to the synthesis described in Intermediate 157, proceeding from 4-{[(2*S*)-1-{[(2*S*)-1-{[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-*tert*-butoxy-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)amino}-3-methylbutan-2-yl]amino}-3-methyl-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 µmol) 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



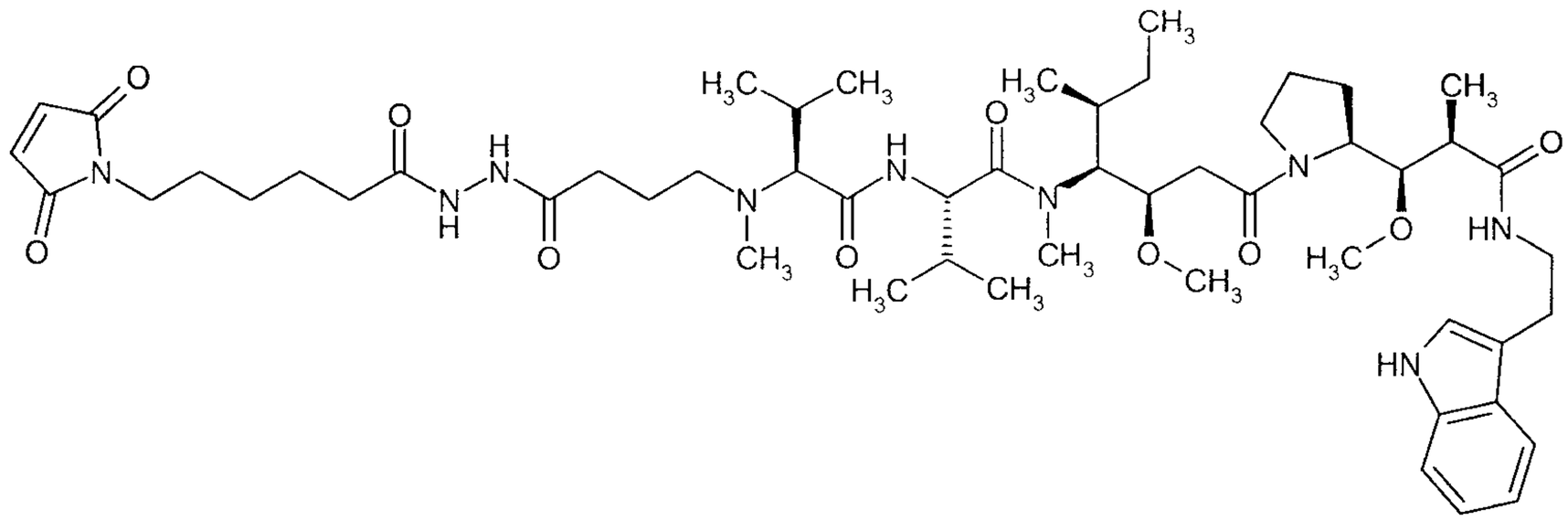
14 mg (16 µmol) of *N*-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 88) were taken up in 750 µl of dioxane and admixed with 1.5 ml of saturated sodium hydrogencarbonate solution and then with 3.2 mg (21 µmol) 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.

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 µmol) 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-

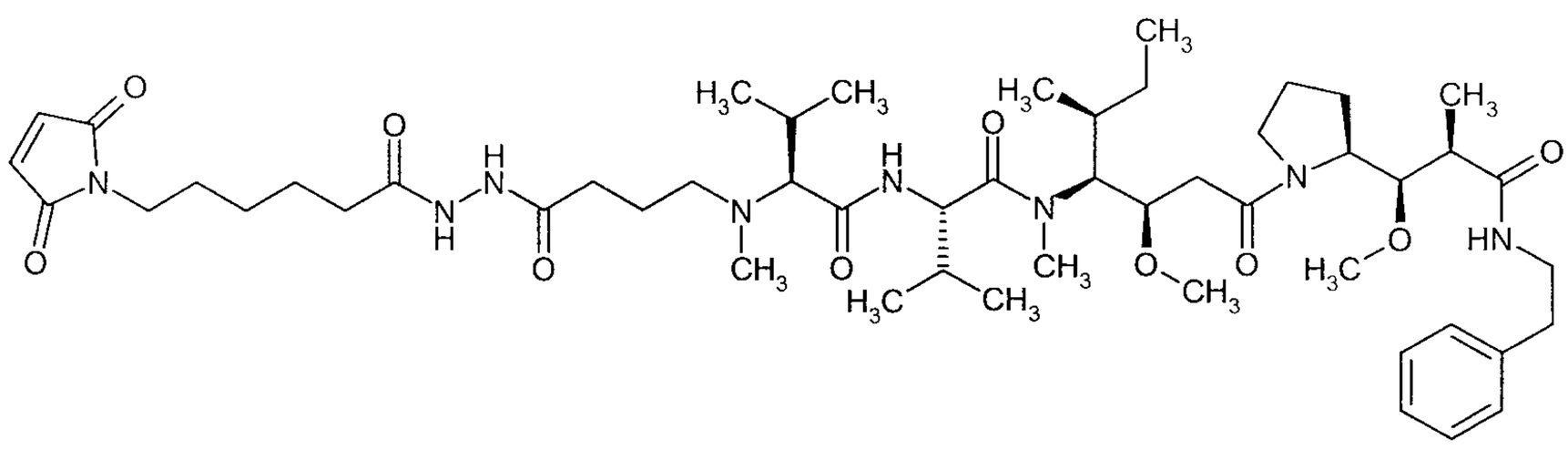
methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 37 ml of DMF and then admixed with 71 mg (187 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 33 µl of *N,N*-diisopropylethylamine and with 37 mg (140 µmol) of commercially available 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 $(M+H)^+$.

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



The compound was prepared in analogy to Intermediate 107.

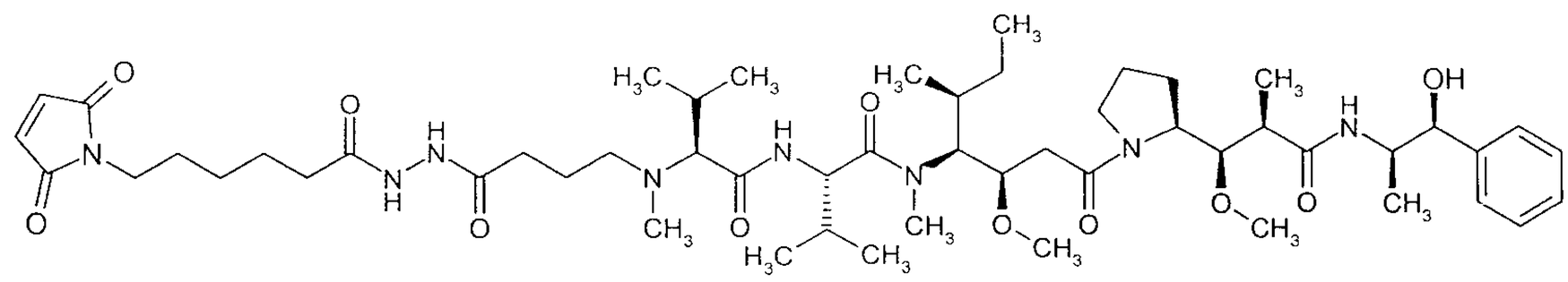
Yield: 2.5 mg (30% of theory)

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

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.

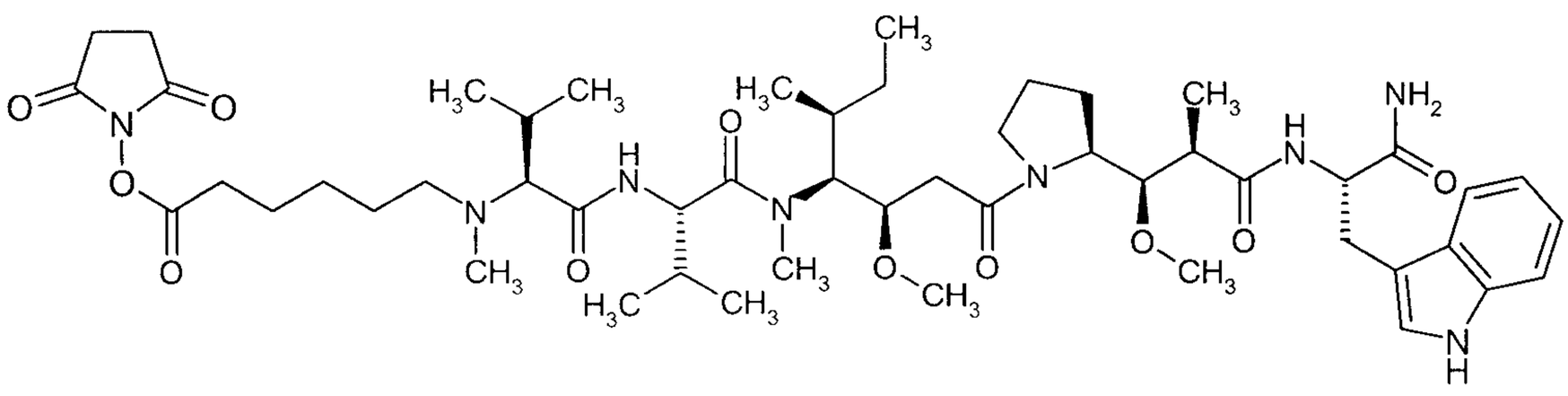
Yield: 35 mg (65% of theory)

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

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



This compound was prepared in analogy to Intermediate 147 from the compound in Intermediate 83.

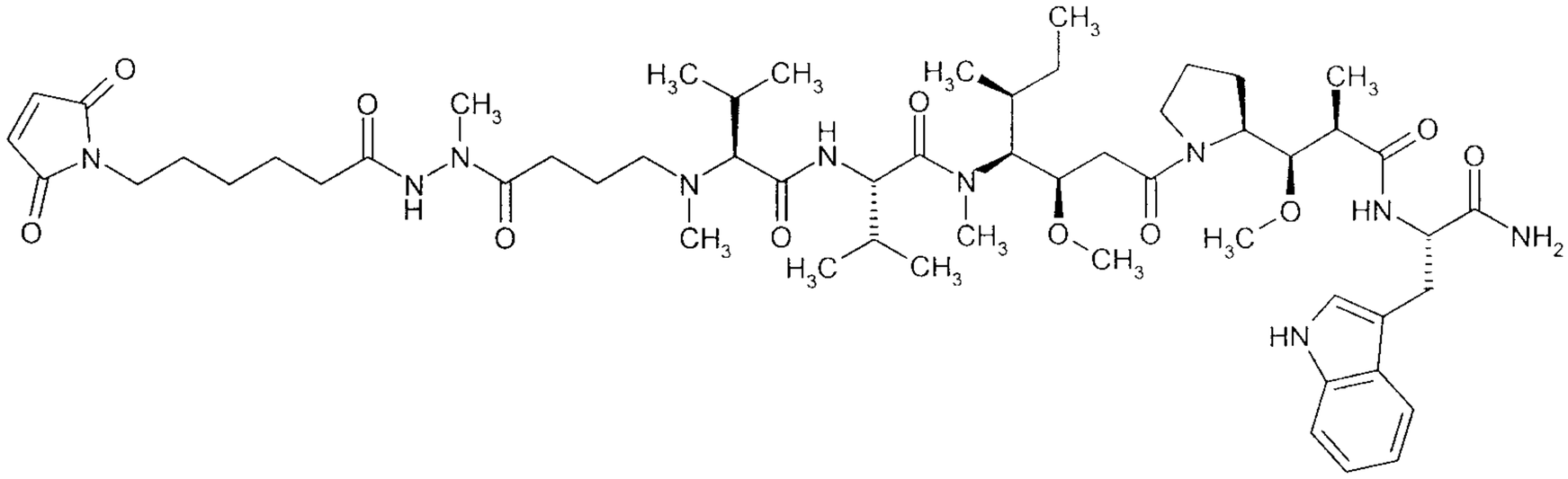
Yield: 2.4 mg (24% of theory)

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-(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 in analogy to Intermediate 140 from Intermediate 82 and Intermediate 22.

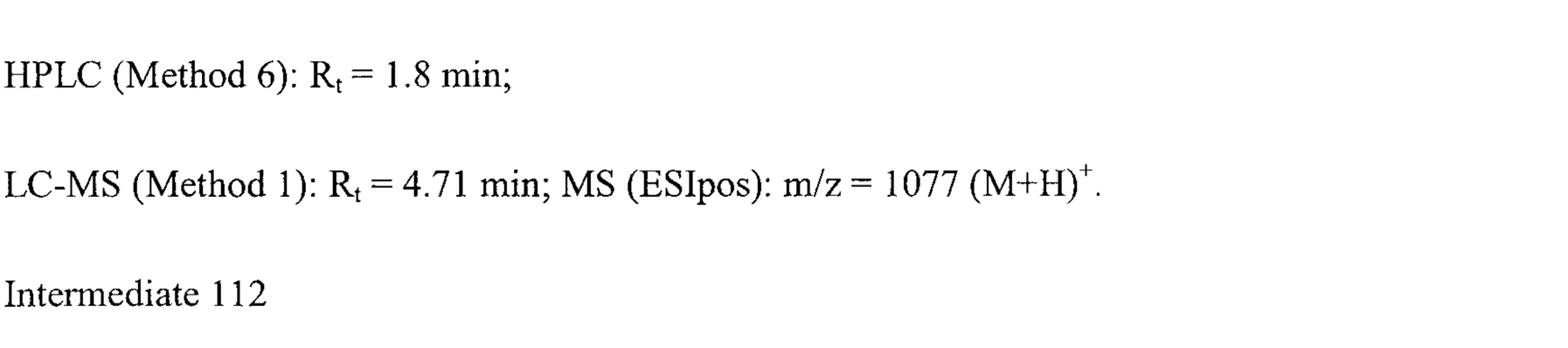
Yield: 6.5 mg (51% of theory)

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.

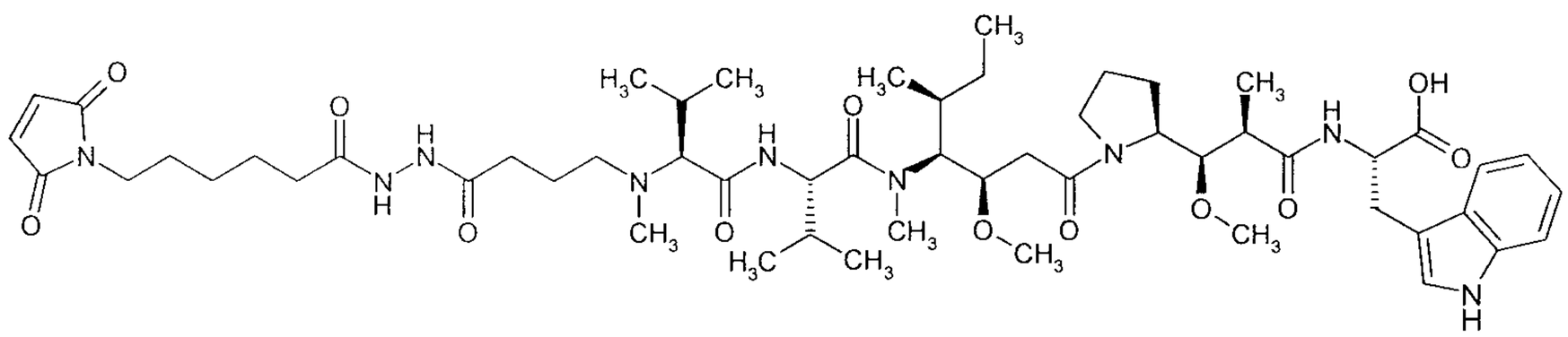
Yield: 5.7 mg (57 % of theory)

HPLC (Method 5): R_t = 1.6 min;

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 µmol) 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 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 73 µl of *N,N*-diisopropylethylamine and with 68 mg (261 µmol) 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.

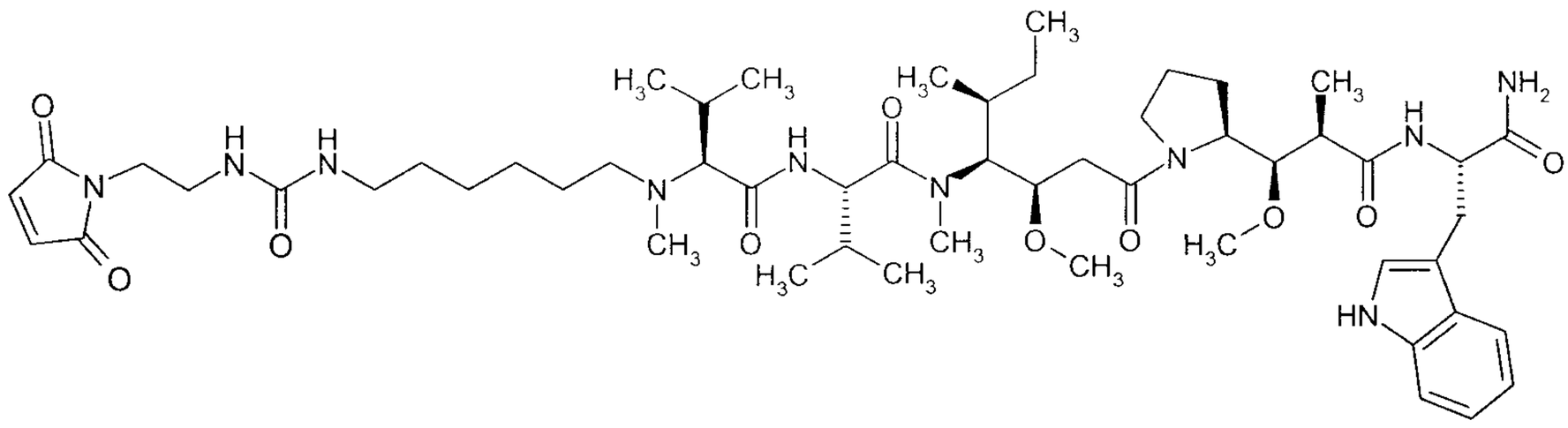
Thus, 61 mg (62 % of theory) of the title compound were obtained as a colourless foam.

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 µmol) of *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 were taken up in 885 µl of DMF and admixed with 5.3 mg (8 µmol) of 4-nitrophenyl-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]carbamate and 2.8 µl 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.

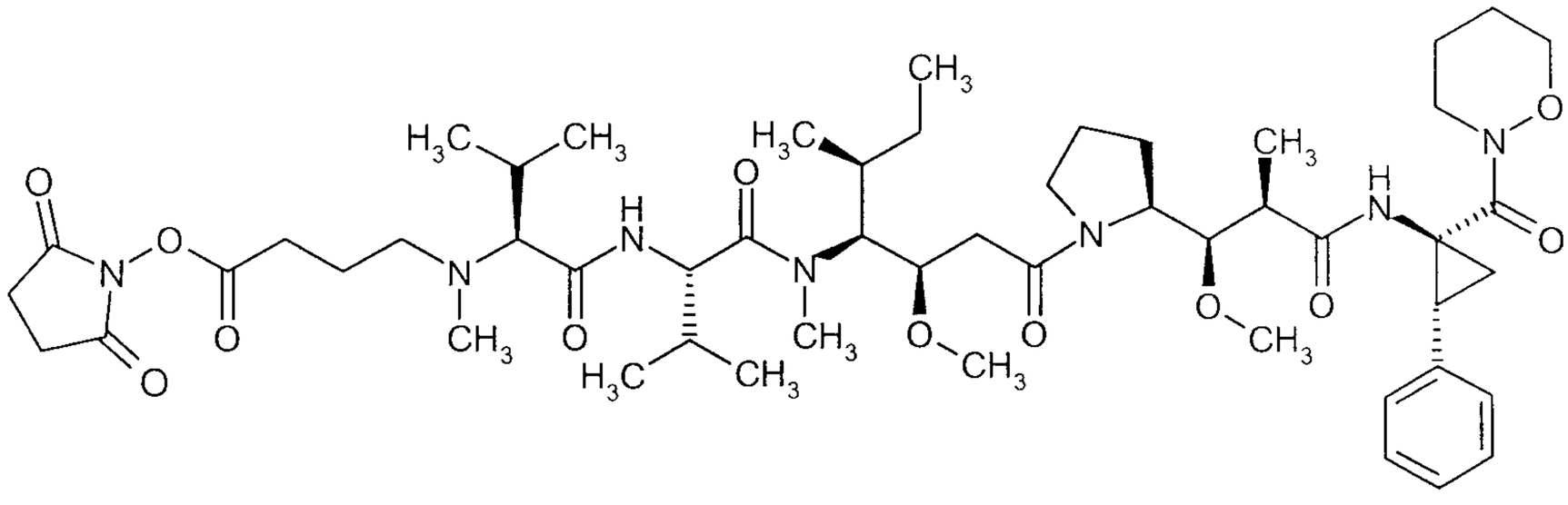
Yield: 0.58 mg (11% of theory) of a colourless foam

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 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. 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.

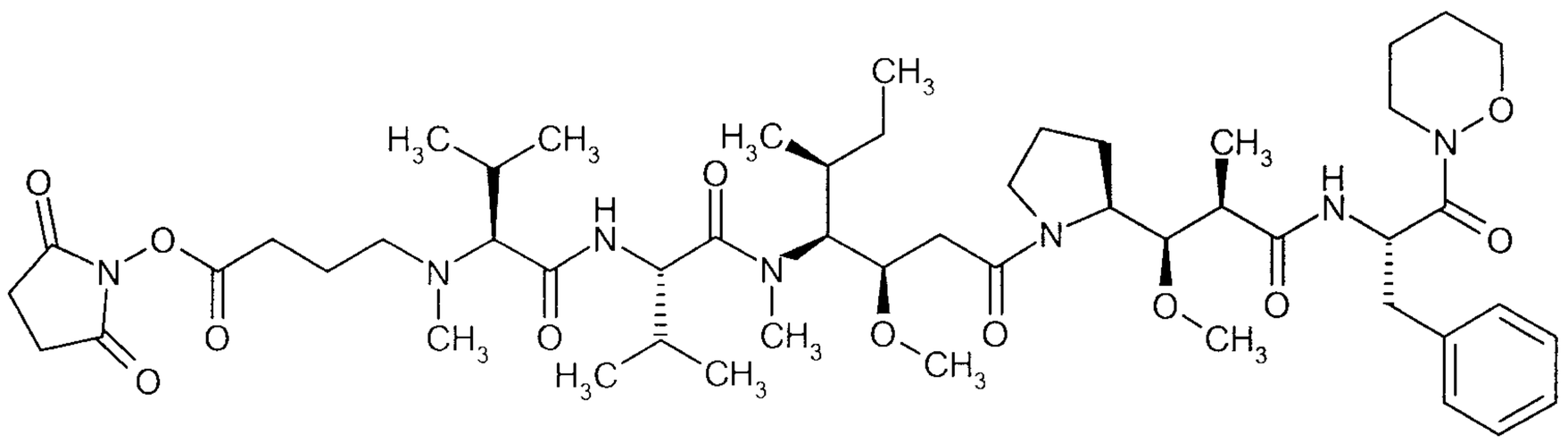
Yield: 3 mg (27% of theory) (hydrolysis-sensitive)

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 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. 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.

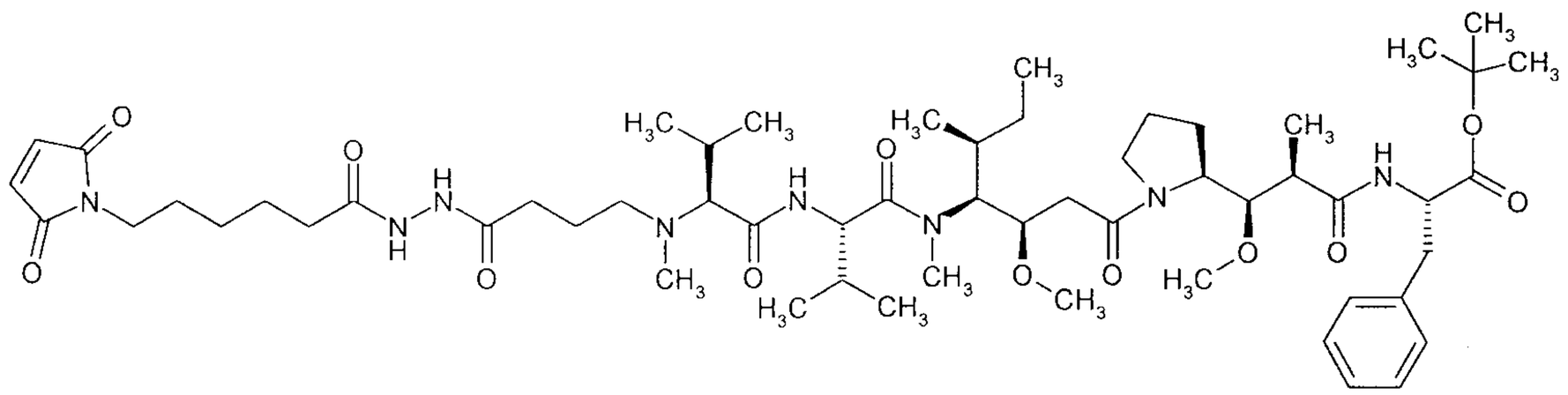
Yield: 3.2 mg (43% of theory) (hydrolysis-sensitive)

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.

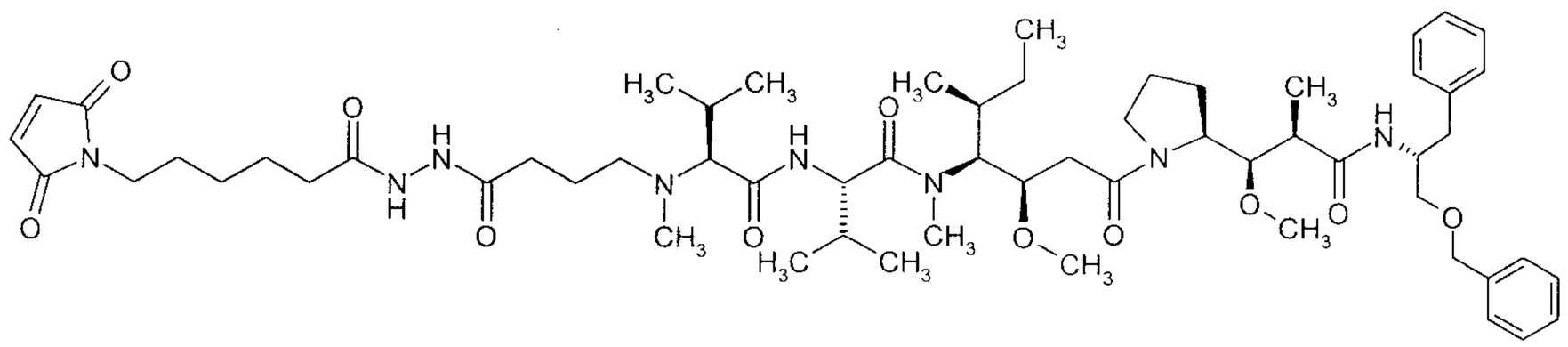
Yield: 7 mg (42% of theory)

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

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

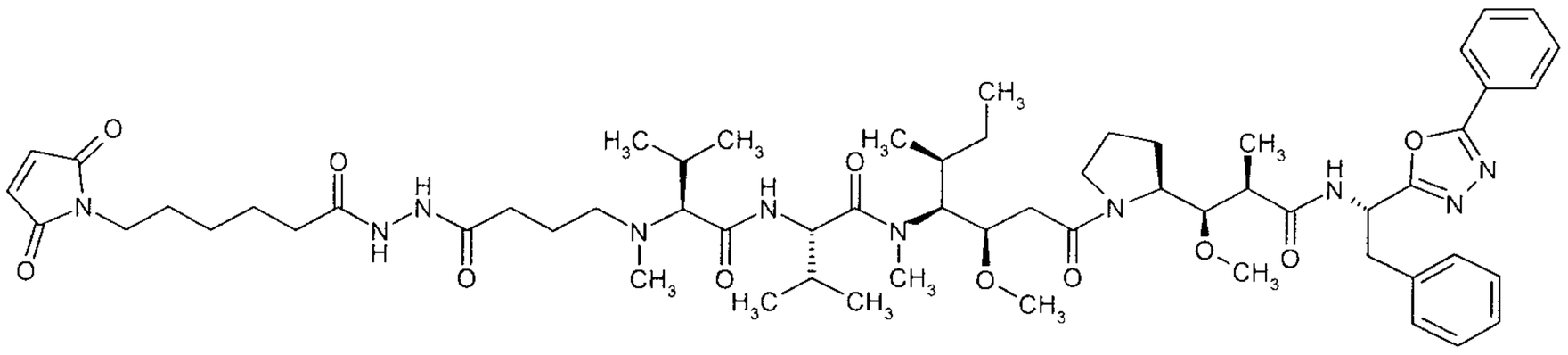


The target compound was prepared in analogy to Intermediate 157 from 7 mg (7.8 µmol) of the compound in Intermediate 68. Yield: 6.3 mg (53% of theory)

LC-MS (Method 1): R_t = 1.00 min; MS (ESIpos): m/z = 1102 $(M+H)^+$.

Intermediate 119

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

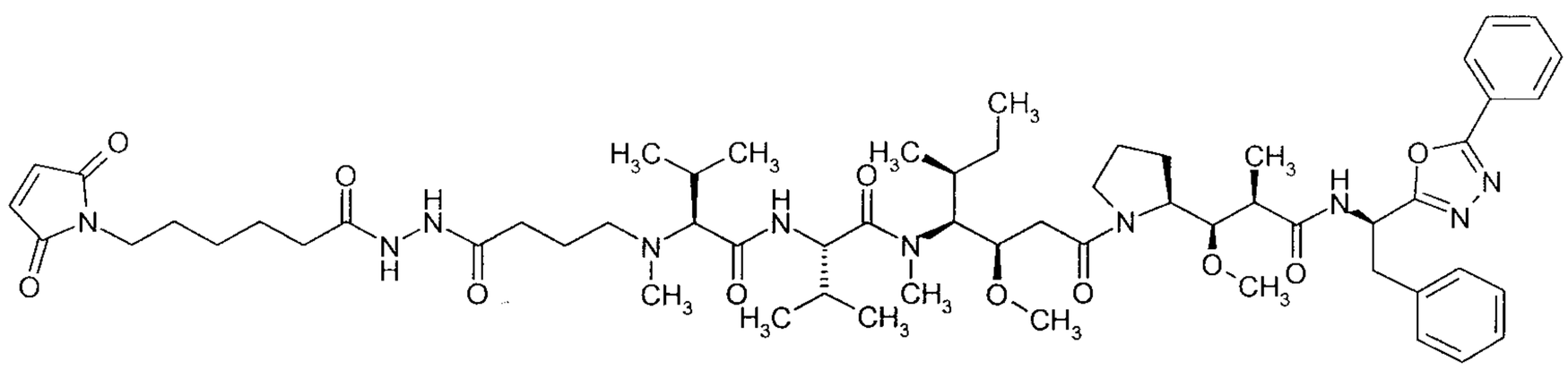


7.4 mg (8.1 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 6.3 mg (24.2 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

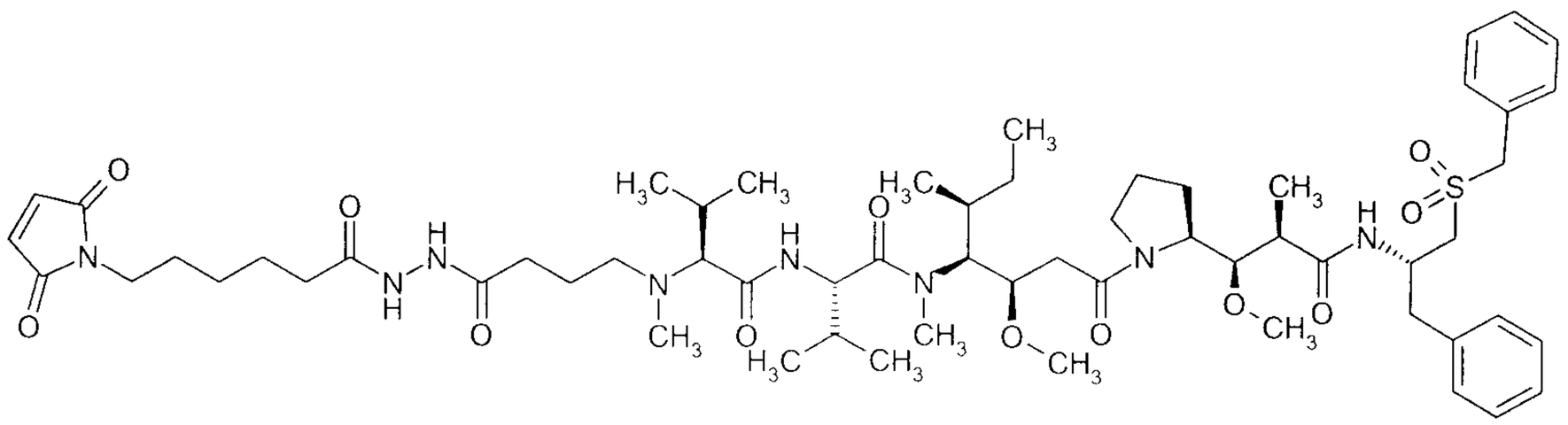


12.8 mg (13.9 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*R*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 10.9 mg (41.8 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

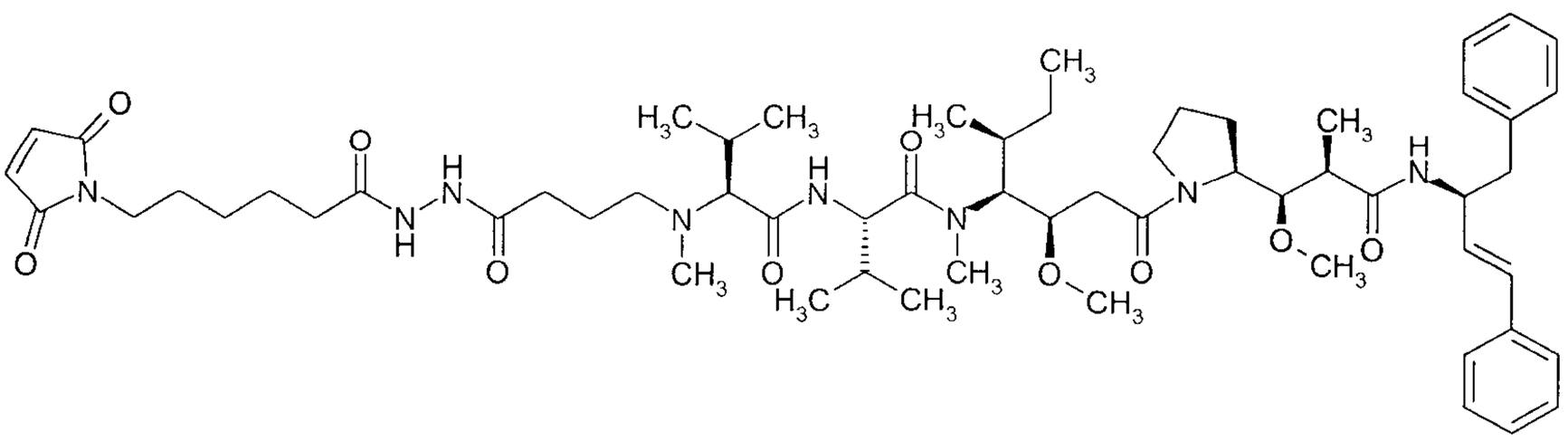


7.4 mg (7.9 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylsulphonyl)-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 6.2 mg (23.5 mmol) of 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 $(M+H)^+$

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



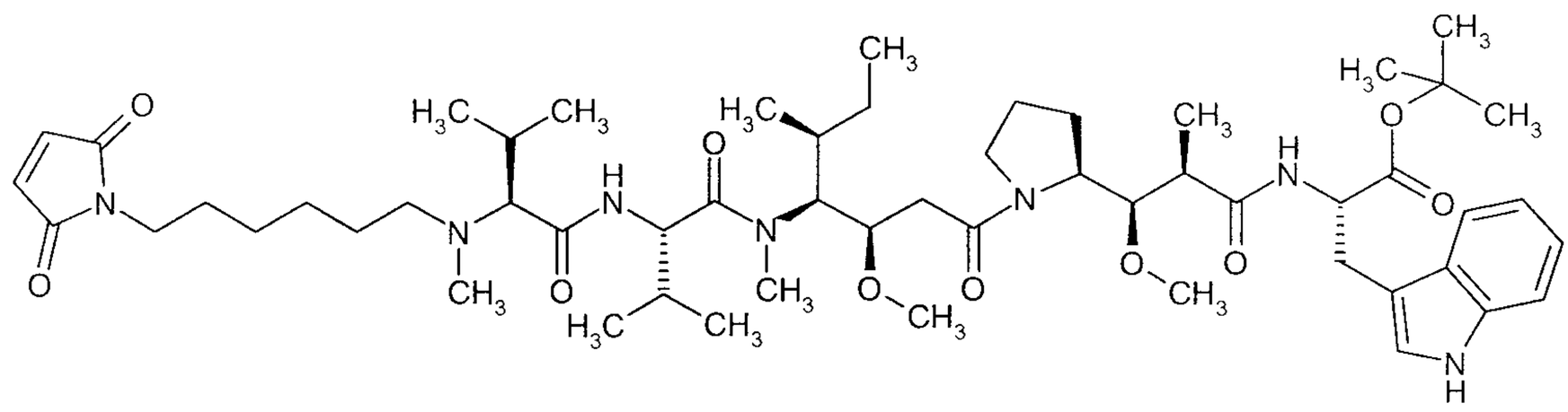
8 mg (9.1 mmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*E*)-1,4-diphenylbut-3-en-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and 7.2 mg (27.4 mmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide 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.

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



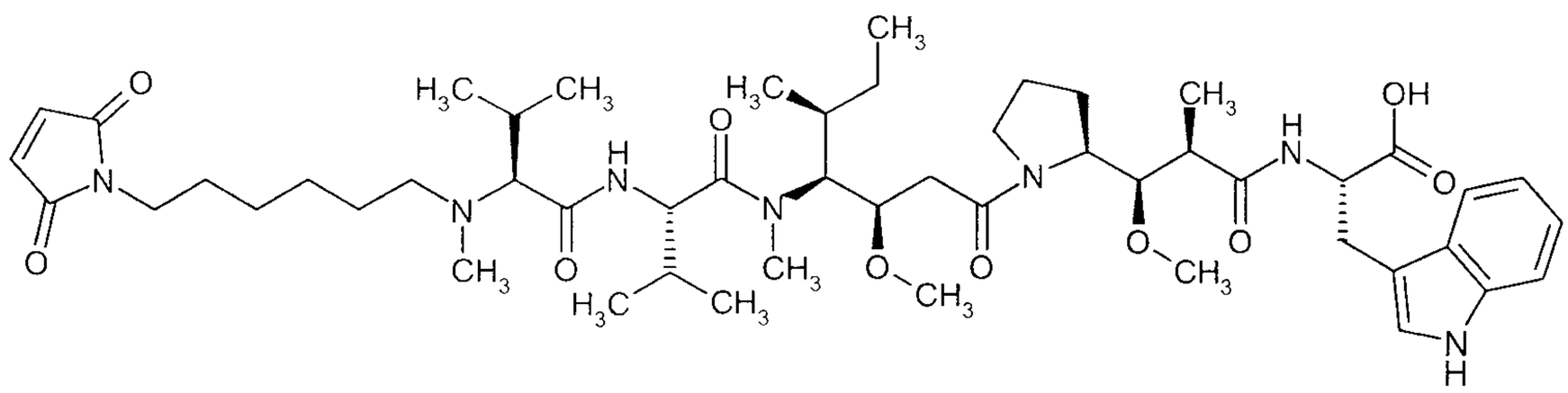
30 mg (30 µmol) of Intermediate 89 were taken up in 2 ml of 1,4-dioxane and admixed with 4 ml of saturated sodium hydrogencarbonate solution and then with 7.5 mg (50 µmol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate. The reaction mixture was stirred at RT for 1 h and then concentrated *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;

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



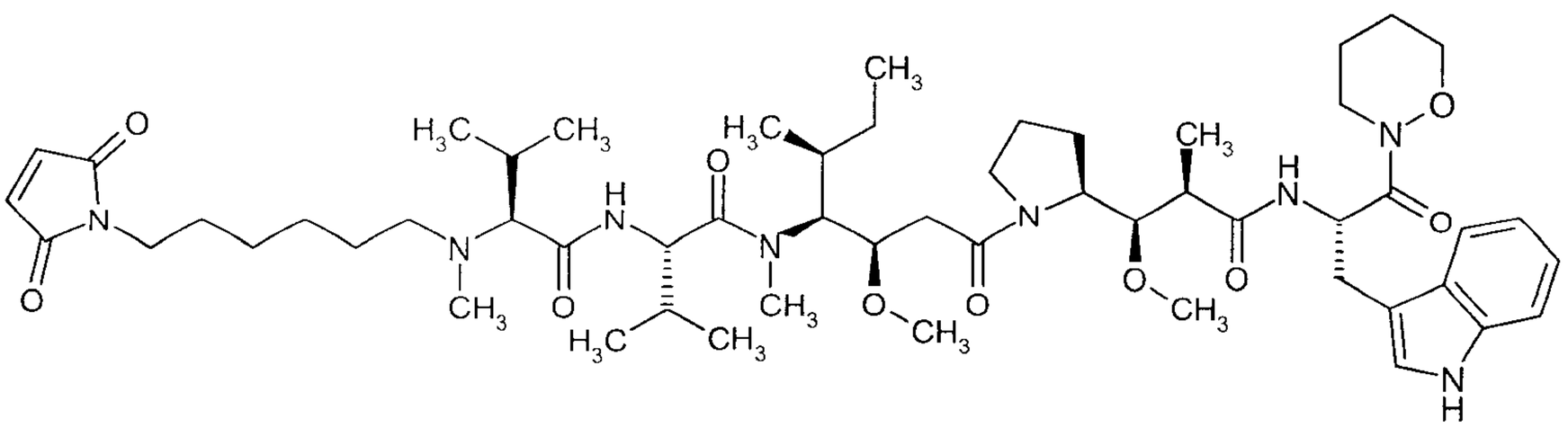
22 mg (20 µmol) of Intermediate 123 were reacted with 4 ml of trifluoroacetic acid in 8 ml of dichloromethane at RT for 1 h. Thereafter, the reaction mixture was concentrated *in vacuo*. The remaining residue was purified by means of preparative HPLC. After lyophilization, 11 mg (54% of theory) of the title compound were obtained.

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-{[(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



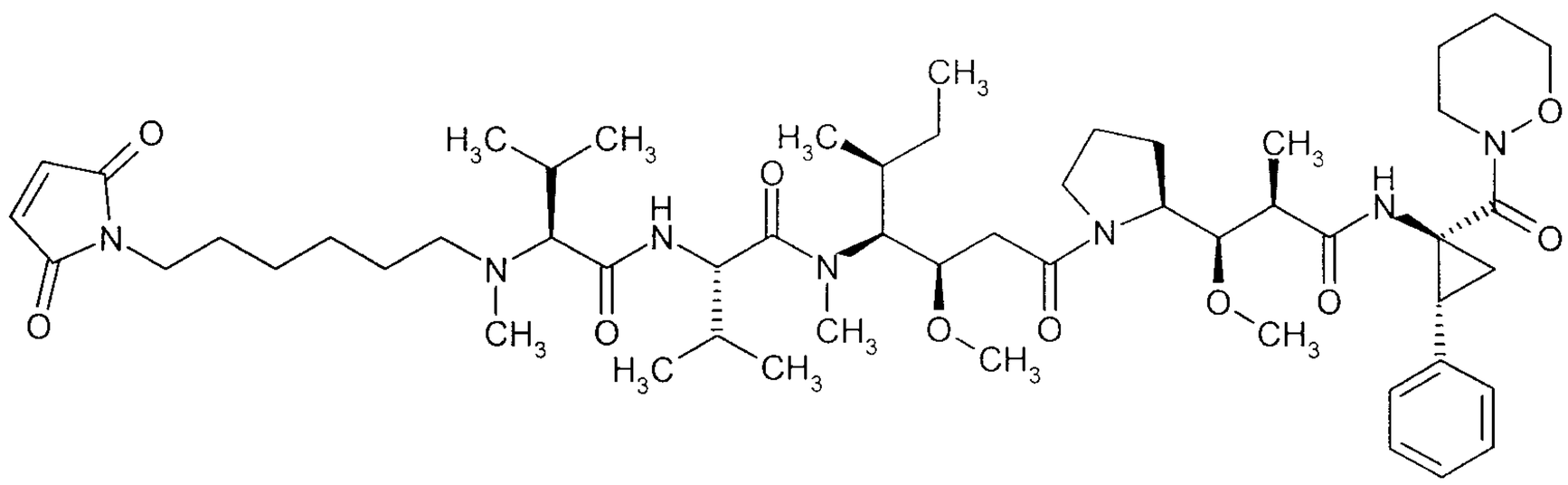
22.5 mg (20 µmol) of Intermediate 101 were taken up in 2 ml of 1:1 dioxane/water and then admixed with 5.6 mg (40 µmol) of methyl 2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate and with 0.25 ml of saturated sodium hydrogencarbonate solution. The reaction mixture was stirred at RT for 30 min. Then another 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, 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-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



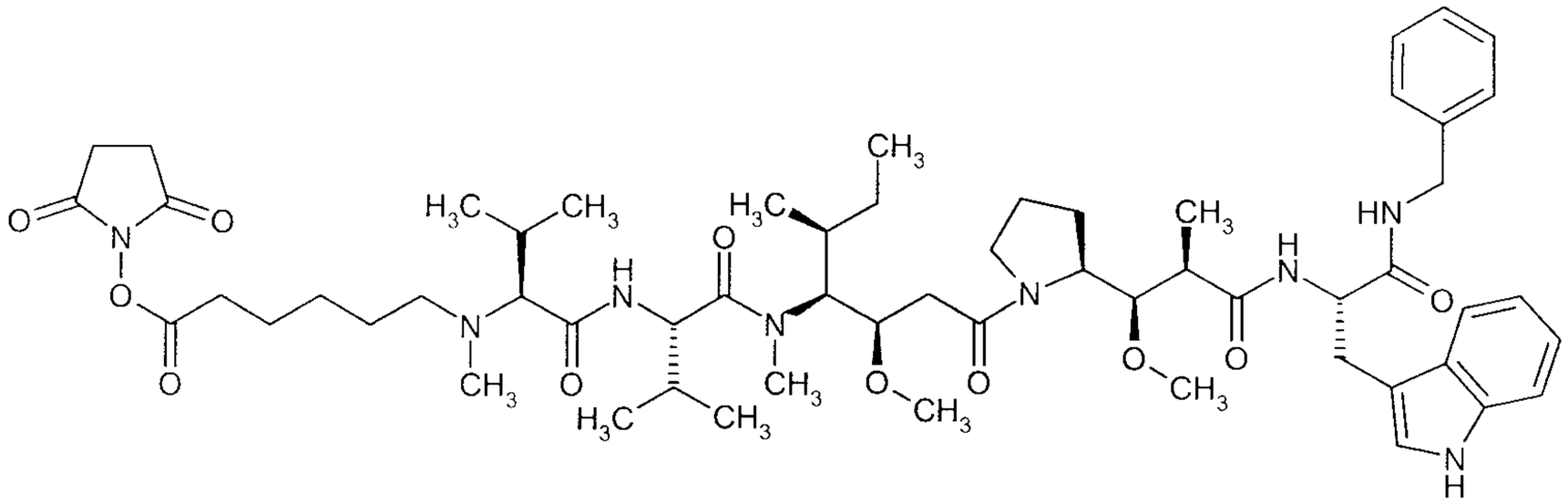
64 mg (70 µmol) of *N*-(6-aminohexyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 97) 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 µmol) 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 µmol) 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 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 µl 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.

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-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide



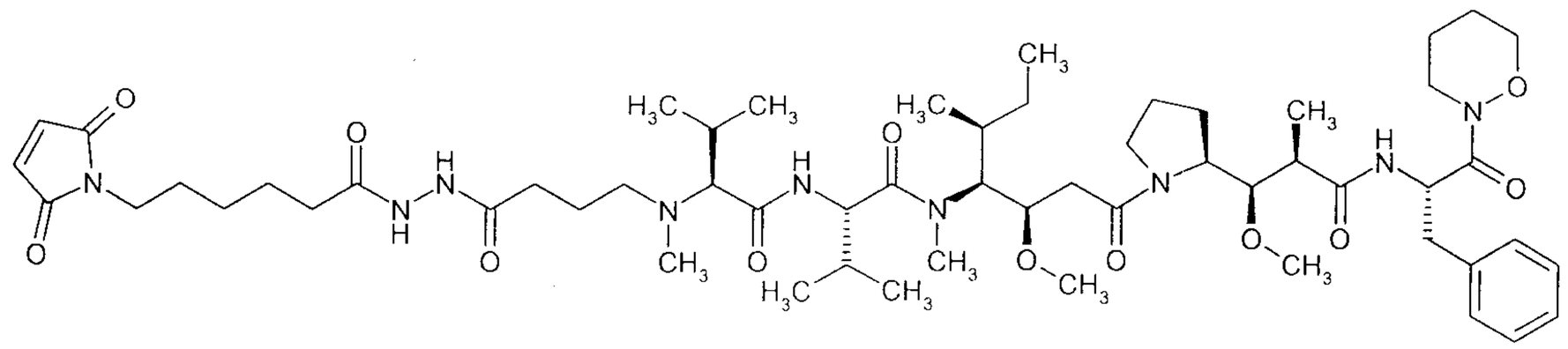
17 mg (18 µmol) of *N*-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 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 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.21 mg (0.17 µmol) 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 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-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



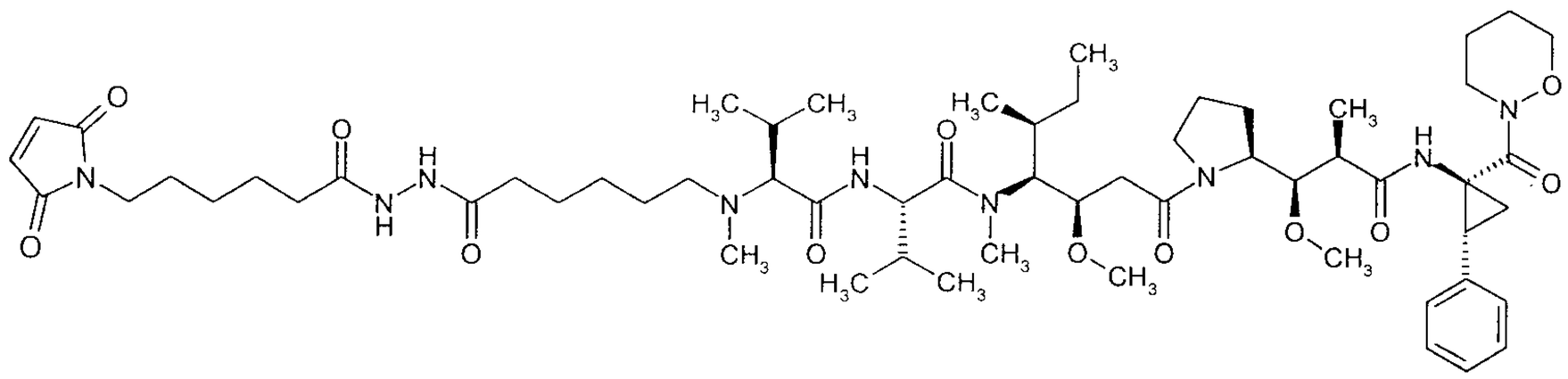
5 mg (5.6 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 845 µl of DMF and then admixed with 3.2 mg (17 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 2.6 mg (17 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 1.96 µl of *N,N*-diisopropylethylamine and with 5.9 mg (22.5 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, 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 µmol) 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]

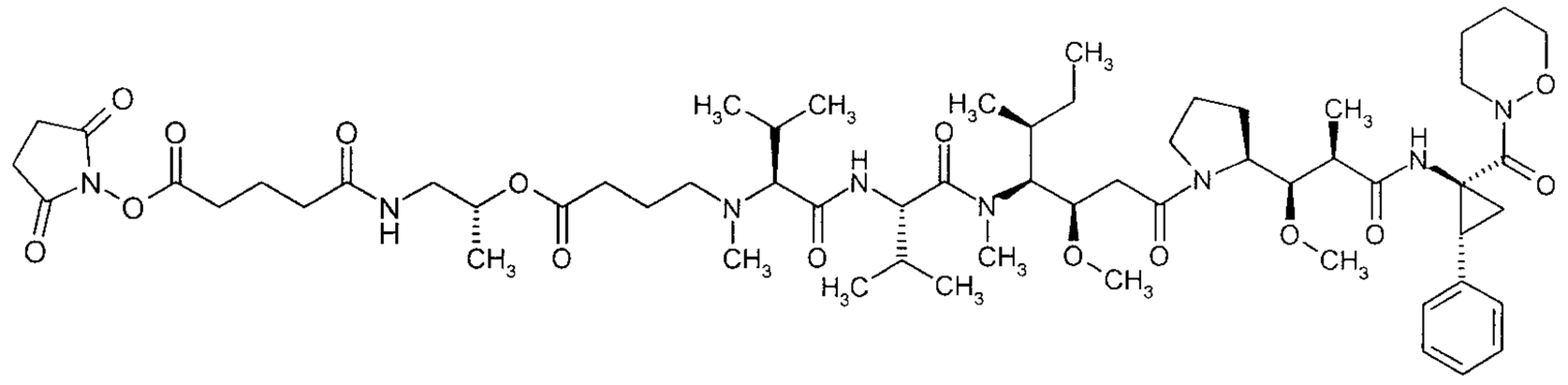
amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 646 µl of DMF and then admixed with 2.5 mg (13 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 2.0 mg (13 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 2.25 µl of *N,N*-diisopropylethylamine and with 4.5 mg (17 µmol) 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 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 3.7 ml of dichloromethane and then admixed with 6.7 mg (35 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 0.7 mg (5.8 µmol) of 4-dimethylaminopyridine and with 8.2 mg (47 µmol) 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 $(M+H)^+$.

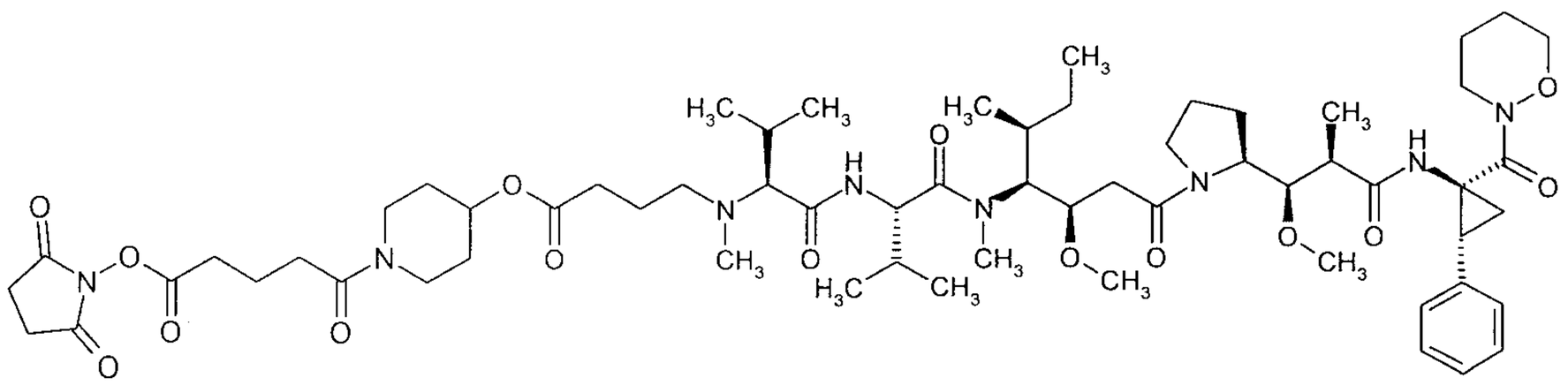
Subsequently, the Boc protecting group was cleaved with trifluoroacetic acid. 4.9 mg (0.005 mmol) of the deprotected crude product were then, without further purification, taken up in 1.8 ml of dichloromethane and admixed with 3.7 mg (0.011 mmol) of 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, 2.4 µl (0.014 mmol) of *N,N*-diisopropylethylamine and 0.6 mg (5 µmol) 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 $(M+H)^+$.

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 mg (11 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 2 ml of dichloromethane and then admixed with 4.3 mg (22 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 0.88 mg (6 µmol) of 4-dimethylaminopyridine and with 5.2 mg (22 µmol) 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.

HPLC (Method 5): R_t = 2.1 min;

LC-MS (Method 1): R_t = 1.04 min; MS (ESIpos): m/z = 1116 $(M+H)^+$.

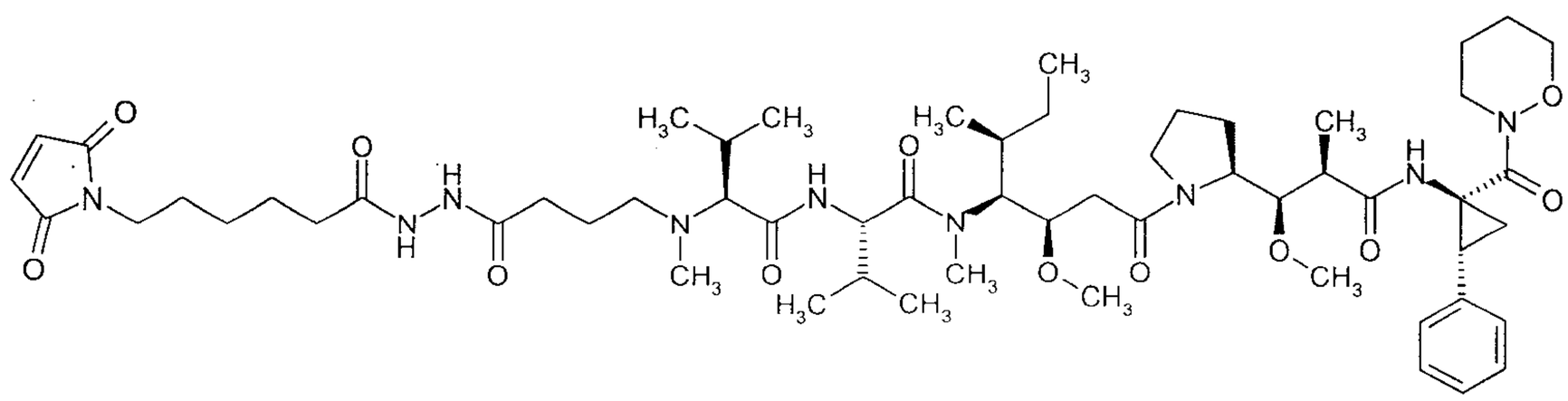
Subsequently, the Z protecting group was cleaved by hydrogenolytic means in ethanol over palladium/activated carbon. 4.6 mg (0.005 mmol) of the deprotected crude product were then, without further purification, taken up in 1.8 ml of dichloromethane and admixed with 3.8 mg (0.012 mmol) of 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, 0.8 µl (0.005 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.

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

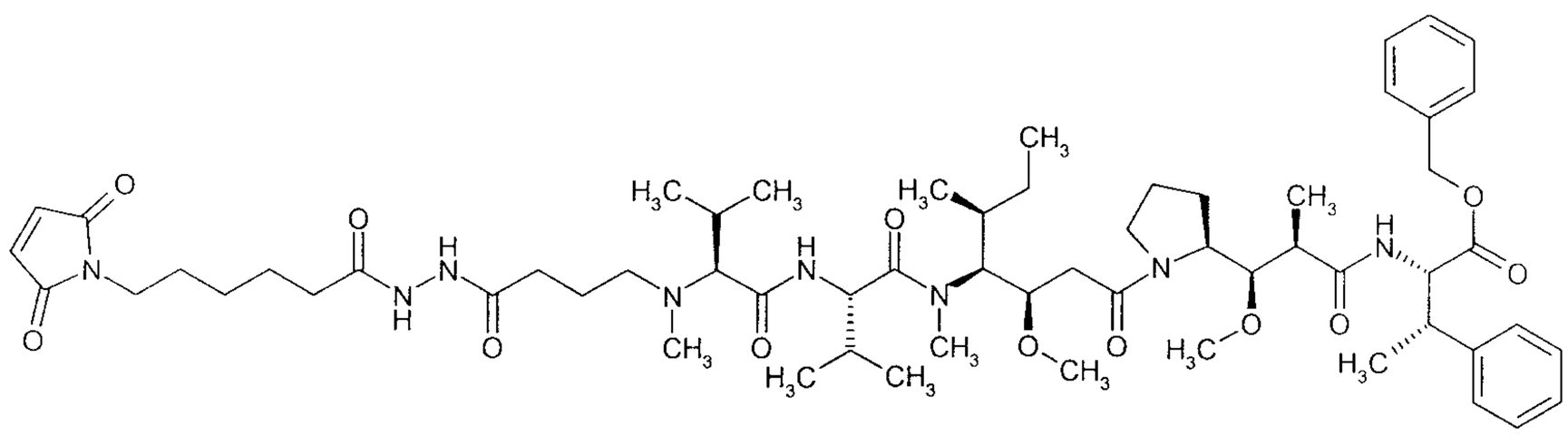
residue was purified by means of preparative HPLC. Thus, 11.2 mg (52% of theory) of the title compound were obtained as a colourless foam.

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



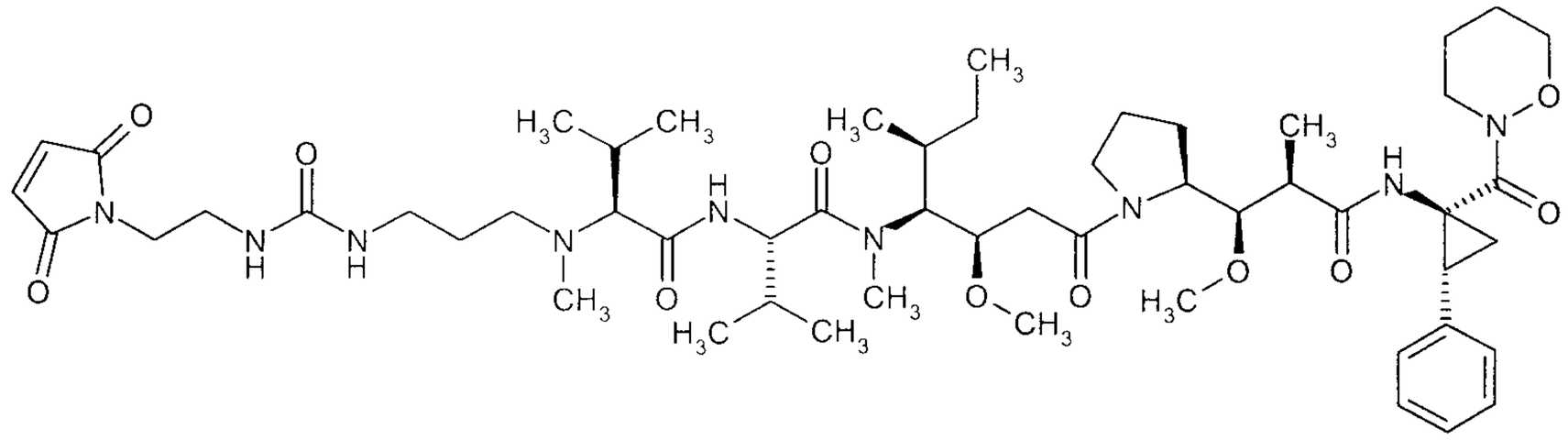
5.8 mg (6.3 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 943 µl of DMF and then admixed with 3.6 mg (19 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 2.9 mg (19 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 2.2 µl of *N,N*-diisopropylethylamine and with 6.6 mg (25 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, 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 $(M+H)^+$.

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.

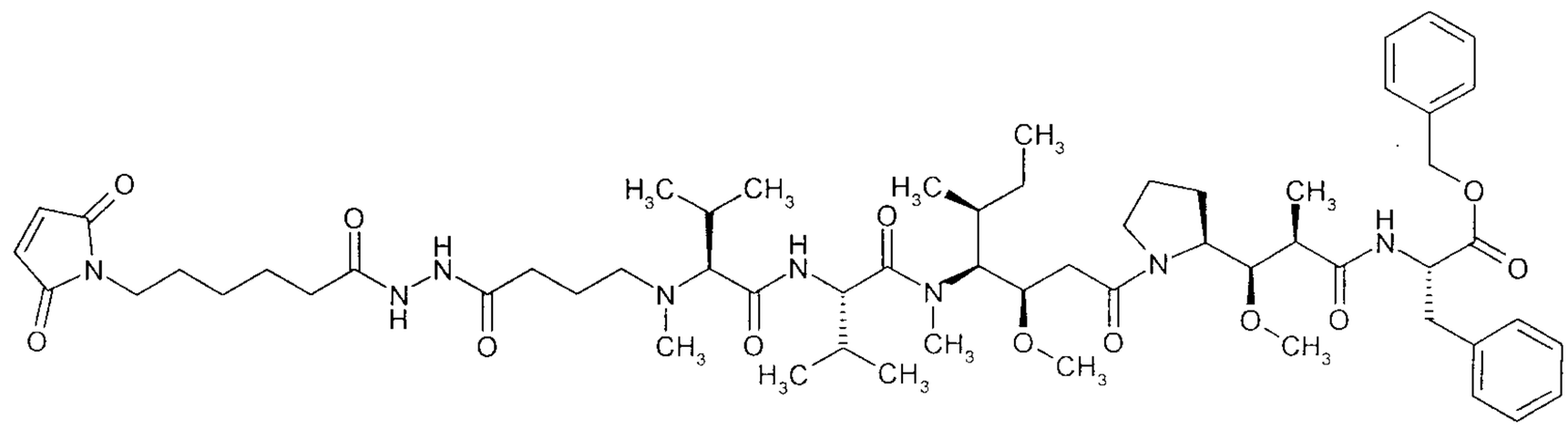
5 mg (6 µmol) of *N*-(3-aminopropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 1000 µl of DMF and then admixed with 2 µl of *N,N*-diisopropylethylamine and with 2.2 mg (9 µmol) of 4-nitrophenyl-[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]carbamate. 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 $(M+H)^+$.

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



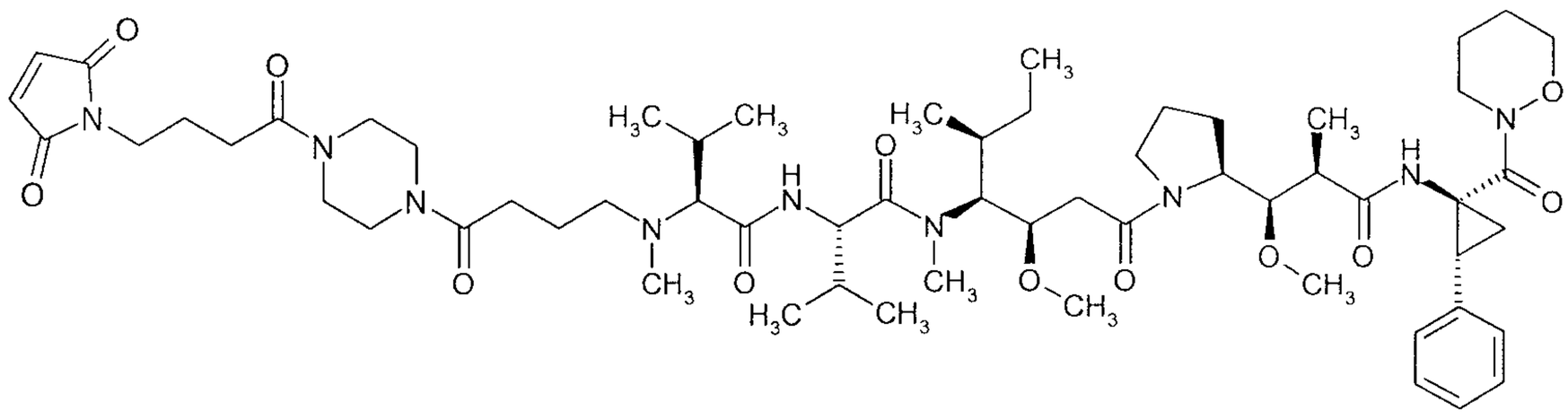
10 mg (11 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 4000 µl of DMF and then admixed with 6.3 mg (33 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 4.5 mg (33 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 5.7 µl of *N,N*-diisopropylethylamine and with 11.5 mg (44 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, 2.6 mg (14% of theory) of the title compound 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*-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, 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.

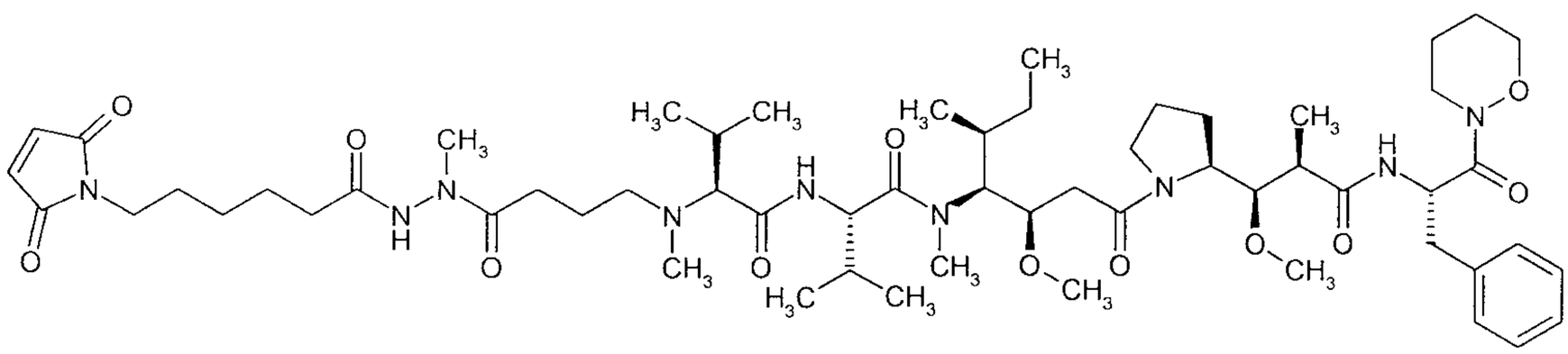
5 mg (5.6 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 1000 µl of DMF and then admixed with 2.1 mg (11 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1.7 mg (11 µmol) 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.

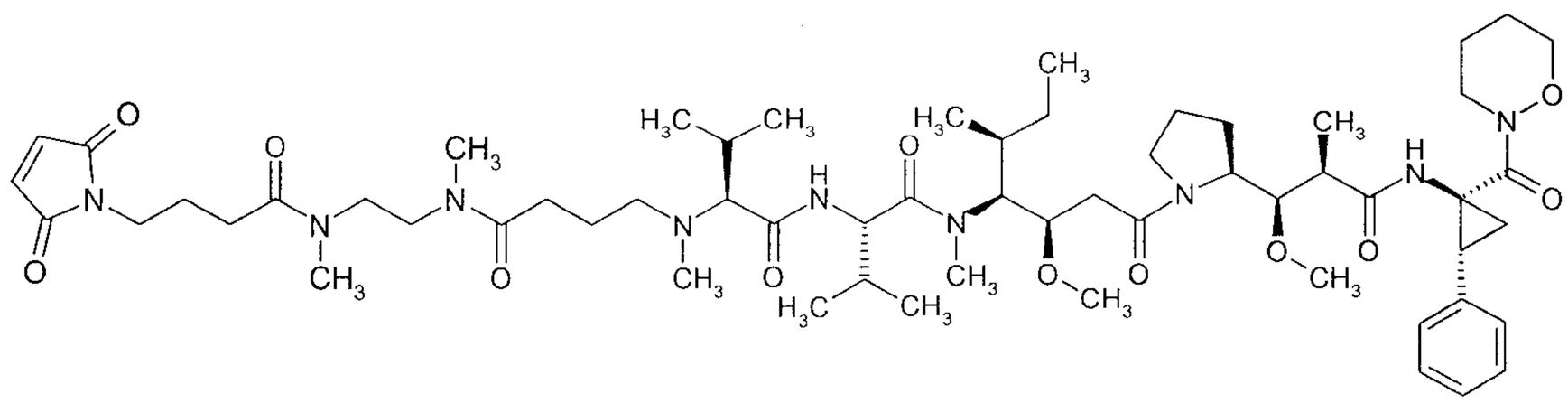
6.9 mg (8 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 2540 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;

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 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-

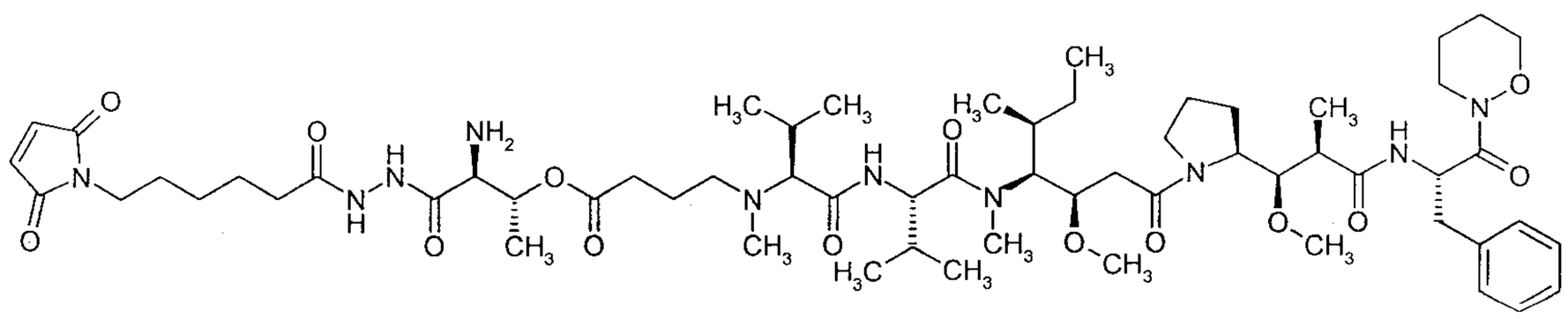
phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 2000 µl of DMF and then admixed with 5.6 mg (14.7 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 2.6 µl of *N,N*-diisopropylethylamine and with 4.1 mg (9 µmol) 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



13 mg (14.7 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-{[(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 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 5.4 mg (44 µmol) of 4-dimethylaminopyridine and with 9 mg (29.3 µmol) 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 with5 mg (26.2 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 4 mg (26.2 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 54.6 µl of *N,N*-diisopropylethylamine and with 9.1 mg (34.9 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 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 $(M+H)^+$.

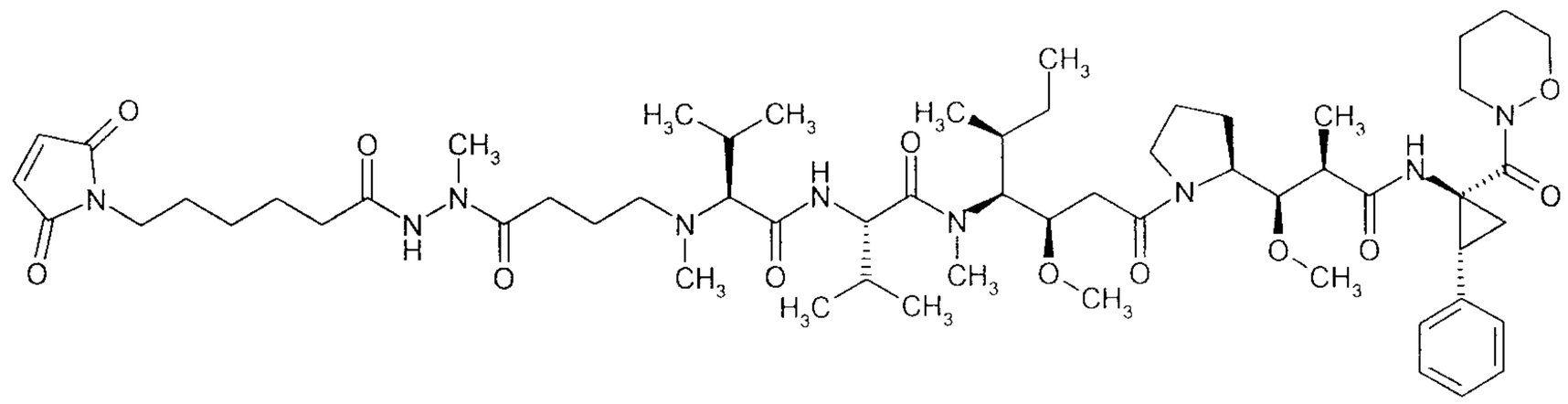
Subsequently, 9.5 mg (7.3 µmol) were deprotected with 0.5 ml of trifluoroacetic acid in 2 ml of dichloromethane of the Boc-protected intermediate and, after lyophilization from dioxane, 9 mg (82% of theory) of the title compound were obtained as a colourless foam.

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



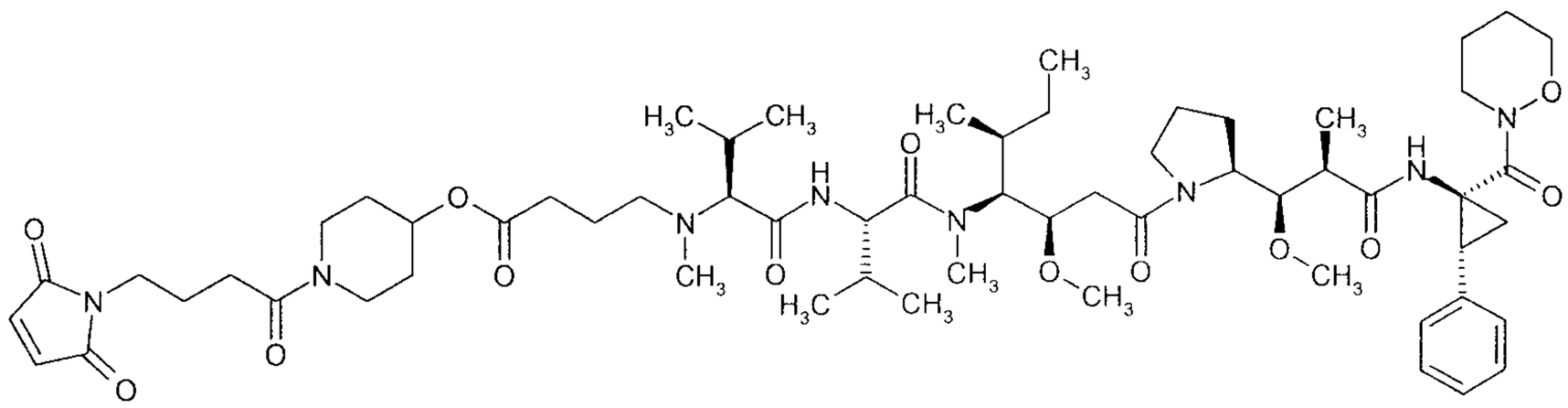
4.1 mg (12 µmol) of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*'-methylhexanehydrazide trifluoroacetate (Intermediate 22) were dissolved together with 6.9 mg (8 µmol) of the compound from Intermediate 61 in 2.5 ml of DMF and then admixed with 3.5 mg (9 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 3 µl 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 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 2 ml of dichloromethane and then admixed with 18.8 mg (98 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 3.8 mg (24 µmol) of 4-

dimethylaminopyridine and with 23 mg (98 µmol) 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.

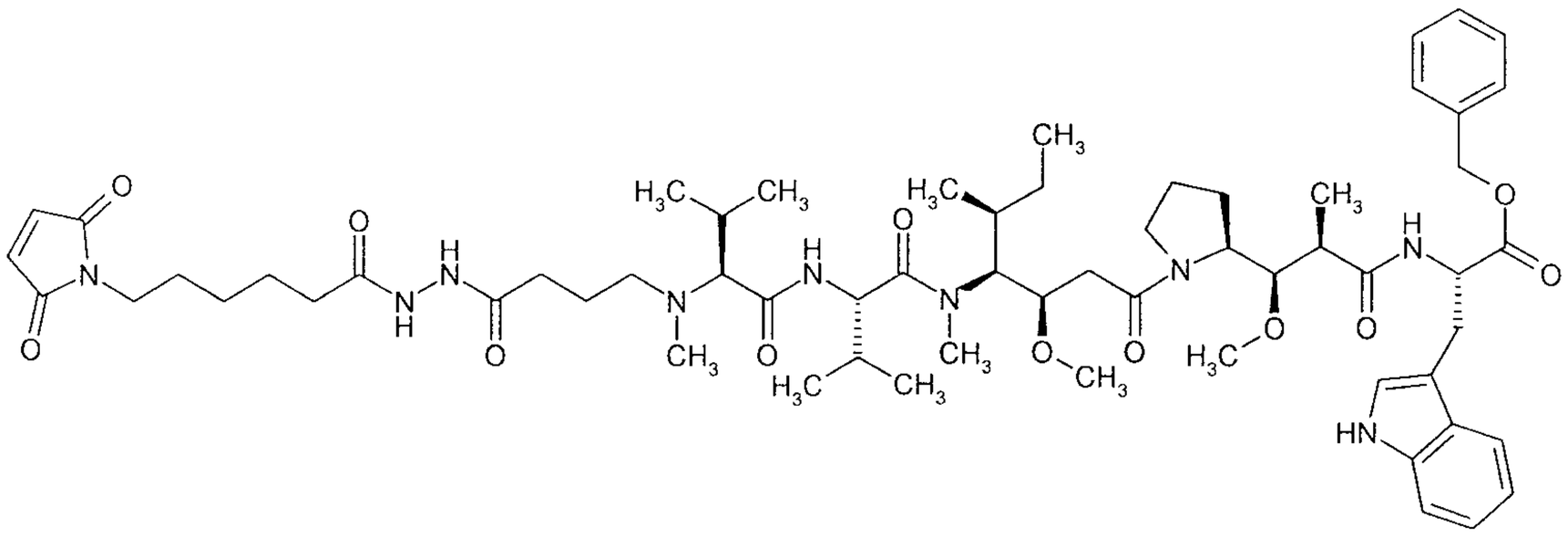
19 mg (19 µmol) of the deprotected crude product were then, without further purification, taken up in 4 ml of DMF and admixed with 7 mg (39 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoic acid, 11 mg (29 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 5 µl 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;

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



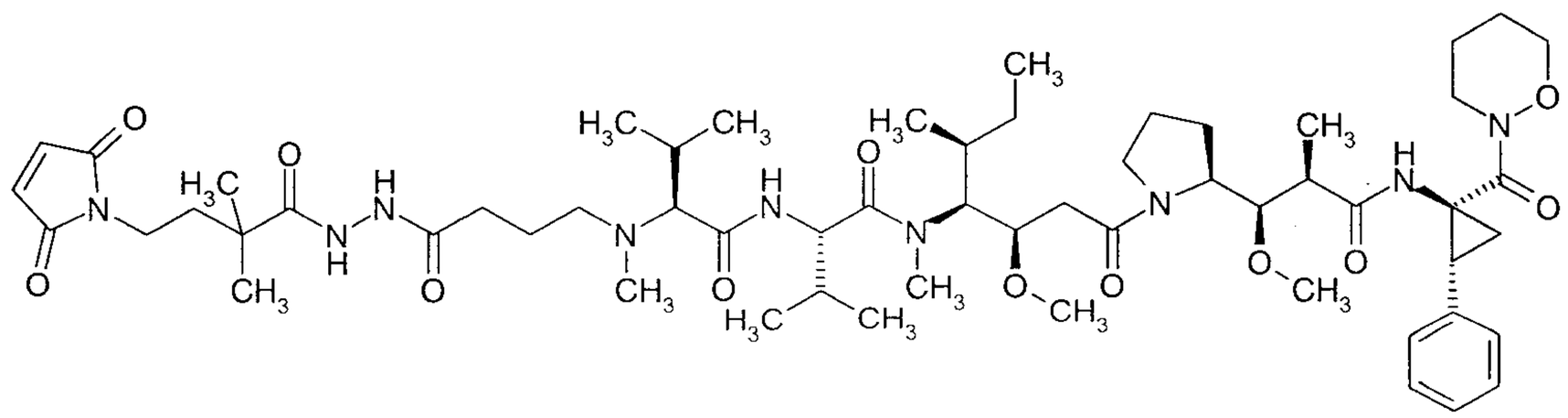
9 mg (9.5 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-(benzyloxy)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 72) were dissolved in 1000 µl of DMF and then admixed with 10 mg (38 µmol) of commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide, 7.2 mg (19 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 8 µl 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



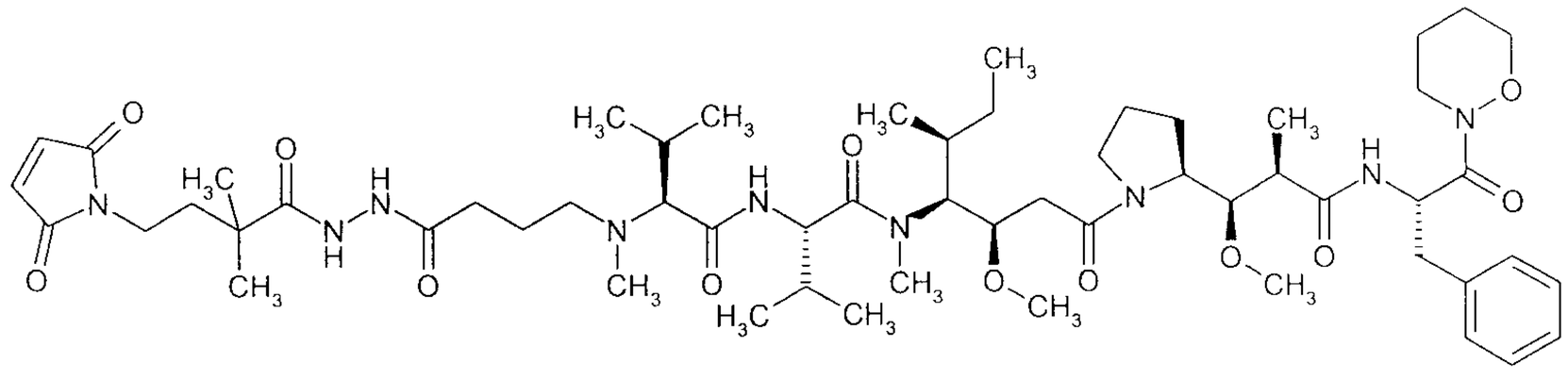
6 mg (6.7 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 61) were reacted with 3 mg (8.7 µmol) 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

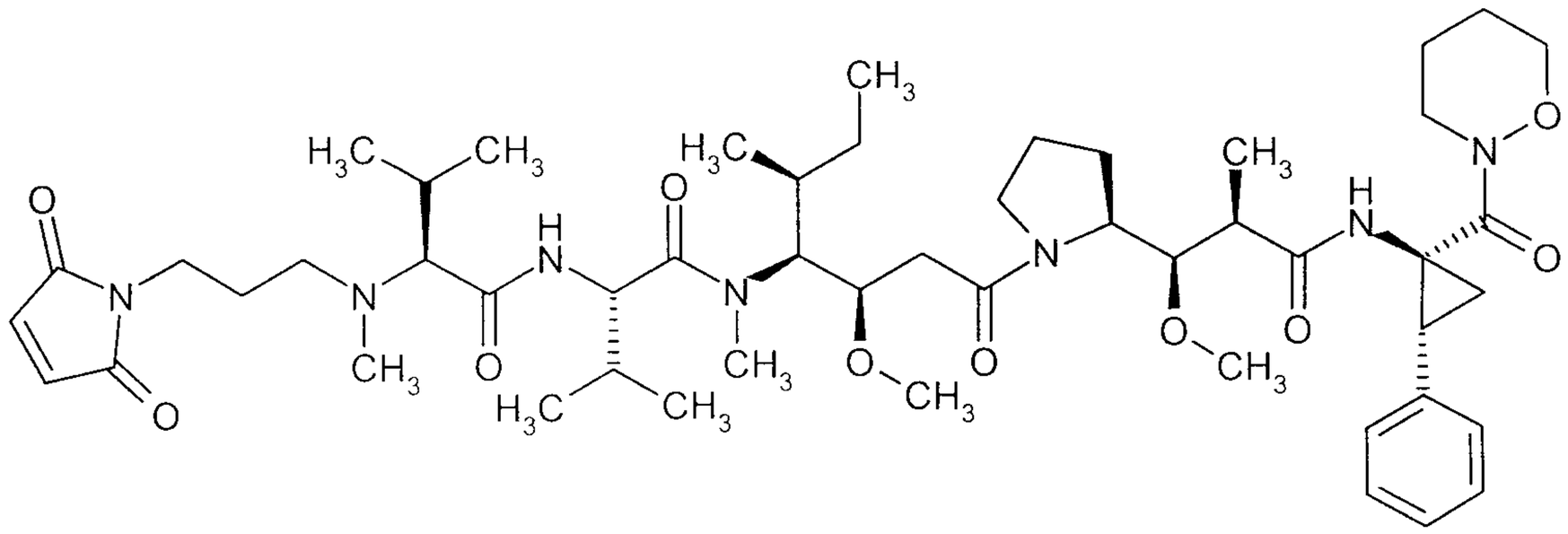


To a solution of 5 mg (5.6 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide in 1 ml of DMF were added 7.65 mg (22.5 µmol) of 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,2-dimethylbutanehydrazide trifluoroacetate, 3.2 mg (16.9 µmol) of EDC, 1.96 µl (11.3 µmol) of diisopropylethylamine and 2.6 mg (16.9 µmol) of HOBT. The reaction mixture was stirred at RT for 3 h. Subsequently, a further 0.95 mg (2.8 µmol) of 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,2-dimethylbutanehydrazide trifluoroacetate were added. After stirring overnight, the reaction mixture was concentrated and purified by means of preparative HPLC. 3.5 mg (85% purity, 48% of theory) of the title compound were obtained.

LC-MS (Method 3): R_t = 1.86 min; m/z = 1094 $(M+H)^+$.

Intermediate 145

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



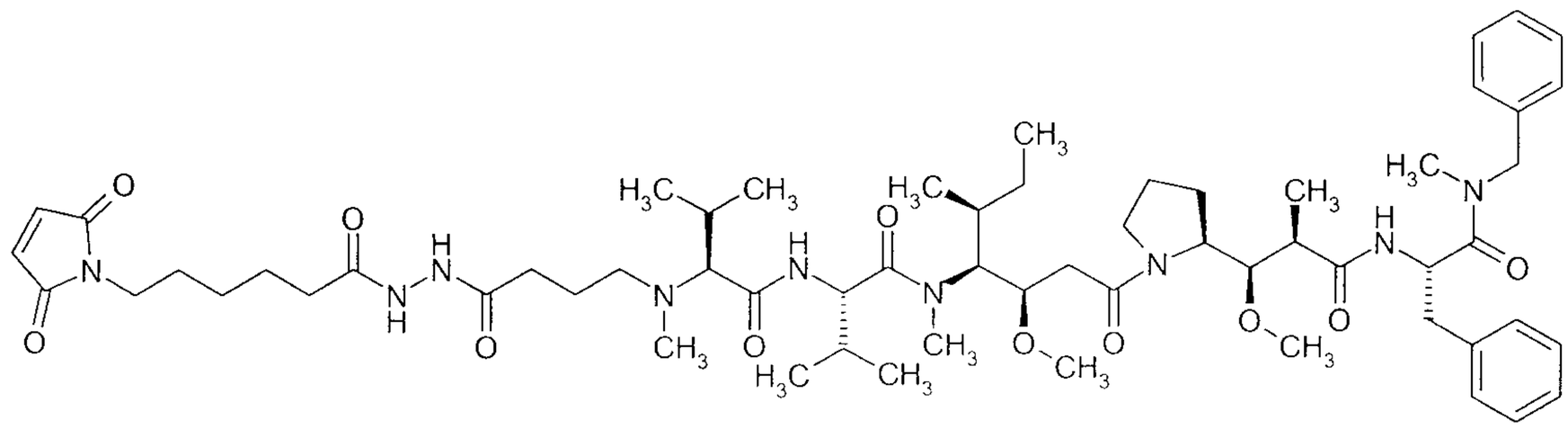
12 mg (14 µmol) of *N*-(3-aminopropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 66) were taken up in 750 µl of dioxane and admixed with 1.5 ml of saturated sodium hydrogencarbonate solution and then with 3.2 mg (21 µmol) 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 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 $(M+H)^+$.

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



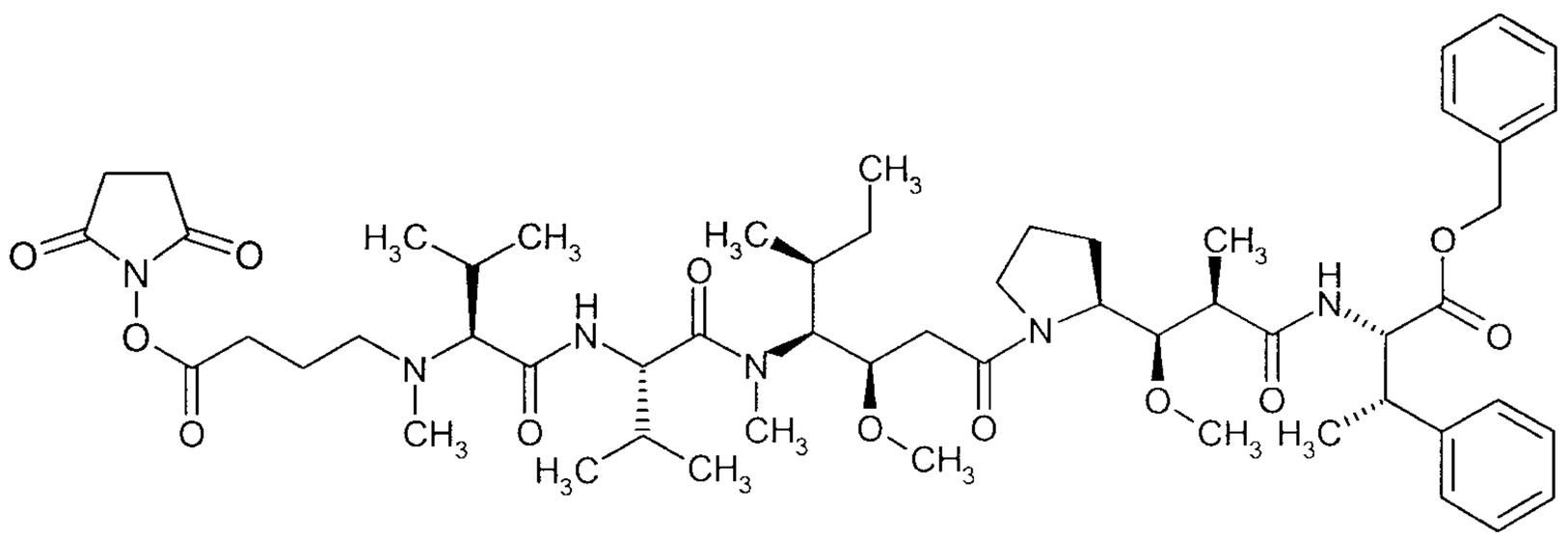
9 mg (9.8 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-({(2*S*)-1-[benzyl(methyl)amino]-1-oxo-3-phenylpropan-2-yl}amino)-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 73) were reacted in analogy to Intermediate 133 with 10 mg (39 µmol) 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.

HPLC (Method 12): R_t = 2.2 min;

LC-MS (Method 9): R_t = 5.11 min; MS (ESIpos): m/z = 1128 $(M+H)^+$.

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



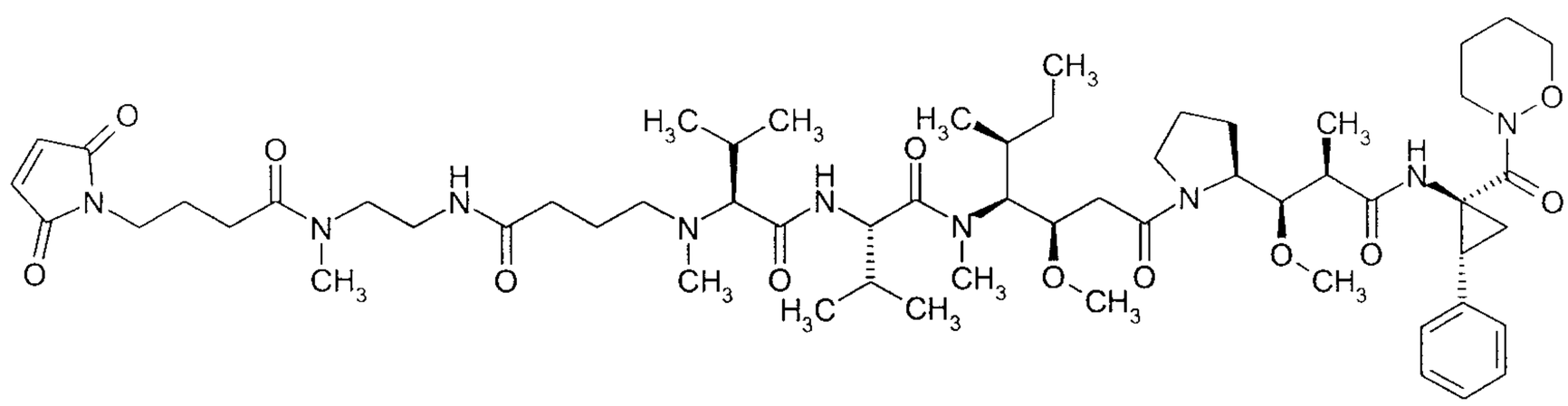
16 mg (17 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*,3*S*)-1-(benzyloxy)-1-oxo-3-phenylbutan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 70) were 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 µmol) 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 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



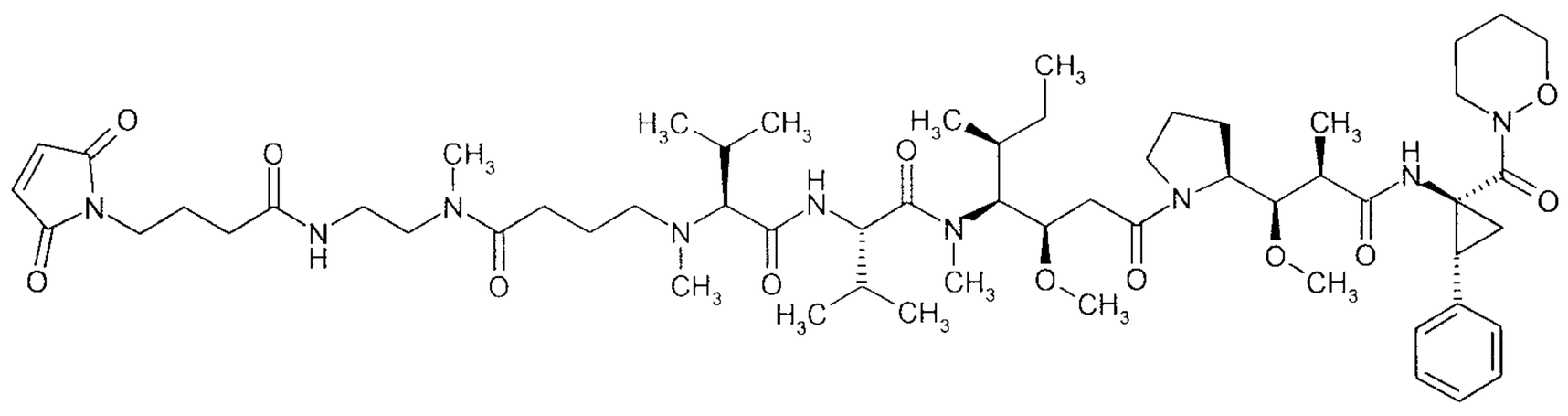
6 mg (7 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 61) were combined with 2.8 mg (8 µmol) of *N*-(2-aminoethyl)-4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-methylbutanamide trifluoroacetate, 10.1 mg (27 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 5 µl of *N,N*-diisopropylethylamine in 2 ml of DMF and stirred at RT overnight. Then another 5 mg (23.5 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate and 3 µl 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



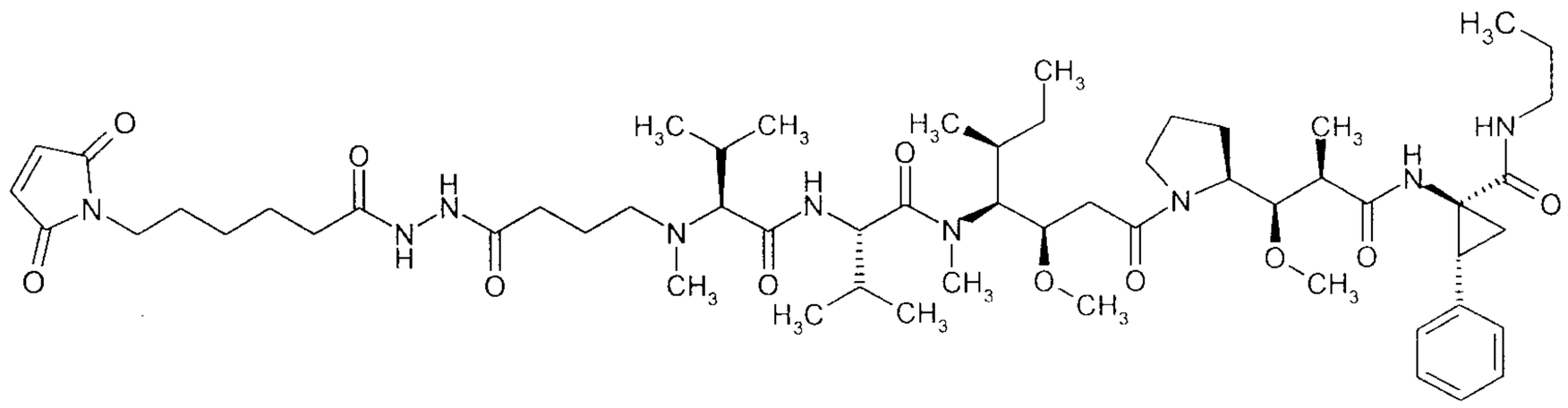
6 mg (7 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 61) were combined with 3.1 mg (9 µmol) of 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-[2-(methylamino)ethyl]butanamide trifluoroacetate, 10.1 mg (27 µmol) of O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate and 5 µl of *N*,*N*-diisopropylethylamine in 2 ml of DMF, and the mixture was stirred at RT for 4 h. Then 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 mg (13.4% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 2.1 min;

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



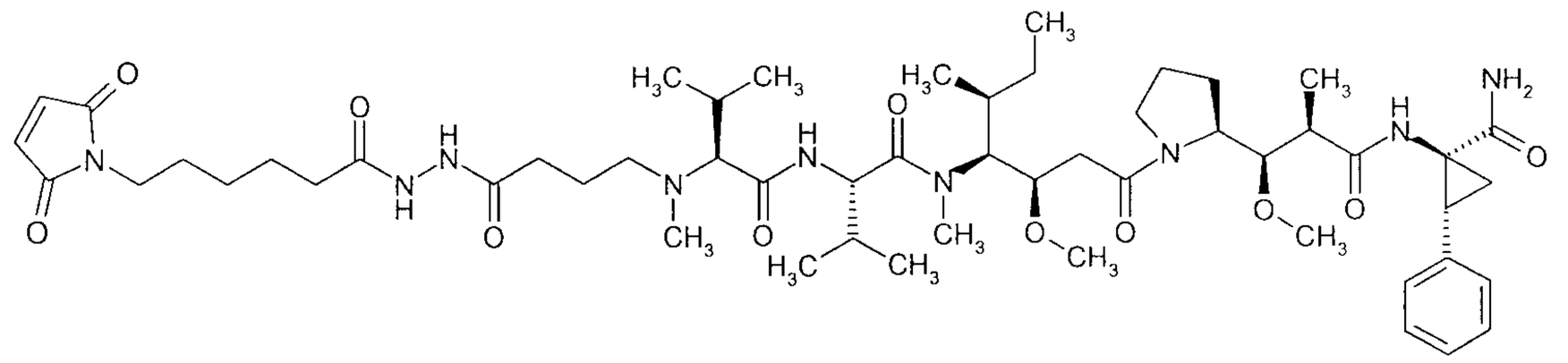
7.9 mg (9 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*,2*R*)-2-phenyl-1-(propylcarbamoyl)cyclopropyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide were dissolved in 3 ml of DMF and then admixed with 10.4 mg (54 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 8.3 mg (54 µmol) of 1-hydroxy-1*H*-benzotriazole hydrate, 9 µl of *N,N*-diisopropylethylamine and with 9.5 mg (36 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, 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



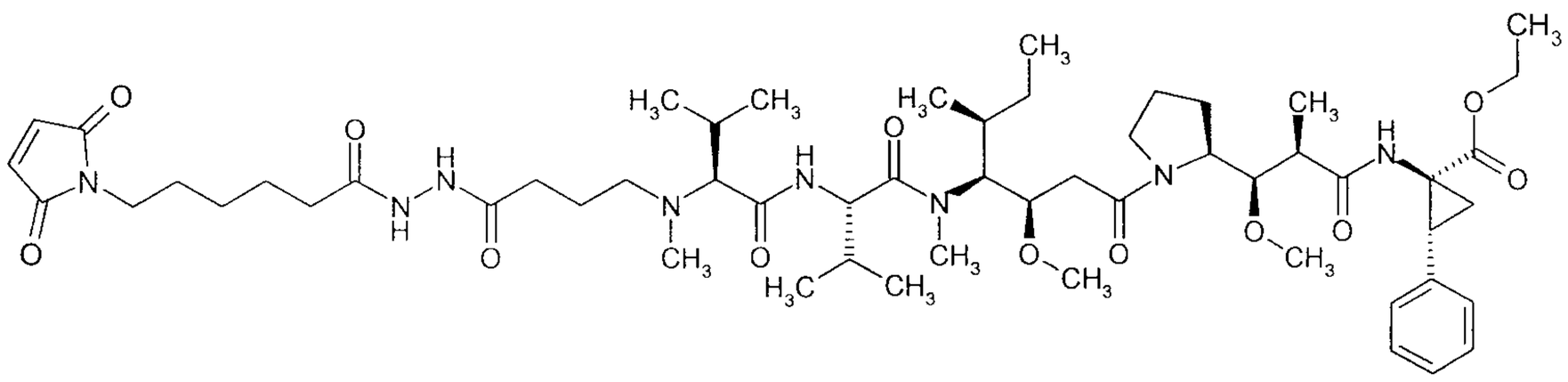
The compound was prepared in analogy to Intermediate 150 starting from the compound in Intermediate 81.

HPLC (Method 5): R_t = 1.7 min;

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 µmol) 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 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate, 10 µl of *N*,*N*-diisopropylethylamine and with 12 mg (47 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, 5.8 mg (37 % of theory) of the title compound were obtained as a colourless foam.

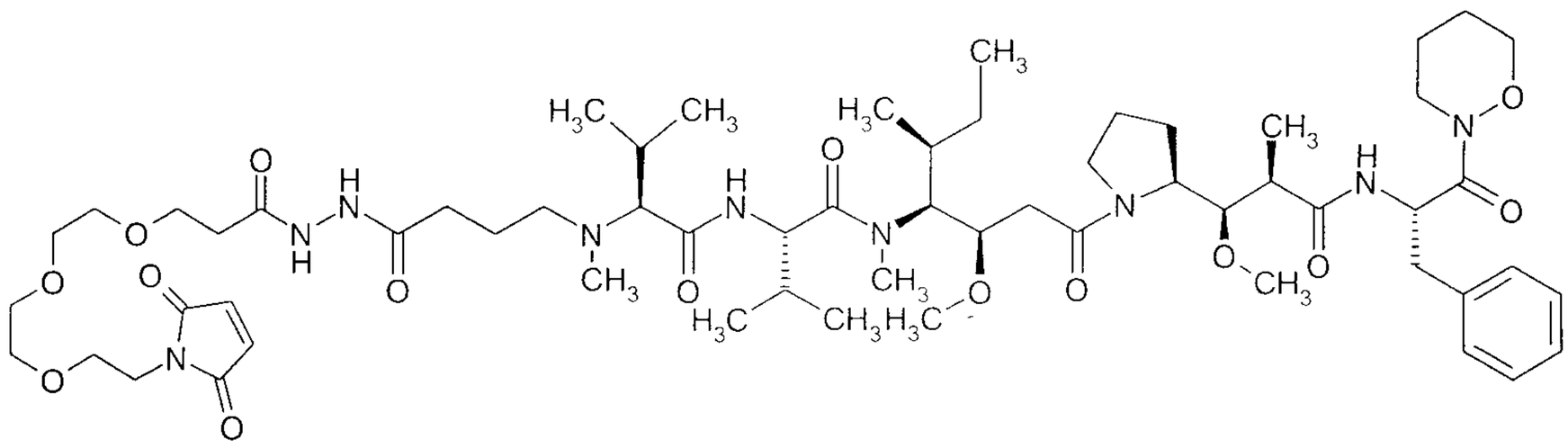
HPLC (Method 6): R_t = 2.0 min;

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-

{[(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



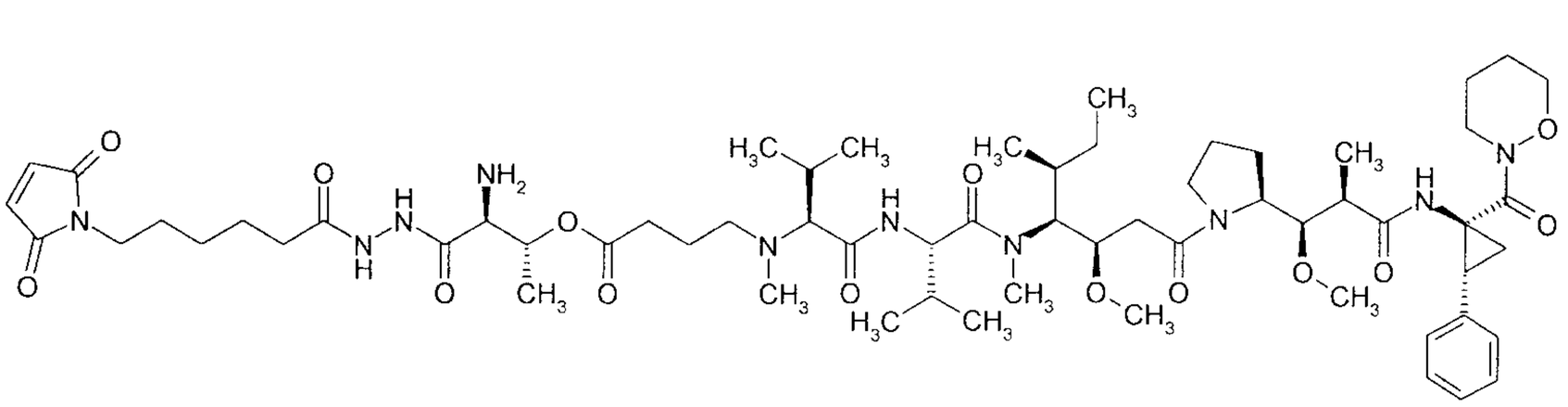
To a solution of 5 mg (5.6 µmol) of *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(2*S*)-1-(1,2-oxazinan-2-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide in 1 ml of DMF were added 9.7 mg (22.5 µmol) 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 µmol) of EDC, 1.96 µl (11.3 µmol) of N,N-diisopropylethylamine and 2.6 mg (16.9 µmol) of HOBT. The reaction mixture was stirred at RT for 3 h. Subsequently, another 1.2 mg (2.8 µmol) of 3-(2-{2-[2-(2,5-dioxo-2,5-dihydro-1H-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 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*)-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 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 10 mg (83 µmol) of 4-dimethylaminopyridine and with 10.3 mg (33 µmol) 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 $(M+H)^+$.

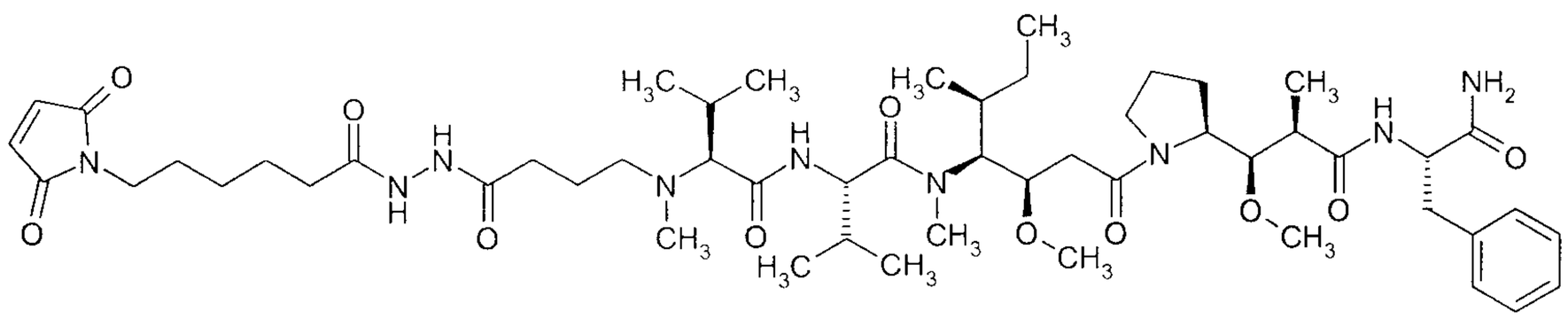
Subsequently, the benzyl ester protecting group was removed by hydrogenation under standard hydrogen pressure in methanol over 10% palladium/activated carbon, and the acid thus obtained, as described in Intermediate 151, was coupled to 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide. In a last 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

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



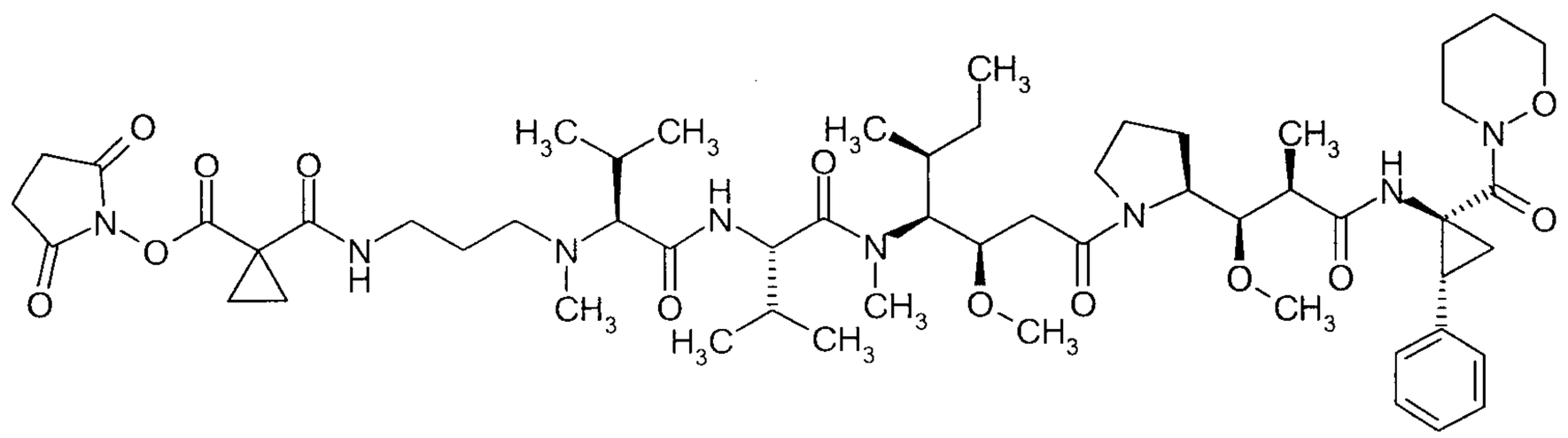
This compound was prepared in analogy to the synthesis described in Intermediate 152 from *N*-(3-carboxypropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-{[(2*S*)-1-amino-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide and commercially available 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanehydrazide.

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

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



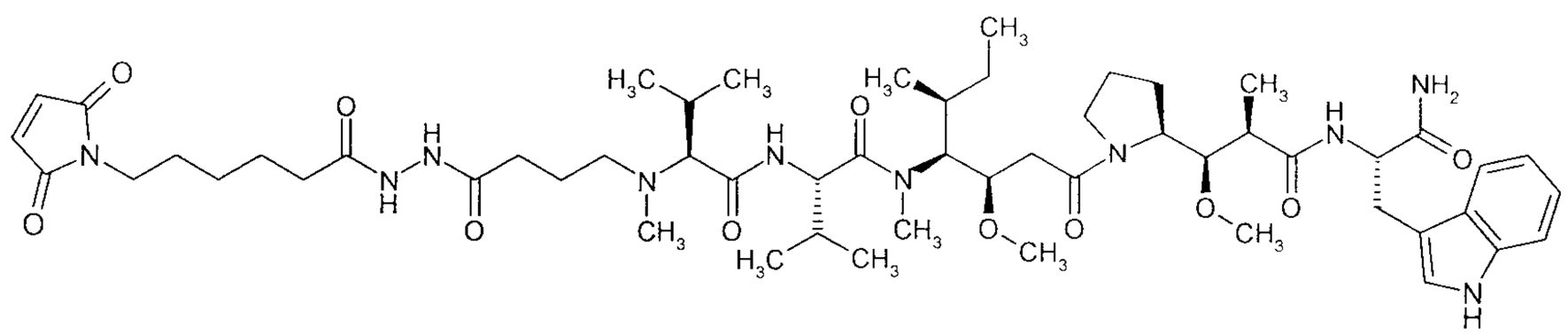
This compound was prepared in analogy to the synthesis described in the last stage of Intermediate 131 from *N*-(3-aminopropyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-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 µmol) 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 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate, 12 µl of *N*,*N*-diisopropylethylamine and with 14 mg (53 µmol) 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;

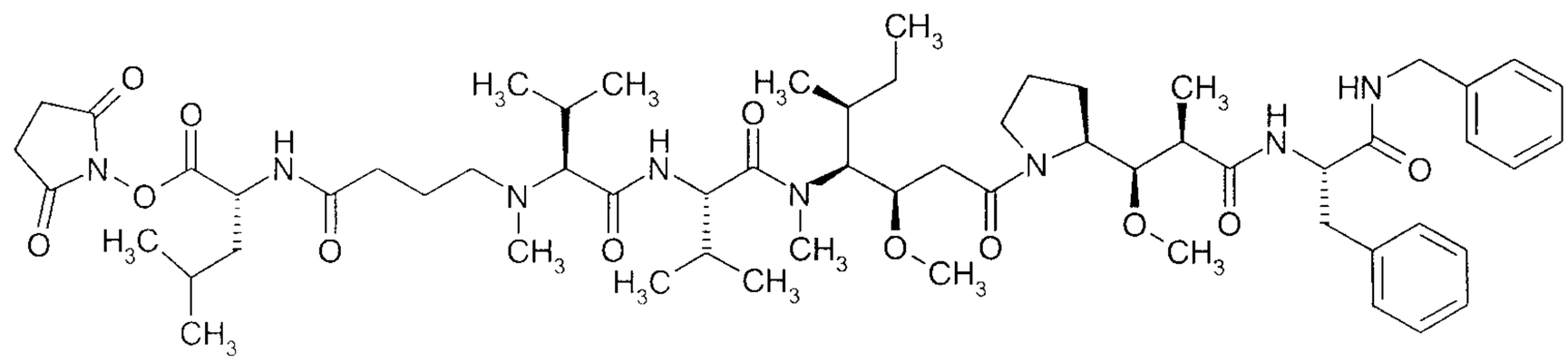
LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 1063 $(M+H)^+$.

^{1}H-NMR (500 MHz, DMSO-d_6, characteristic signals): δ = 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).

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



13 mg (14.7 µmol) 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 were dissolved in 4 ml of dimethylformamide and then admixed with 9.4 mg (25 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, 6 µl of *N,N*-diisopropylethylamine and with 7 mg (31 µmol) 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 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;

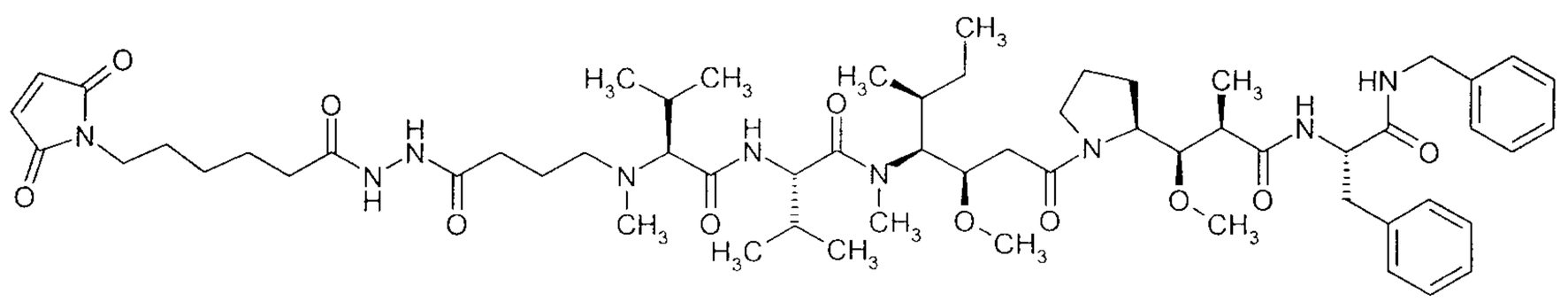
LC-MS (Method 1): R_t = 1.21 min; MS (ESIpos): m/z = 1076 $(M+H)^+$.

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 µmol) of this intermediate were taken up in 1.5 ml of dichloromethane and reacted with 0.8 mg (7 µmol) of *N*-hydroxysuccinimide, in the presence of 1.2 mg (6 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.16 mg (1 µmol) 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.

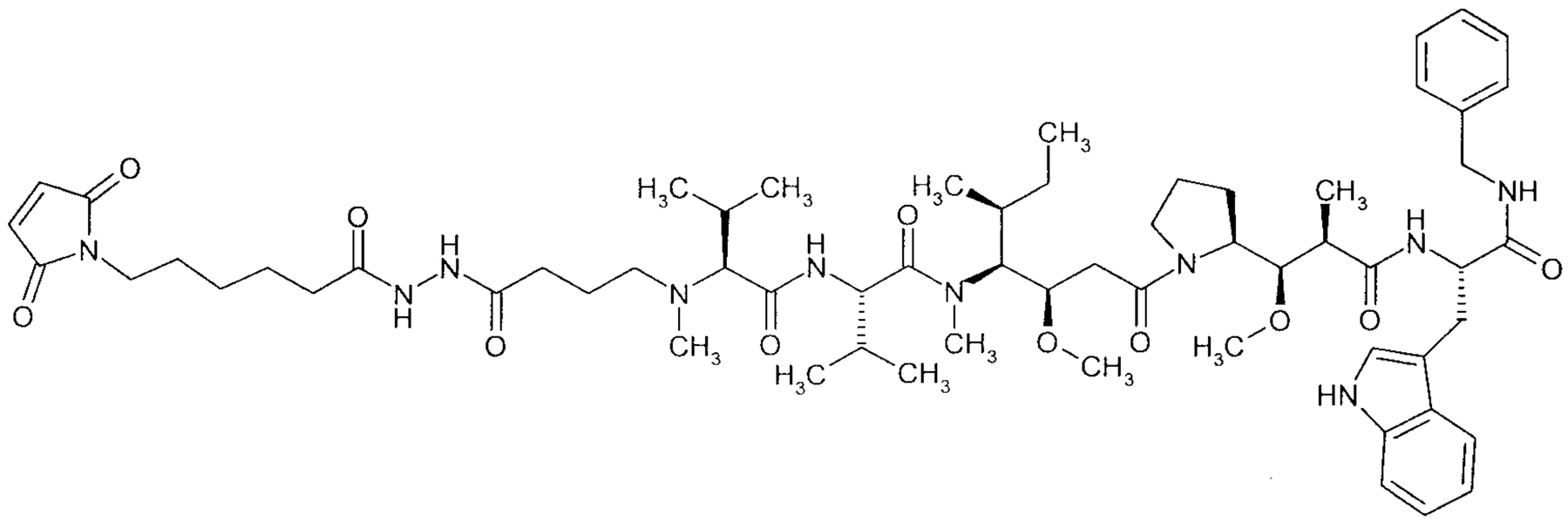
Yield: 6 mg (53% of theory)

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 mg (21 µmol) 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.

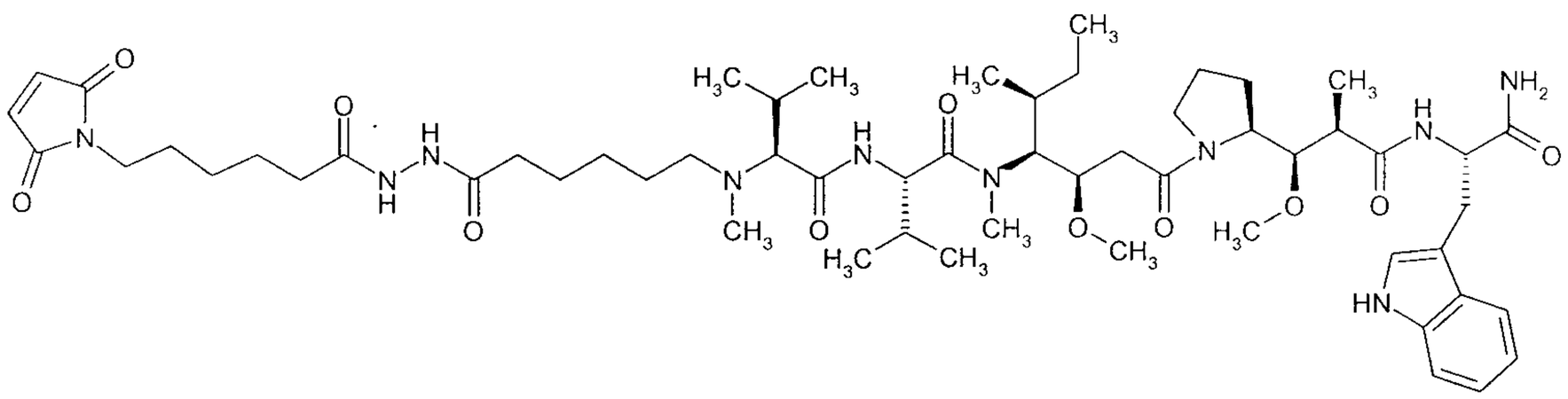
Yield: 13 mg (52% of theory)

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-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*-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.

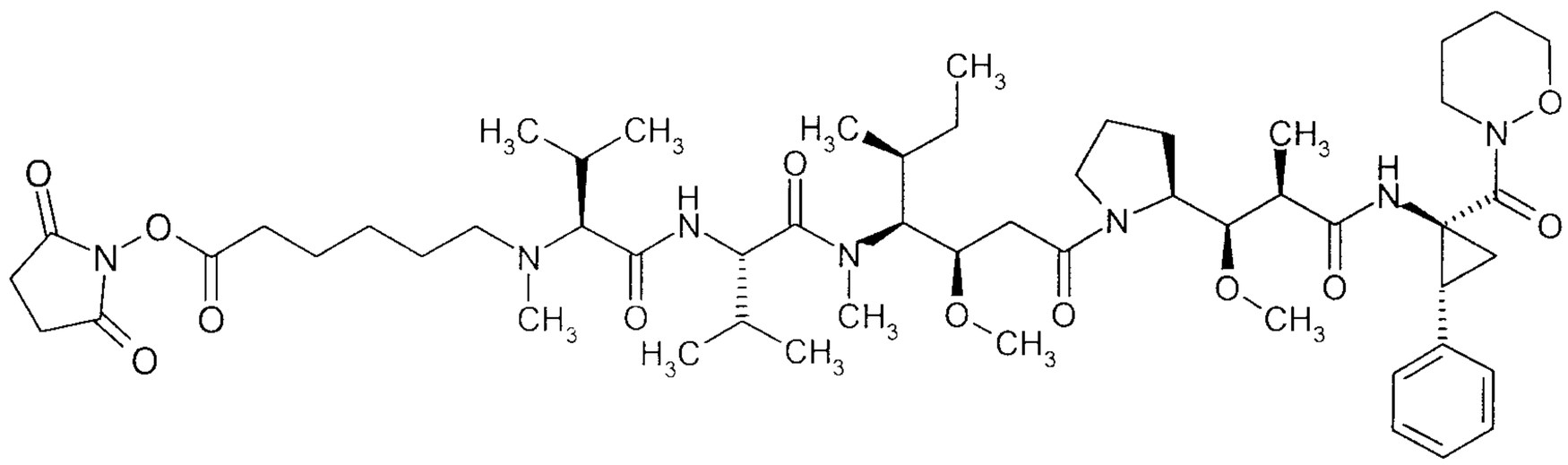
Yield: 0.8 mg (16% of theory)

HPLC (Method 5): R_t = 1.6 min;

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



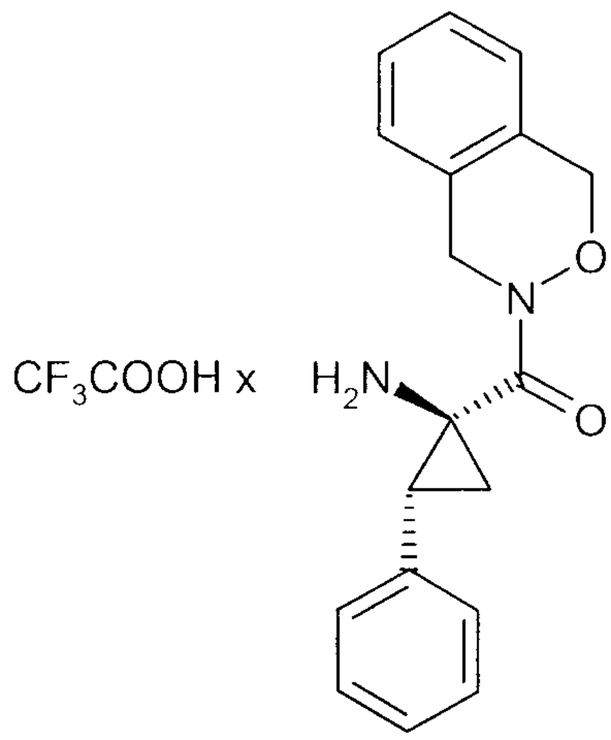
18 mg (20 µmol) of *N*-(5-carboxypentyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-{[(1*S*,2*R*)-1-(1,2-oxazinan-2-ylcarbonyl)-2-phenylcyclopropyl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 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 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.24 mg (0.17 µmol) of DMAP. After stirring at RT for 2 h, another 22 mg (190 mmol) of 1-hydroxypyrrolidine-2,5-dione, 11 mg (60 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.24 mg (0.17 µmol) 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.

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



First, starting with 265 mg (0.82 mmol) of *tert*-butyl (1*S*,2*R*)-1-(hydroxycarbamoyl)-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-[(1S,2R)-1-(1,4-dihydro-3H-2,3-benzoxazin-3-ylcarbonyl)-2-phenylcyclopropyl]carbamate was prepared.

Yield: 108 mg (34% of theory)

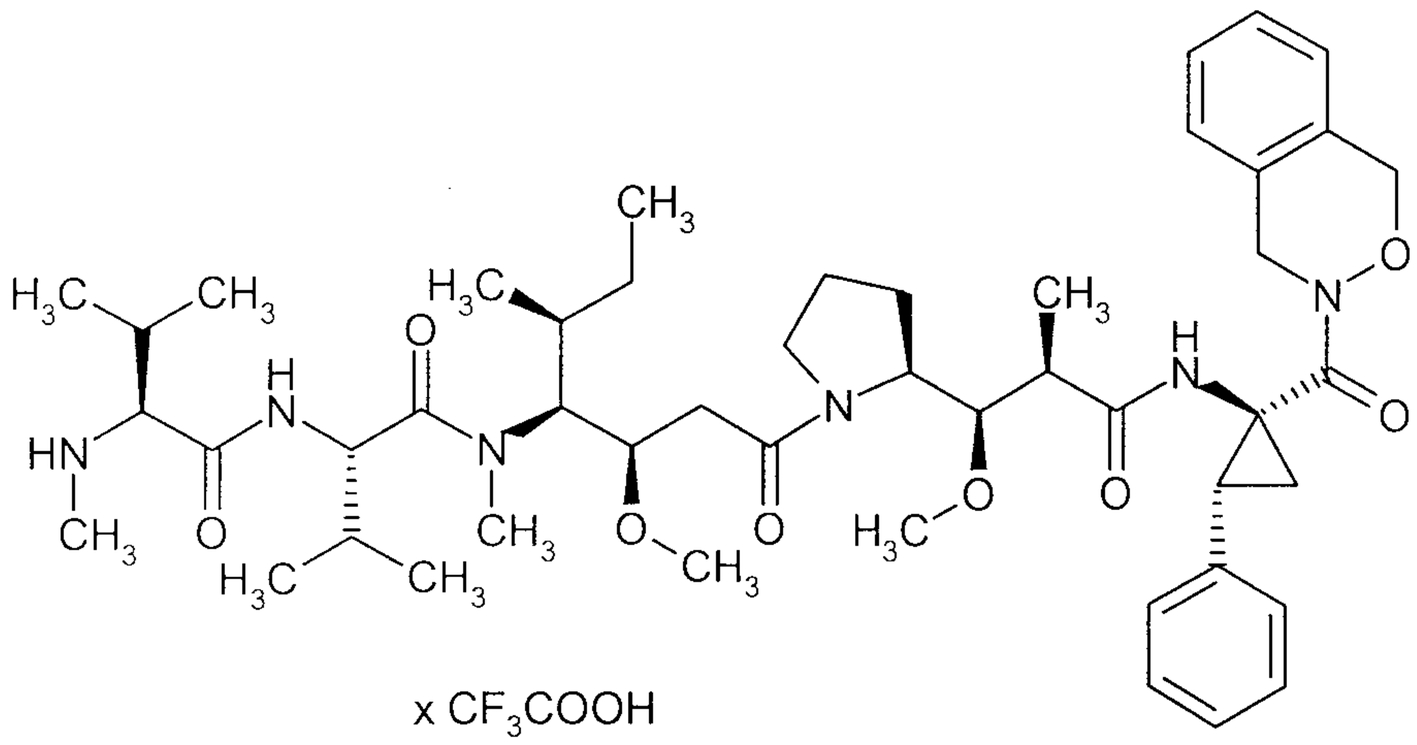
LC-MS (Method 2): R_t = 1.3 min; MS (ESIpos): m/z = 395 $(M+H)^+$.

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.

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-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*-[(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*'-tetramethyluronium hexafluorophosphate (HATU) and 682 µl (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 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.

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 µl 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

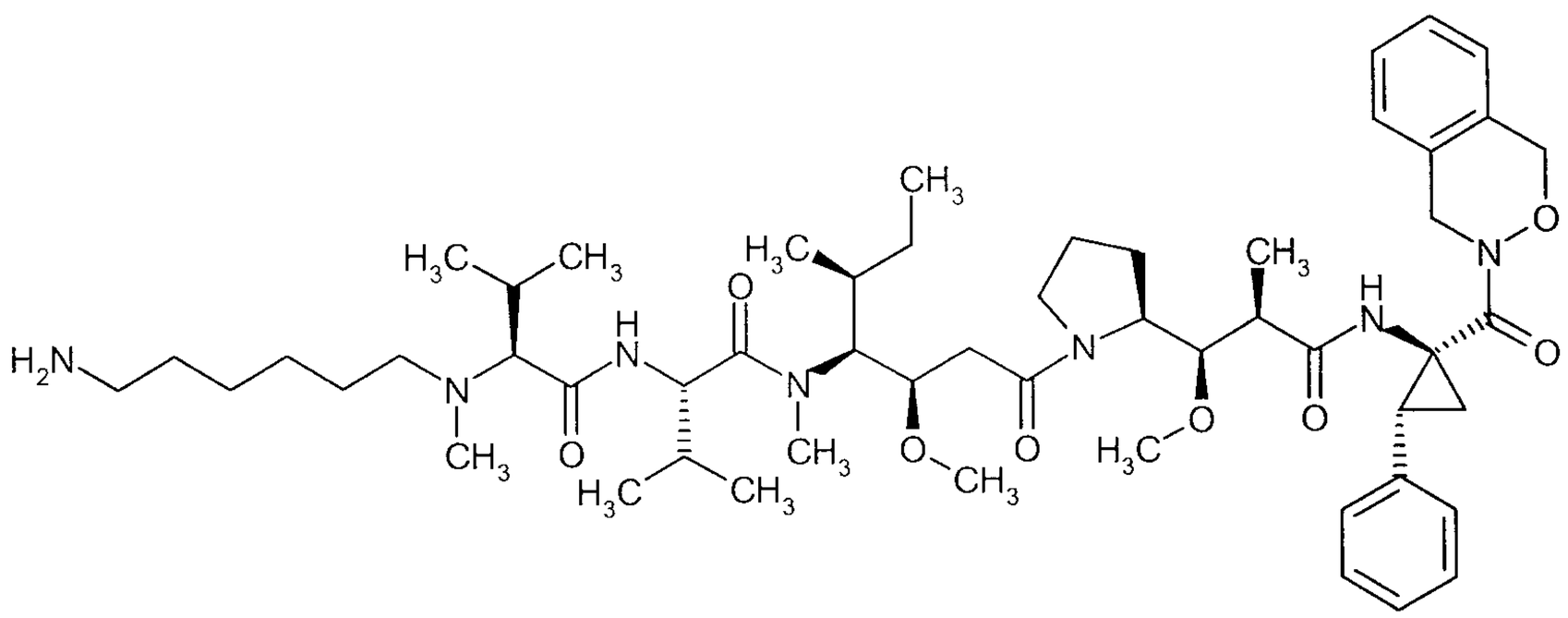
from dioxane/water. 12 mg (92% of theory) of the title compound were obtained as a colourless foam.

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.

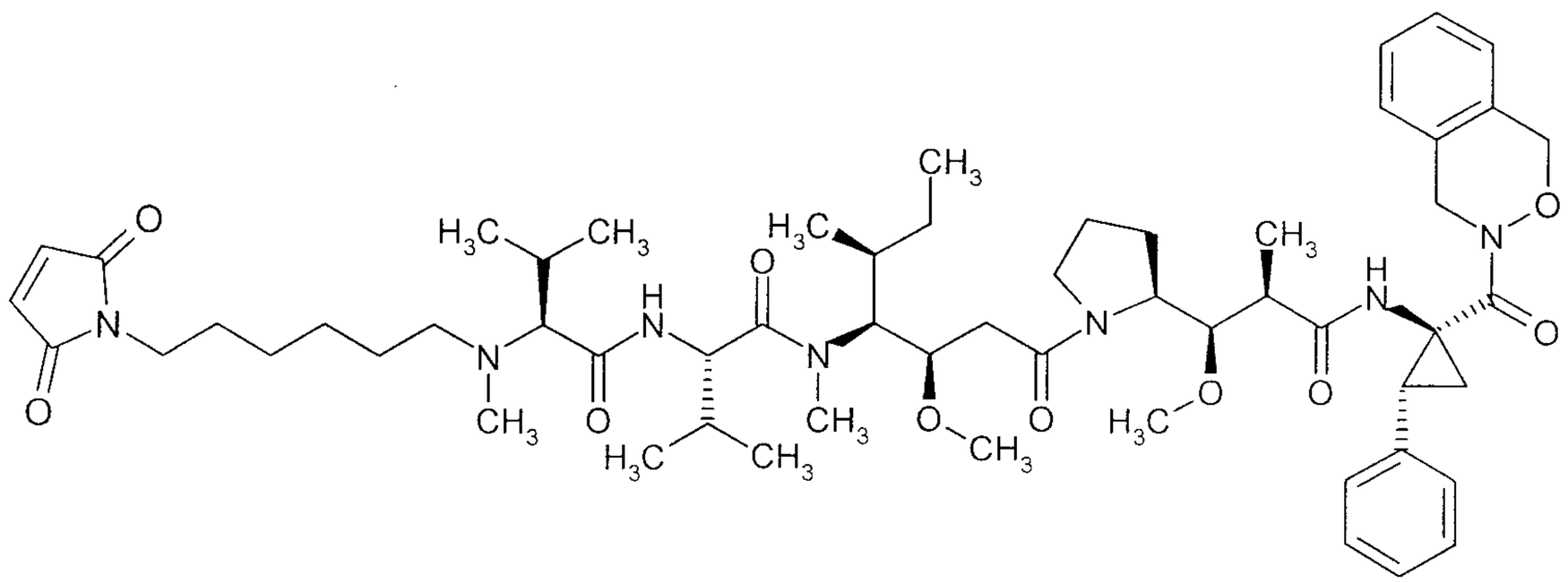
Yield: 4.5 mg (23% of theory over 2 stages)

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



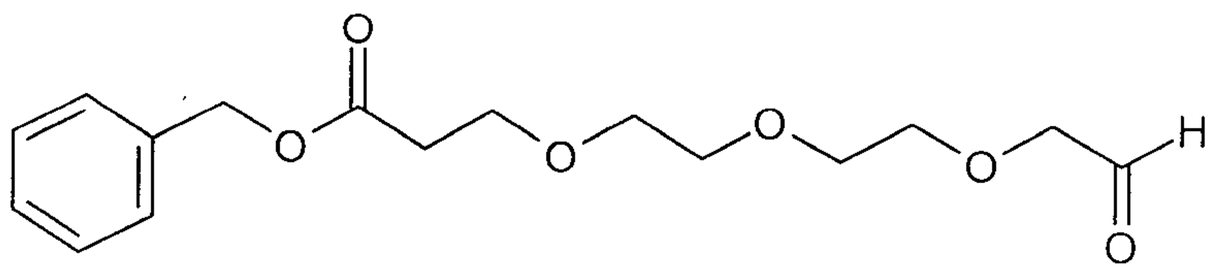
4.4 mg (4.5 µmol) of Intermediate 165 were taken up in 1 ml of 1:1 dioxane/water and then admixed with 1 mg (6.8 µmol) of methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate and with 50 µl of saturated aqueous sodium hydrogencarbonate solution. The reaction mixture was stirred at RT for 30 min. Then another 50 µl of the saturated aqueous sodium hydrogencarbonate solution were added, and the reaction mixture was stirred at RT for a further 15 min and then 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

Benzyl 3-{2-[2-(2-oxoethoxy)ethoxy]ethoxy}propanoate



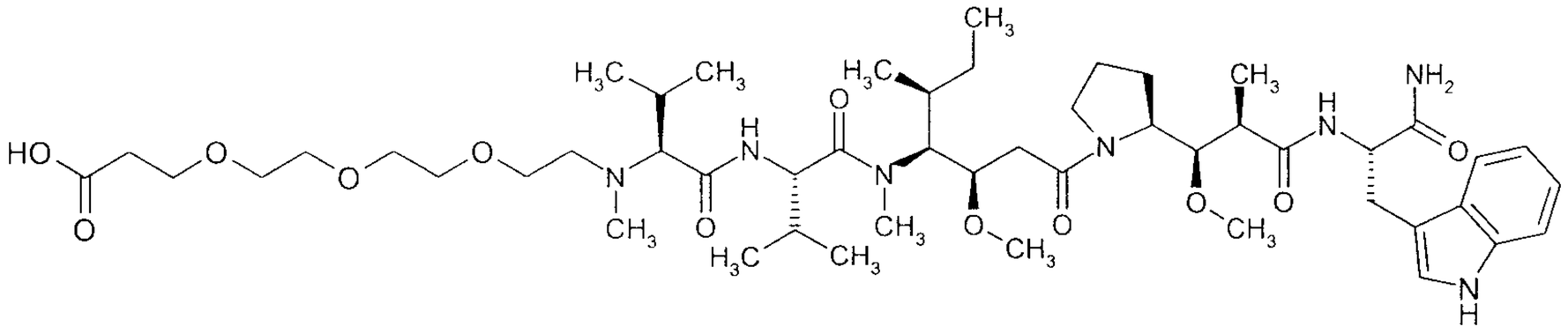
The title compound was prepared from 6 g (21.55 mmol) of commercially available 3-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}propanoic acid under standard conditions, first by esterification with benzyl chloride and caesium carbonate and subsequent oxidation with sulphur trioxide-pyridine complex.

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-[(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 *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.

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

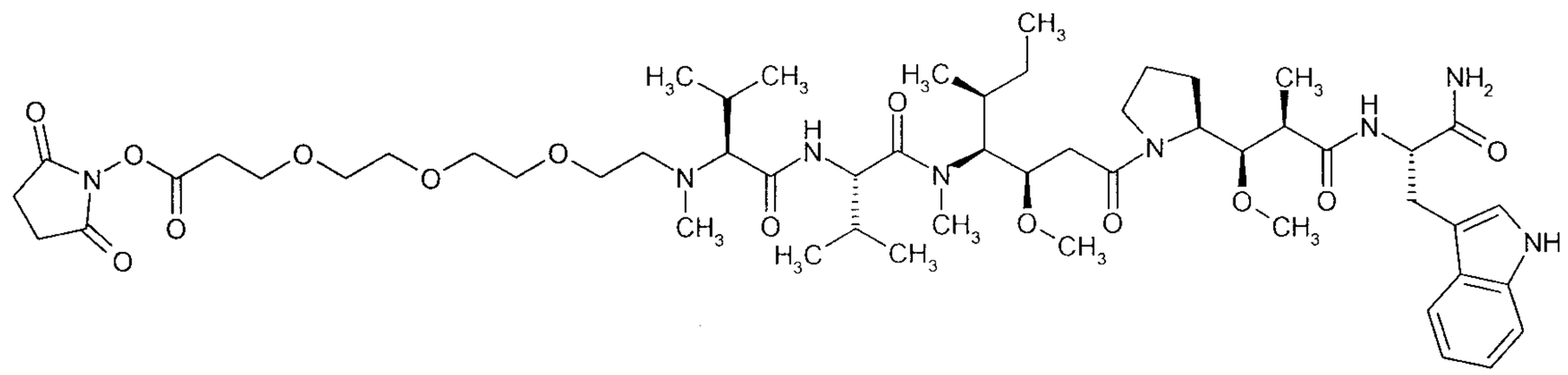
catalyst was then filtered off, and the solvent was removed *in vacuo*. After lyophilization from dioxane, 19.7 mg (85% of theory) of the title compound were obtained.

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



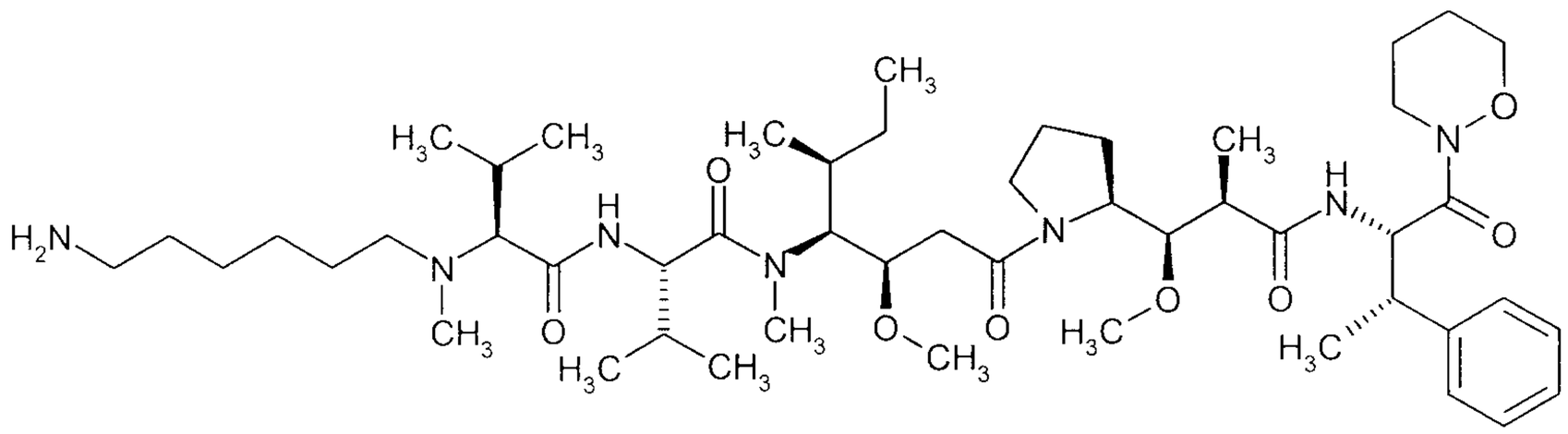
10 mg (10 µmol) of Intermediate 168 were dissolved in 3 ml of DMF and admixed with 3.5 mg (30 mmol) of 1-hydroxypyrrolidine-2,5-dione and then with 2.4 mg (10 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 5 µl of N,N-diisopropylethylamine. After stirring at RT for 20 h, 8 mg (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.

HPLC (Method 12): Rt = 1.9 min;

LC-MS (Method 11): Rt = 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



This compound was prepared in analogy to Intermediate 101 over 2 stages, starting from 26 mg (0.028 mmol) of Intermediate 15.

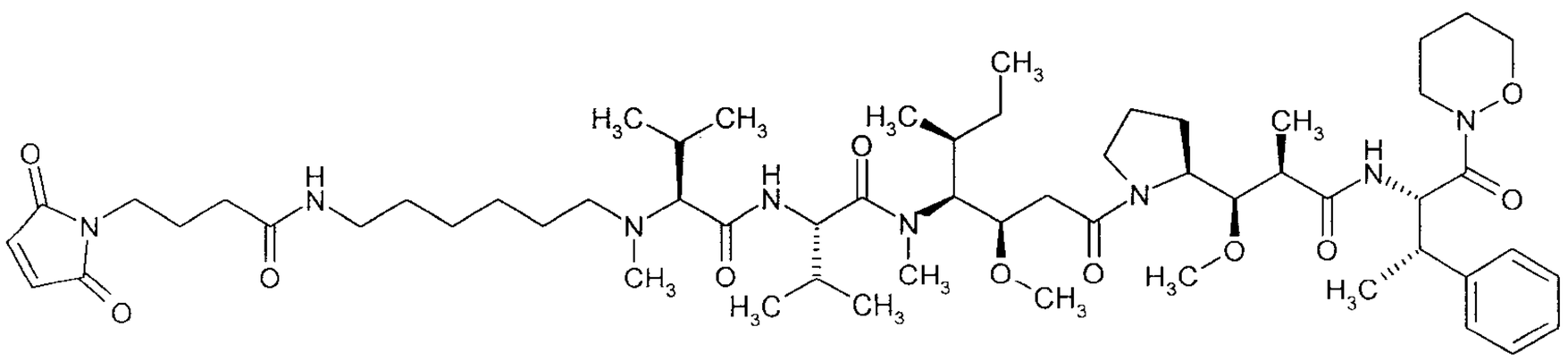
Yield: 16.7 mg (63% of theory over 2 stages)

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.81 min; MS (ESIpos): m/z = 914 $(M+H)^+$.

Intermediate 171

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



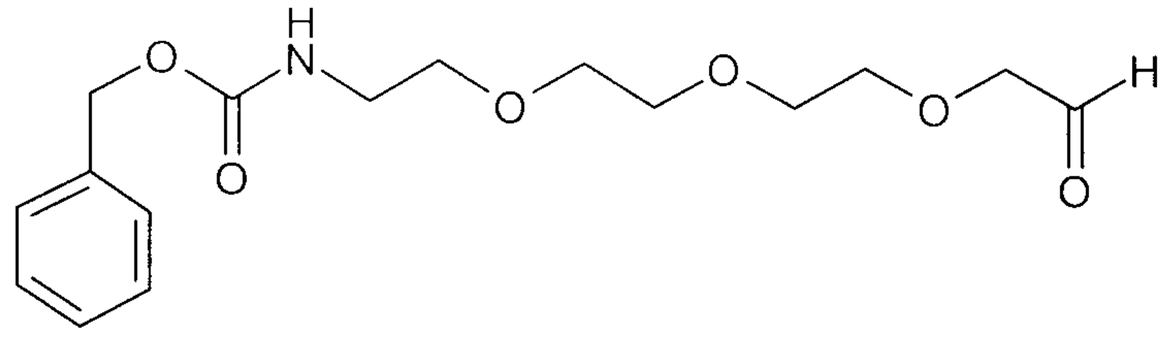
6.7 mg (7.3 µmol) of the compound from Intermediate 170 and 3 mg (14.7 µmol) of commercially available 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoic acid were taken up in 2 ml of DMF and admixed with 5.6 mg (14.7 µmol) of *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (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 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



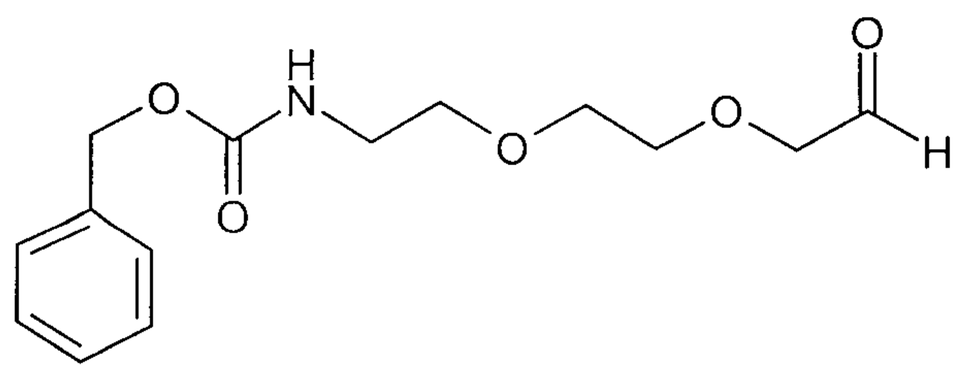
The title compound was prepared from commercially available 2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethanol under standard conditions by first introducing the Z protecting group and then oxidizing with sulphur trioxide-pyridine complex.

HPLC (Method 12): R_t = 1.4 min;

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



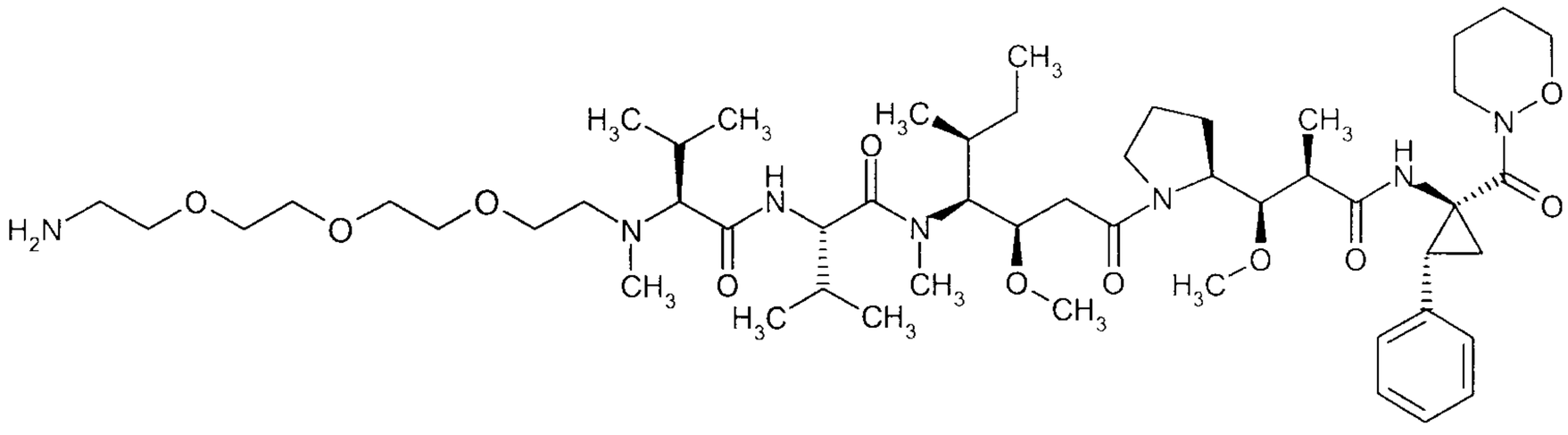
The title compound was prepared in analogy to Intermediate 172 from commercially available 2-[2-(2-aminoethoxy)ethoxy]ethanol under standard conditions by first introducing the Z protecting group and then oxidizing with sulphur trioxide-pyridine complex.

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



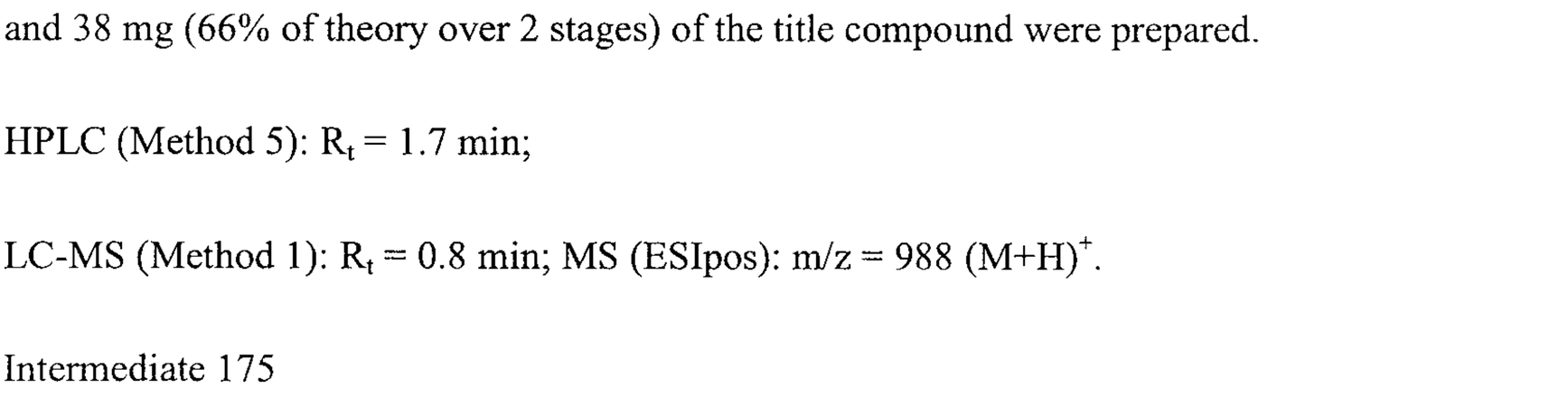
47 mg (0.05 mmol) of Intermediate 16 were reductively aminated in analogy to the preparation of Intermediate 167 with benzyl-(2-{2-[2-(2-oxoethoxy)ethoxy]ethoxy}ethyl)carbamate in the presence of borane-pyridine complex. Subsequently, the Z protecting group was removed by hydrogenolytic means with 5% 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.

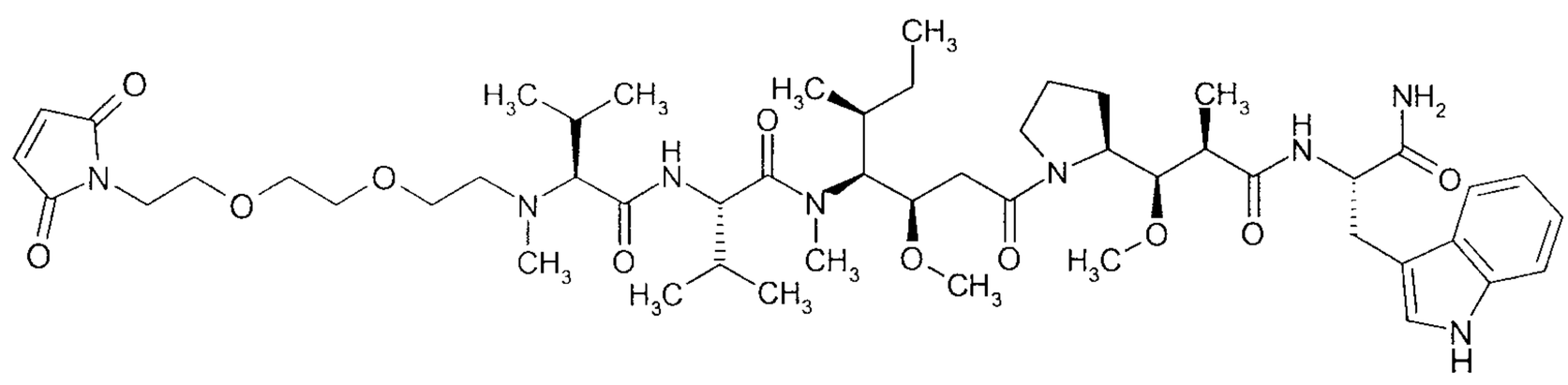
Yield: 8.3 mg (23% of theory)

HPLC (Method 5): R_t = 1.9 min;

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-methyl-L-valinamide



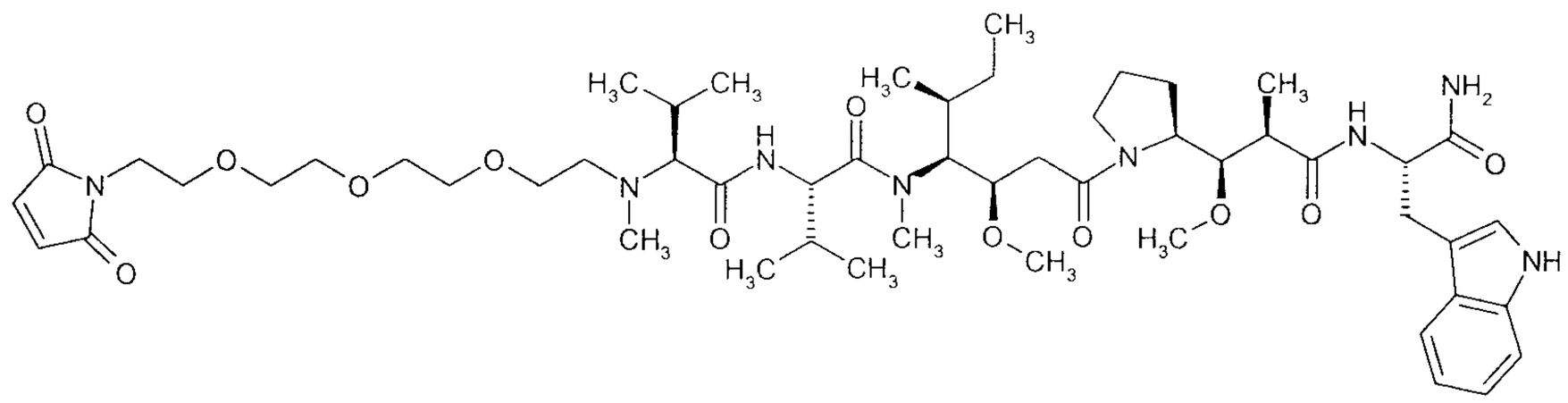
The preparation was done in analogy to Intermediates 174 and 175 starting with the reductive amination of Intermediate 16 with Intermediate 173, subsequent deprotection and formation of the maleimide.

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



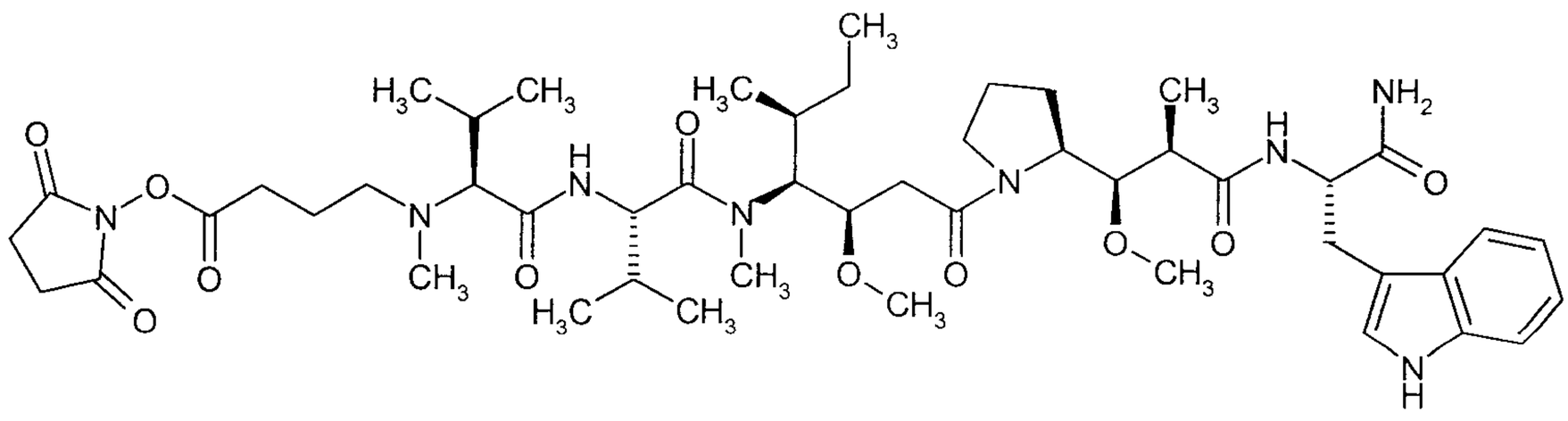
The preparation was done in analogy to Intermediates 174 and 175 starting with the reductive amination of Intermediate 16 with Intermediate 172, subsequent deprotection and formation of the maleimide.

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-[(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

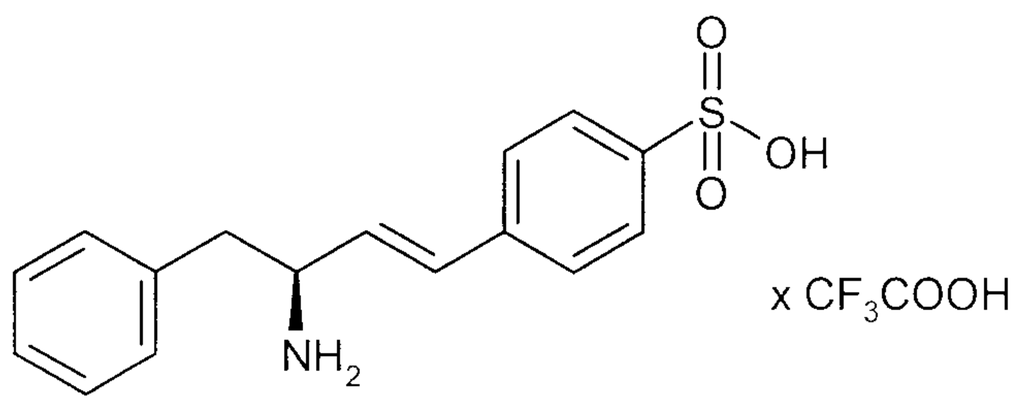


The preparation was done in analogy to Intermediate 162 starting from 6 mg of Intermediate 82.

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



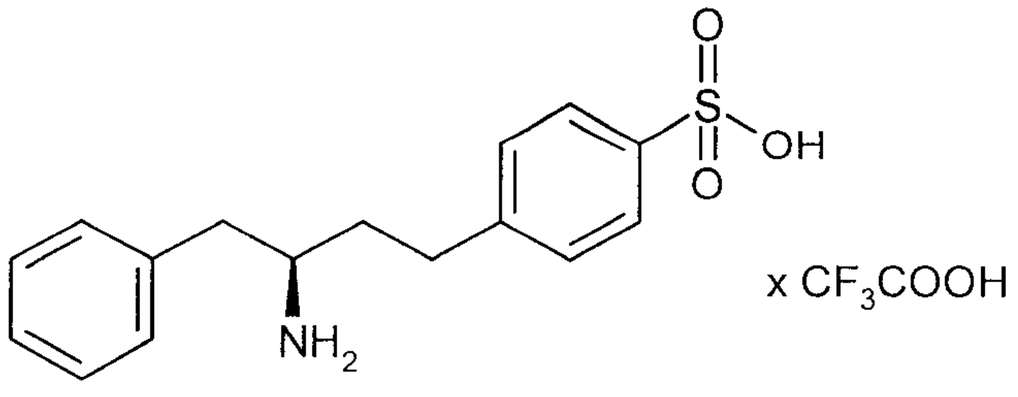
A mixture of 13.6 mg (0.06 mmol) of palladium(II) acetate, 469 mg (1.46 mmol) of potassium 4-iodobenzenesulphonate, 300 mg (1.21 mmol) of (S)-tert-butyl-1-phenylbut-3-en-2-yl-carbamate, 16.5 mg (0.12 mmol) of phenylurea and 167.6 mg (1.21 mmol) of potassium carbonate in 7.5 ml of DMF was heated in a 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 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



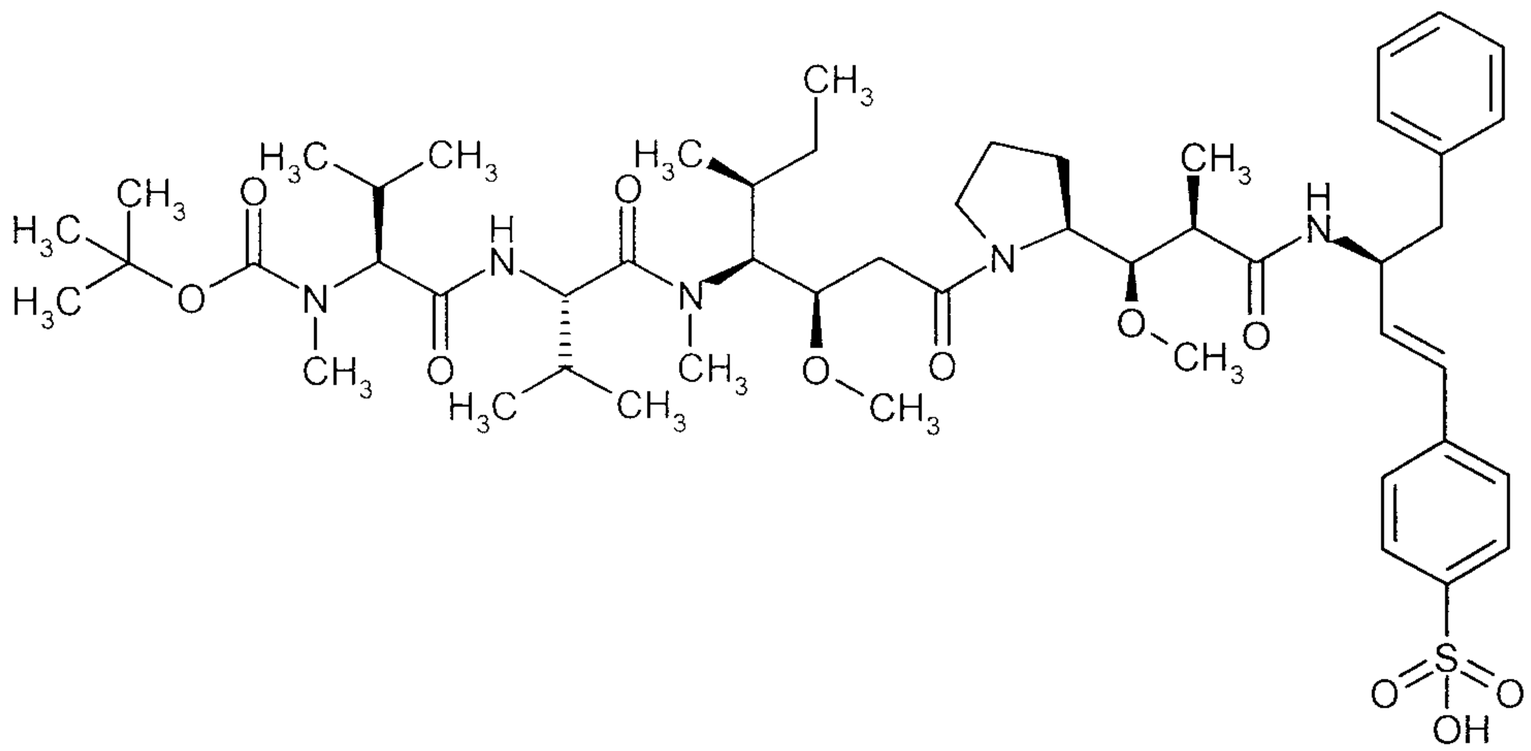
100 mg (0.25 mmol) of 4-[(1E,3S)-3-amino-4-phenylbut-1-en-1-yl]benzenesulphonic acid trifluoroacetate were suspended in 10 ml of acetic acid and a few drops of DMF and water, admixed with 70 mg (0.07 mmol) of palladium on carbon (10%) and hydrogenated at hydrogen pressure 2.2 bar for 24 h. The solution was filtered and the filtrate purified by means of 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

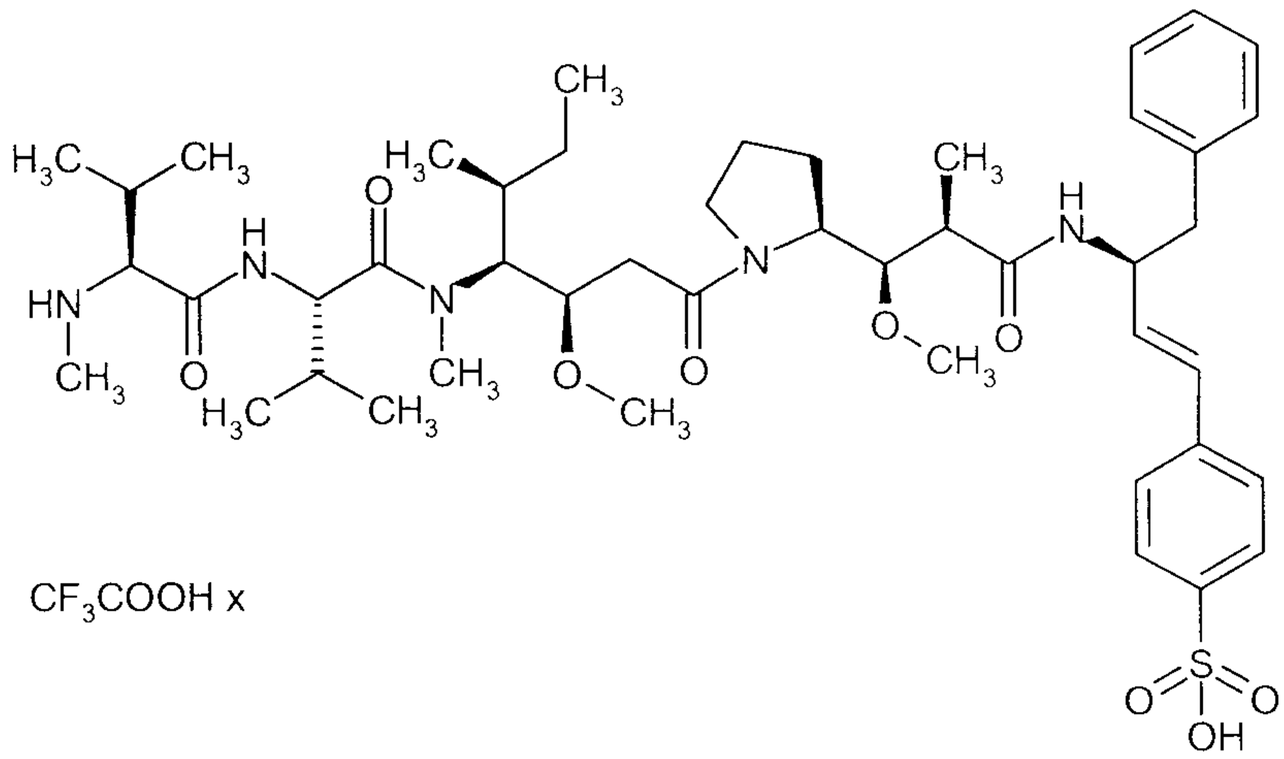


To a solution of 90 mg (0.13 mmol) of N-(tert-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 4 ml of DMF were added 60 mg (0.16 mmol) of HATU and 69 µl of (0.39 mmol) Hünig's base. 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



CF_3COOH x

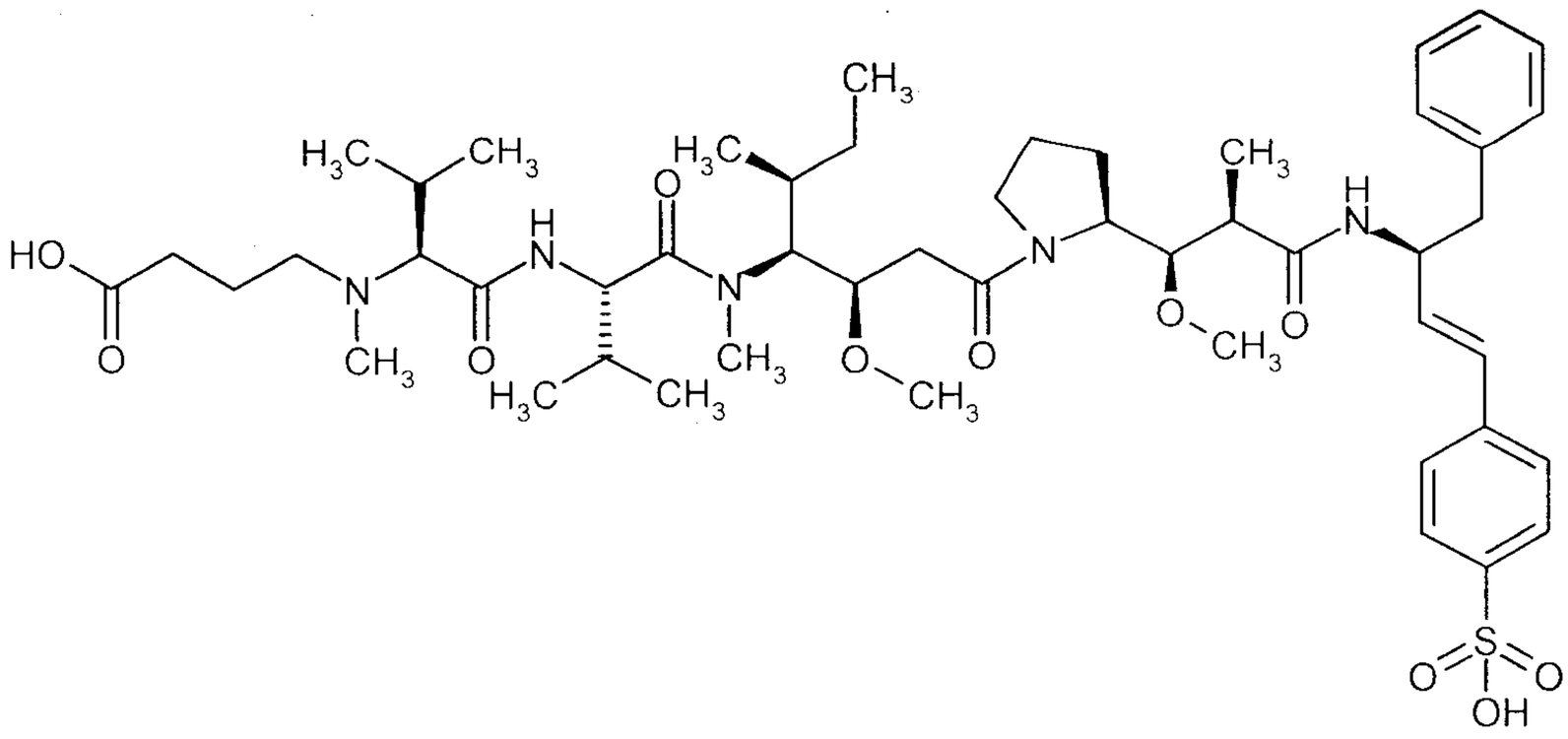
90 mg of Intermediate 180 were dissolved in 4.6 ml of dichloromethane, and 0.92 ml of trifluoroacetic acid were added. The reaction mixture was stirred at RT for 30 min and then concentrated. The obtained crude product was purified by means of preparative HPLC.

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-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-

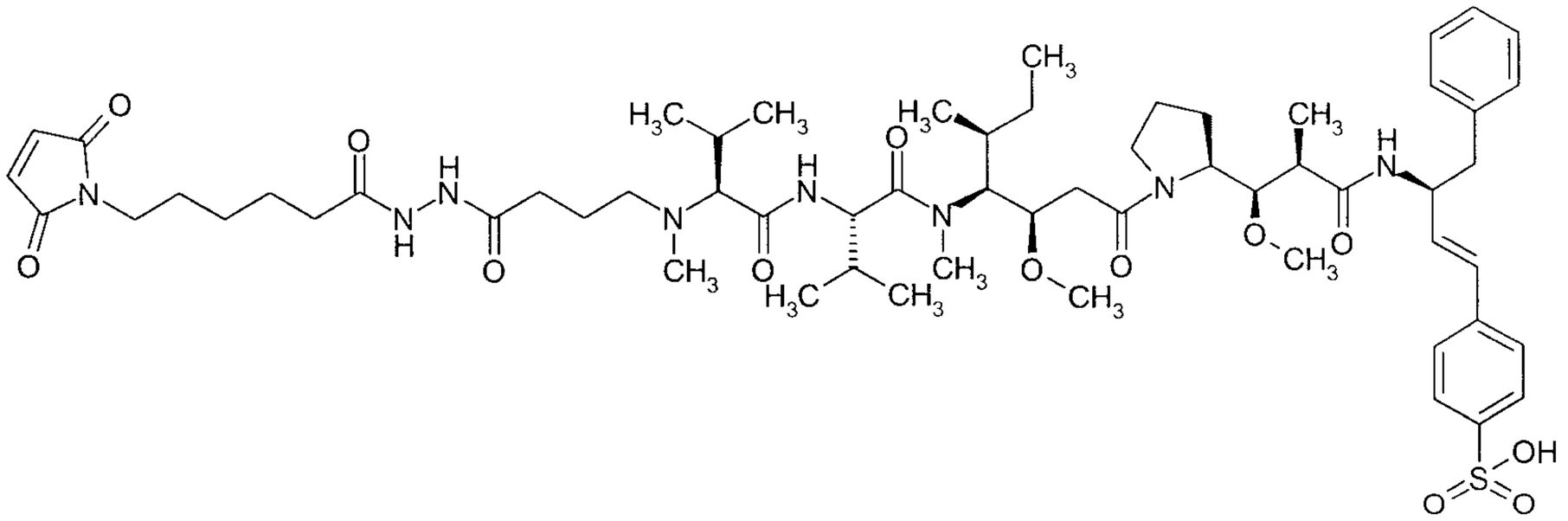
methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate (Intermediate 181) and 1.1 µl (0.02 mmol) of acetic acid. The reaction mixture was stirred for 5 min 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 $(M+H)^+$

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



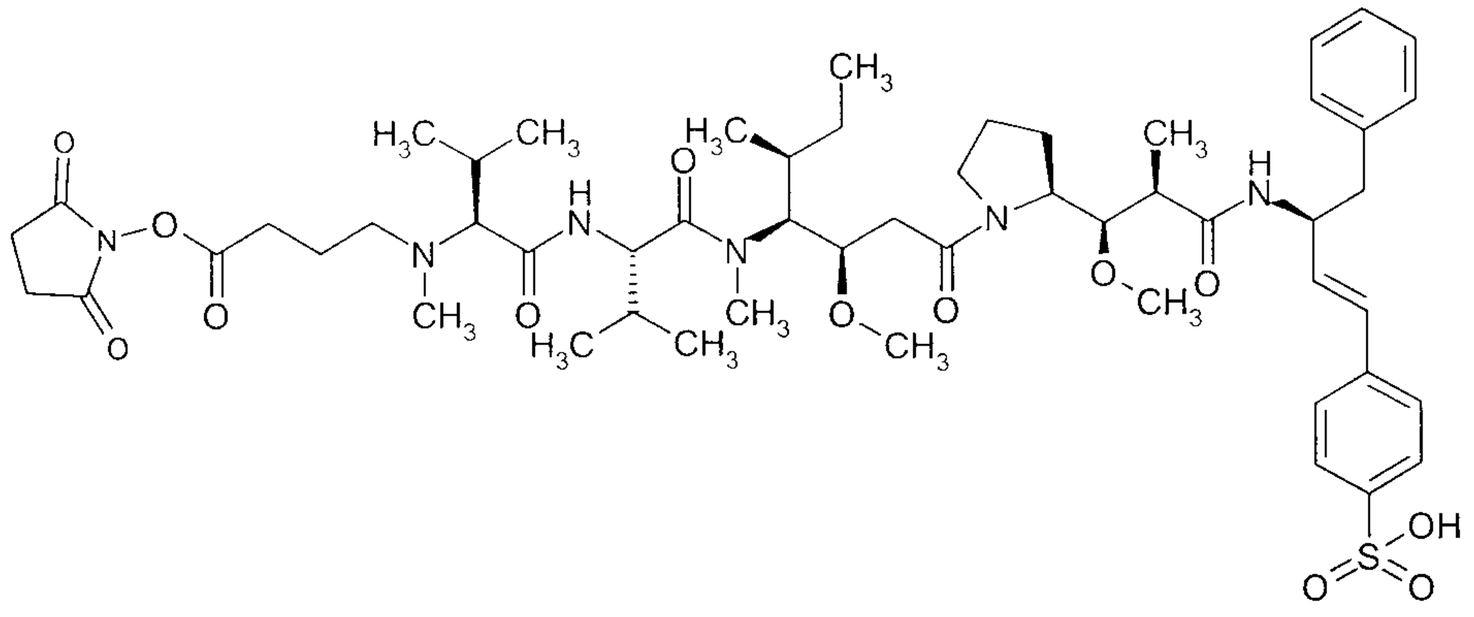
8 mg (7.5 µmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide, 2.8 mg (8.2 µmol) of 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanehydrazide 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

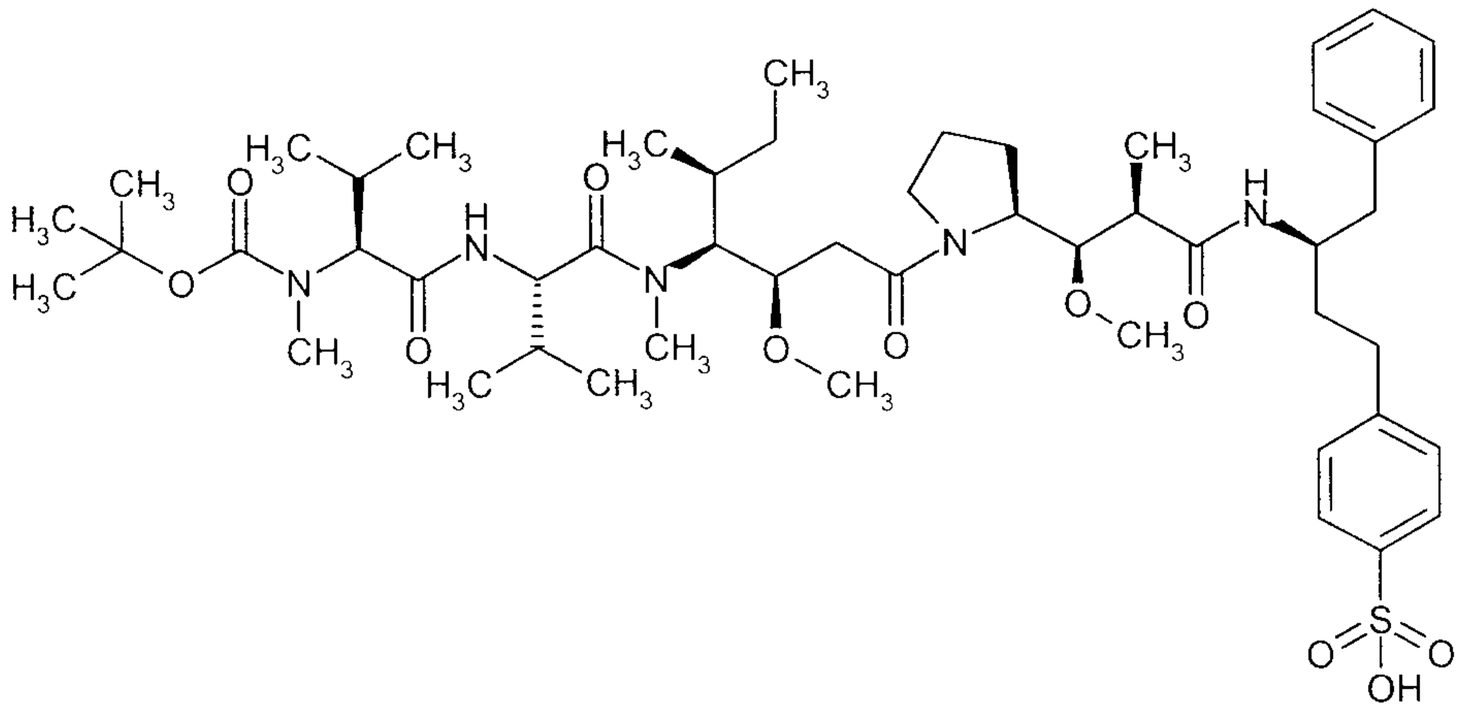


To a solution of 8 mg (7.5 µmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2S,3E)-1-phenyl-4-(4-sulphophenyl)but-3-en-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 2 ml of DMF were added 8.6 mg (74.8 µmol) of N-hydroxysuccinimide, 8.5 mg (22.4 µmol) of EDCI and 0.1 mg (0.75 µmol) of DMAP. The reaction mixture was stirred at RT for 20 h. Subsequently, 1.3 µl (7.5 µmol) 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.

LC-MS (Method 1): R_t = 0.89 min; MS (ESIpos): m/z = 1054 $(M+H)^+$

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

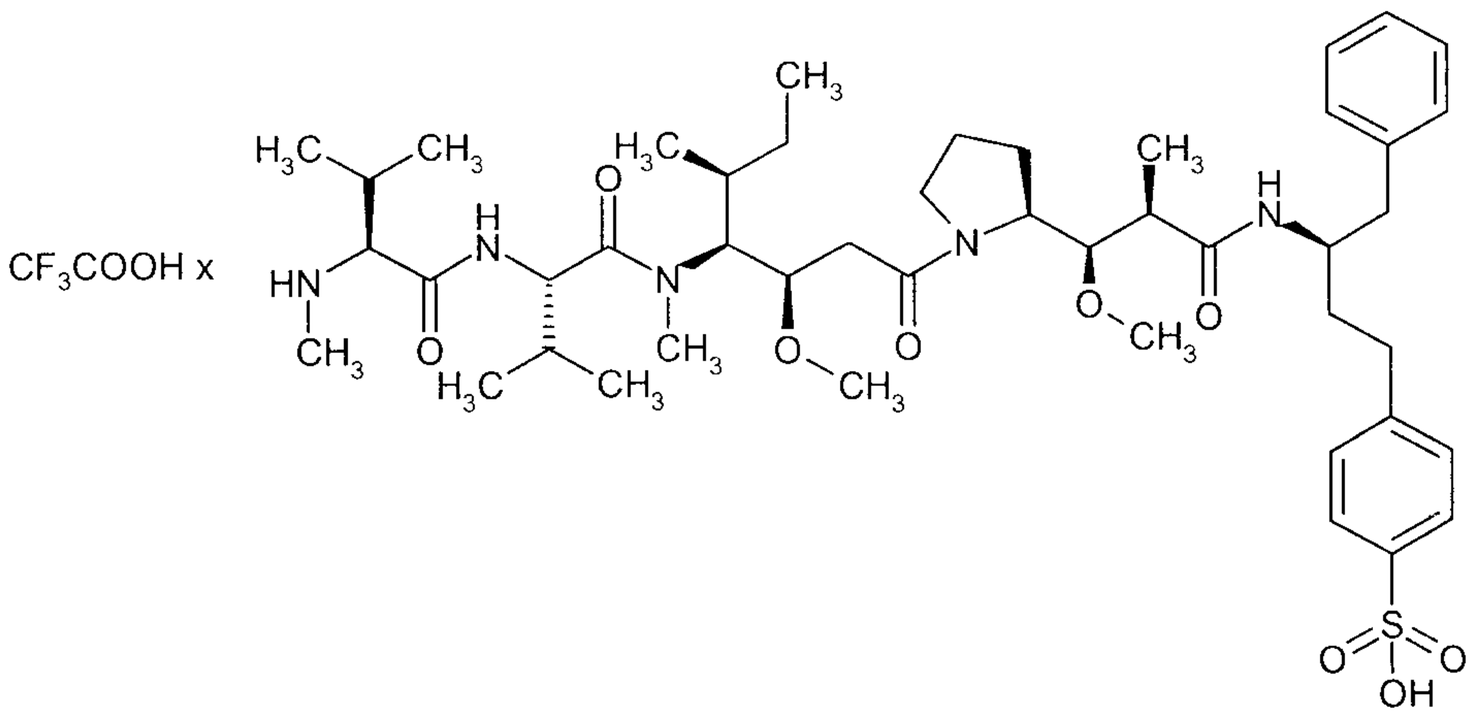


To a solution of 43 mg (0.06 mmol) of N-(tert-butoxycarbonyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 1.9 ml of DMF were added 29 mg (0.07 mmol) of HATU and 33 µl (0.19 mmol) of Hünig's 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



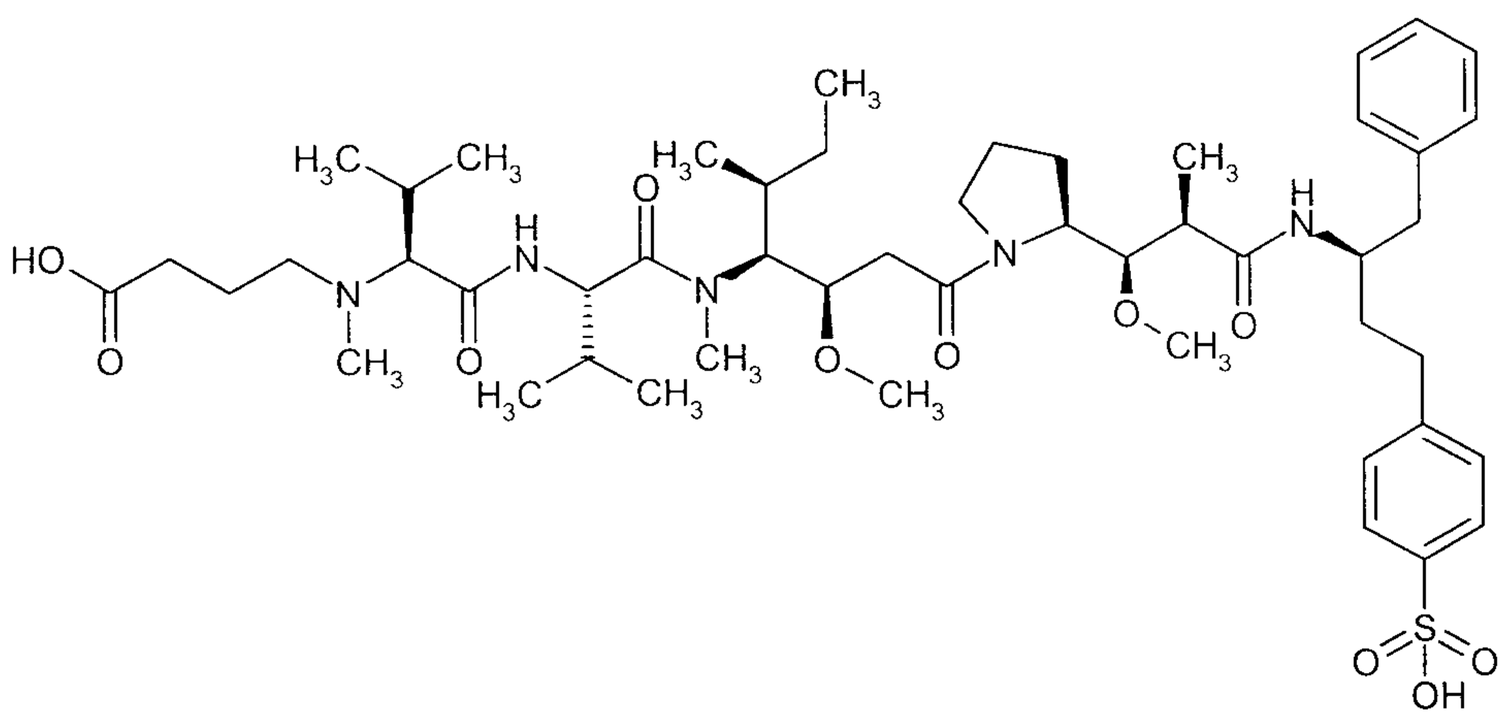
58 mg of Intermediate 186 were dissolved in 4.1 ml of dichloromethane, 0.41 ml of trifluoroacetic acid were added, and the mixture was stirred at RT for 30 min. After concentration *in vacuo*, the crude product was purified by means of preparative HPLC.

50 mg (90% purity, 85% of theory) of the title compound were obtained.

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



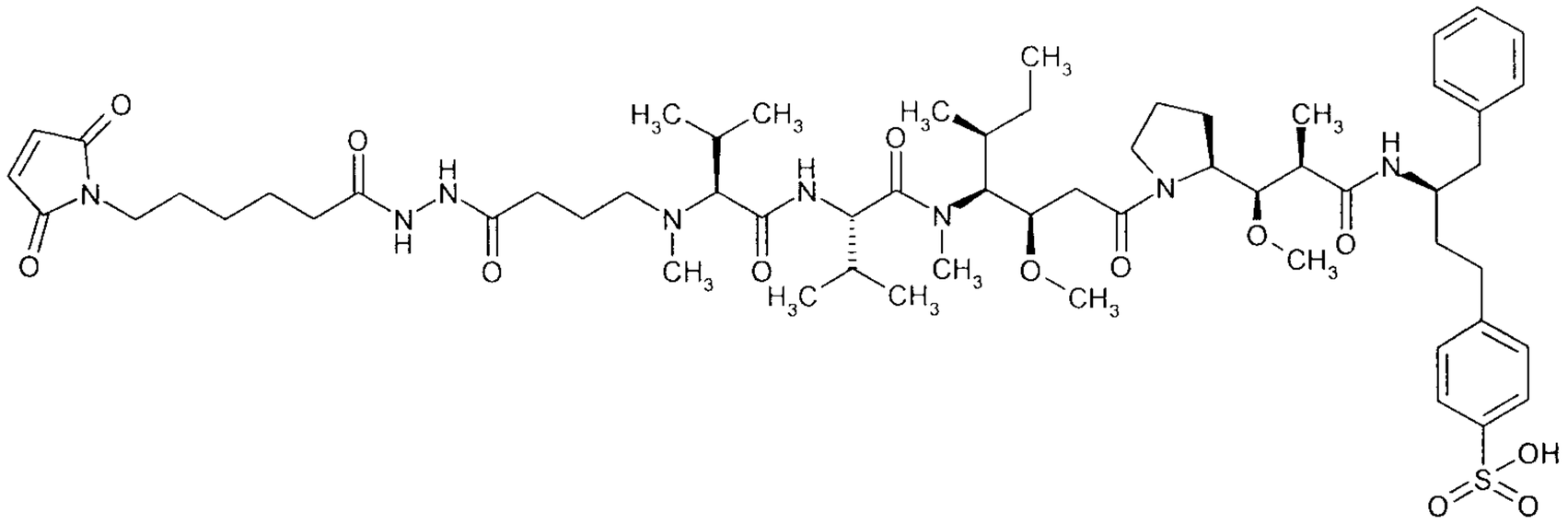
171 µl (0.26 mmol) of a 15% aqueous succinaldehyde solution were initially provided in 2.5 ml of methanol and admixed with 50 mg (0.05 mmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate and 11.6 µl (0.2 mmol) of acetic acid. The reaction mixture was stirred for 5 min at RT, and then 30 µl (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.

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



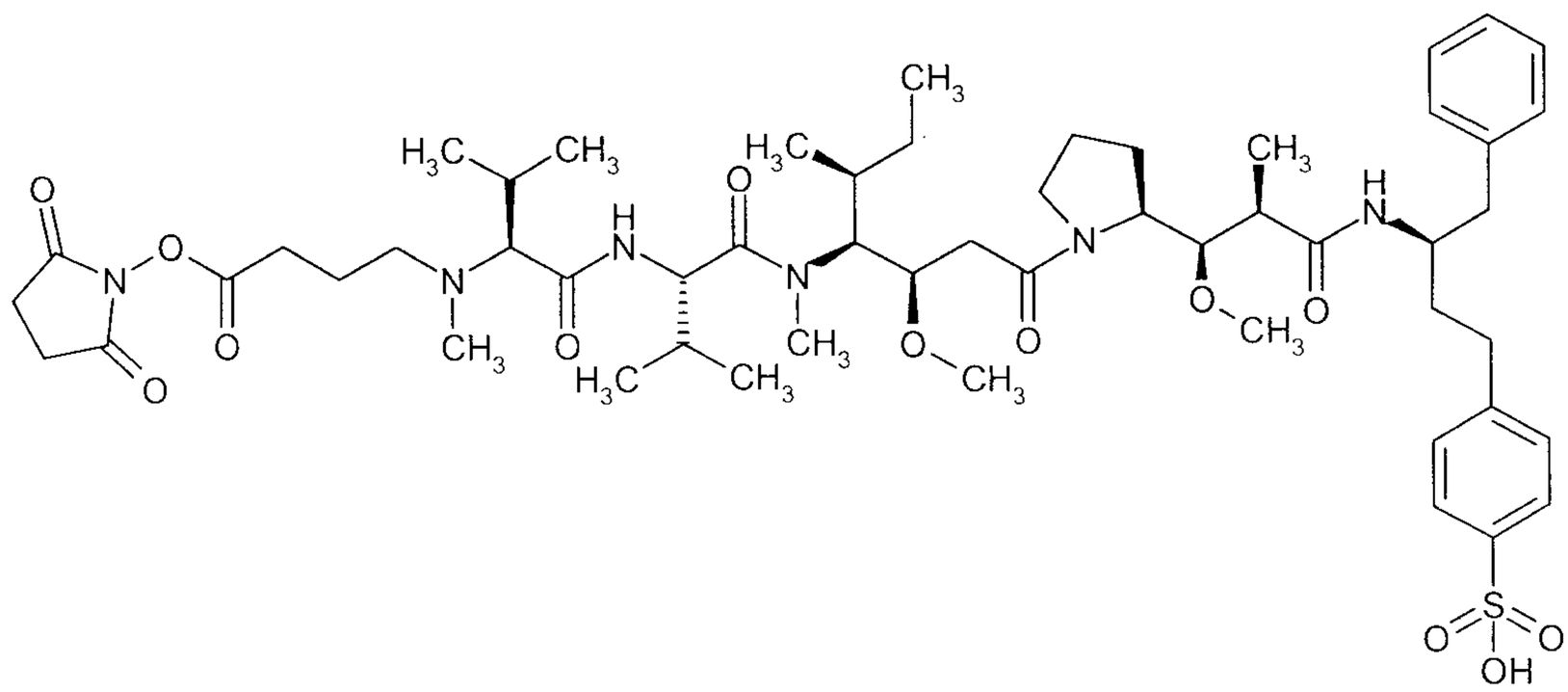
10 mg (9.3 µmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide, 3.5 mg (10.3 µmol) of 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanehydrazide 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 $(M+H)^+$

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



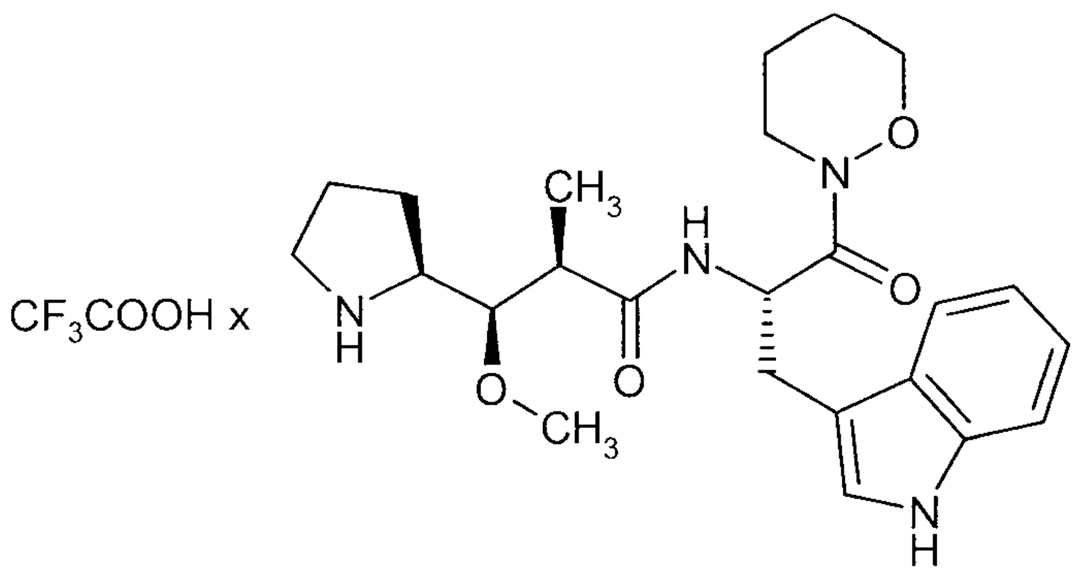
To a solution of 10 mg (9.3 µmol) of N-(3-carboxypropyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(2R)-1-phenyl-4-(4-sulphophenyl)butan-2-yl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide in 2.5 ml of DMF were added 10.7 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 $(M+H)^+$

Intermediate 191

(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 trifluoroacetate



The title compound was prepared in analogy to the synthesis of Intermediate 7 over two stages from Starting Compound 1 and (2*S*)-2-amino-3-(1*H*-indol-3-yl)-1-(1,2-oxazinan-2-yl)propan-1-one trifluoroacetate (Intermediate 99).

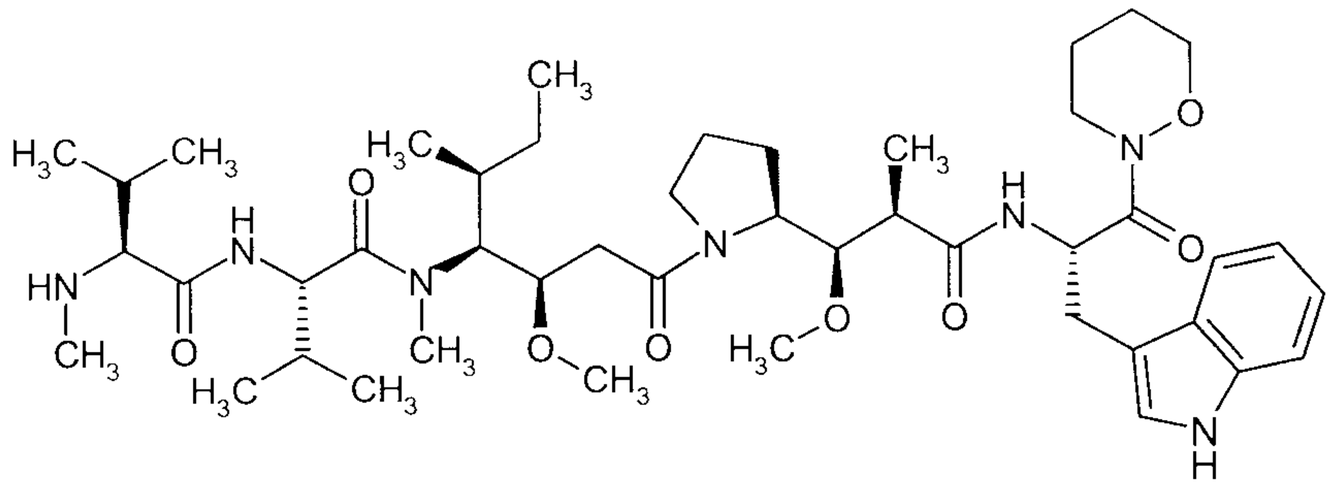
Yield over 2 stages: 62 mg (67% of theory)

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

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*-[(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 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, without further purification, taken up in 20 ml of acetonitrile, 2 ml of piperidine were added tho 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 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.

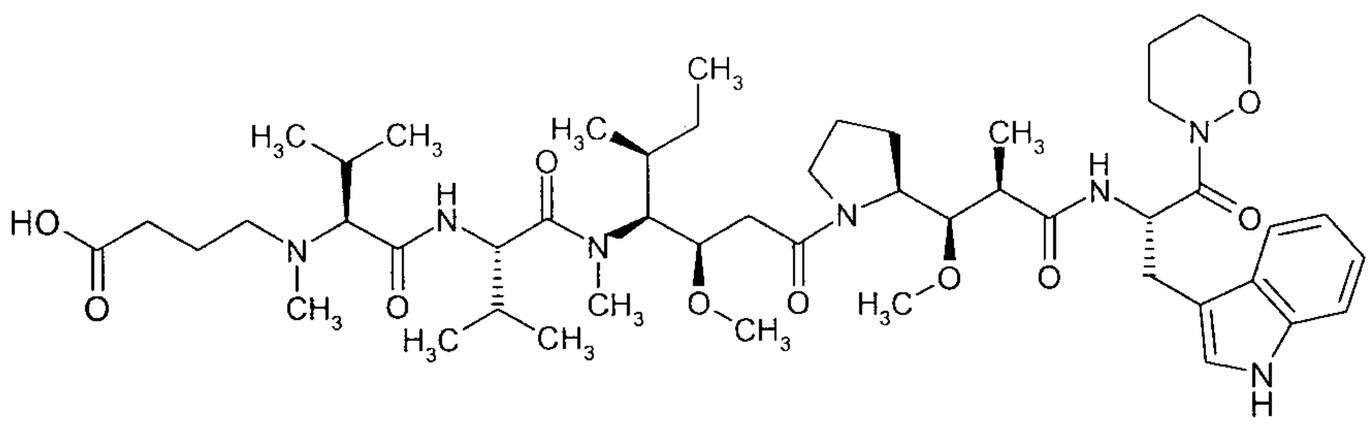
HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 840 $(M+H)^+$.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 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), 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 H_2O peak].

Intermediate 193

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



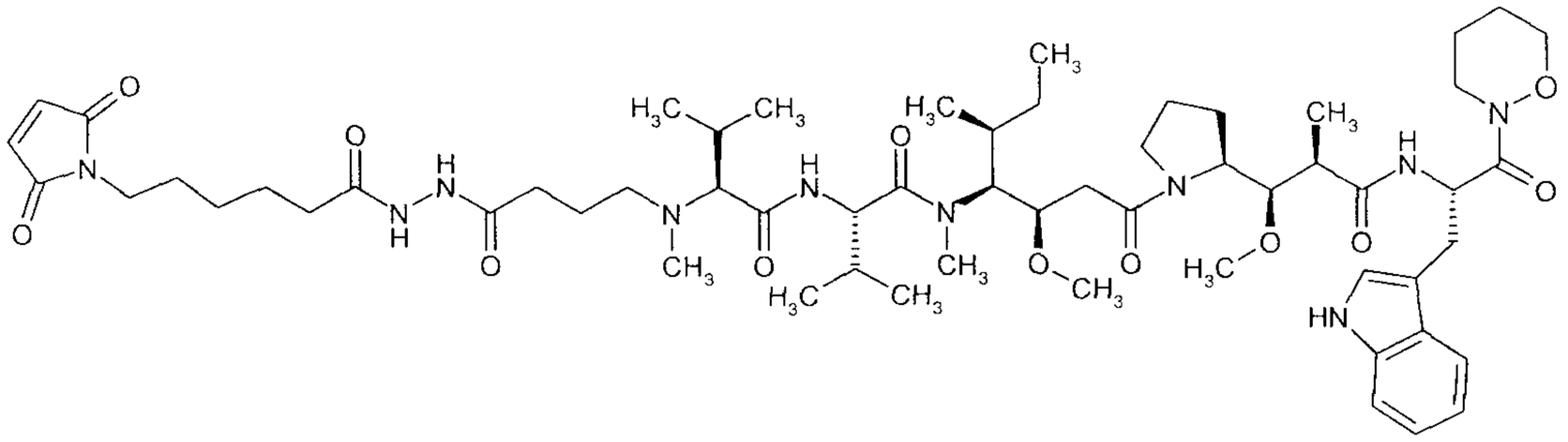
50 mg (0.052 mmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 192) and 204 µl einer of a 15% aqueous 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 µl of acetic acid. The reaction mixture was stirred at RT overnight. This was 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 µmol) 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.

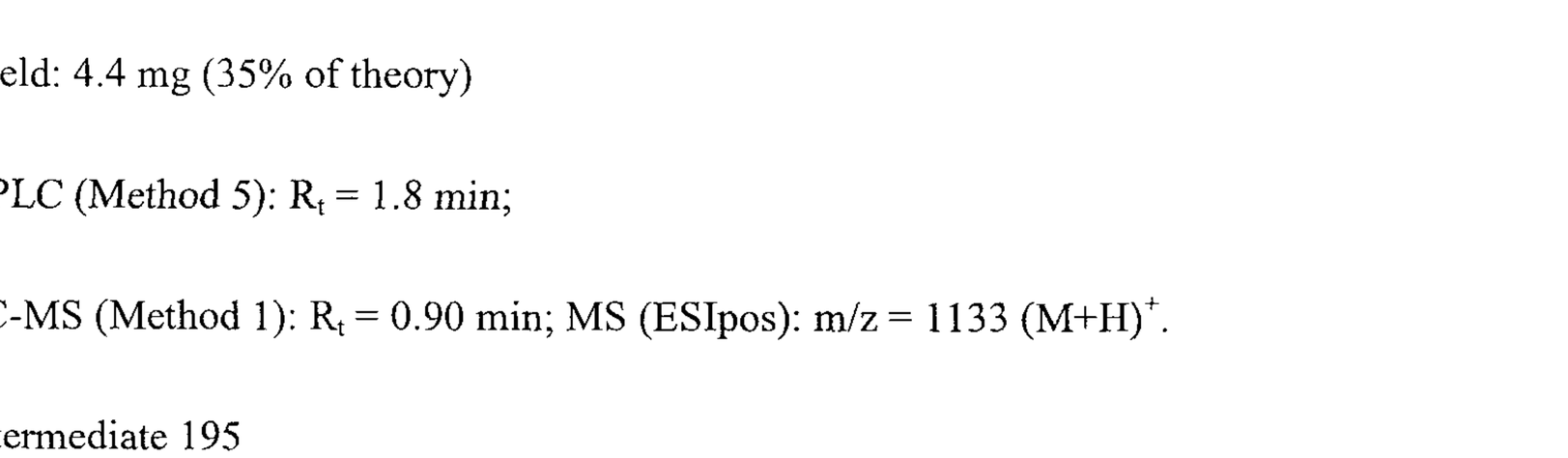
Yield: 4.4 mg (35% of theory)

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.

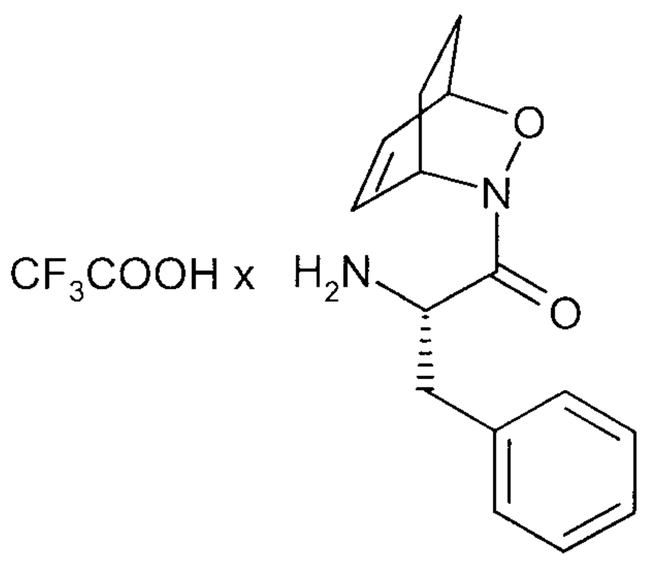
Yield: 1.1 mg (10% of theory)

HPLC (Method 12): R_t = 2.0 min;

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 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 µl (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 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.

LC-MS (Method 1): R_t = 1.1 min; MS (ESIpos): m/z = 359 $(M+H)^+$.

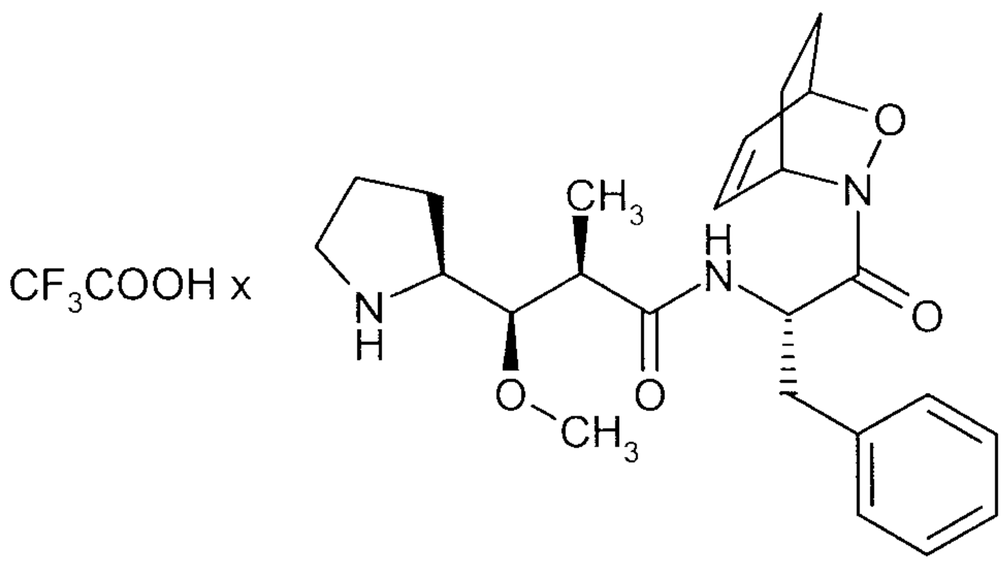
64 mg (0.18 mmol) of this intermediate were taken up in 10 ml of dichloromethane, 1 ml of trifluoroacetic acid was added, and the mixture was stirred at RT for 30 min. This was followed by concentration *in vacuo* and lyophilization of the remaining residue from water/dioxane. In this way, 66 mg (quant.) 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 µl (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

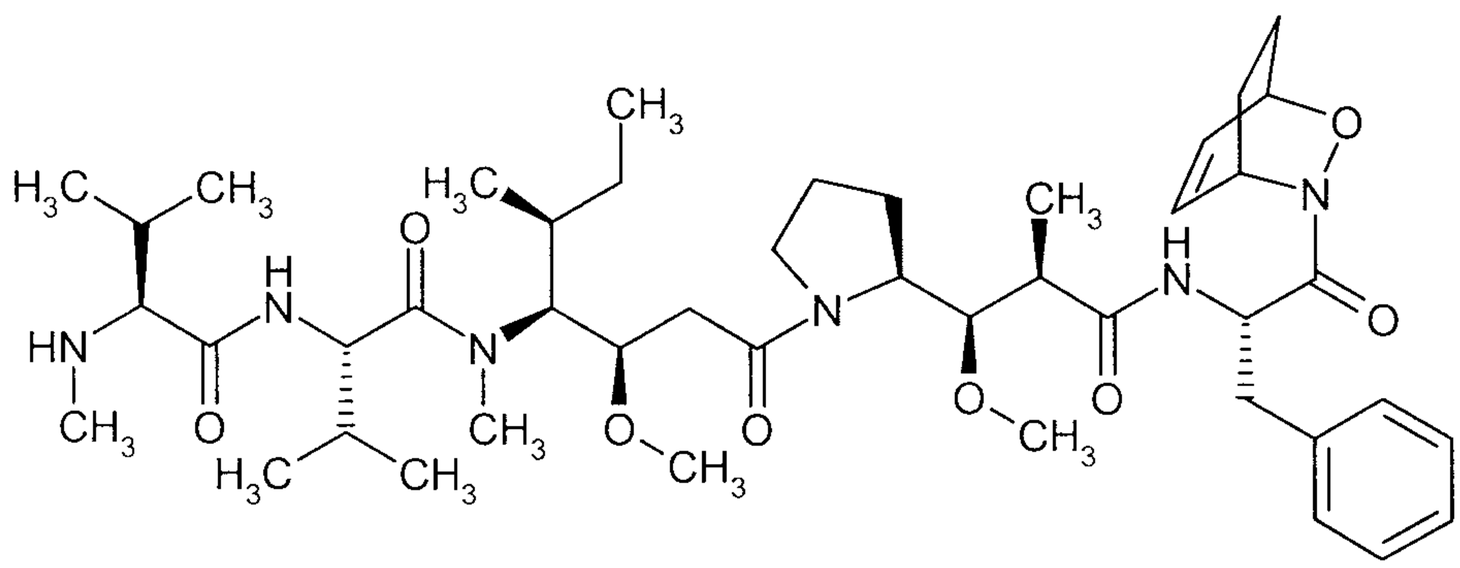
10 min, the mixture was filtered, and the filter residue was dried under high vacuum. In this way, 39 mg (72% of theory) of the title compound were obtained.

HPLC (Method 6): R_t = 1.62 min;

LC-MS (Method 1): R_t = 0.68 min; MS (ESIpos): m/z = 428 $(M+H)^+$.

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



x CF_3COOH

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), 32.5 mg (0.086 mmol) of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and 41 µl (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 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.

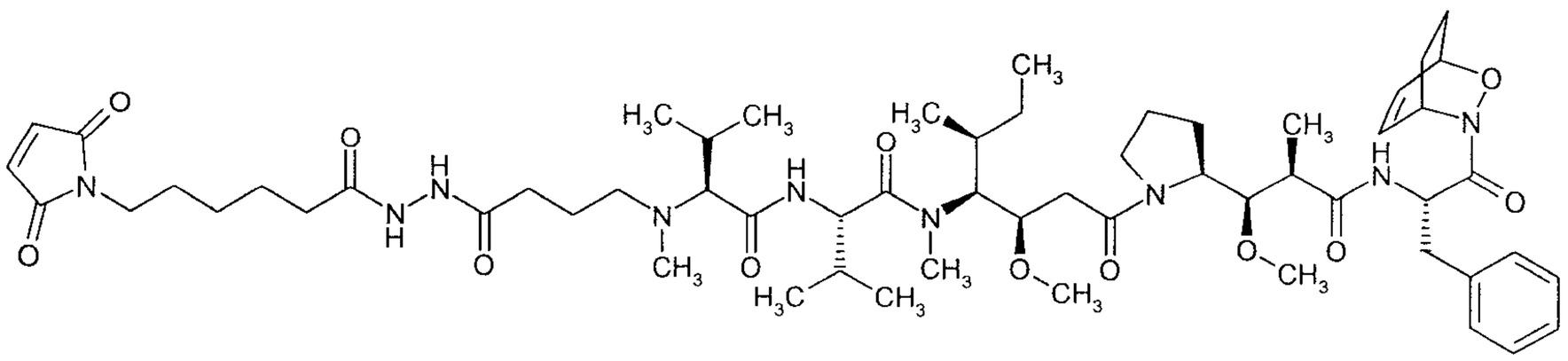
HPLC (Method 6): R_t = 1.94 min;

LC-MS (Method 3): R_t = 1.71 min; MS (ESIpos): m/z = 825 $(M+H)^+$

^{1}H NMR (400 MHz, DMSO-d_6): δ = 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 H_2O 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



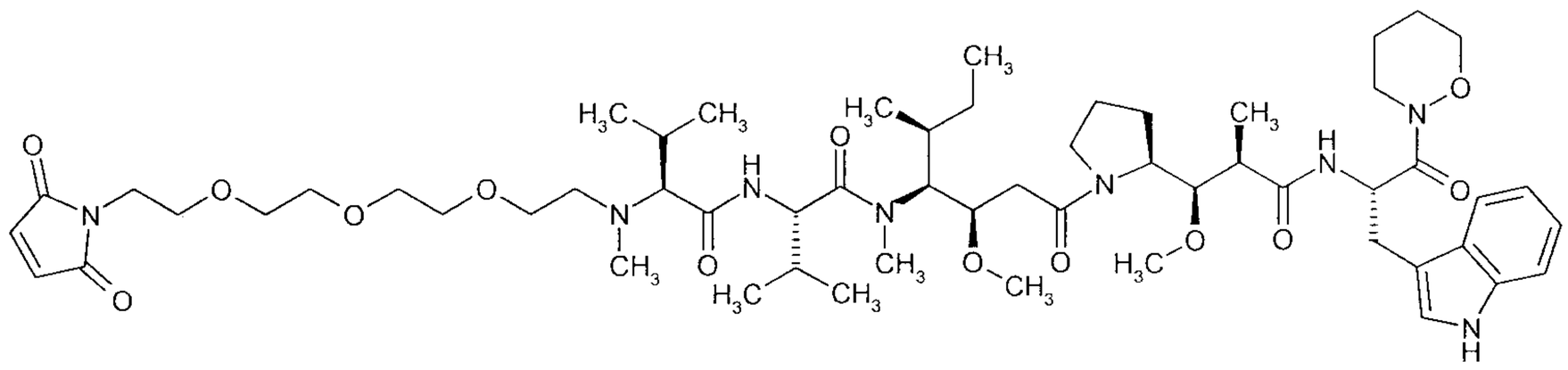
The title compound was prepared in analogy to Intermediates 193 and 194 starting from 23 mg (24 µmol) of N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-{[(2S)-1-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-1-oxo-3-phenylpropan-2-yl]amino}-3-oxopropyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide trifluoroacetate (Intermediate 198).

HPLC (Method 12): Rt = 1.9 min;

LC-MS (Method 2): Rt = 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



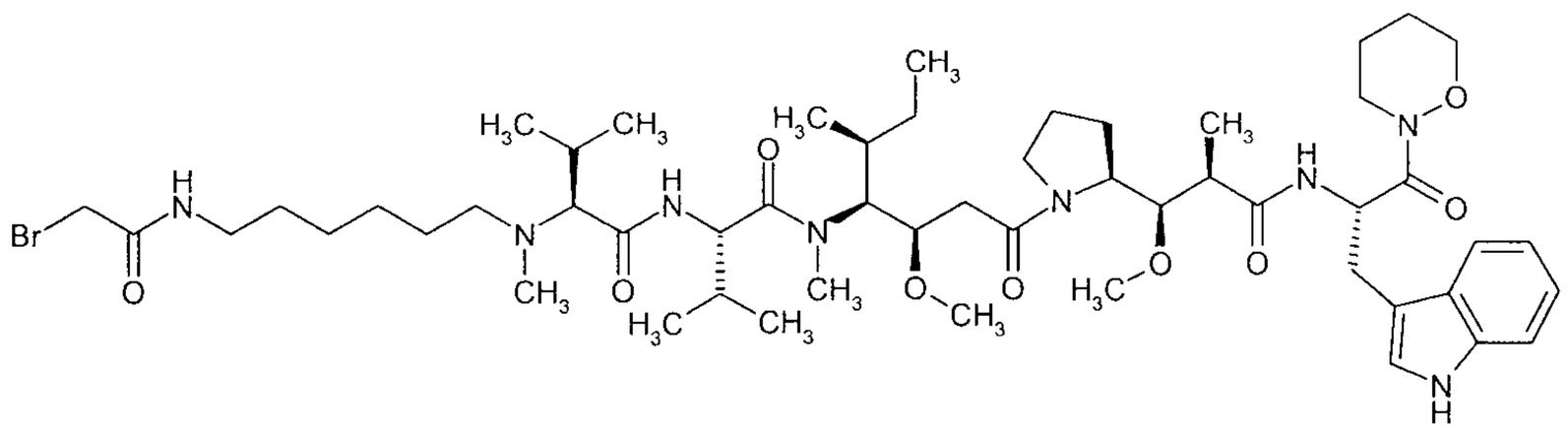
The preparation was done in analogy to Intermediates 174 and 175 starting with the reductive alkylation of Intermediate 192 with Intermediate 172, subsequent deprotection and formation of the maleimide.

HPLC (Method 12): Rt = 1.9 min;

LC-MS (Method 1): Rt = 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-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-

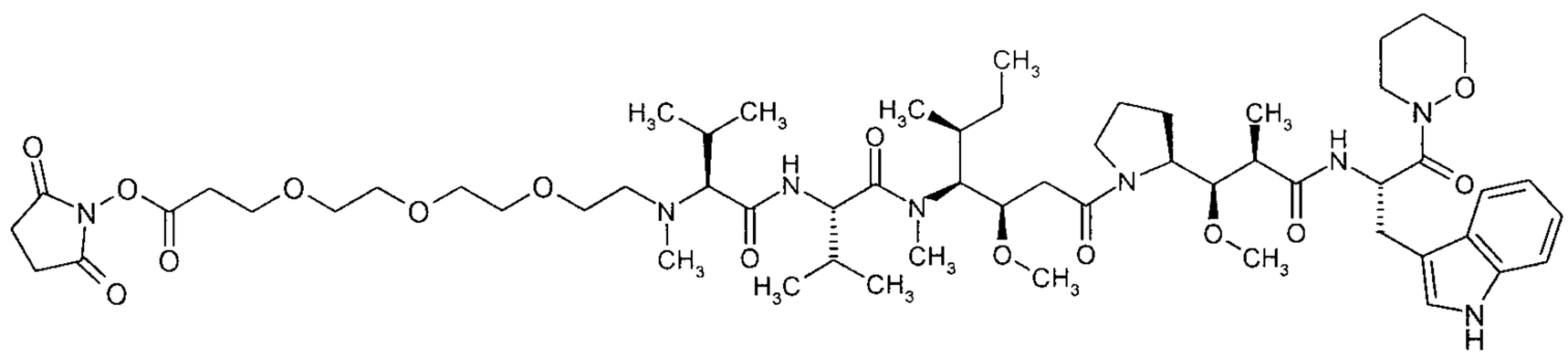
methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 101) were dissolved in 9.5 ml of THF and admixed at 0°C with 4.2 µl of triethylamine. A solution of bromoacetyl chloride in THF was added dropwise, and the reaction mixture was stirred at 0°C 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.

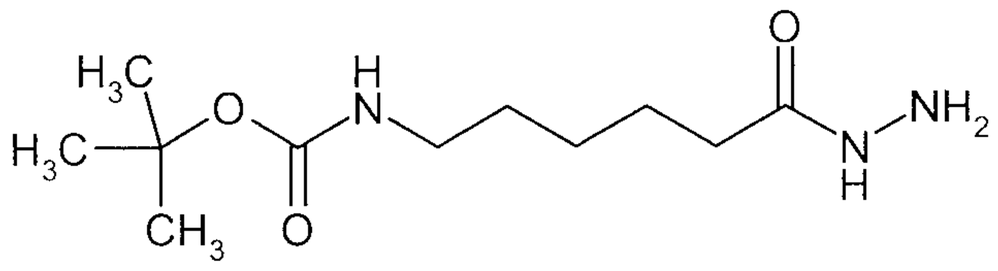
13 mg (10 µmol) of this intermediate were dissolved in 5 ml of DMF and admixed with 2.1 mg (20 mmol) of 1-hydroxypyrrolidine-2,5-dione, 6.5 µl of *N,N*-diisopropylethylamine and 7.1 mg (0.02 mmol) of HATU. The reaction mixture was stirred at RT overnight and then concentrated *in vacuo*. The remaining 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

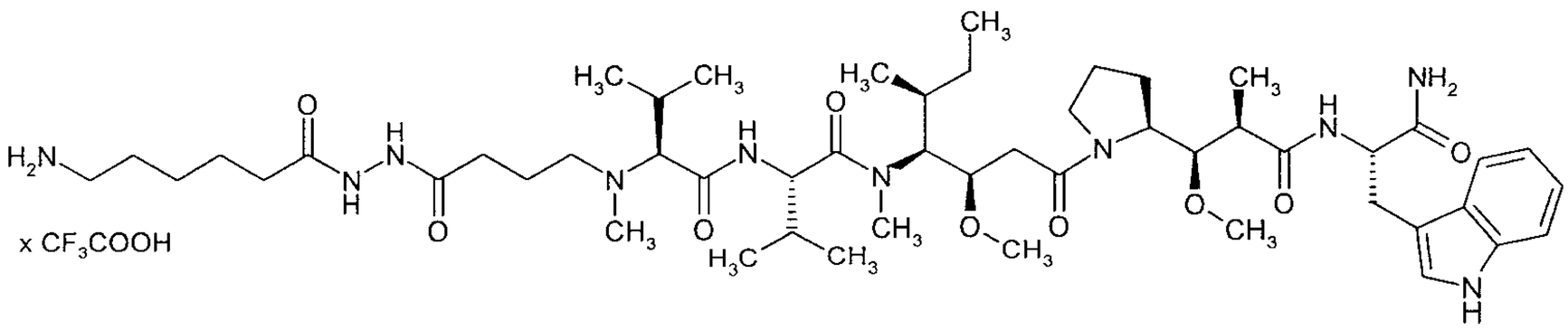


This compound was prepared by standard peptide chemistry methods by coupling of 6-[(*tert*-butoxycarbonyl)amino]hexanoic acid with benzyl hydrazinecarboxylate in the presence of EDCI and HOBT and subsequent hydrogenolytic cleavage of the benzyloxycarbonyl protecting group.

LC-MS (Method 11): R_t = 0.59 min; MS (ESIpos): m/z = 246 $(M+H)^+$.

Intermediate 204

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



146 mg (50 µmol) 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 µmol) of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate, 19 µl of N,N-diisopropylethylamine and with 22.4 mg (60 µmol) 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

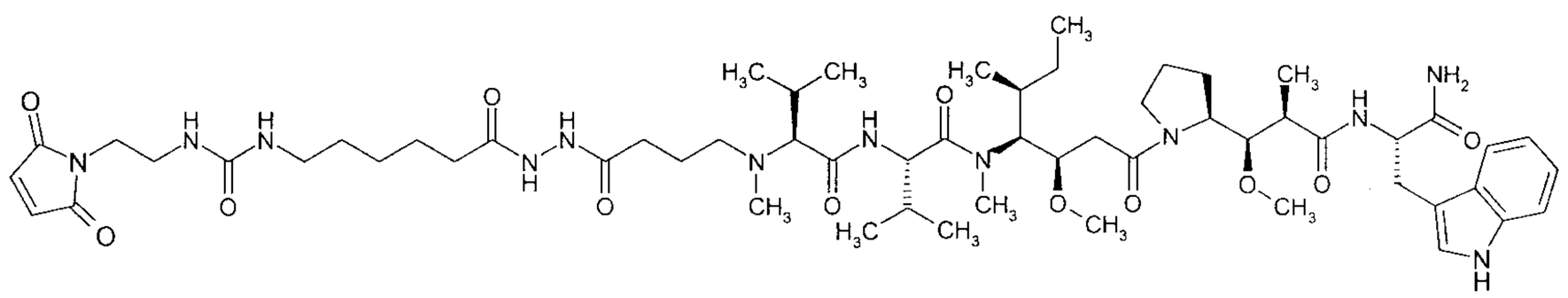
trifluoroacetic acid. The reaction mixture was concentrated, and the residue was stirred in with dichloromethane, and the solvent was removed again *in vacuo*. Thus, 45 mg (68% of theory over 2 stages) of the title compound were obtained.

HPLC (Method 12): R_t = 1.6 min;

LC-MS (Method 11): R_t = 0.66 min; MS (ESIpos): m/z = 983 $(M+H)^+$.

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



This compound was prepared in analogy to Intermediate 114 starting from Intermediates 50 and 204.

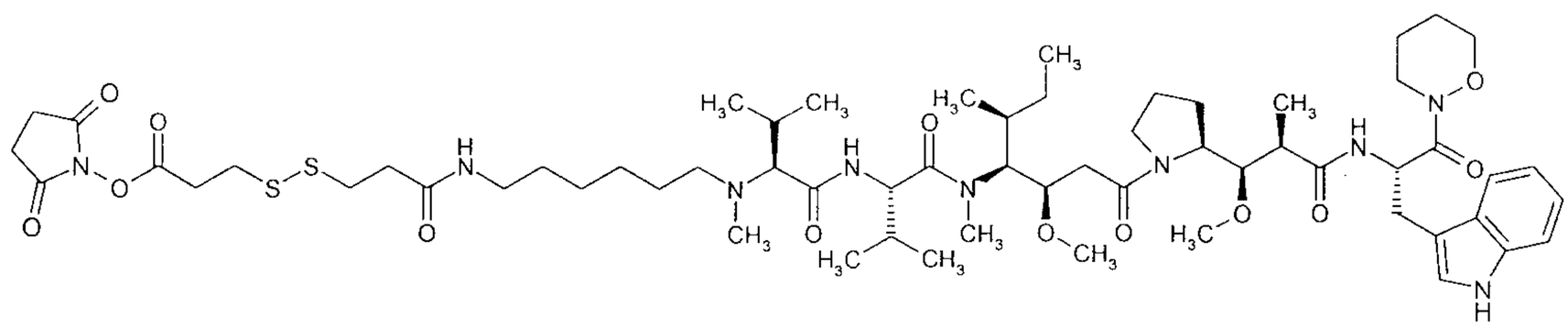
Yield: 4 mg (78% of theory)

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}disulphanyl)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



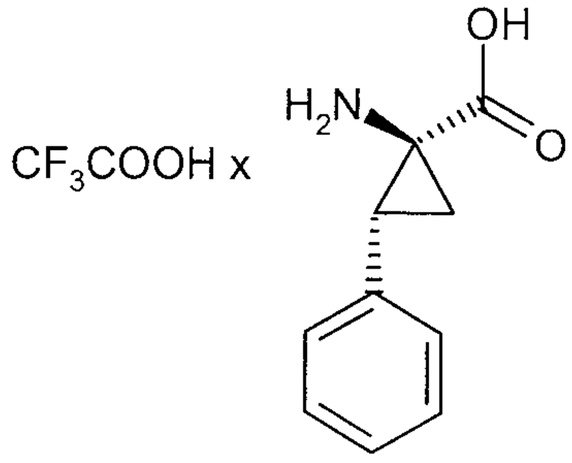
8 mg (10 µmol) of Intermediate 101 were dissolved in 2 ml of DMF and admixed with 8.6 mg (20 µmol) of 1,1'-{disulphanediylbis[(1-oxopropane-3,1-diyl)oxy]}dipyrrolidine-2,5-dione and 3.7 µl of *N,N*-diisopropylethylamine. The reaction mixture was stirred at RT for 2 h, and then the solvent was evaporated 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 [½ $(M+2H^+]$

Intermediate 207

(1S,2R)-1-amino-2-phenylcyclopropanecarboxylic acid trifluoroacetate

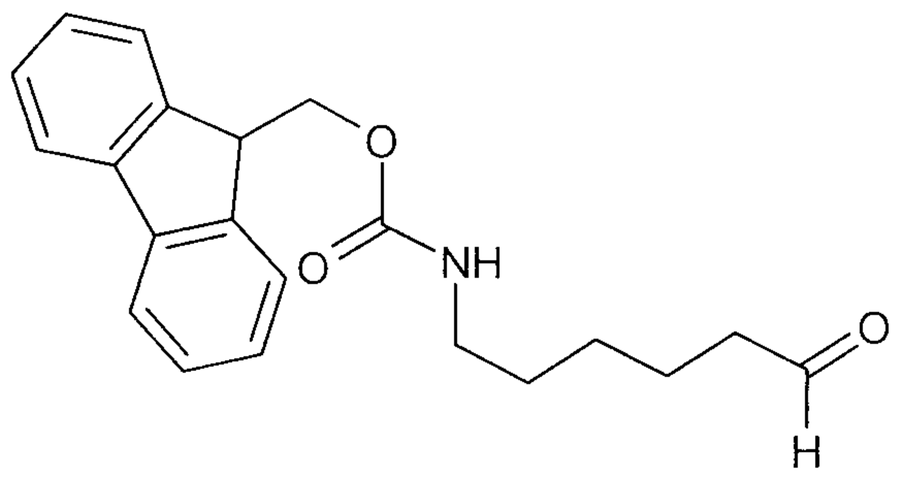


The title compound was obtained in quantitative yield by deprotecting 210 mg (0.76 mmol) of commercially available (1*S*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-phenylcyclopropanecarboxylic acid with trifluoroacetic acid.

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



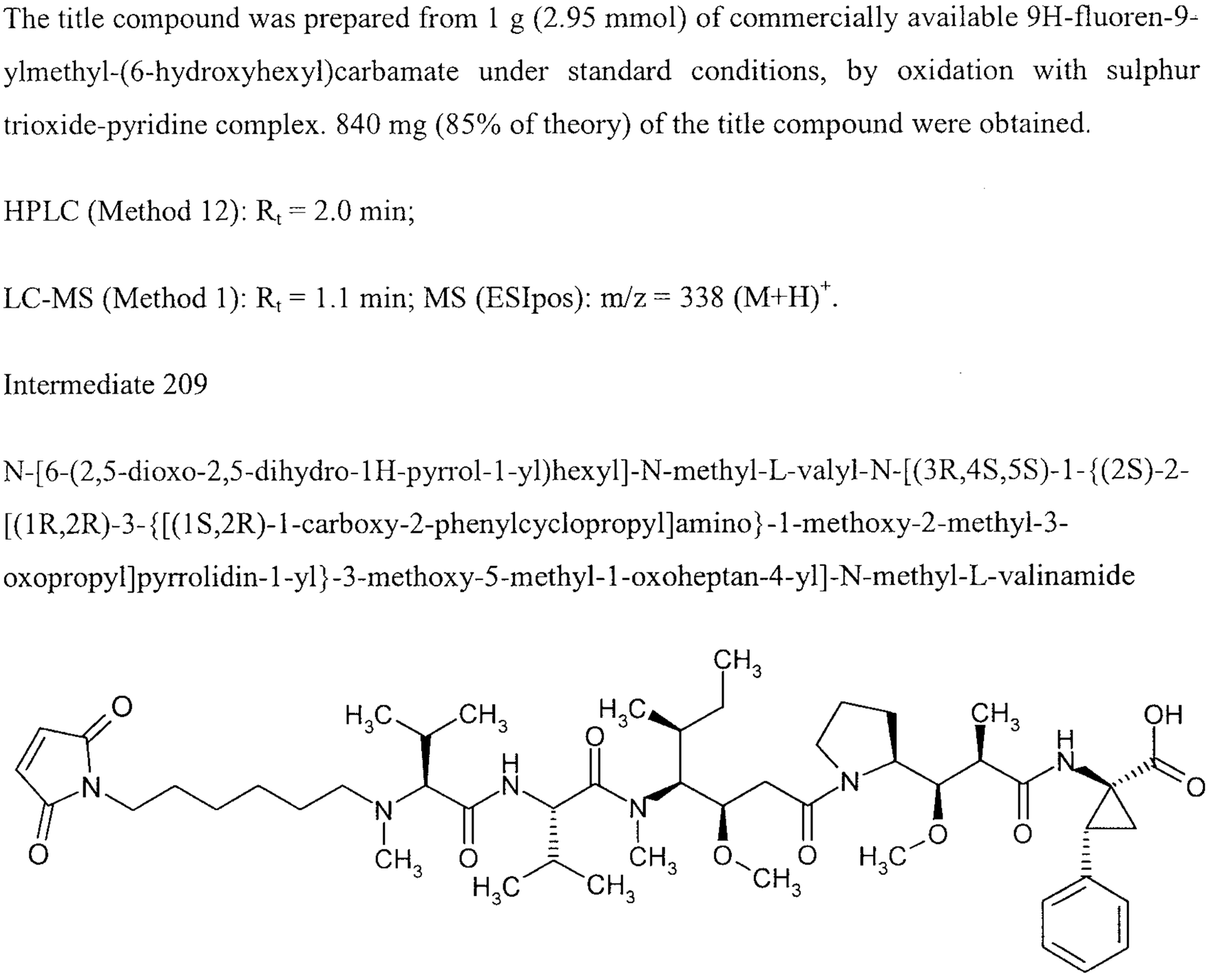
The title compound was prepared from 1 g (2.95 mmol) of commercially available 9H-fluoren-9-ylmethyl-(6-hydroxyhexyl)carbamate under standard conditions, by oxidation with sulphur trioxide-pyridine complex. 840 mg (85% of theory) of the title compound were obtained.

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-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-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)-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 µmol) of this intermediate were taken up in 1 ml of dioxane and then admixed with 6.6 mg (42.7 µmol) of methyl 2,5-dioxo-2,5-dihydro-1H-pyrrole-1-carboxylate and with 5 µl of saturated aqueous sodium hydrogencarbonate solution. The reaction mixture was stirred at RT for 1 h. Then another 3 portions each of 50 µl 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.

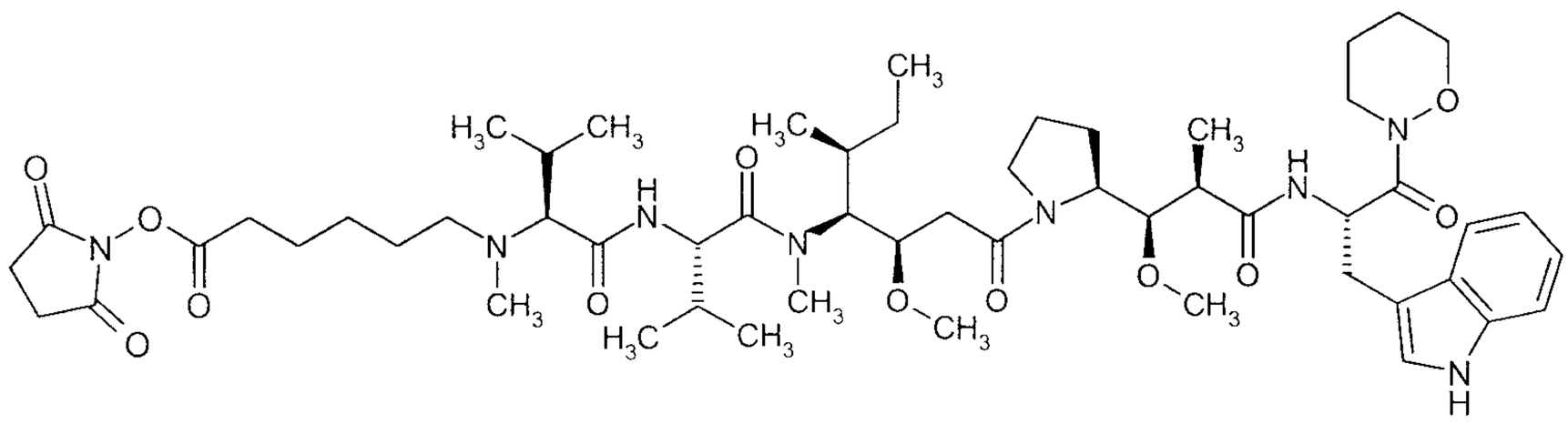
HPLC (Method 12): R_t = 1.9 min;

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



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 (Intermediate 192) and 65.4 mg (0.5 mmol) of 6-oxohexanoic acid were combined in 9 ml of methanol and admixed with 10 µl 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 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.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 955 $(M+H)^+$.

^{1}H 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).

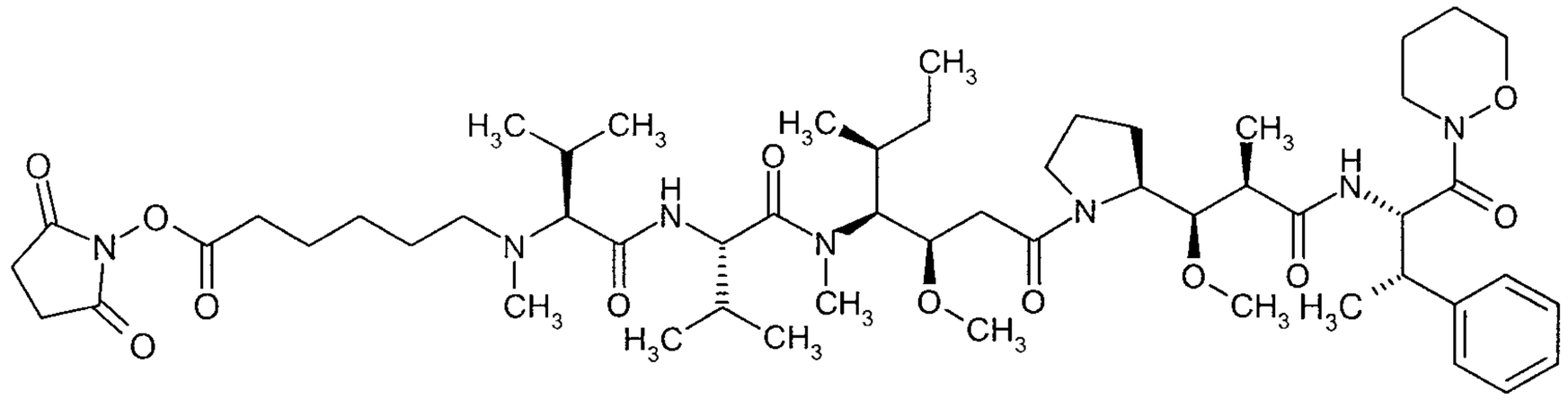
22 mg (23 µmol) of this intermediate were dissolved in 1.8 ml of dichloromethane and admixed with 13.2 mg (70 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 26.5 mg (230 µmol) of 1-hydroxypyrrolidine-2,5-dione and 0.28 mg (2 µmol) of dimethylaminopyridine, and the reaction mixture 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 $(M+H)^+$.

Intermediate 211

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-{[(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 mg (20 µmol) 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.

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 µmol) of this intermediate were dissolved in 3 ml of DMF and admixed with 5.6 mg (48 µmol) of 1-hydroxypyrrolidine-2,5-dione, 5 µl of *N,N*-diisopropylethylamine and 5.5 mg

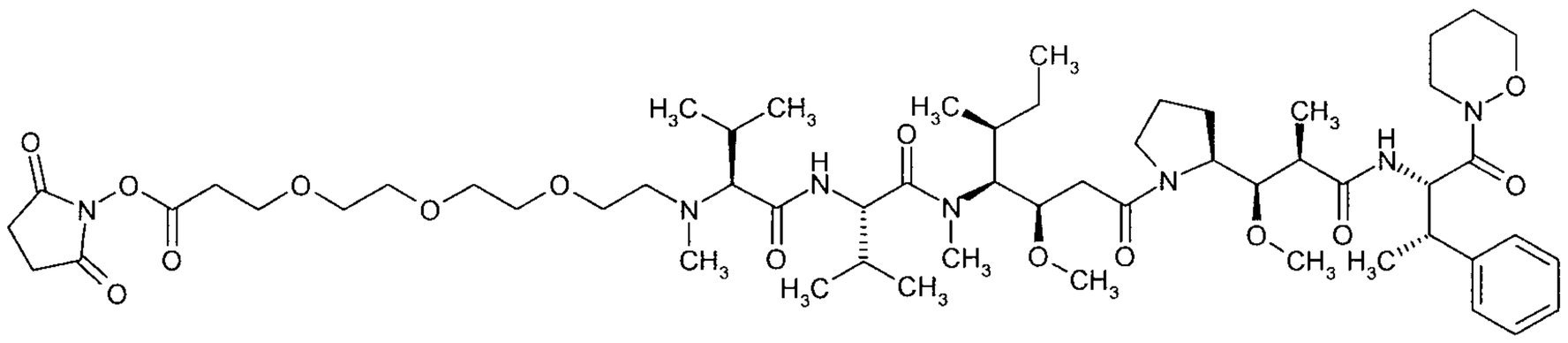
(0.015 mmol) of HATU, and the reaction mixture was treated in an ultrasound bath for 6 h. In the course of this, 5.5 mg of HATU were re-added every hour. Subsequently, the reaction mixture was concentrated *in vacuo*, and the residue was taken up in acetonitrile/water and adjusted to pH 2 with trifluoroacetic 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



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 µmol) of this intermediate were dissolved in 3 ml of DMF and admixed with 9.5 mg (80 µmol) of 1-hydroxypyrrolidine-2,5-dione, 10 µl of *N,N*-diisopropylethylamine and 9.4 mg (25 µmol) 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

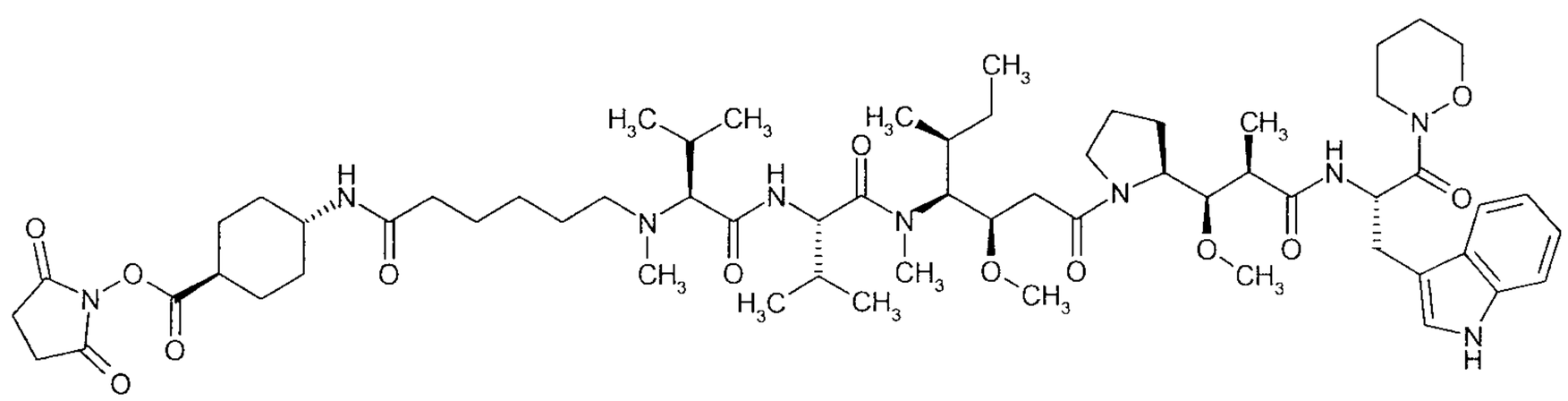
lyophilization from acetonitrile/water, 4 mg (32% of theory) of the title compound were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 0.96 min; MS (ESIpos): m/z = 1117 $(M+H)^+$.

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



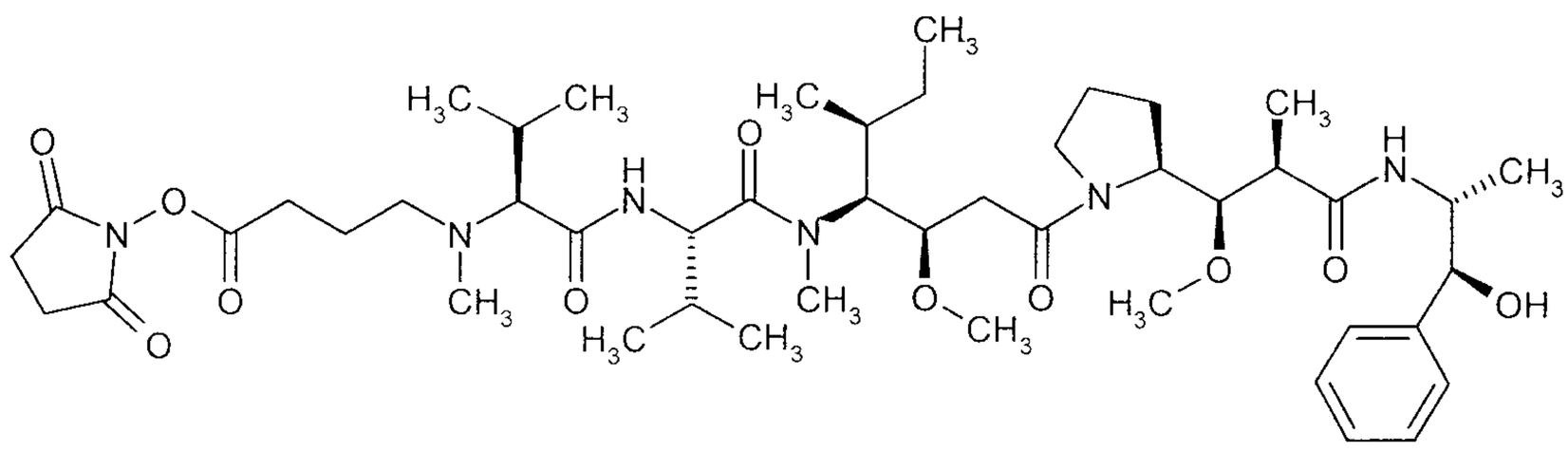
This compound was prepared in analogy to Intermediate 104 starting from N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide, the synthesis of which was described under Intermediate 210. 9.3 mg of the title compound (37% of theory over 3 stages) were obtained.

HPLC (Method 12): R_t = 1.9 min;

LC-MS (Method 1): R_t = 0.9 min; MS (ESIpos): m/z = 1177 $(M+H)^+$.

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



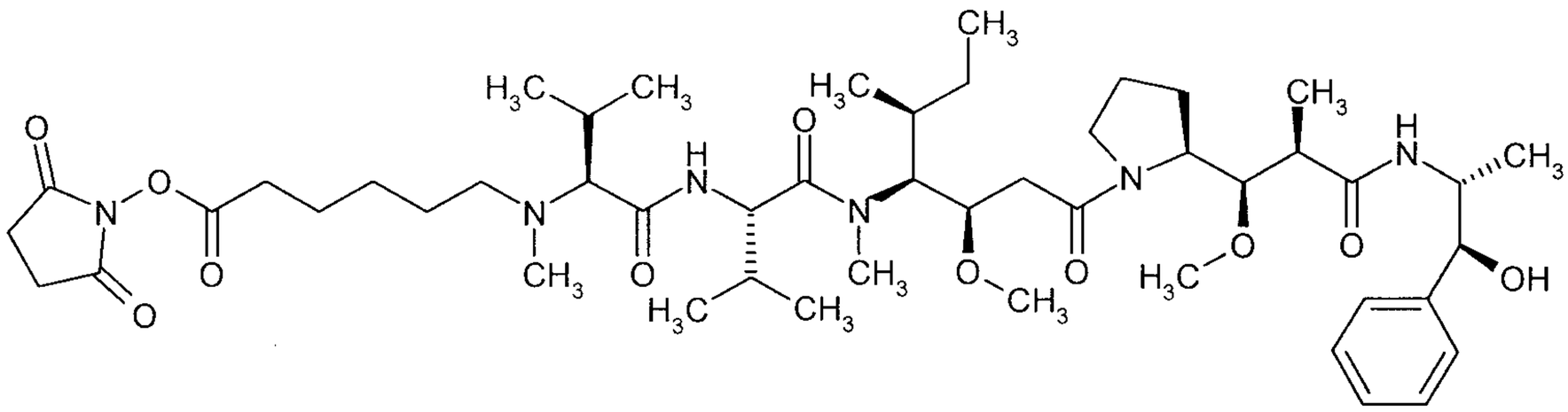
This compound was prepared in analogy to Intermediate 210 by conversion of Intermediate 92 to the active ester.

HPLC (Method 5): R_t = 1.6 min;

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



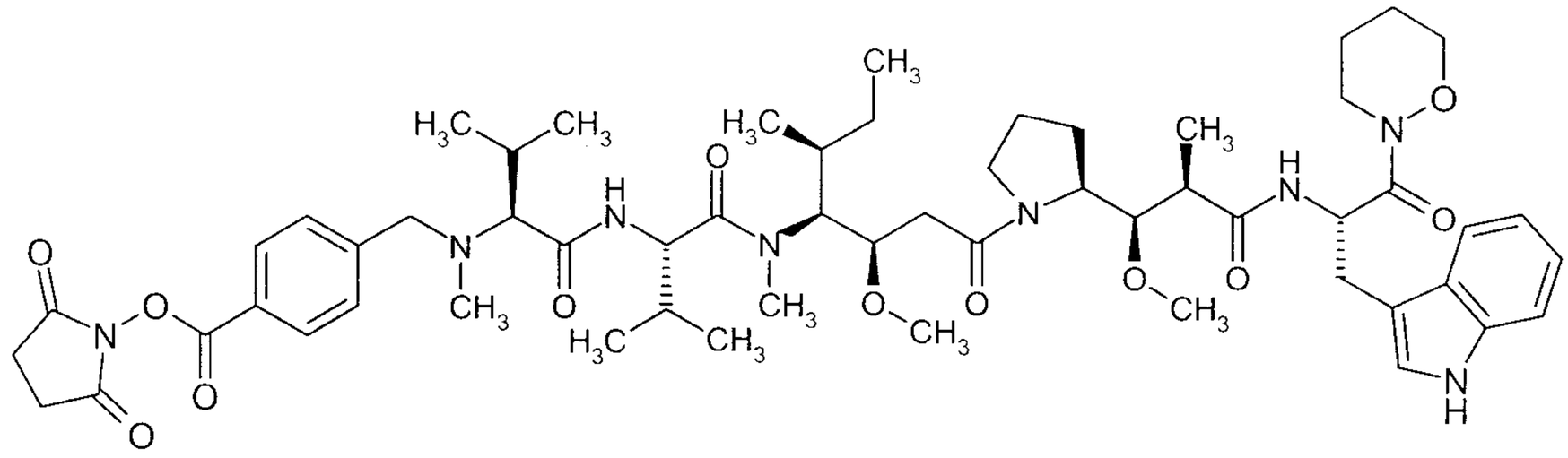
First, from Intermediate 40, in analogy to Intermediate 183 with borane-pyridine complex, was prepared N-(5-carboxypentyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(1S,2R)-1-hydroxy-1-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide. 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-methyl-L-valinamide



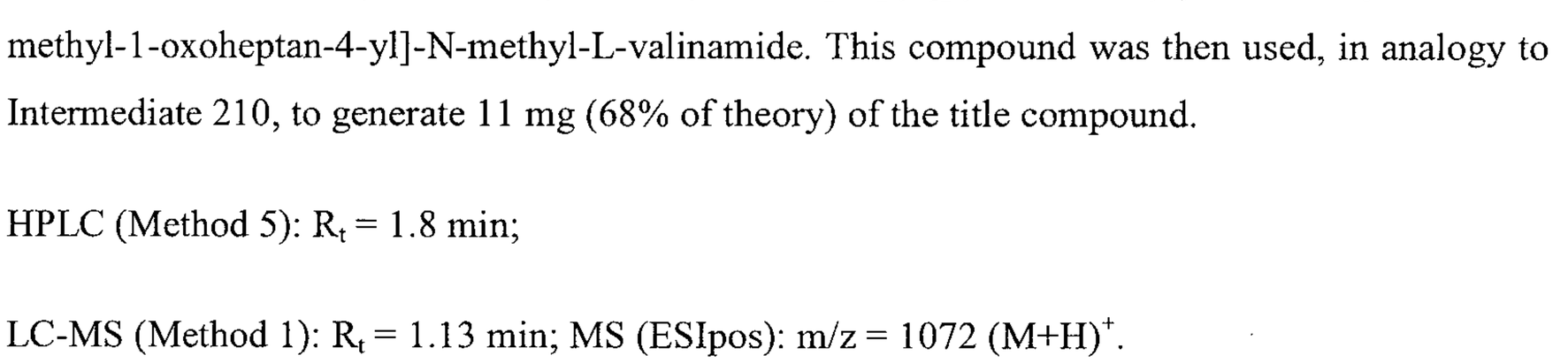
First, in analogy to the preparation of Intermediate 183, Intermediate 192 was reacted with 4-formylbenzoic acid with borane-pyridine complex to yield N-(4-carboxybenzyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(2S)-3-(1H-indol-3-yl)-1-(1,2-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-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;

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 µmol) 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 µmol) 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 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 $(M+H)^+$.

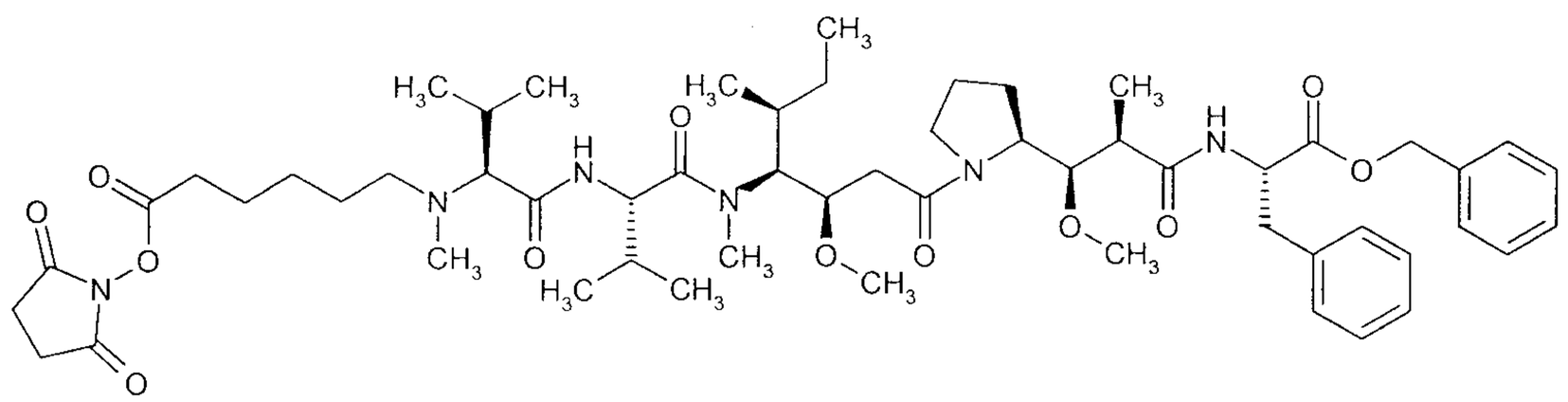
60 mg (0.06 mmol) of this intermediate were reacted, in analogy to Intermediate 210, with 6-oxohexanoic acid in the presence of borane-pyridine complex. 45 mg (75% of theory) of the title compound were obtained as a foam.

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-[(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



This compound was prepared by conversion of 42 mg (0.05 mmol) of Intermediate 217 to the active ester.

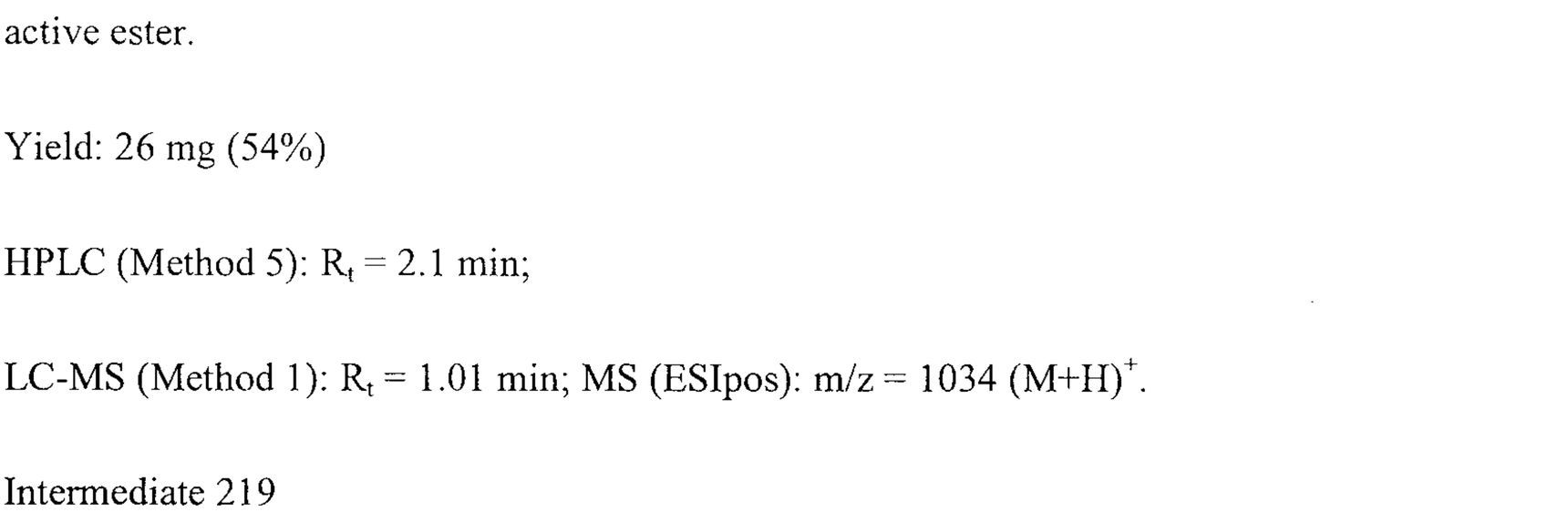
Yield: 26 mg (54%)

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-[(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



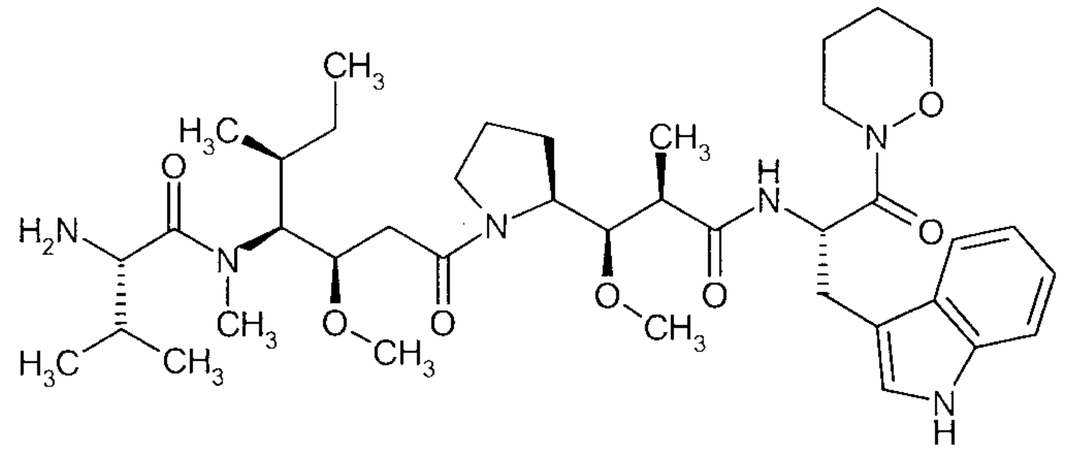
20 mg (0.02 mol) of the compound from Intermediate 218 were taken up in 2.4 ml of methanol and hydrogenated over 5% palladium on activated carbon under standard hydrogen pressure at RT for 30 min. The catalyst was then filtered off, and the solvent was removed *in vacuo*. The residue was lyophilized 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



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.

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 µl (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.

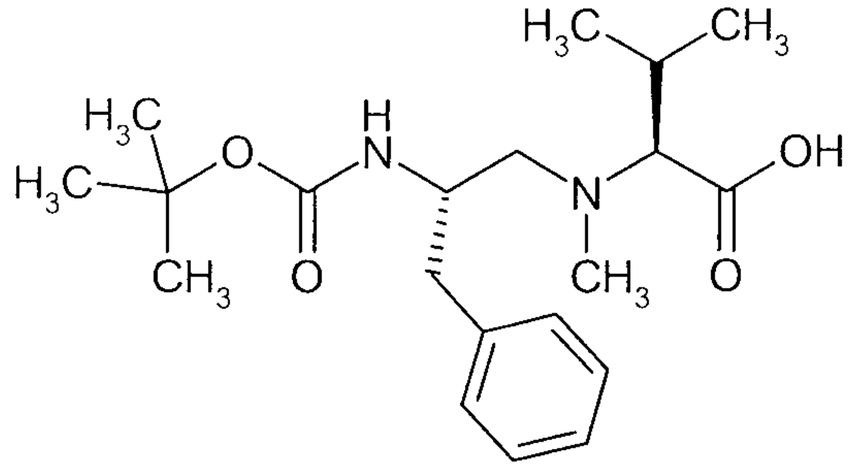
562 mg (0.57 mmol) of this intermediate were taken up in 50 ml of methanol and hydrogenated with 155 mg of 10% palladium on activated carbon under standard hydrogen pressure at RT for 20 min. The catalyst was then filtered off, and the solvent was removed *in vacuo*. The residue was purified by means of preparative HPLC. The corresponding fractions were combined, the solvent was evaporated *in vacuo*, and the residue was lyophilized from dioxane. This yielded 361 mg (87% of theory) of the title compound as a foam.

HPLC (Method 5): double peak with Rt = 1.75 and 1.86 min;

LC-MS (Method 1): double peak at Rt = 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



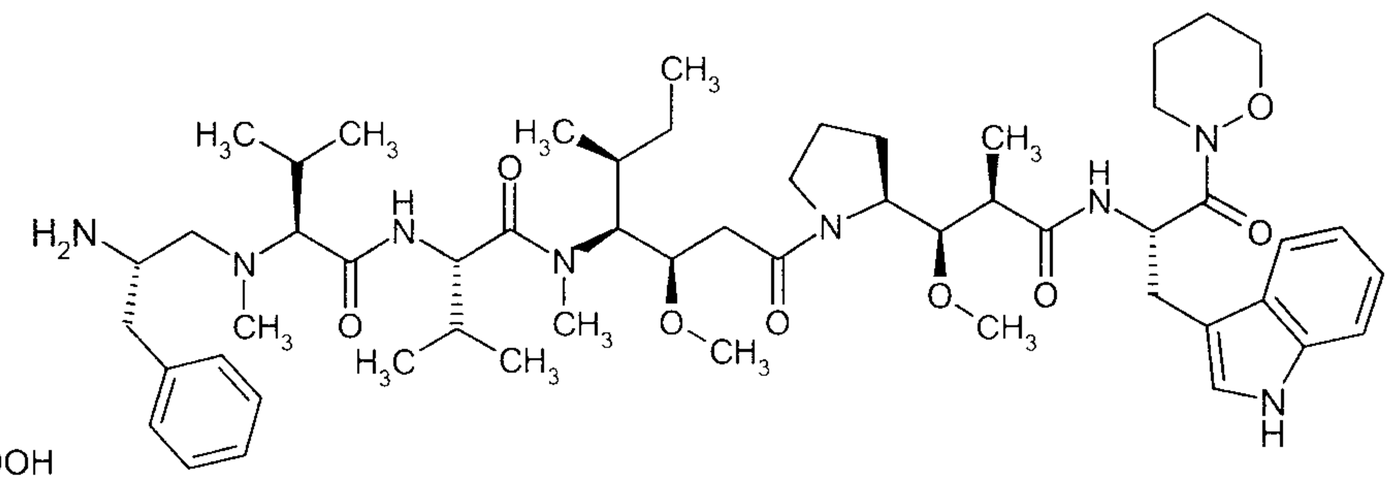
100 mg (0.76 mmol) of commercially available N-methyl-L-valine and 285 mg (1.14 mmol) of commercially available *tert*-butyl (2S)-1-oxo-3-phenylpropan-2-yl carbamate were combined in 22 ml of methanol and admixed with 340 mg (3.66 mmol) of borane-pyridine complex and 70 µl of acetic acid. The reaction 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;

LC-MS (Method 11): R_t = 0.76 min; MS (ESIpos): m/z = 365 $(M+H)^+$.

Intermediate 222

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



x CF_3COOH

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 µl (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.

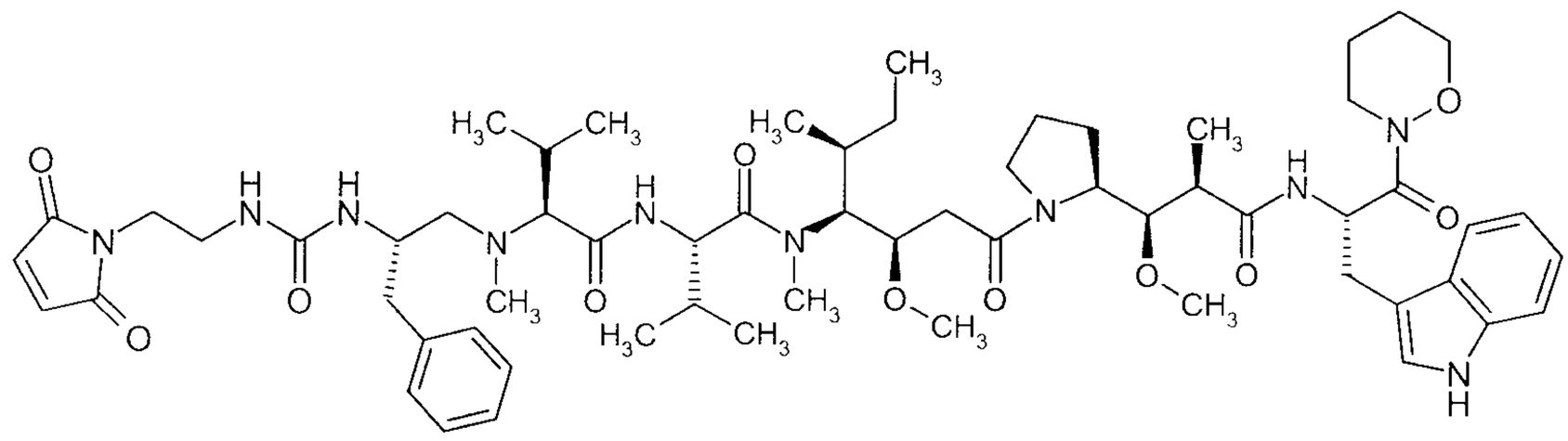
HPLC (Method 12): R_t = 1.9 min;

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



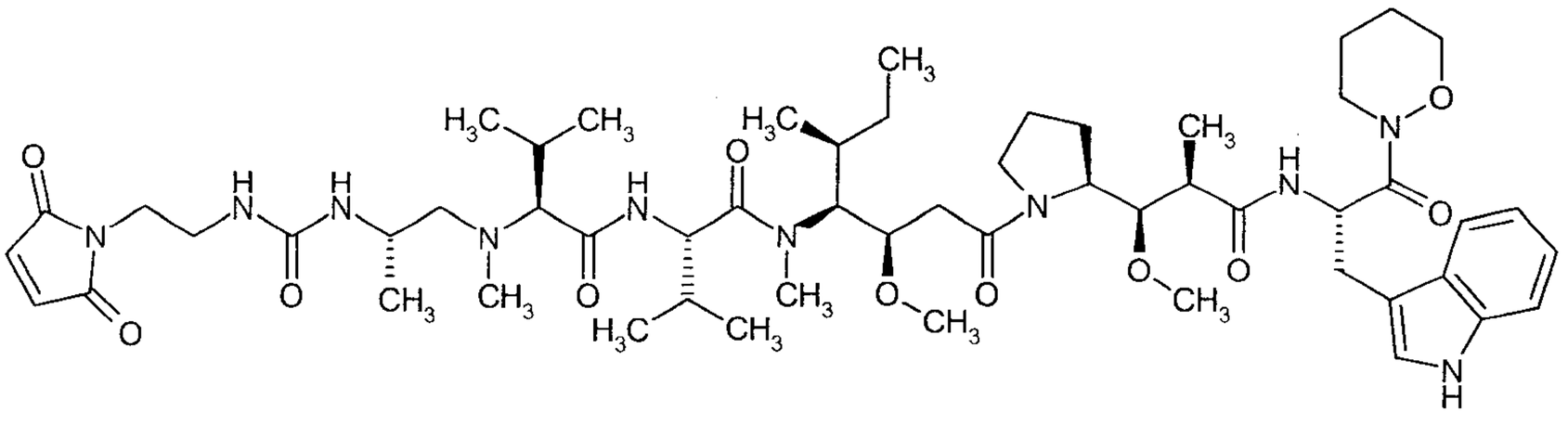
The preparation was done in analogy to Intermediate 134 starting from 5 mg (4.6 µmol) of Intermediate 222. 3.4 mg (65% of theory) of the title compound were obtained.

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]carbamoyl}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



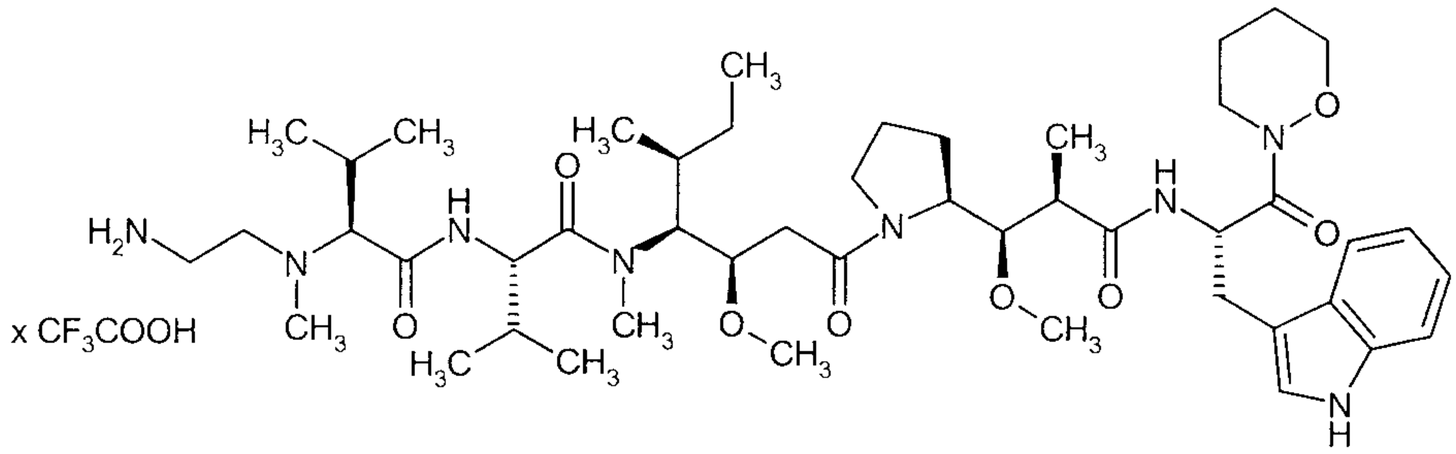
The preparation was done in analogy to the synthesis of Intermediate 223.

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 µl 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 µl (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 $(M+H)^+$.

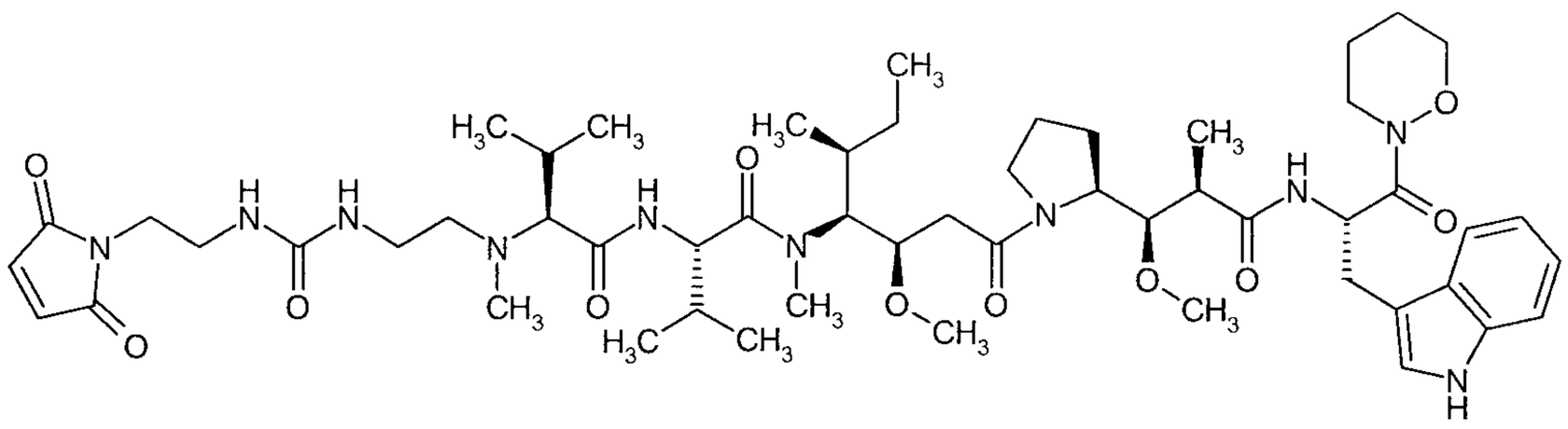
53 mg (0.05 mmol) of this intermediate were taken up in 10 ml of dichloromethane, 2 ml of trifluoroacetic acid were added, and the reaction mixture was stirred at RT for 30 min. Subsequently, the reaction mixture was concentrated *in vacuo* and the remaining residue was purified by means of preparative 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

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



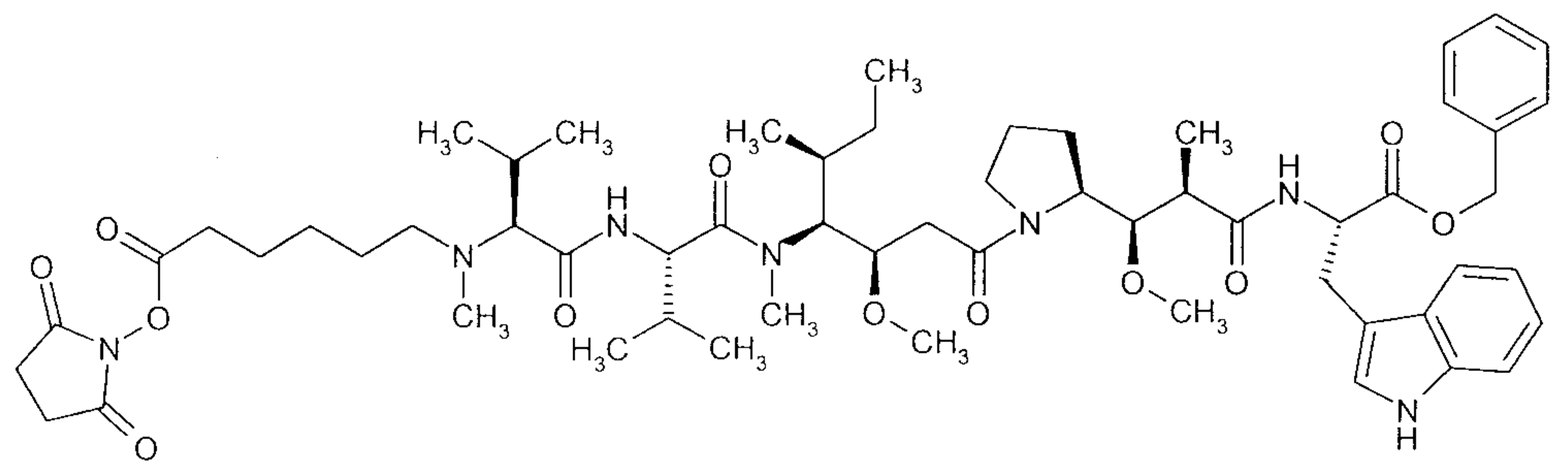
The preparation was done starting from Intermediate 225 in analogy to the synthesis of Intermediate 134. 11.6 mg (59% of theory) of the title compound were obtained.

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

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.

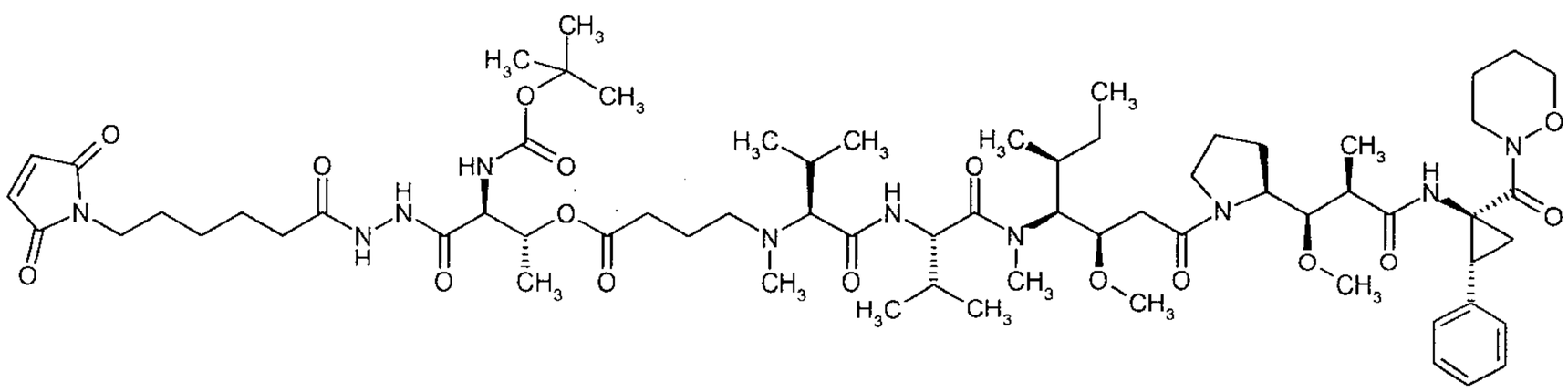
Yield: 18 mg (51% of theory)

HPLC (Method 5): R_t = 2.1 min;

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



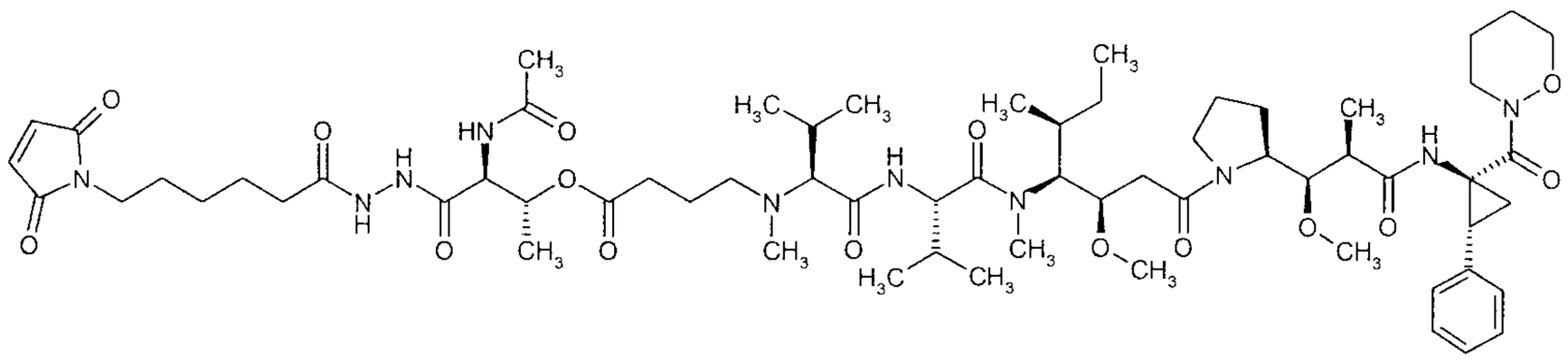
The title compound was prepared by coupling the Boc-protected intermediate obtained from the synthesis of Intermediate 154 with commercially available 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanehydrazide.

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 µmol) of Intermediate 154 by acetylation with 2.3 µl of acetic anhydride in 1 ml of DMF in the presence of 0.4 µl of *N,N*-diisopropylethylamine.

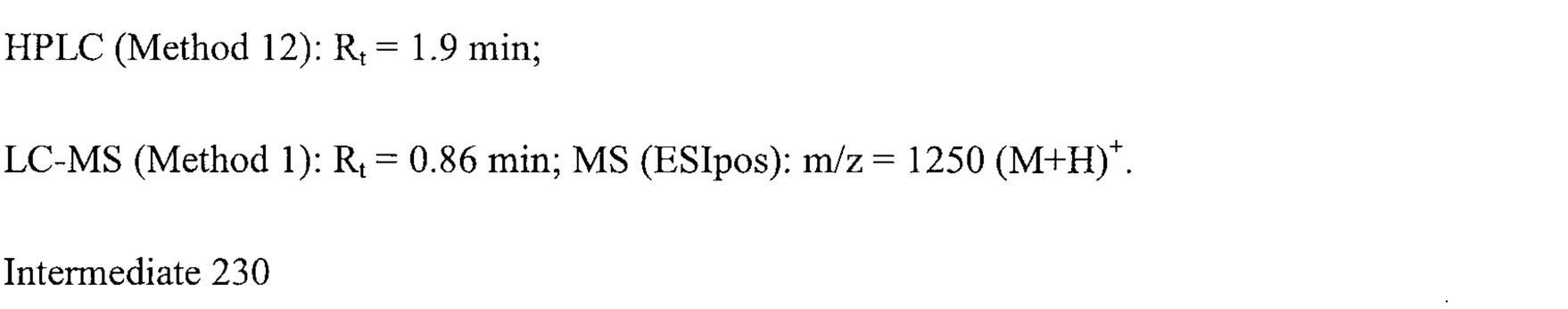
Yield: 1.4 mg (40% of theory)

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



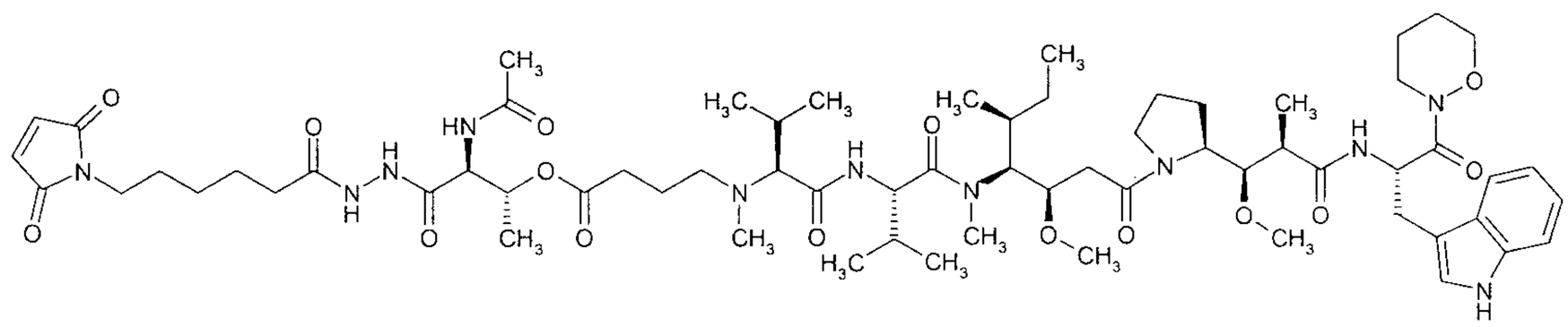
This compound was prepared in analogy to Intermediate 228 starting from Intermediate 193. 16 mg (30% of theory over 3 stages) of the title compound were obtained.

HPLC (Method 12): R_t = 2.0 min;

LC-MS (Method 1): R_t = 1.02 min; MS (ESIpos): m/z = 1335 $(M+H)^+$.

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



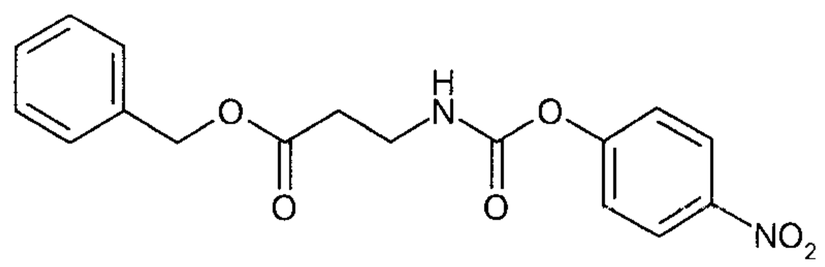
This compound was prepared from 8 mg (6 µmol) of Intermediate 230, first by deprotection with trifluoroacetic acid and subsequent acetylation with acetic anhydride in DMF in the presence of *N,N*-diisopropylethylamine. 2 mg (37% of theory over 2 stages) of the title compound were obtained.

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

Benzyl-N-[(4-nitrophenoxy)carbonyl]-beta-alaninate



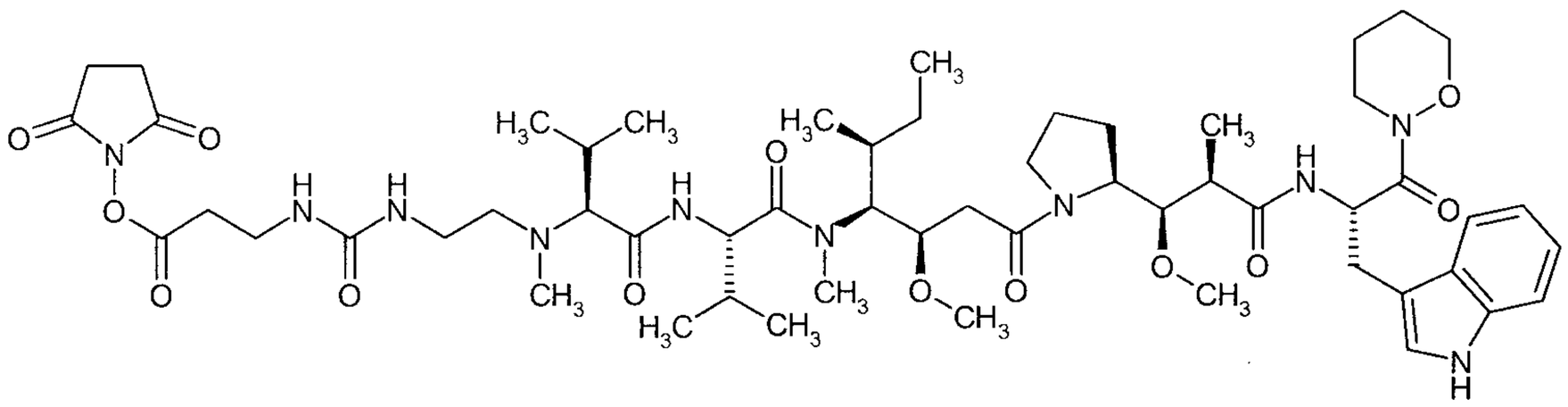
200 mg (0.57 mmol) of commercially available 4-methylbenzenesulphonic acid benzyl beta-alaninate and 229 mg (1.14 mmol) of 4-nitrophenyl chlorocarbonate were taken up in 15 ml of tetrahydrofuran, and the reaction mixture was then heated to reflux for 30 min. Subsequently, the reaction mixture was concentrated *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 µmol) of Intermediate 225 and 6.7 mg (20 µmol) of Intermediate 232 were dissolved in 3 ml of DMF, and then 7 µl 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. 6in; MS (ESIpos): m/z = 1089 $(M+H)^+$.

5.4 mg (5 µmol) 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 he 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 $(M+H)^+$.

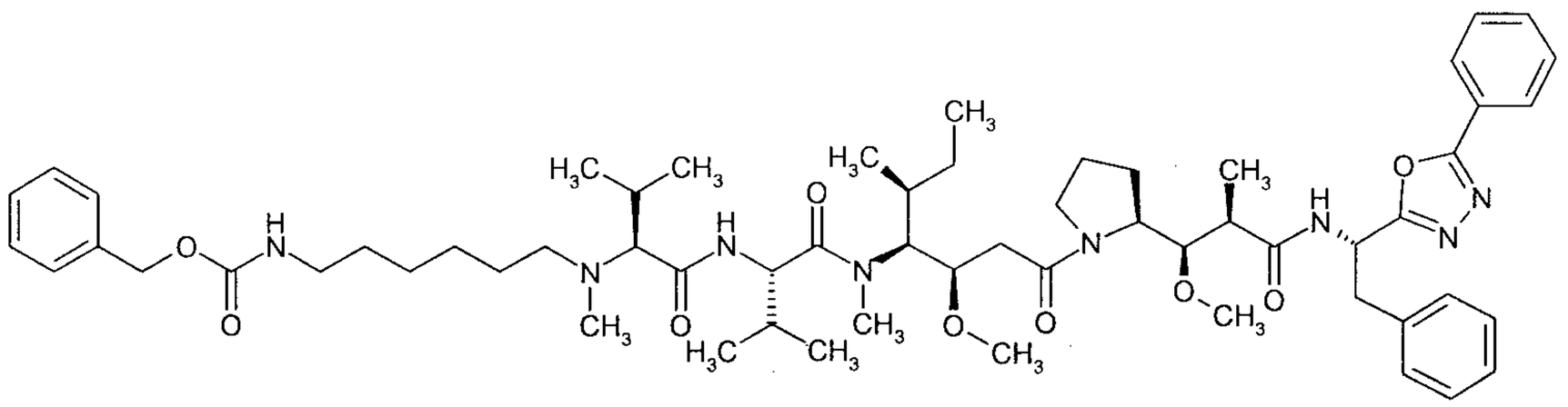
5 mg (10 µmol) of this intermediate were dissolved in 1 ml of DMF and admixed with 5.8 mg (50 mmol) of 1-hydroxypyrrolidine-2,5-dione and then with 2.6 µl of *N,N*-diisopropylethylamine and 3.8 mg (10 µmol) of HATU. After stirring at RT for 20 h, the reaction mixture was concentrated *in vacuo*. The 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;

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-[(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

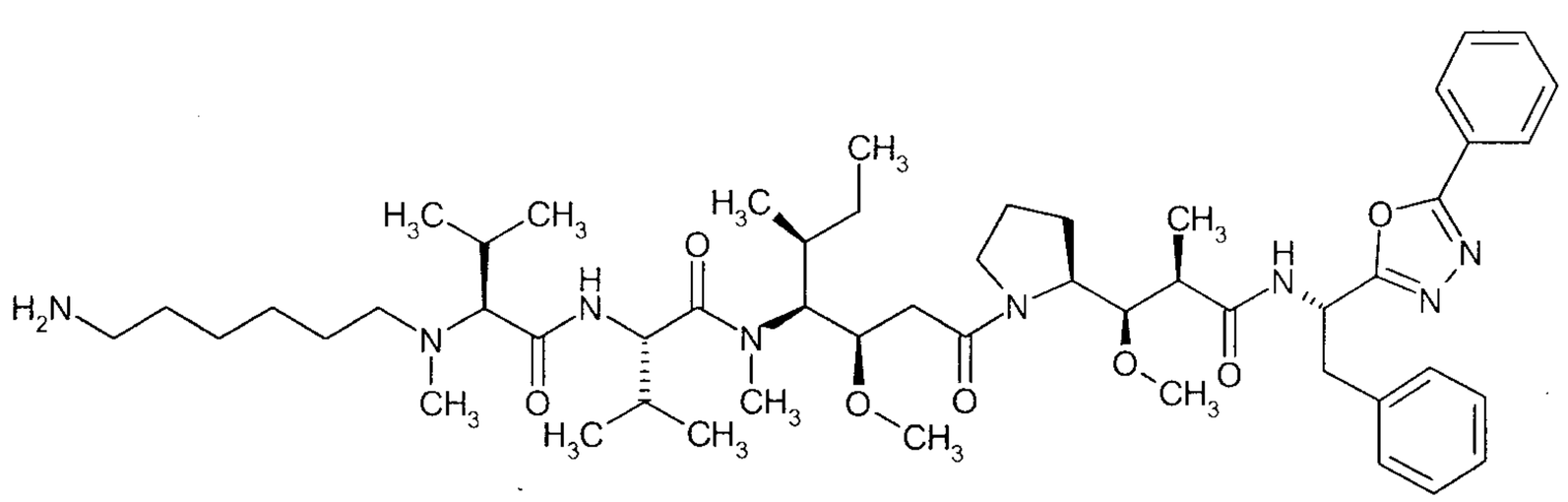


25 mg (30 µmol) of N-methyl-*L*-valyl-N-[(*3R,4S,5S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-*L*-valinamide (Intermediate 55) and 45 mg (180 µmol) of benzyl-(6-oxohexyl)carbamate were taken up in 3 ml of methanol and acidified with acetic acid. At room temperature, 15 µl (144 µmol; 9.4M) of borane-pyridine complex were subsequently added. The batch was subsequently stirred at RT for 24 h, and acetic acid and 15 µl (144 µmol; 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.

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

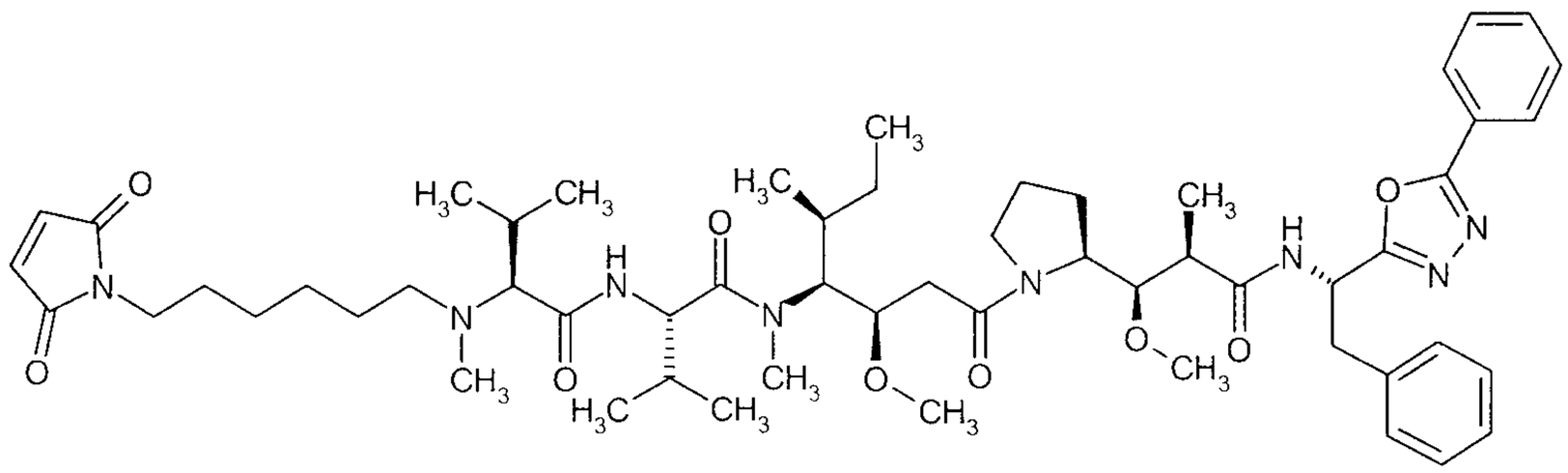


15 mg (14 µmol) of N-(6-{[(benzyloxy)carbonyl]amino}hexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 234) were 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

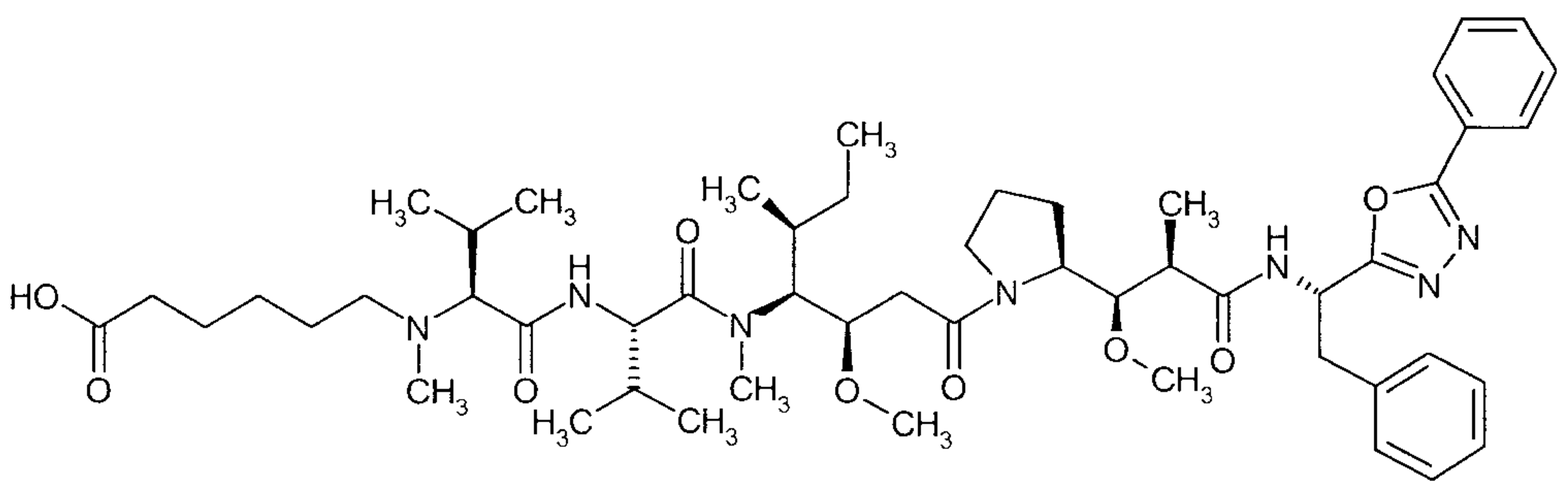


11 mg (12 µmol) of N-(6-aminohexyl)-N-methyl-L-valyl-N-[(3R,4S,5S)-3-methoxy-1-{(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-{[(1S)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide (Intermediate 235) were taken up in 500 µl of 1:1 dioxane/water and admixed with 253 µl of 1M aqueous sodium hydrogencarbonate solution and then with 2.8 mg (18 µmol) 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 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-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide



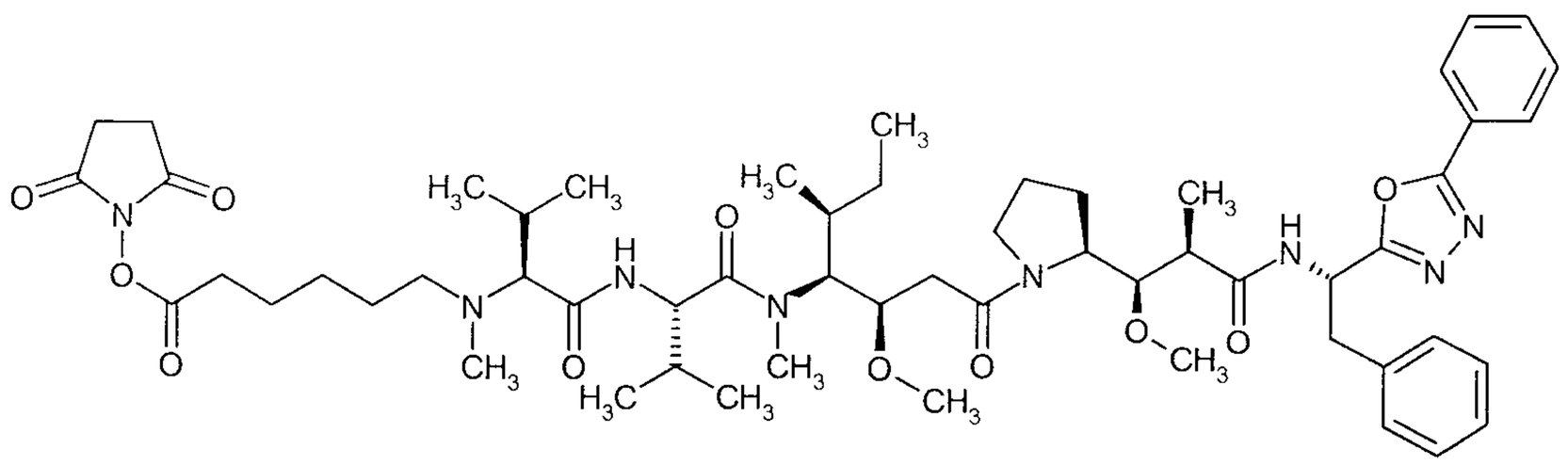
25 mg (30 µmol) of N-methyl-*L*-valyl-N-[(*3R,4S,5S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-{[(1*S*)-2-phenyl-1-(5-phenyl-1,3,4-oxadiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-*L*-valinamide (Intermediate 55) and 23 mg (180 µmol) of 6-oxohexanoic acid were taken up in 3 ml of methanol and acidified with acetic acid. At room temperature, 15 µl (144 µmol; 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 µl (144 µmol; 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

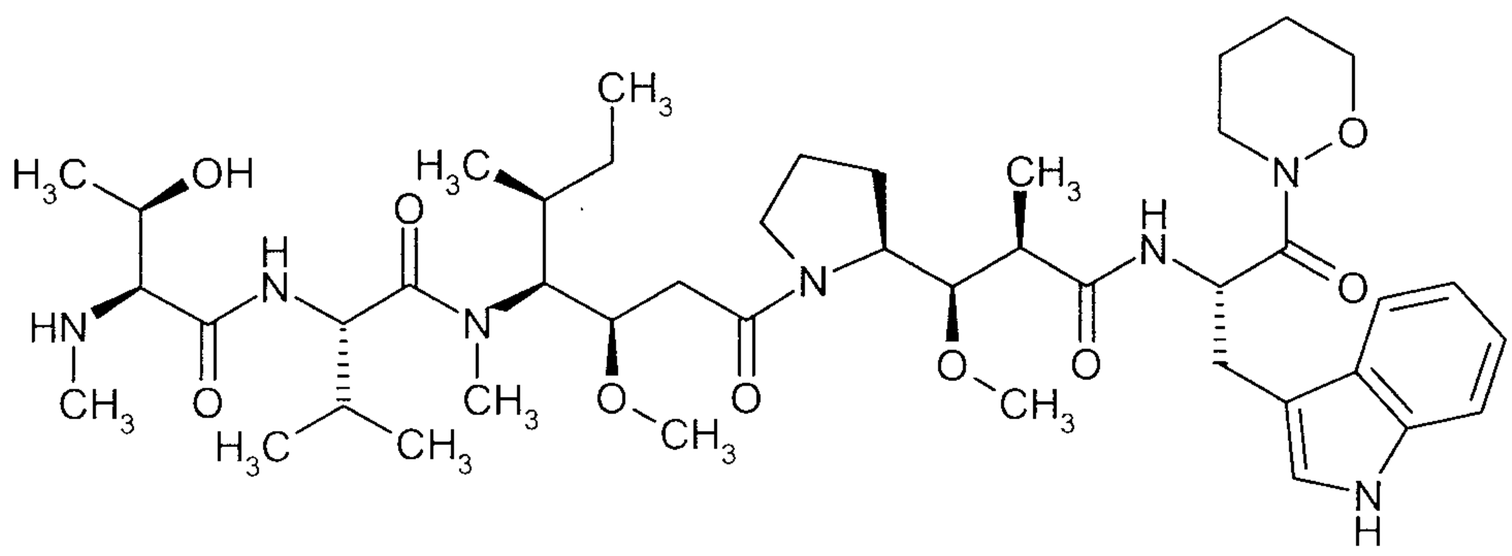


21 mg (22 µmol) of Intermediate 237 were dissolved in 1 ml of DMF and admixed with 38 mg (333 µmol) of 1-hydroxypyrrolidine-2,5-dione and then with 2.4 mg (10 µmol) of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) and 19 µl of N,N-diisopropylethylamine. After stirring 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.

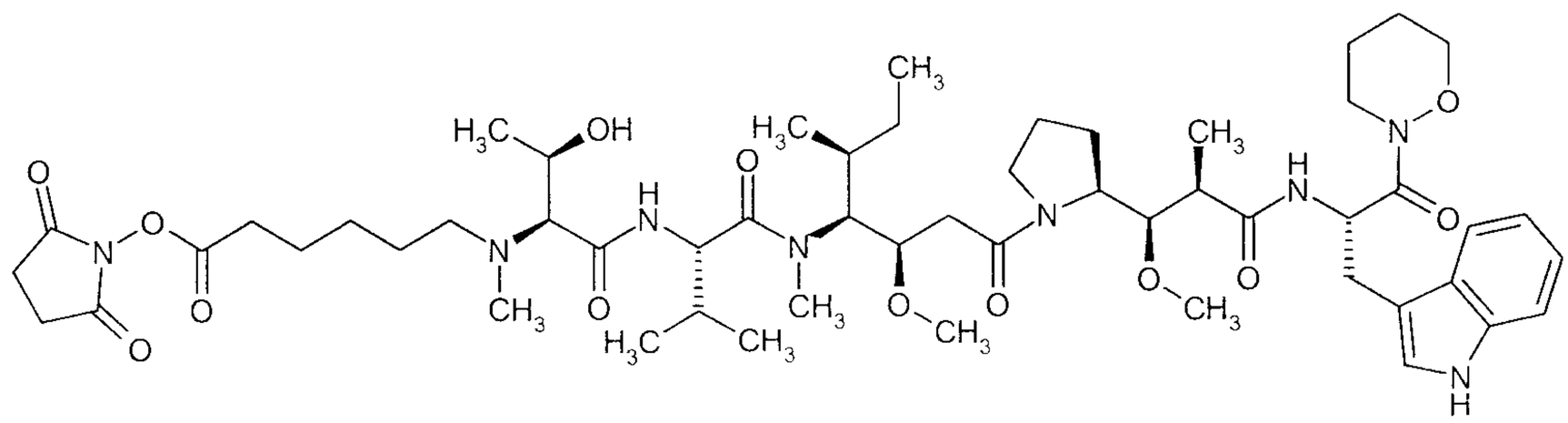
LC-MS (Method 1): R_t = 1.15 min; MS (ESIpos): m/z = 976 $(M+H)^+$.

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-[(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



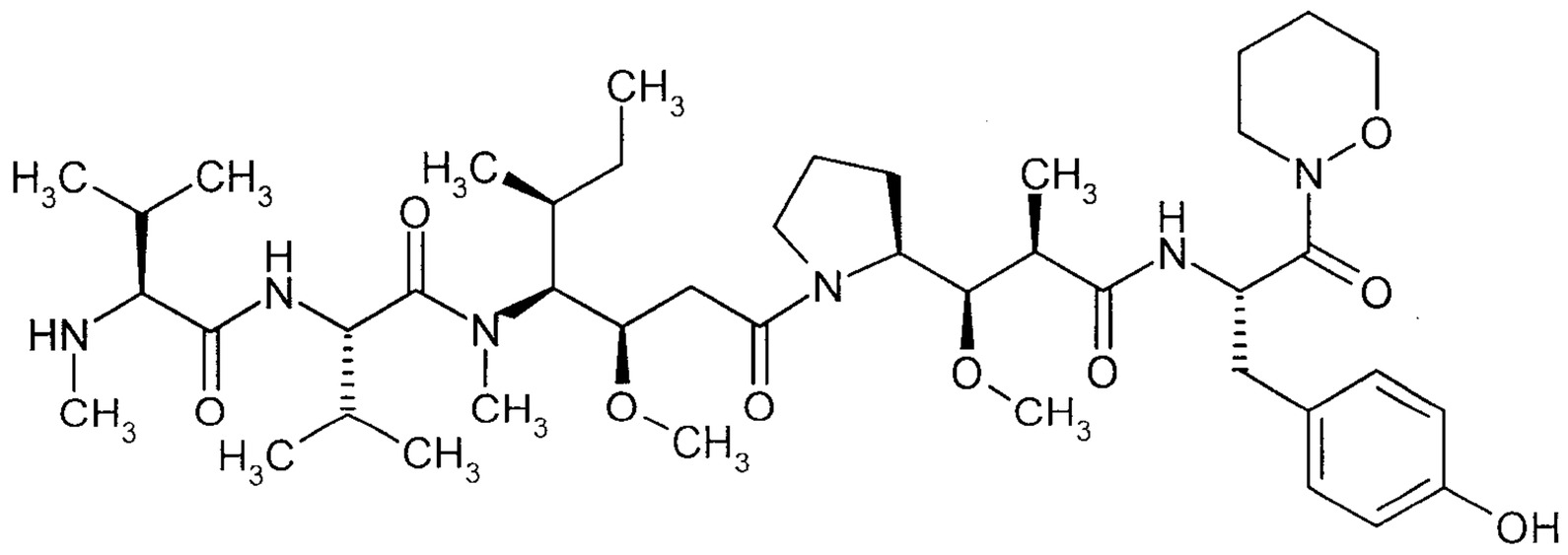
This compound was prepared in analogy to Intermediate 210 from 15.6 mg (0.016 mmol) of Intermediate 239. 10.8 mg (67% of theory over 2 stages) of the title compound were obtained.

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



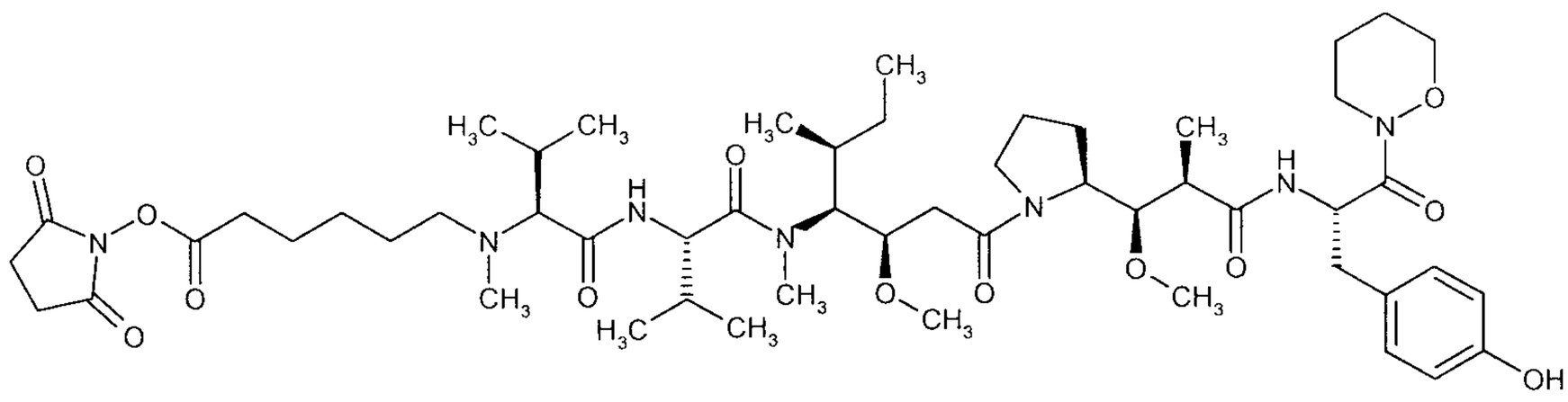
First, in analogy to Intermediate 5, trifluoroacetic acid-(2S)-2-amino-3-(4-hydroxyphenyl)-1-(1,2-oxazinan-2-yl)propan-1-one (1:1) was prepared. This component was then used to obtain the title compound, in analogy to the synthesis described in Intermediate 75, by coupling with *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valyl-*N*-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-2-carboxy-1-methoxypropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-*N*-methyl-L-valinamide (Intermediate 26) 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.

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

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



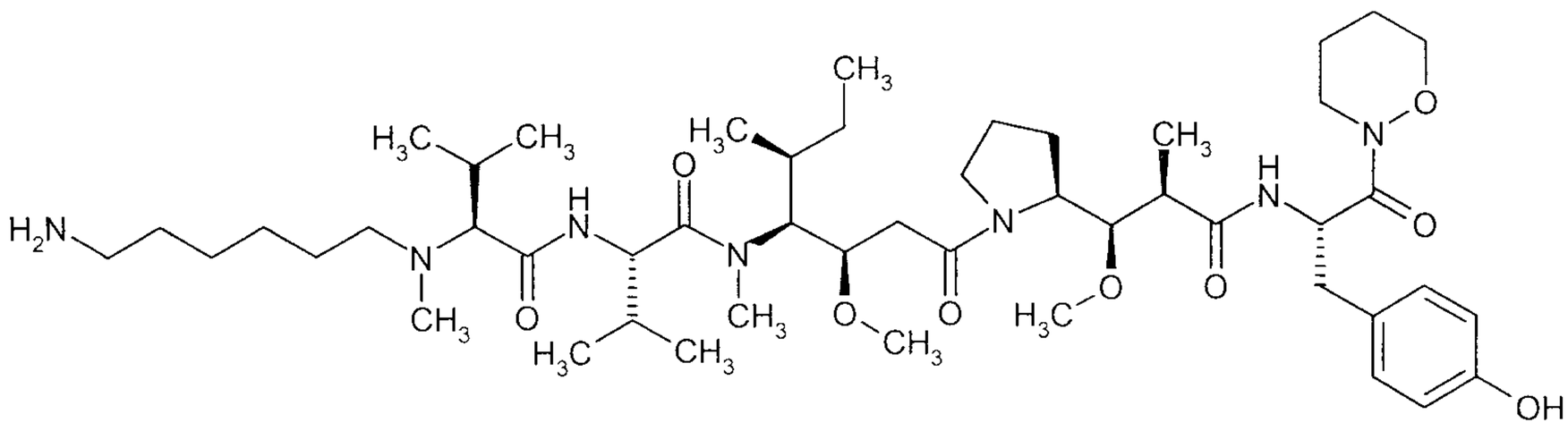
50 mg (0.05 mmol) of Intermediate 241 were reacted, in analogy to Intermediate 210, with 6-oxohexanoic acid in the presence of borane-pyridine complex. Subsequently, 22.5 mg (0.02 mmol) of the obtained acid were converted to the activated ester. 13.5 mg (36% of theory over 2 stages) of the title compound were obtained.

HPLC (Method 12): R_t = 1.8 min;

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1028 $(M+H)^+$.

Intermediate 243

N-(6-aminohexyl)-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 hydrogenation in methanol as the solvent.

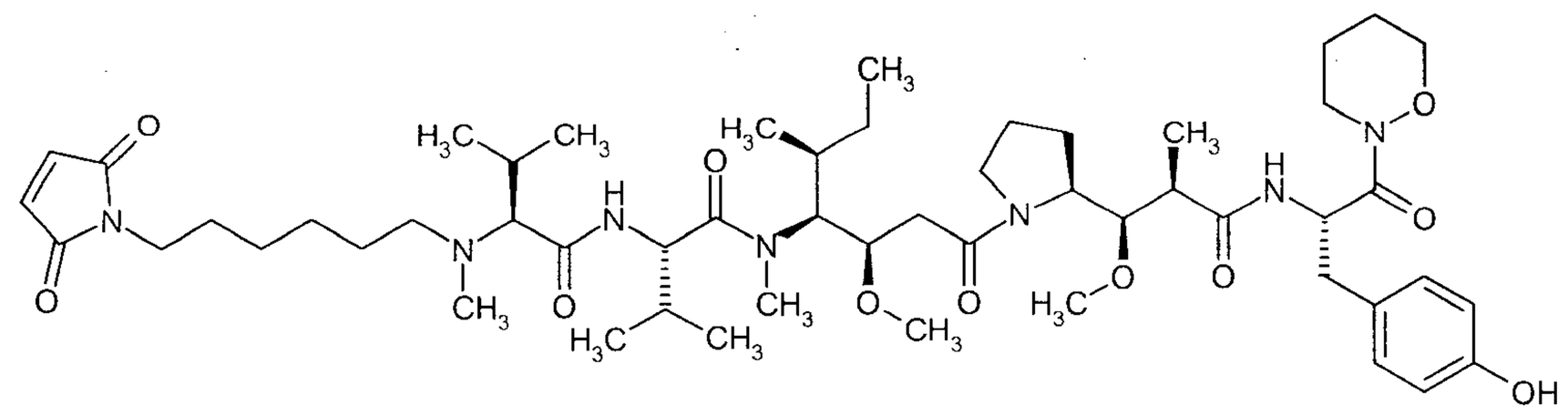
Yield: 17.5 mg (34% of theory over 2 stages)

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

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



The preparation was done in analogy to Intermediate 166 starting from Intermediate 243.

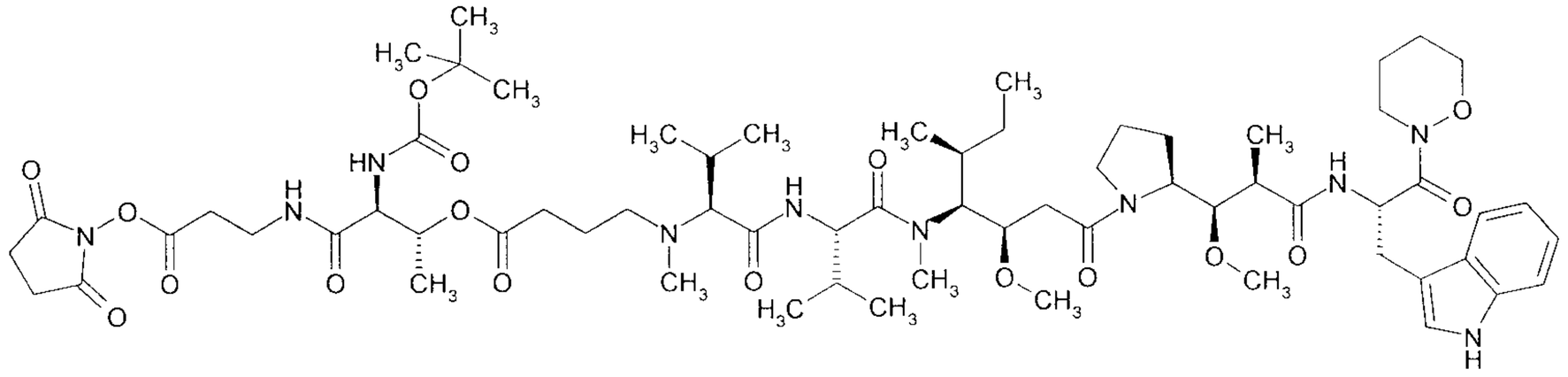
Yield: 1.3 mg (12% of theory)

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



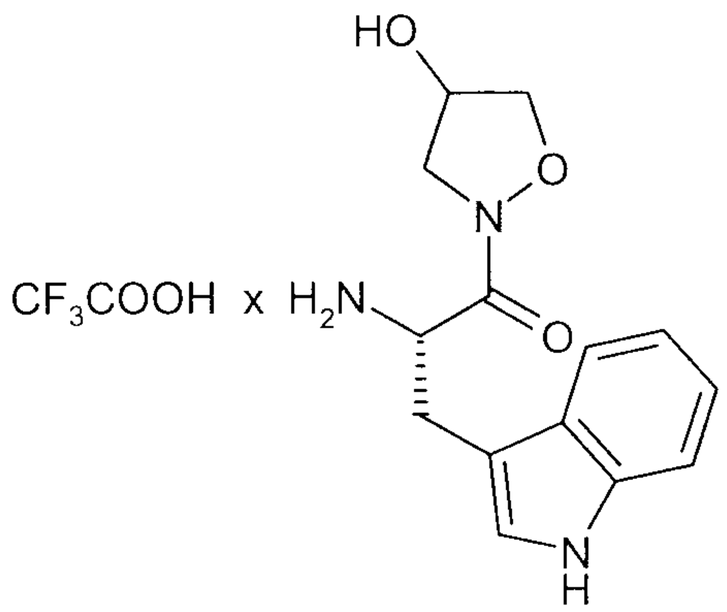
First, Intermediate 193, as described for Intermediate 154, was reacted with benzyl N-(tert-butoxycarbonyl)-L-threoninate, and then the benzyl ester was removed by hydrogenolytic means. 30 mg (0.027 mmol) of the thus obtained N-[4-({(1S,2R)-1-[(tert-butoxycarbonyl)amino]-1-carboxypropan-2-yl}oxy)-4-oxobutyl]-N-methyl-L-valyl-N-[(3R,4S,5S)-1-{(2S)-2-[(1R,2R)-3-{[(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 $(M+H)^+$

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 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 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) of a mixed fraction of the Boc-protected intermediates were obtained.

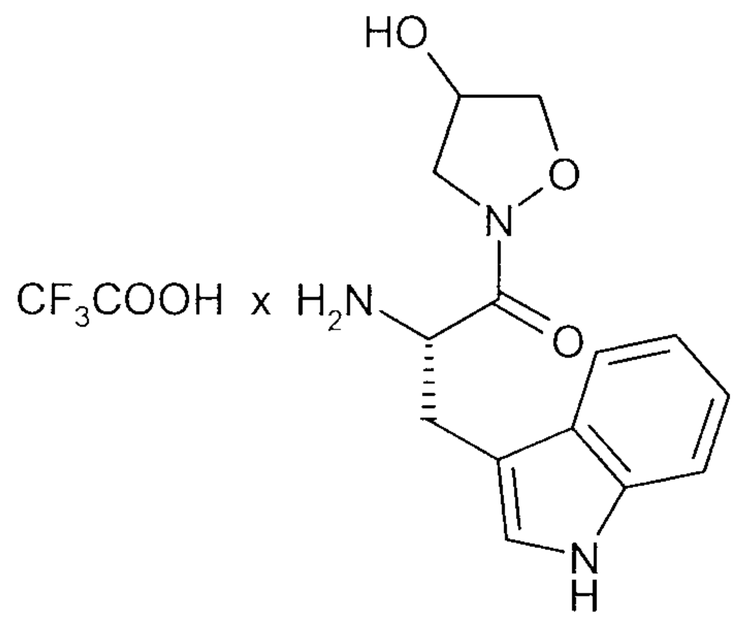
5 ml of trifluoroacetic acid in 20 ml of dichloromethane were used under standard conditions for cleaving the Boc protecting group from 272 mg (725 µmol) of Diastereomer 1 of this intermediate and, after lyophilization from dioxane/water, 290 mg (quant) of the title compound were obtained in 75% purity and used without further purification in the next stage.

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)

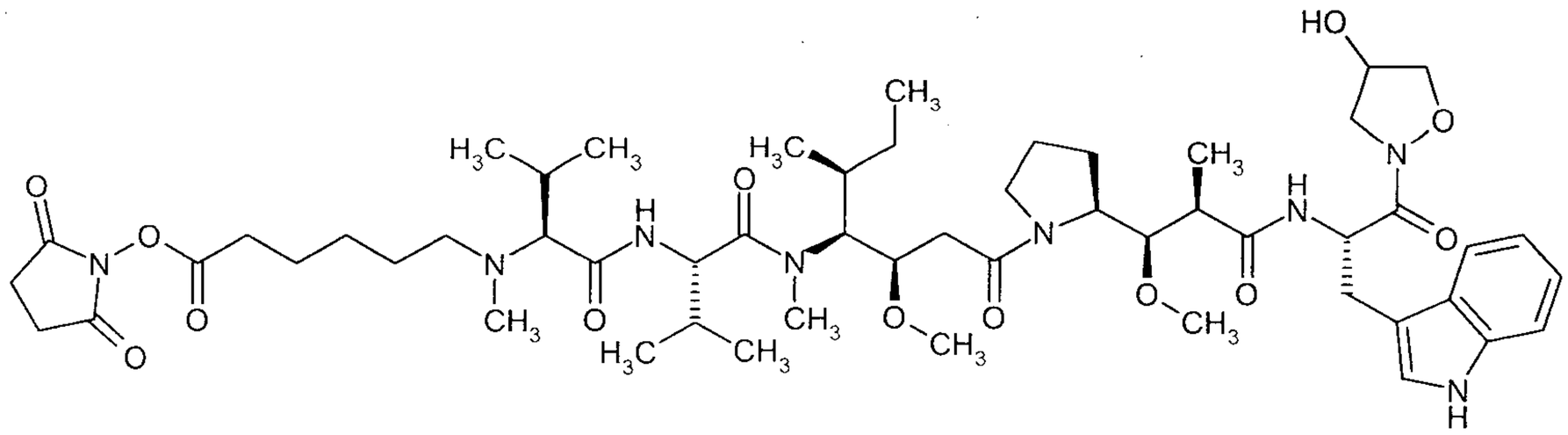


5 ml of trifluoroacetic acid in 20 ml of dichloromethane were used under standard conditions for cleaving the Boc protecting group from 236 mg (630 µmol) of Diastereomer 2 of the intermediate described in 246a and, after concentration, stirring with diethyl ether and drying of the residue under high 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-[(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)



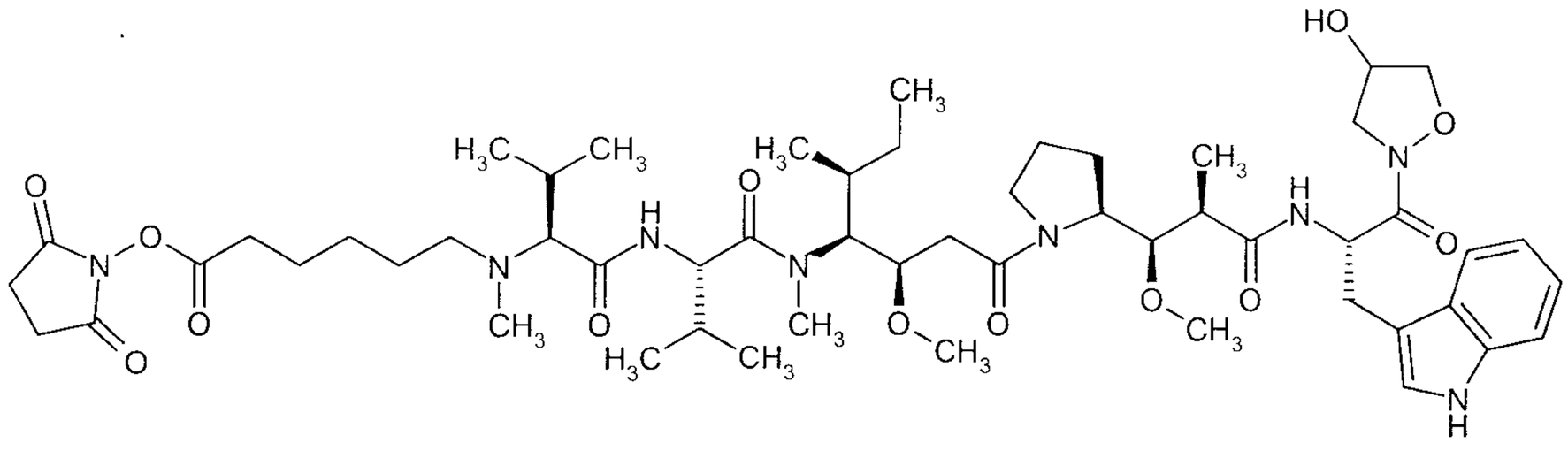
To synthesize this compound, the coupling of Intermediates 26 and 246a with subsequent cleaving of the Boc protecting group was first performed as described for Intermediate 74. Subsequently, the alkylation with 6-oxohexanoic acid in the presence of borane-pyridine complex and subsequent conversion of 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)



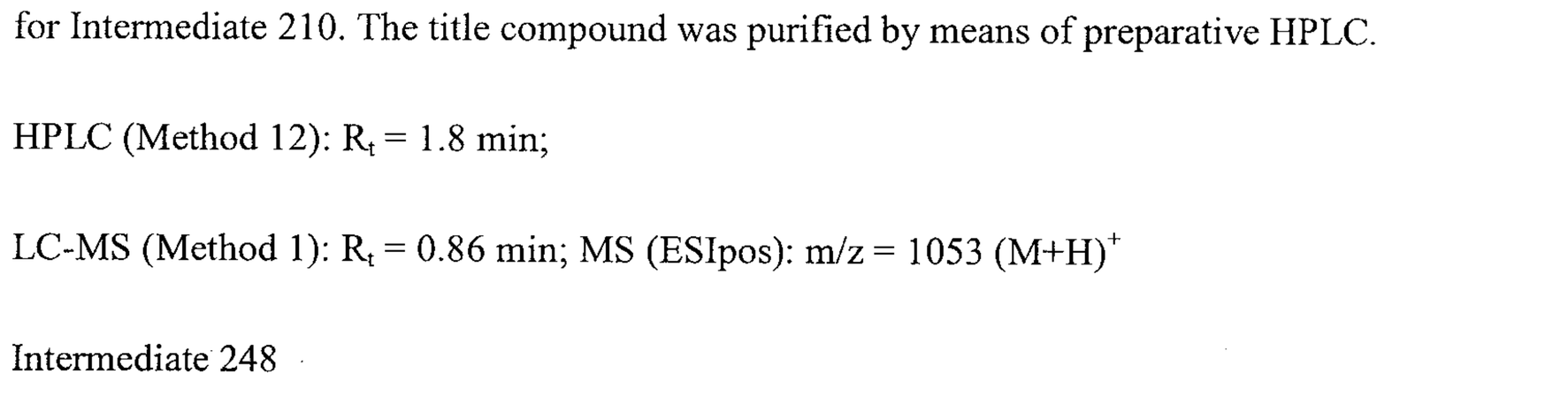
To synthesize this compound, the coupling of Intermediates 26 and 246b with subsequent cleaving of the Boc protecting group was first performed as described for Intermediate 74. Subsequently, the alkylation with 6-oxohexanoic acid in the presence of borane-pyridine complex and subsequent conversion of 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*-[(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-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)

Additional names: IMC-225, C225, EMR-62202, BMS-564717, Fab C225

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:

Light chain (kappa):

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPS
RFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
T LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYN
TPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSAA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
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:

Light chain (kappa):

DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPS
RFSGSGSGTDFTFTISSLQPEDIATYFCQHFDHLPLAFGGGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
T LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy chain:

QVQLQESGPGLVKPSETLSLTCTVSGGSVSSGDYYWTWIRQSPGKGLEWIGHIYYSGNTN
YNPSLKSRLTISIDTSKTQFSLKLSSVTAADTAIYYCVRDRVTGAFDIWGQGTMVTVSSA
STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV
VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPG

Nimotuzumab (INN No. 8545)

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).

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

Erbitux commercial product (Erbitux® 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 CIMAher commercial product (CIMAher® 50 mg AMP 4 × 10 mL, imported from Cuba, YM BioSciences 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 rebuffered 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:

Anti-EGFR1 antibodies:

cetuximab

nimotuzumab

panitumumab

Other antibodies:

anti-PDL1

anti-ICOSLG

anti-FGFR3

herceptin

anti-TYRP1

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 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 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 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_{1A}-AK_{1J} have the following meanings:

AK_{1A} = cetuximab (partially reduced)-S§[1]

AK_{1B} = nimotuzumab (partially reduced)-S§[1]

AK_{1C} = panitumumab (partially reduced)-S§[1]

AK_{1D} = anti-PDL1 (partially reduced)- S§[1]

AK_{1E} = anti-ICOSLG (partially reduced)- S§[1]

AK_{1F} = anti-FGFR3 (partially reduced)- S§[1]

AK_{1G} = herceptin (partially reduced)- S§[1]

AK_{1H} = anti-TYRP1 (partially reduced)- S§[1]

AK_{1J} = anti-glypican-3 (partially reduced)- S§[1]

wherein

§[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-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:

anti-PDL1

anti-ICOSLG

anti-FGFR3

herceptin

anti-TYRP1 hIgG1-kapp

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 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 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 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.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 530

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 530

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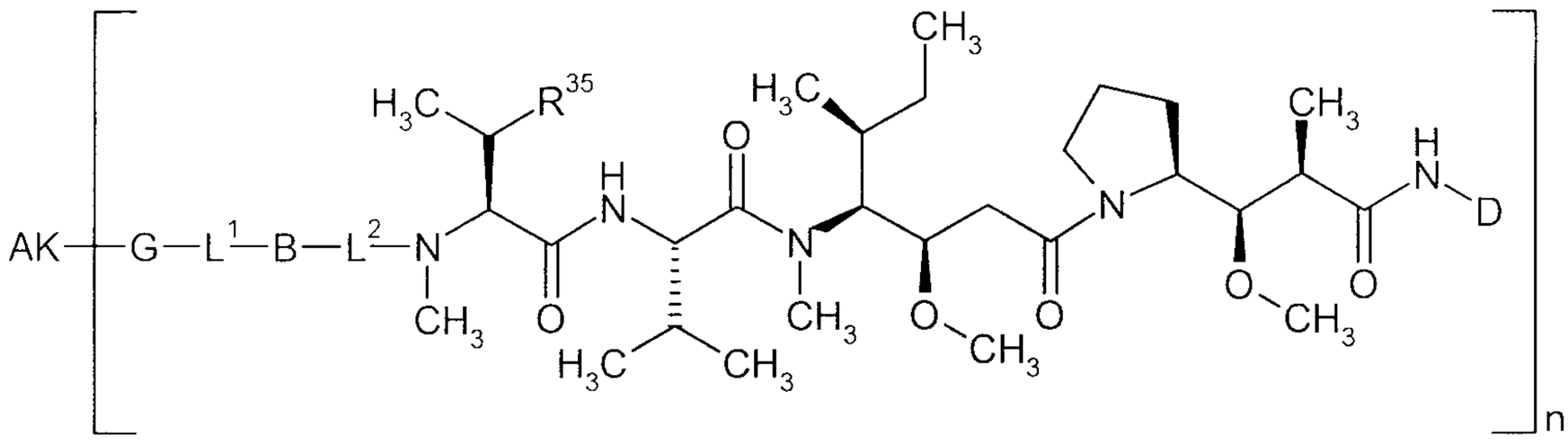
NOTE POUR LE TOME / VOLUME NOTE:

Claims

1. Binder-drug ingredient conjugates of general formula (Ia)



(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,

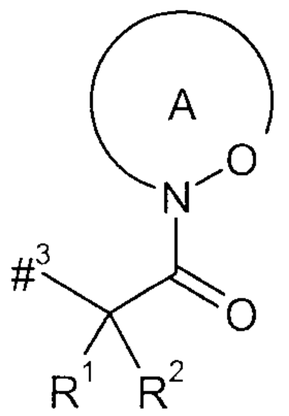
the group §-G-L1-B-L2-§§ stands for a linker,

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



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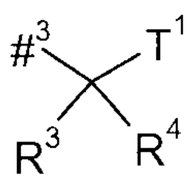

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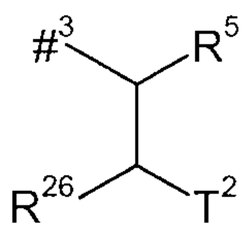


wherein

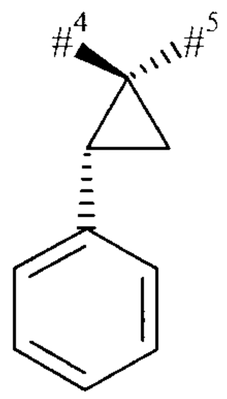
$\#^3$ denotes the linkage site to the nitrogen atom,

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

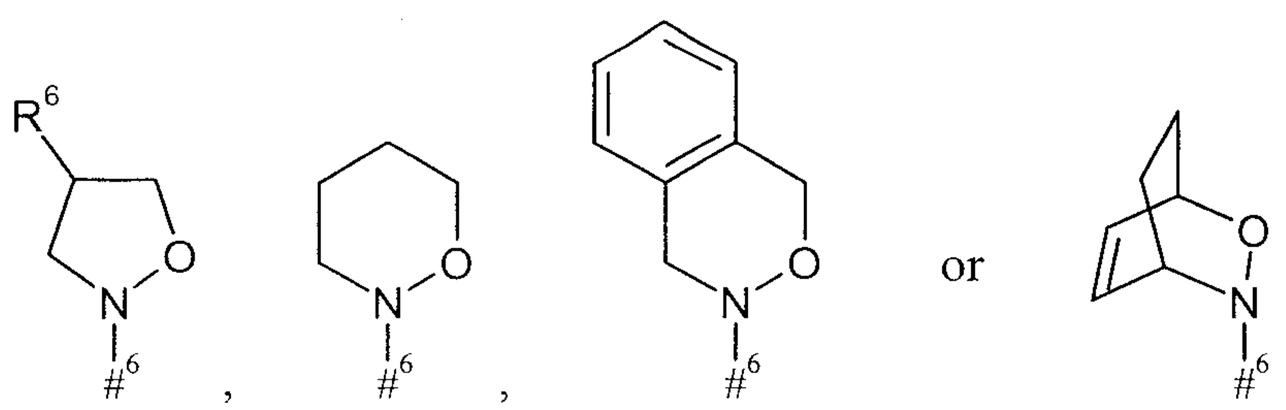


wherein

$\#^4$ 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,

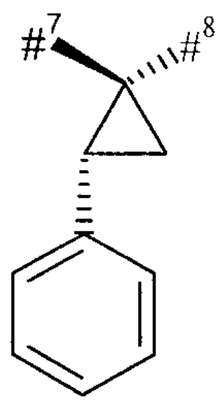
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-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



wherein

$\#^7$ denotes the linkage site with 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,

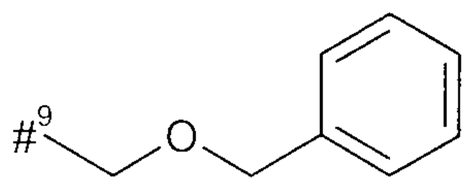
or

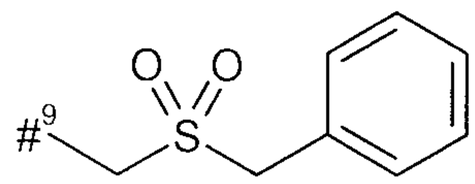
R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

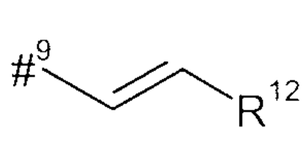
R^{10} stands for benzoyl,

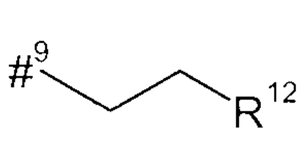
R^{11} stands for benzyl which may be substituted in the phenyl group with methoxycarbonyl or carboxyl,

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









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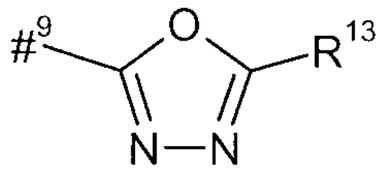


wherein

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

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^{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, solvates and solvates of the salts.

2. Binder-drug conjugates of general formula (Ia) according to claim 1, wherein

n stands for a number from 1 to 50,

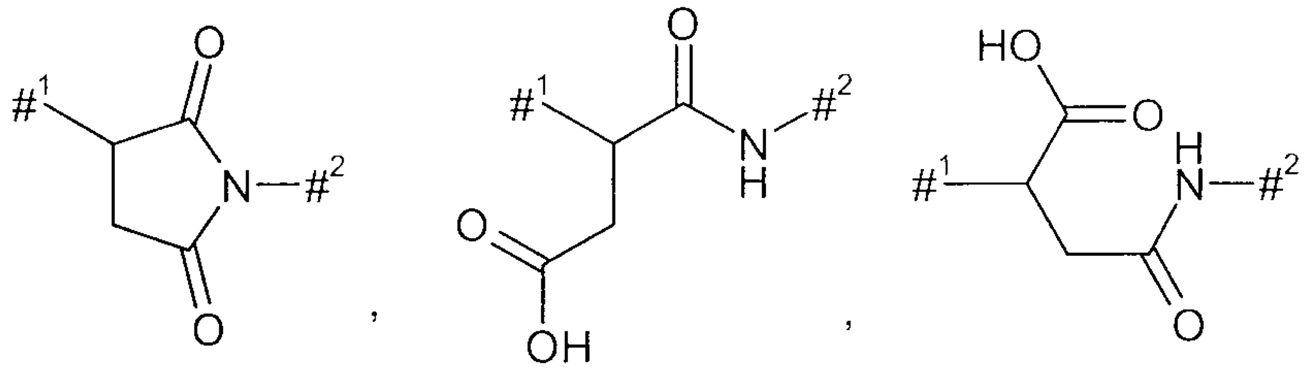
AK stands for AK_1 or AK_2

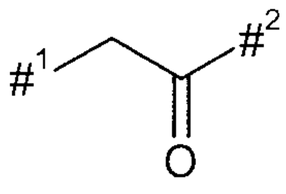
wherein

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,

AK_2 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 $AK = AK_1$ stands for a group of the formula



or 

wherein

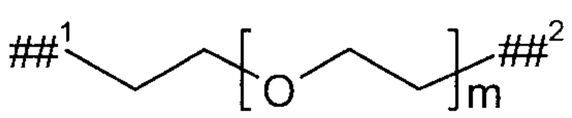
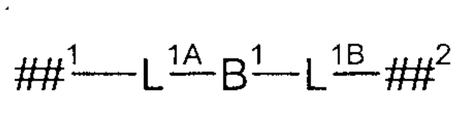
$\#^1$ denotes the linkage site to the sulfur atom of the binder,

$\#^2$ 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 $(C_1\text{-}C_{10})$-alkanediyl, a group of the formula

 or 

wherein

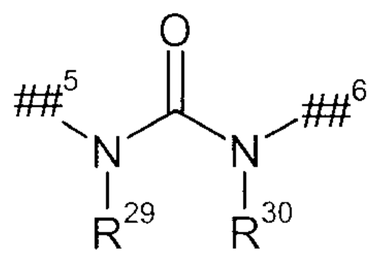
m stands for a number from 2 to 6,

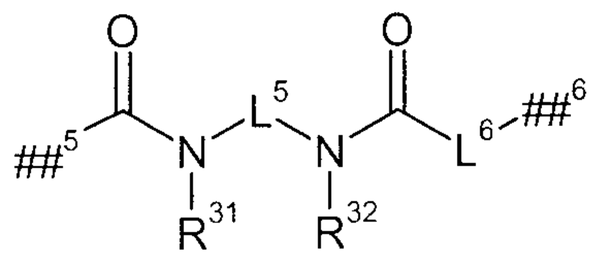
$\#\#^1$ denotes the linkage site to the group G,

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

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

B^1 stands for a group of the formula





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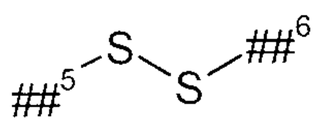


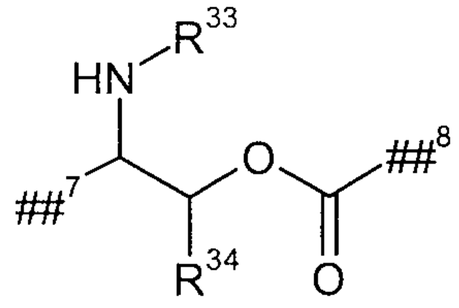
wherein

$##^5$ 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 $(C_2\text{-}C_4)$-alkanediyl,

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



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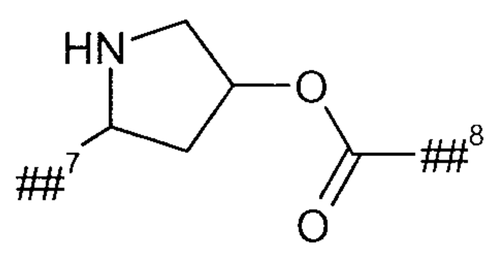


wherein

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

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

R^{33} stands for hydrogen, $(C_1\text{-}C_4)$-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

R^{34} stands for hydrogen or methyl,

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

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

or

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

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,

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

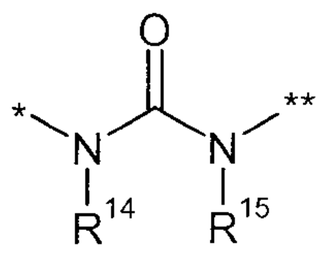
and

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

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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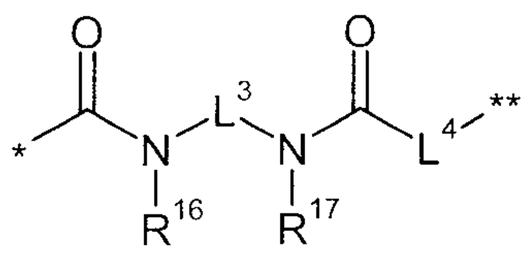
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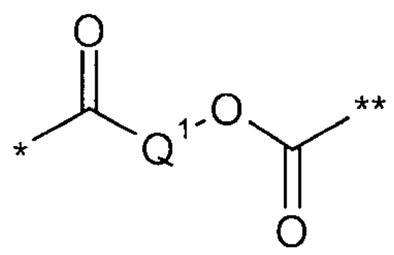
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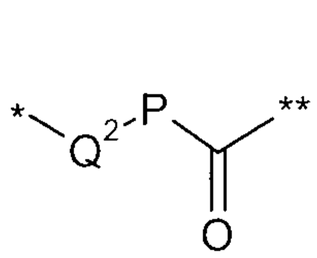
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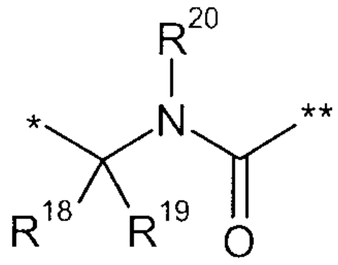
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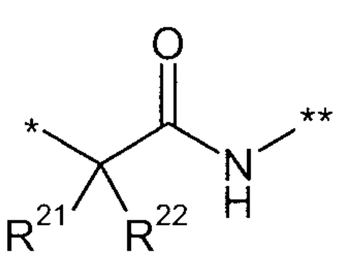
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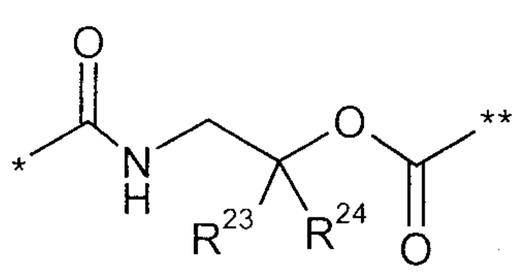
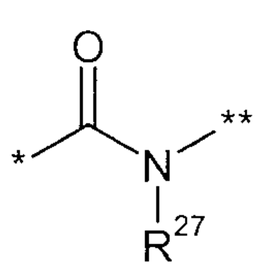
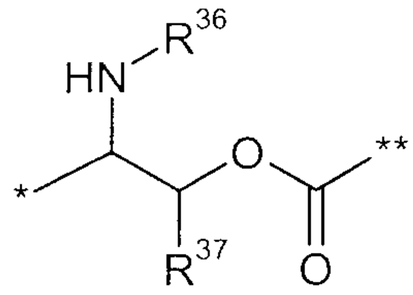
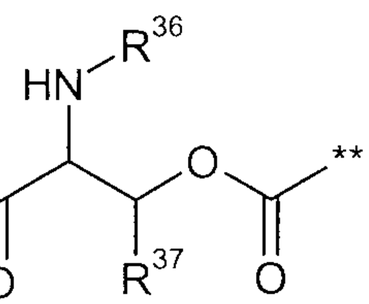






wherein

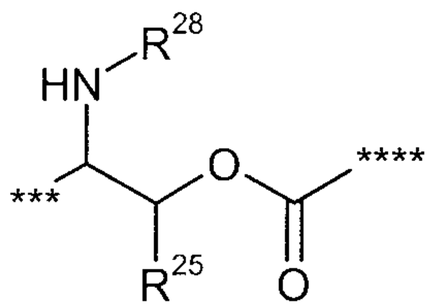
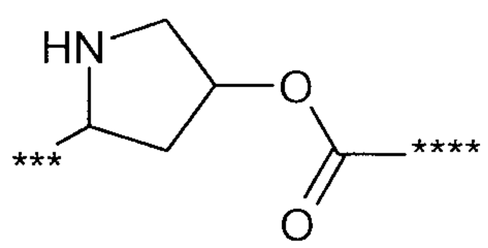
* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

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

*** 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,

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 $(C_1$-$C_4)$-alkyl,

R^{15} stands for hydrogen or $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-alkyl,

R^{17} stands for hydrogen or $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-alkyl,

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 $(C_1$-$C_4)$-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,

R^{23} stands for $(C_1$-$C_4)$-alkyl,

R^{24} stands for hydrogen or (C_1-C_4)-alkyl,

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

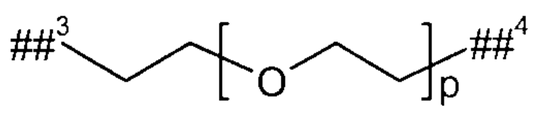
R^{36} stands for hydrogen, (C_1-C_4)-alkylcarbonyl, *tert*-butyloxycarbonyl or benzyloxycarbonyl,

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



wherein

p stands for a number from 2 to 6,

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

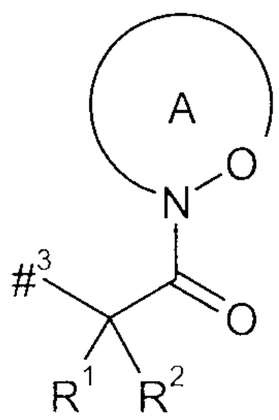
$##^4$ denotes the linkage site to the nitrogen atom,

wherein (C_2-C_{10})-alkanediyl may be substituted with 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,

D stands for a group of the formula



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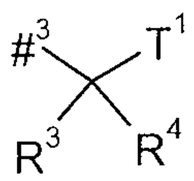

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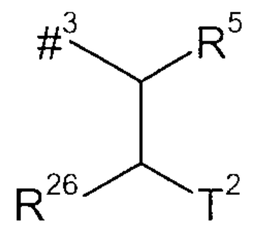


wherein

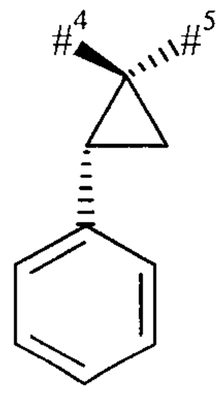
$\#^3$ denotes the linkage site to the nitrogen atom,

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 (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

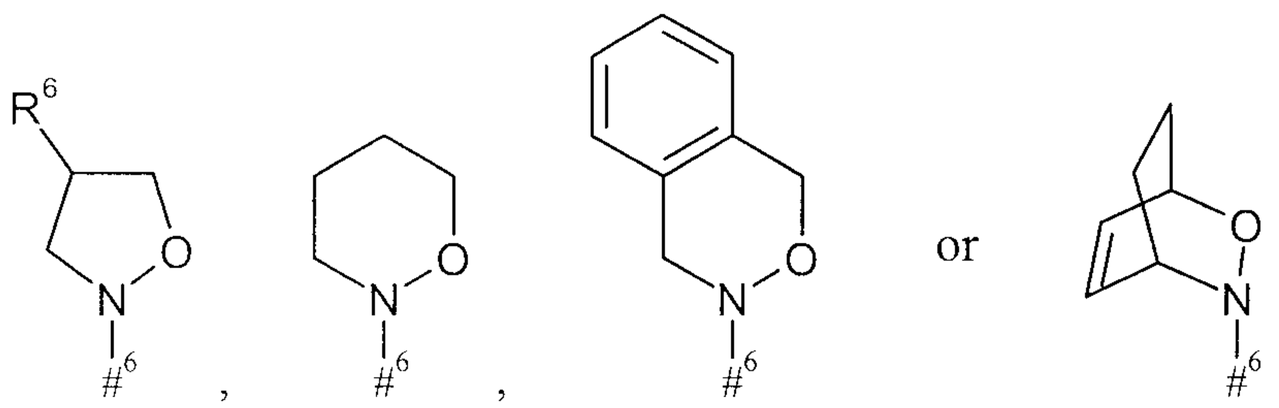


wherein

$\#^4$ 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 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,

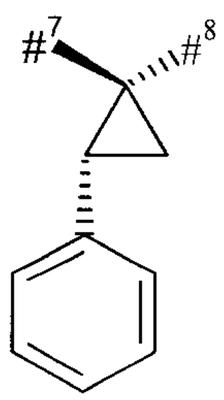
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

R^4 stands for isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-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^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 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,

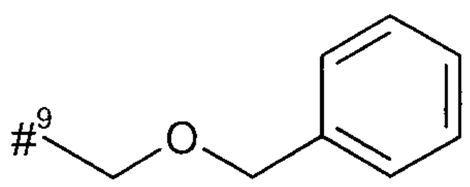
or

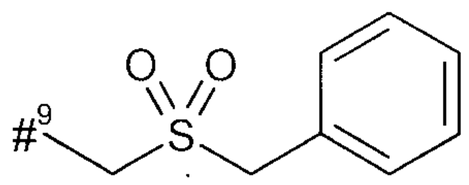
R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

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





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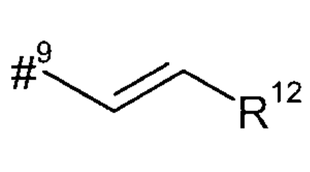

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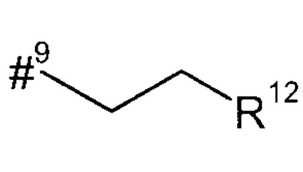

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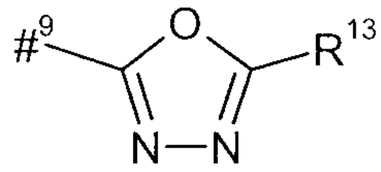


wherein

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

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^{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.

3. Binder-drug conjugates of general formula (Ia) according to claim 1 or 2, wherein

n stands for a number from 1 to 20,

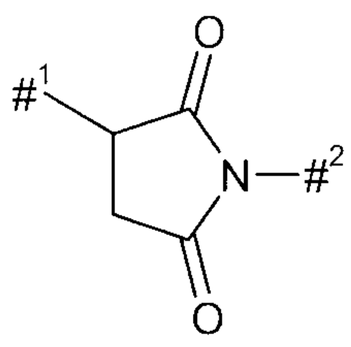
AK stands for AK_1 or AK_2

wherein

AK_1 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,

AK_2 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 = AK_1, stands for a group of the formula



wherein

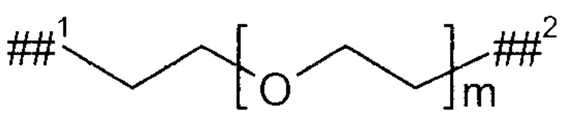
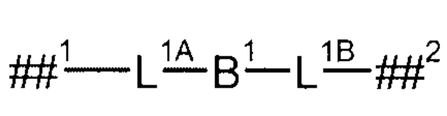
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

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

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

 or 

wherein

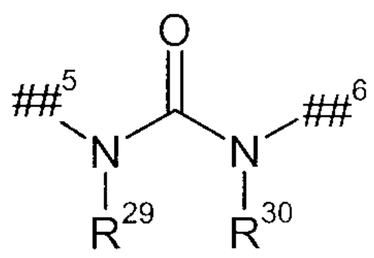
m stands for a number from 2 to 6,

$##^1$ 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,

B^1 stands for a group of the formula



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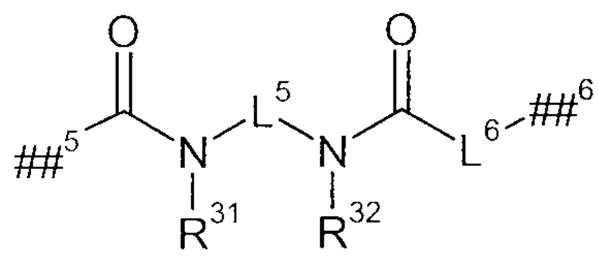

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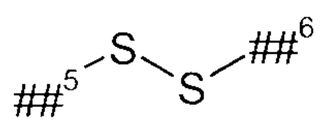


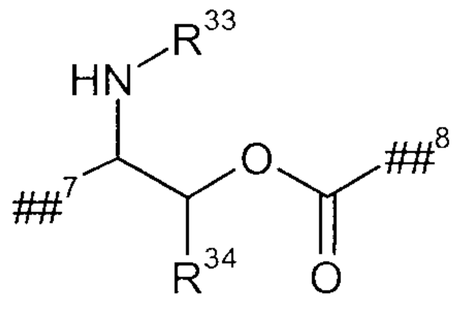
wherein

$##^5$ 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,

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



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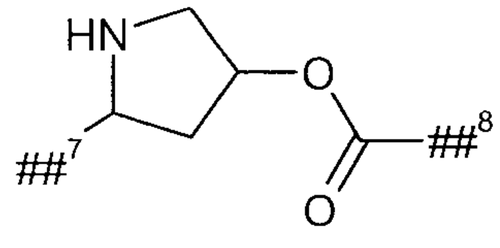


wherein

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

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

R^{33} 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,

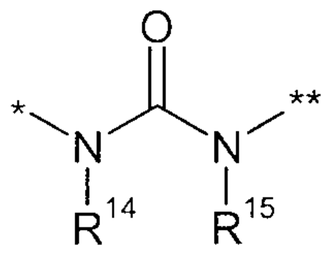
R^{32} stands for hydrogen or methyl,

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,

B stands for a bond or a group of the formula



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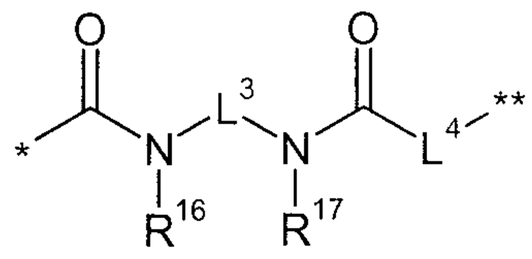

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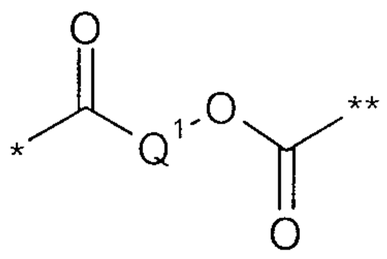

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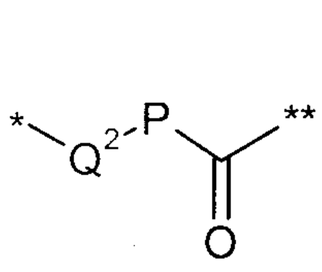

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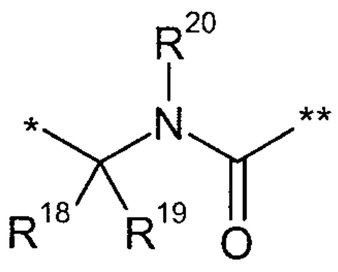

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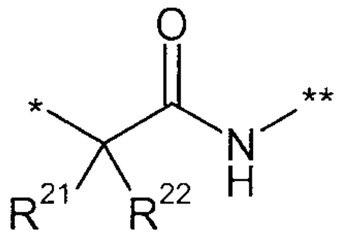

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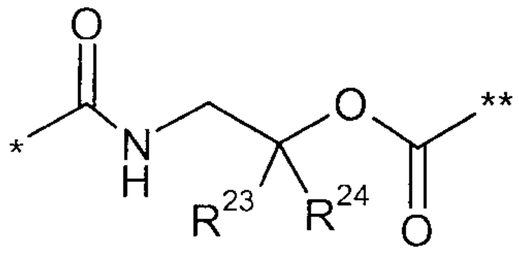

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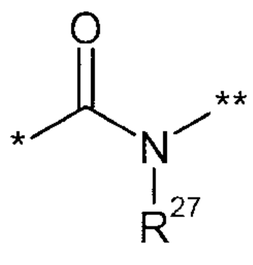

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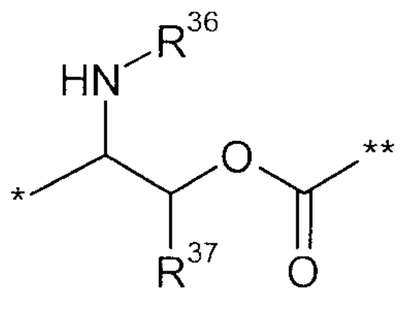


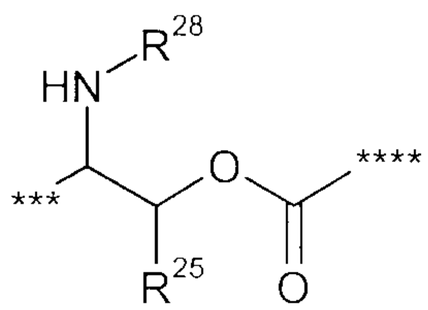
wherein

* 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



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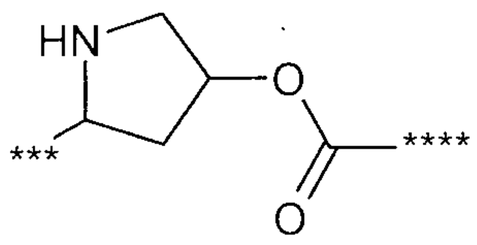


wherein

*** 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,

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,

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 1-methyl-propan-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,

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,

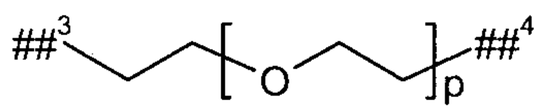
R^{36} stands for hydrogen, methylcarbonyl or *tert*-butyloxycarbonyl,

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,

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



wherein

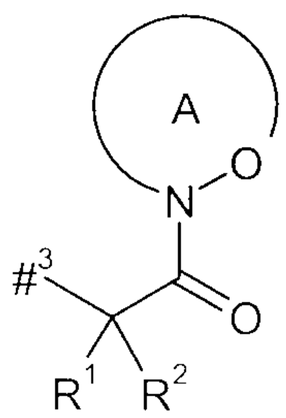
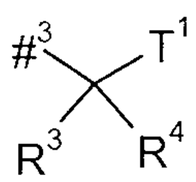
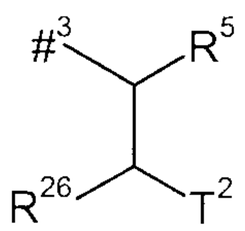
p stands for a number from 2 to 6,

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

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

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

D stands for a group of the formula



or


wherein

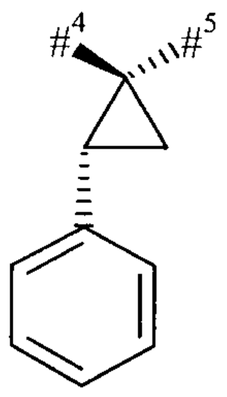
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

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

or

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

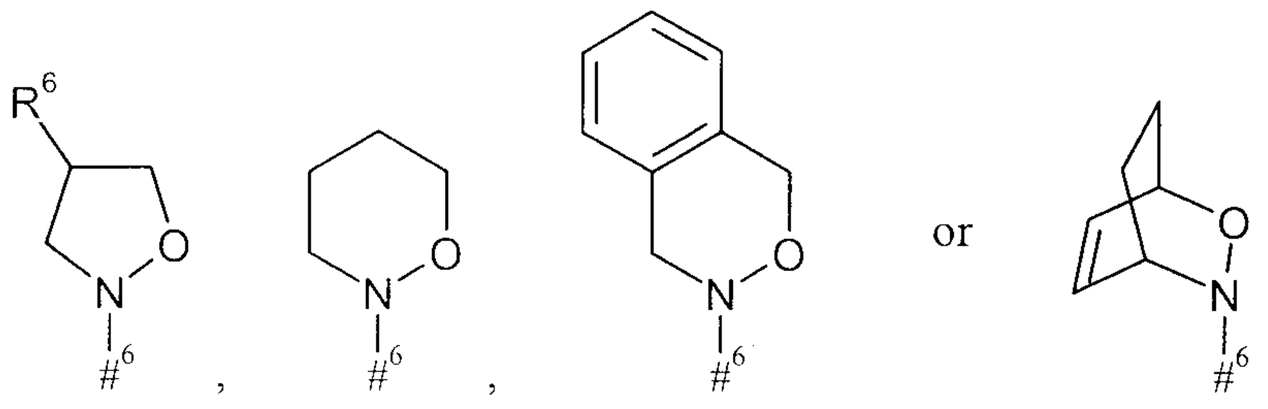


wherein

$\#^4$ 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



wherein

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

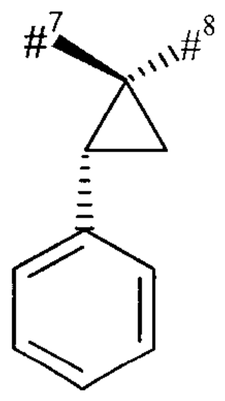
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 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 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 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,

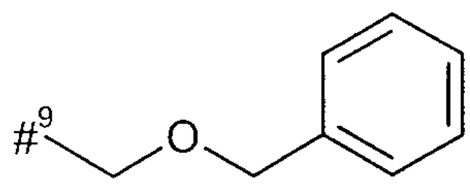
or

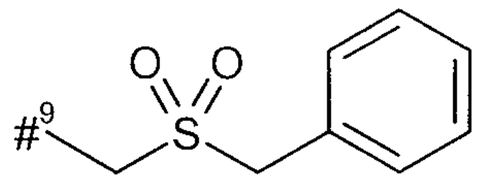
R^8 and R^9 together with the nitrogen atom which they are bound may form a four- to seven-membered heterocycle,

R^{10} stands for benzoyl,

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





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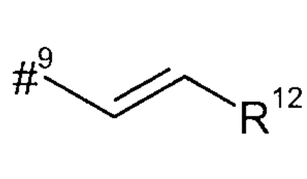

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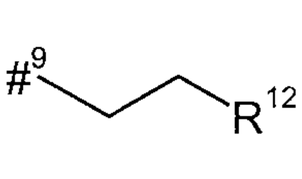

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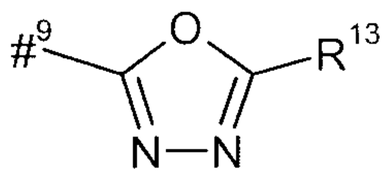


wherein

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

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^{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.

4. Binder-drug conjugates of general formula (Ia) according to claims 1 to 3, wherein

n stands for a number between 1 and 10,

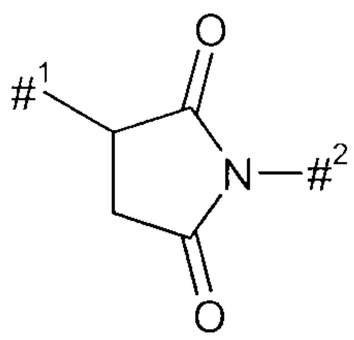
AK stands for AK_1 or AK_2

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,

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

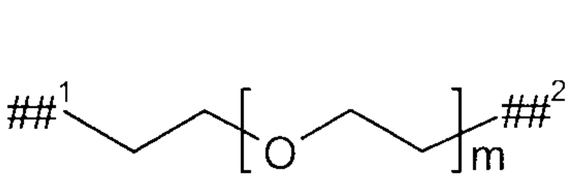
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ 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

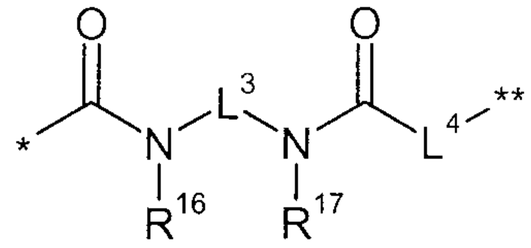
m stands for a number of 2 or 3,

##[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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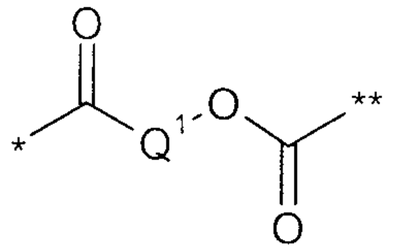
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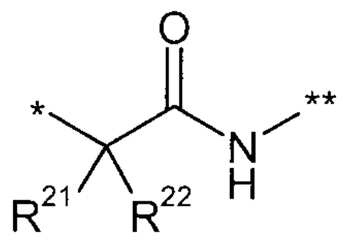
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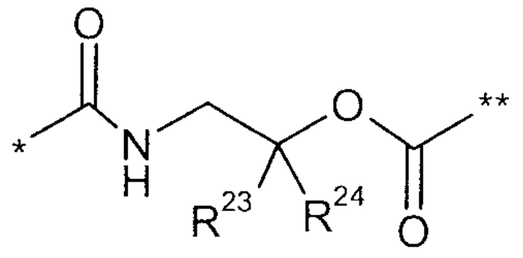
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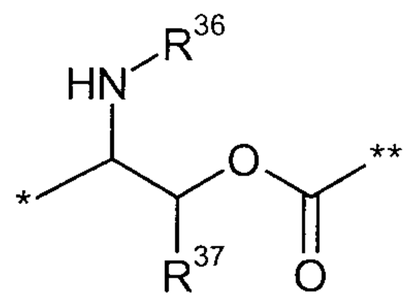
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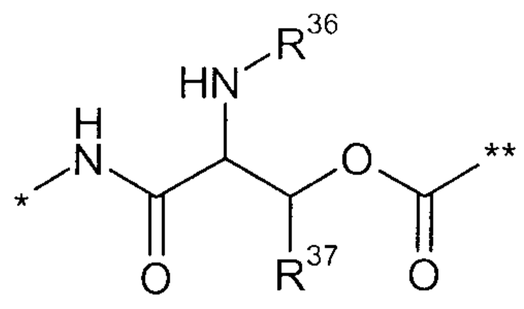


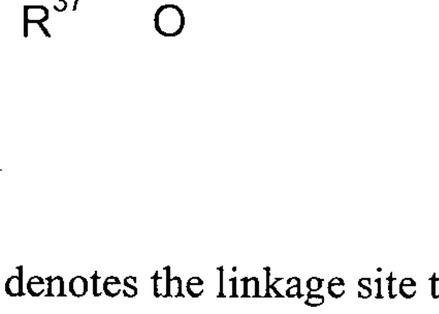
wherein

* 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


or

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,

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

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

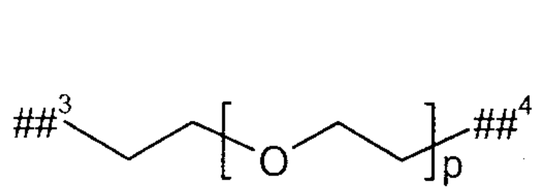
R^{23} stands for methyl,

R^{24} stands for hydrogen,

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

R^{37} stands for hydrogen or methyl,

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



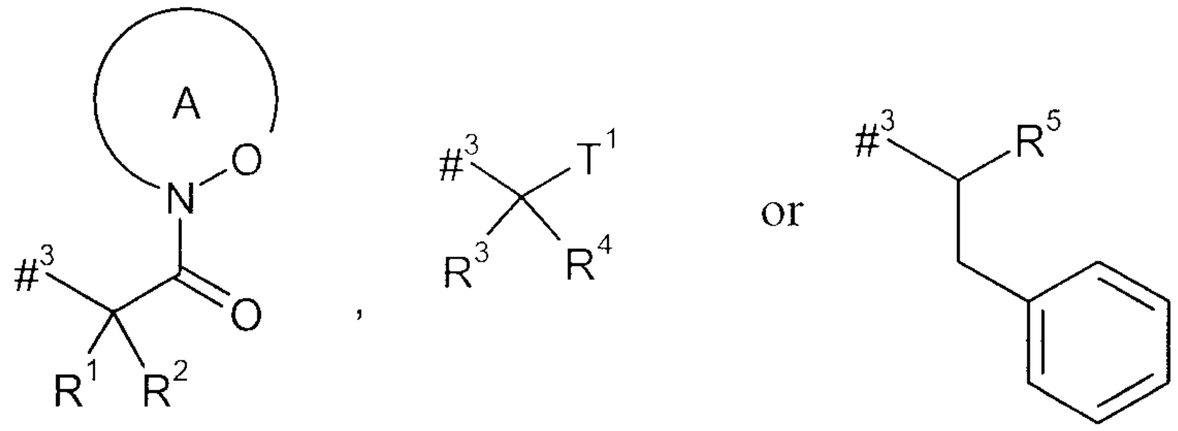
wherein

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

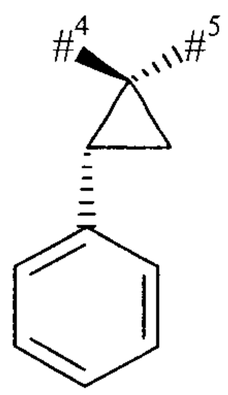
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 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

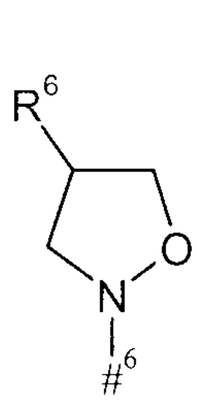
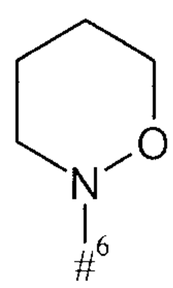
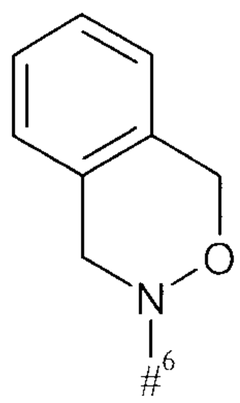


wherein

$\#^4$ 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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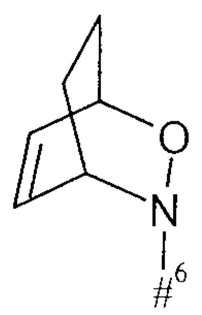


wherein

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

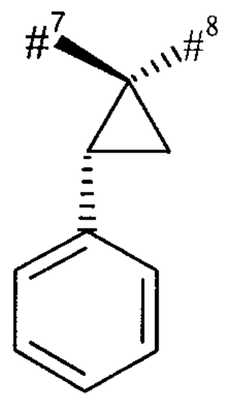
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 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 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

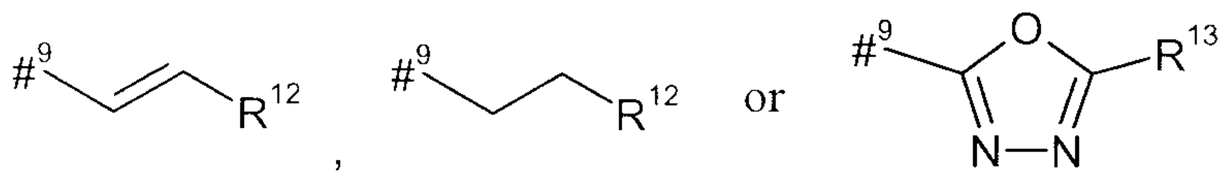
R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

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

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,

R^{35} stands for methyl or hydroxyl,

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

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 AK_2,

wherein

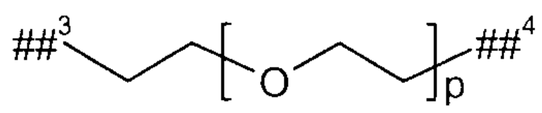
AK_2 stands for cetuximab, pantitumumab or nimutuzumab, which is bound to the group G via the NH side group of a lysine radical of the binder,

G stands for carbonyl,

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



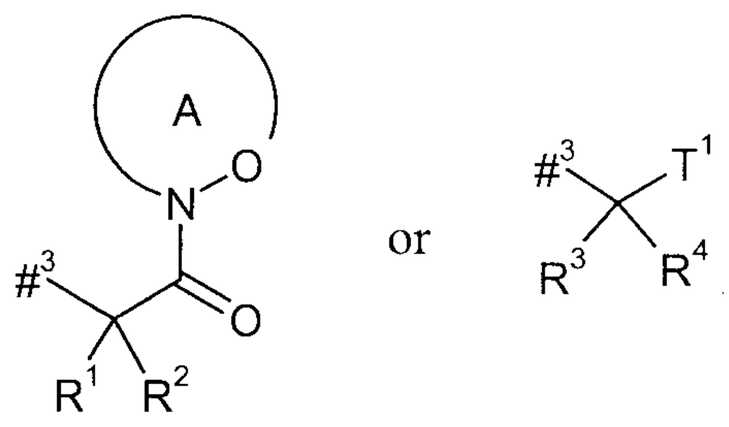
wherein

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

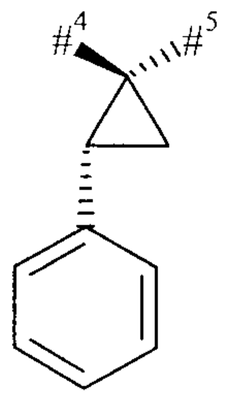
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for benzyl, 4-hydroxybenzyl 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

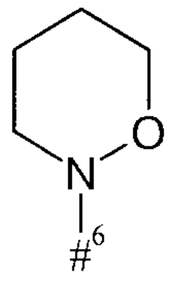


wherein

$\#^4$ 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

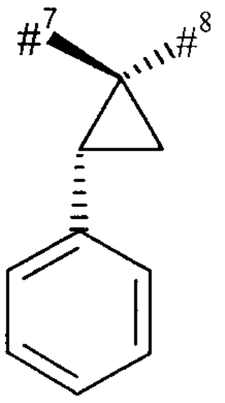
$\#^6$ denotes the linkage site to the carbonyl group,

R^3 stands for hydrogen,

R^4 stands for benzyl, 4-hydroxybenzyl 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 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, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen,

R^9 stands for hydrogen or benzyl,

R^{35} 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

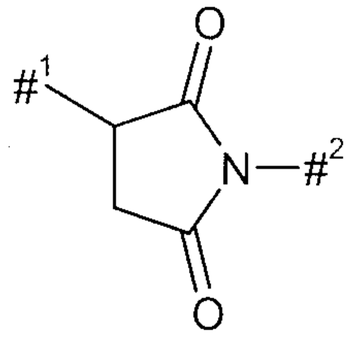
n stands for a number from 1 to 10,

AK stands for AK_1

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,

G stands for a group of the formula

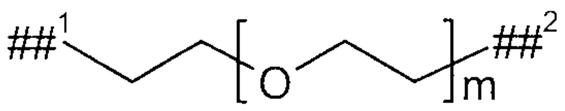


wherein

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

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

L^1 stands for a bond, linear $(C_3$-$C_5)$-alkanediyl or a group of the formula



wherein

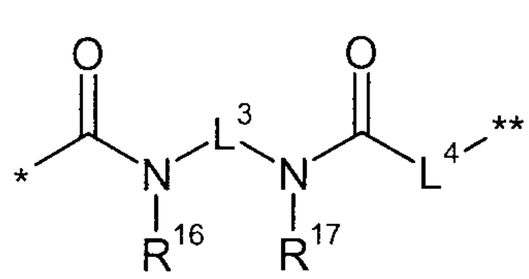
m stands for a number of 2 or 3,

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

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

wherein $(C_3$-$C_5)$-alkanediyl may be substituted with one or two methyl substituents,

B stands for a bond or a group of the formula



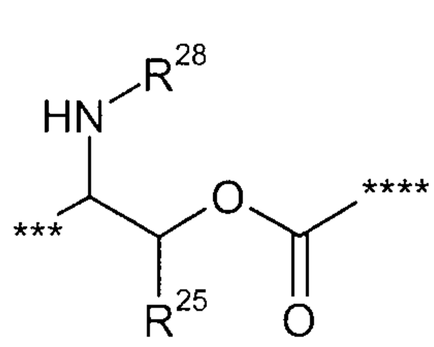
wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

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,

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

R^{25} stands for methyl,

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

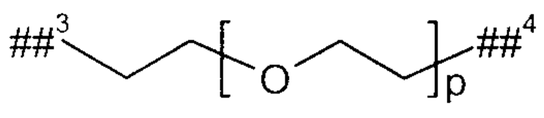
R^{16} stands for hydrogen or methyl,

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



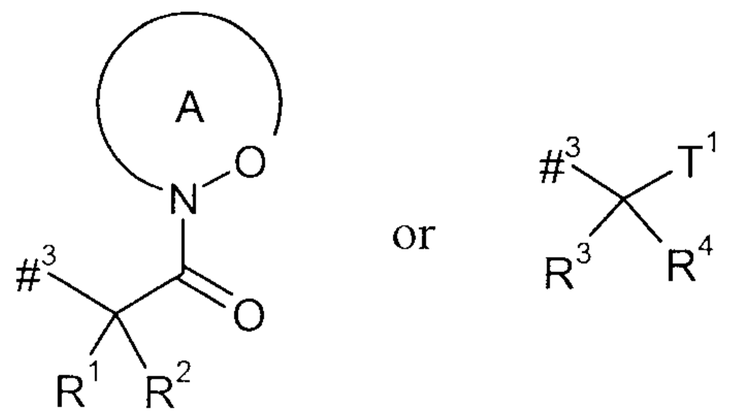
wherein

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,

D stands for a group of the formula



wherein

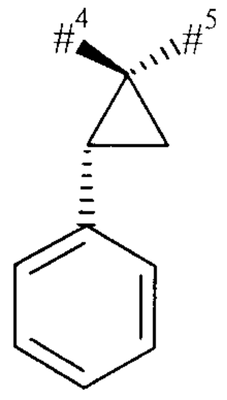
#3 denotes the linkage site to the nitrogen atom,

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

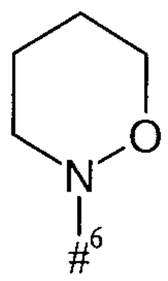


wherein

$\#^4$ 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

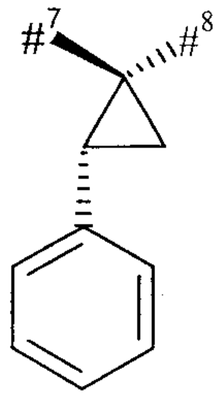
$\#^6$ denotes the linkage site to the carbonyl group,

R^3 stands for hydrogen,

R^4 stands for benzyl 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 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, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

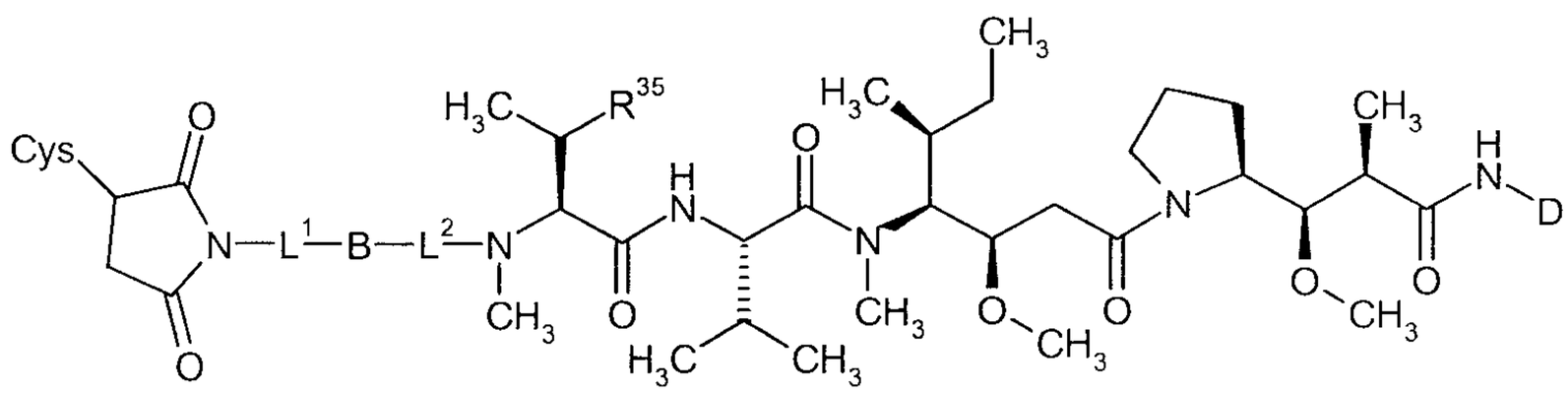
R^8 stands for hydrogen,

R^9 stands for hydrogen or benzyl,

R^{35} stands for methyl,

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

7. Compounds of formula (XXXa)

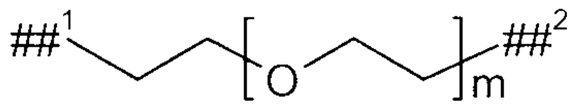


(XXXa),

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,

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



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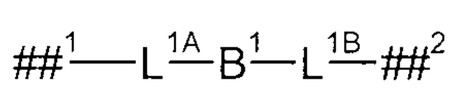


wherein

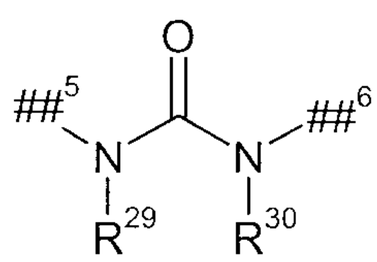
m stands for a number from 2 to 6,

$##^1$ 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_{10})-alkanediyl,

B^1 stands for a group of the formula



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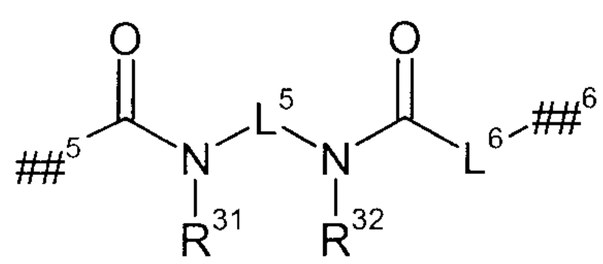


wherein

$##^5$ 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,

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,

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,

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

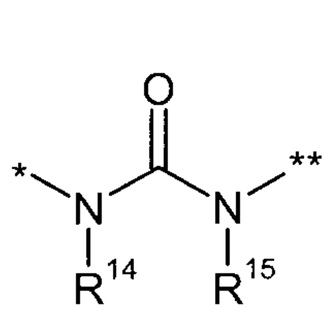
and

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

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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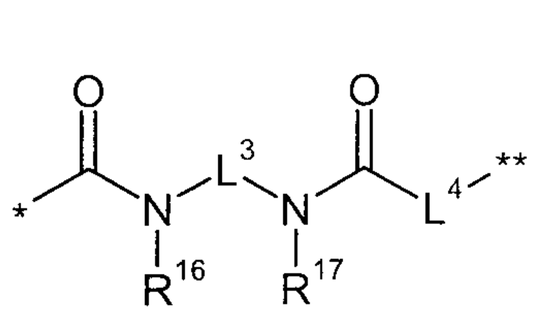
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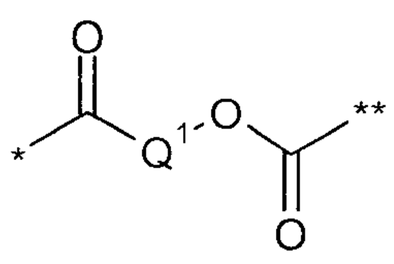
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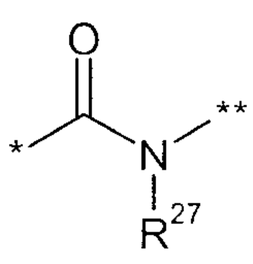


wherein

* 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,

R^{14} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

R^{15} stands for hydrogen or $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-alkyl,

R^{17} stands for hydrogen or $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-alkyl,

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 $(C_1$-$C_4)$-alkyl,

R^{22} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

or

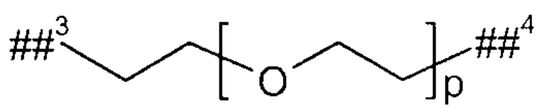
R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{23} stands for $(C_1\text{-}C_4)$-alkyl,

R^{24} stands for hydrogen or $(C_1\text{-}C_4)$-alkyl,

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

L^2 stands for linear $(C_2\text{-}C_{10})$-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,

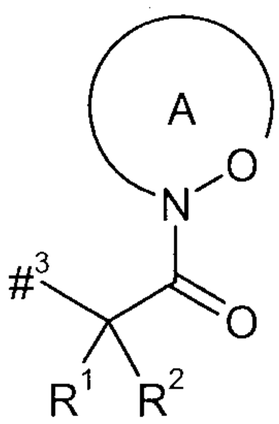
$##^4$ denotes the linkage site to the nitrogen atom,

wherein $(C_2\text{-}C_{10})$-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 $(C_3\text{-}C_6)$ cycloalkyl ring or a phenyl ring,

D stands for a group of the formula



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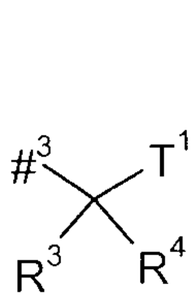

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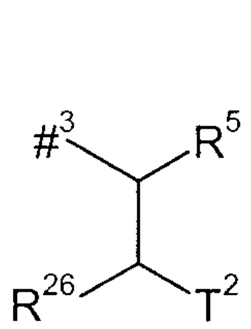


wherein

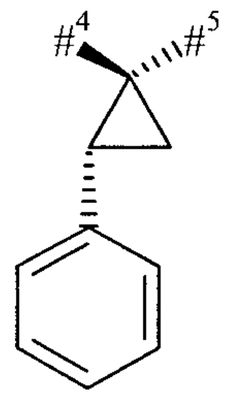
$\#^3$ denotes the linkage site to the nitrogen atom,

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-amino-benzyl, 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 (1S,2R)-2- phenylcyclopropane-1,1-diyl group of the formula

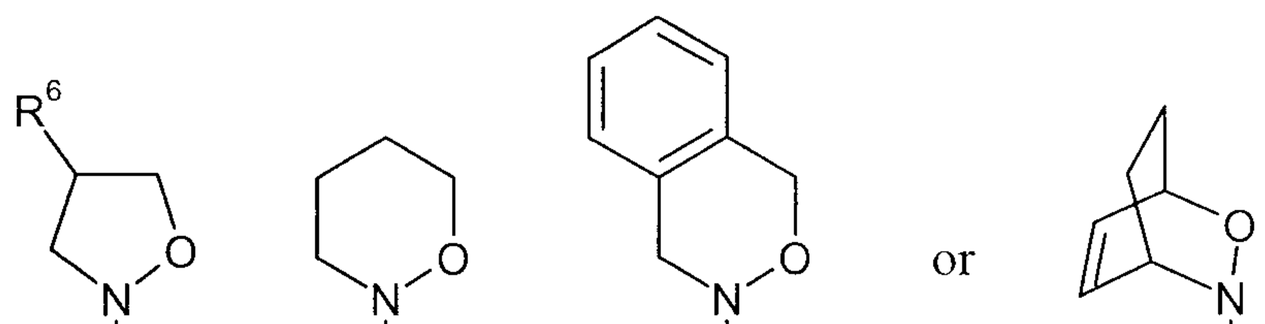


wherein

$\#^4$ 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,

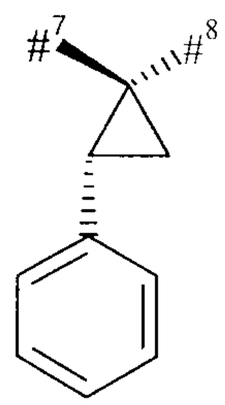
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

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, 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 (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 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,

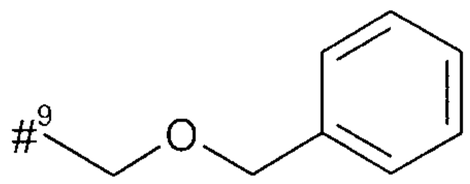
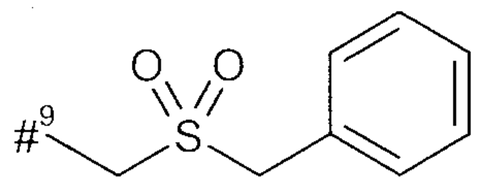
or

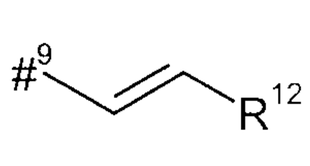
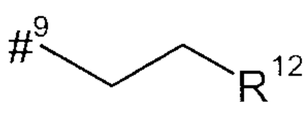
R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

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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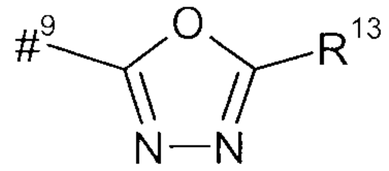


wherein

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

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^{26} stands for hydrogen or hydroxyl,

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

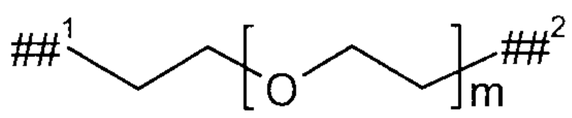
R^{35} stands for methyl or hydroxyl,

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

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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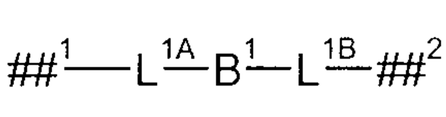


wherein

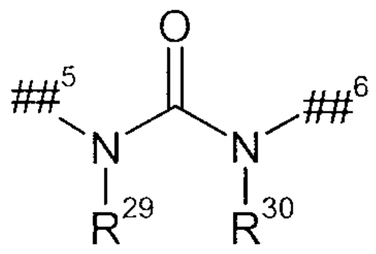
m stands for a number of 2 or 3,

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

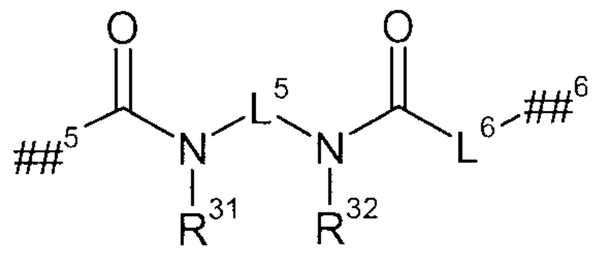
$##^2$ denotes the linkage site to the group B,

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

B^1 stands for a group of the formula



or



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,

L^6 stands for a bond,

R^{29} stands for hydrogen,

R^{30} stands for hydrogen,

R^{31} stands for hydrogen or methyl,

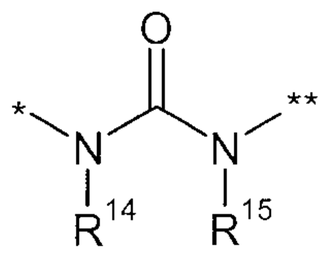
R^{32} stands for hydrogen or methyl,

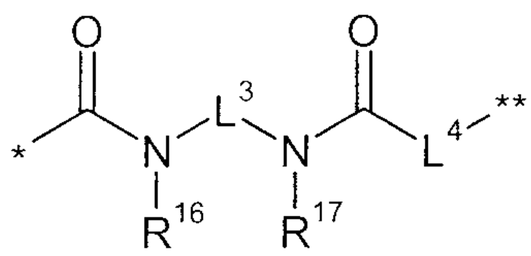
L^{1B} stands for linear (C_2-C_6)-alkanediyl,

and

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

B stands for a bond or a group of the formula





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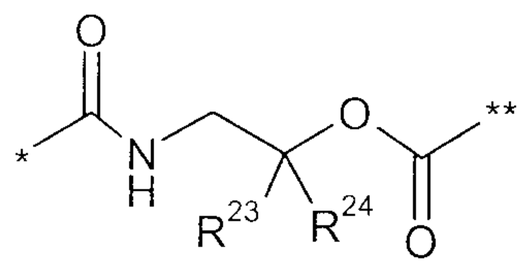


wherein

* 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,

R^{14} stands for hydrogen,

R^{15} stands for hydrogen,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

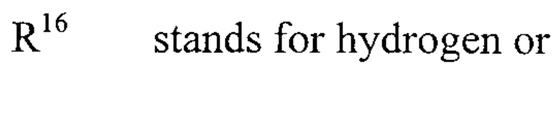
or

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,

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



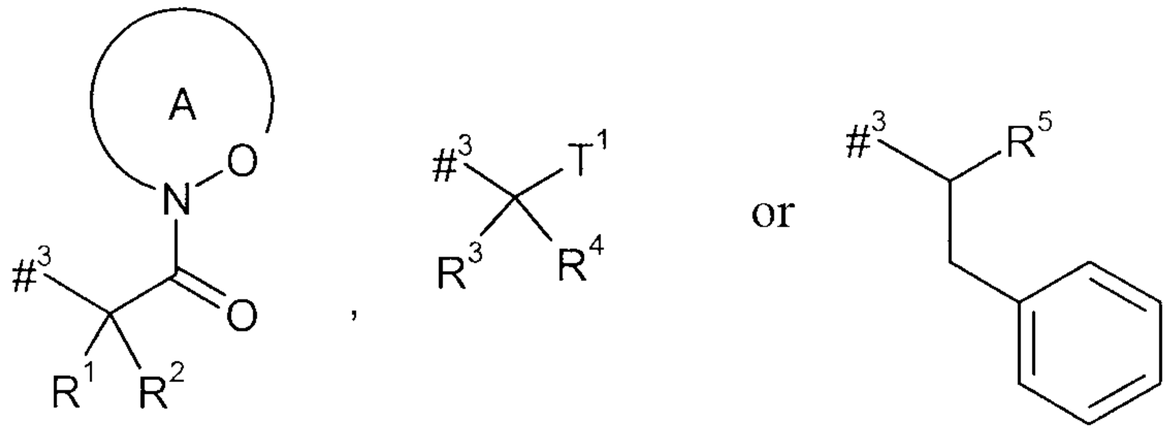
wherein

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



wherein

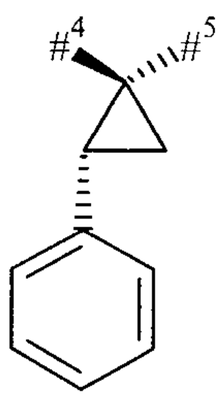
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

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

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

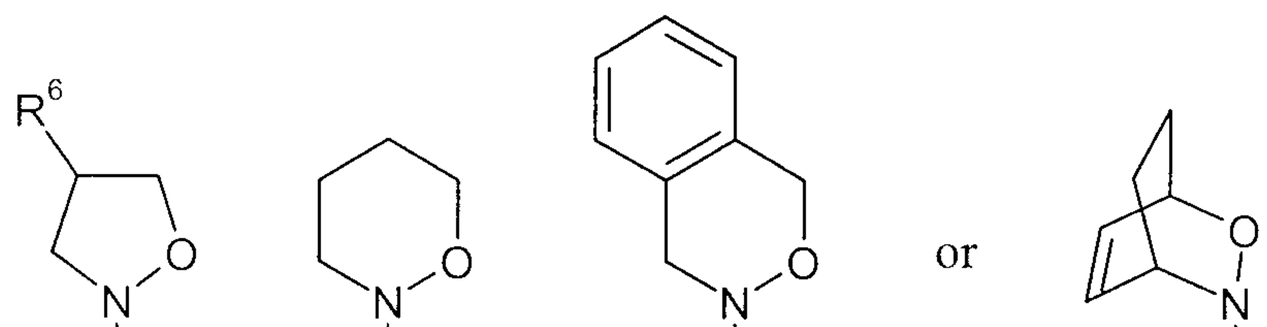


wherein

$\#^4$ 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,

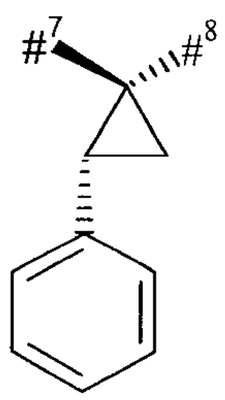
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 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 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,

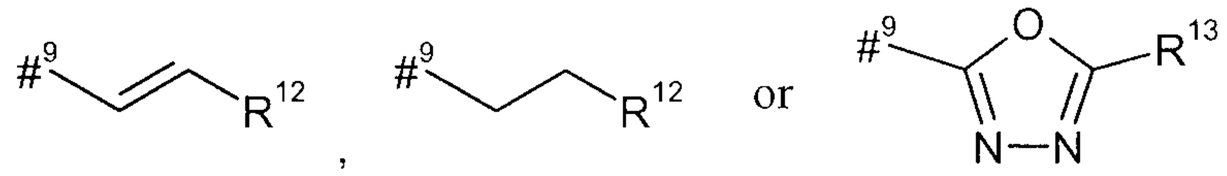
or

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



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)$_2$OH,

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

R^{35} stands for methyl or hydroxyl,

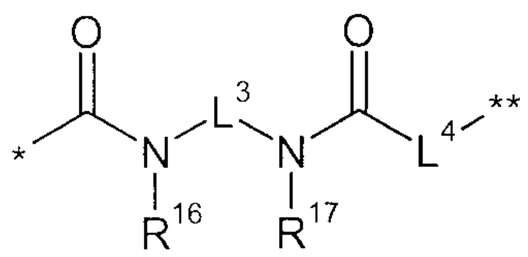
as well as their salts, solvates and solvates of the salts.

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 (C_2-C_6)-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,

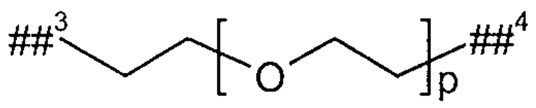
L^3 stands for a bond,

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



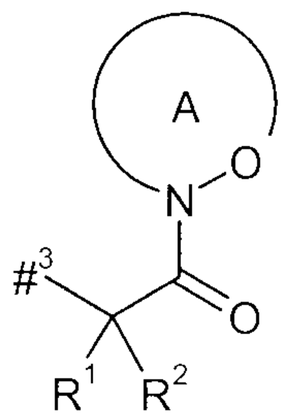
wherein

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



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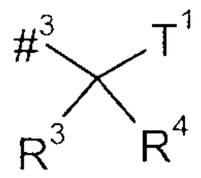


wherein

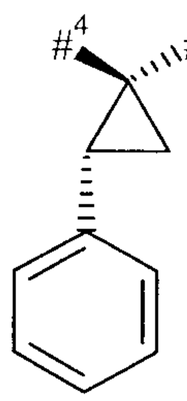
$\#^3$ denotes the linkage site to the nitrogen atom,

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

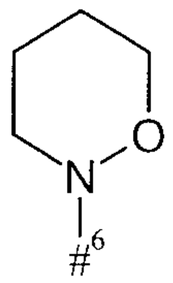


wherein

$\#^4$ 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

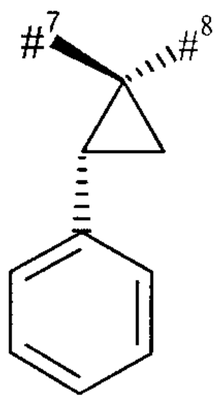
$\#^6$ denotes the linkage site to the carbonyl group,

R^3 stands for hydrogen,

R^4 stands for benzyl 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 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,

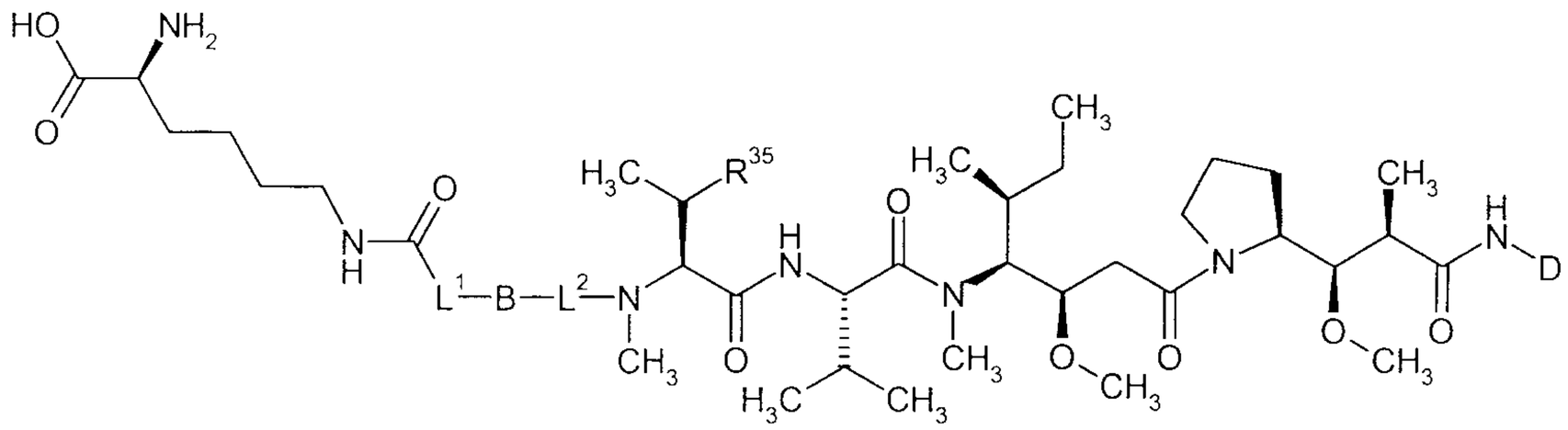
R^8 stands for hydrogen,

R^9 stands for hydrogen,

R^{35} stands for methyl,

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

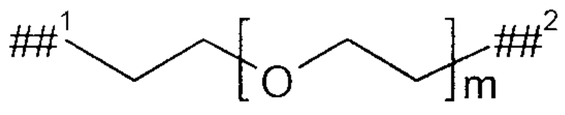
10. Compounds of formula (XXXI)



(XXXI),

in which

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



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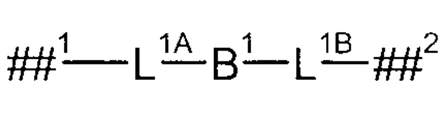


wherein

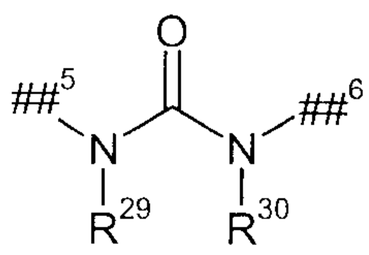
m stands for a number from 2 to 6,

$##^1$ 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_{10})-alkanediyl,

B^1 stands for a group of the formula



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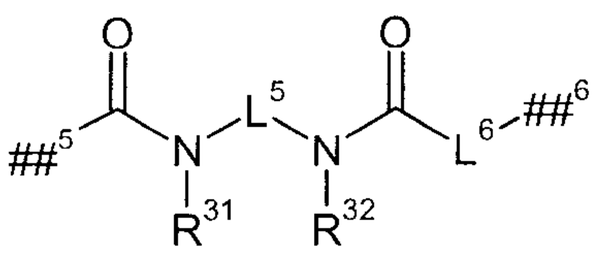


wherein

$##^5$ 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,

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,

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,

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

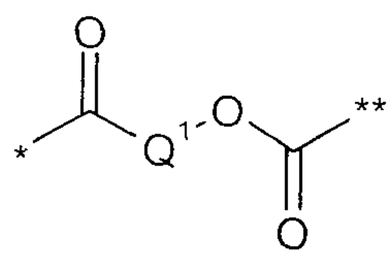
and

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

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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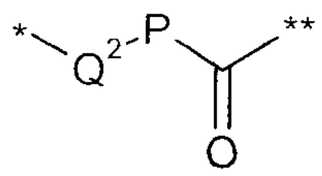
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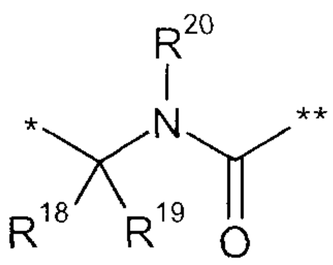
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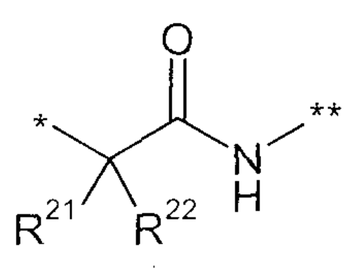
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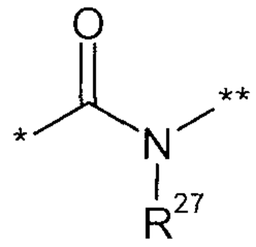


wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

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 (C_1-C_4)-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 (C_1-C_4)-alkyl,

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 (C_1-C_4)-alkyl,

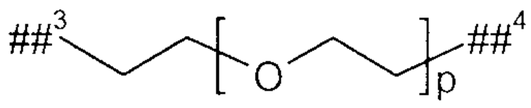
R^{22} stands for hydrogen or (C_1-C_4)-alkyl,

or

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

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



wherein

p stands for a number from 2 to 6,

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

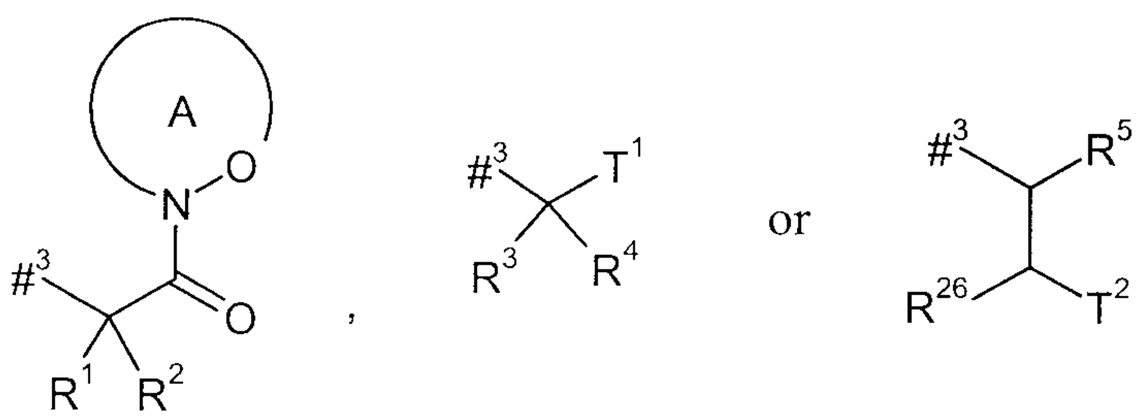
$\#\#^4$ denotes the linkage site to the nitrogen atom,

wherein $(C_2$-$C_{10})$-alkanediyl may be substituted with 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,

D stands for a group of the formula



wherein

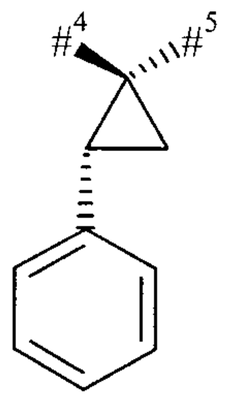
$\#^3$ denotes the linkage site to the nitrogen atom,

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-amino-benzyl, 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 (1S,2R)-2- phenylcyclopropane-1,1-diyl group of the formula

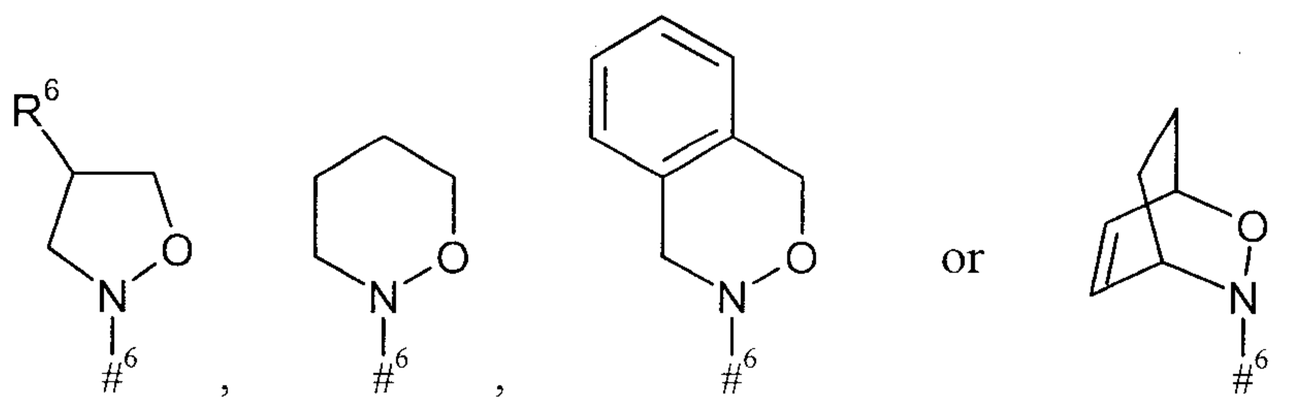


wherein

$\#^4$ 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,

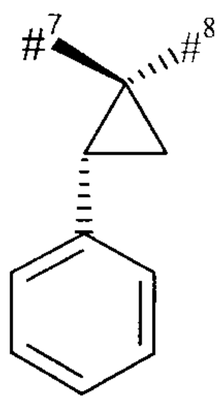
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen or methyl,

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, 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 (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 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,

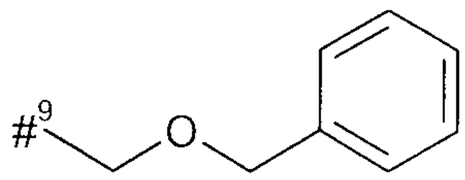
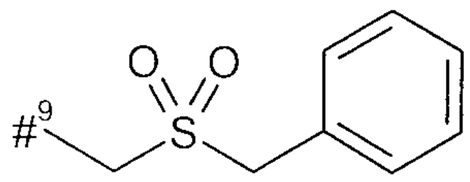
or

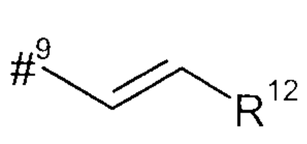
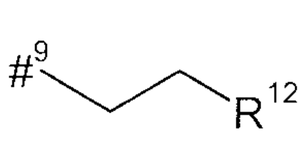
R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

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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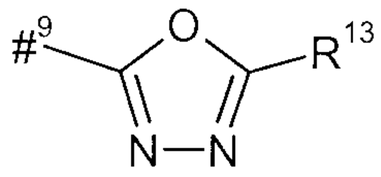


wherein

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

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^{26} stands for hydrogen or hydroxyl,

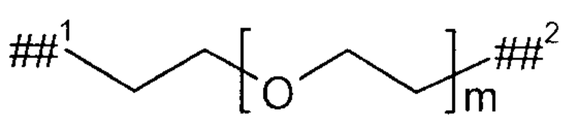
T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

R^{35} stands for methyl or hydroxyl,

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

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

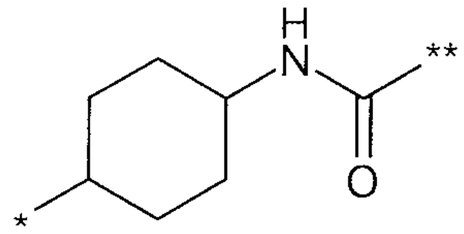
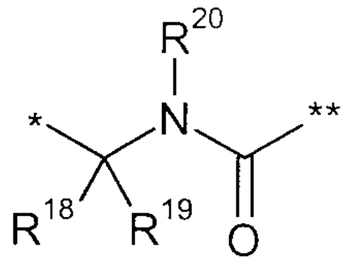
m stands for a number of 2 or 3,

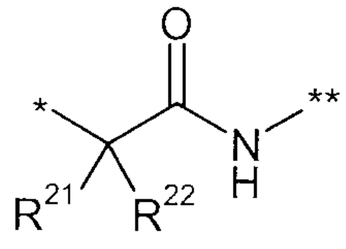
##[1] denotes the linkage site to the group G,

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

wherein (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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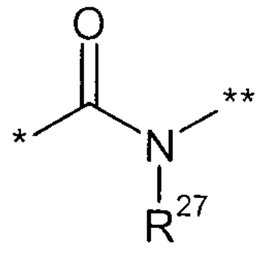


wherein

* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

R^{18} stands for hydrogen,

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

R^{20} stands for hydrogen or (C_1-C_4)-alkyl,

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,

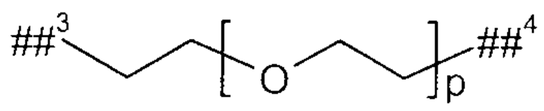
R^{22} stands for hydrogen or methyl,

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,

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,

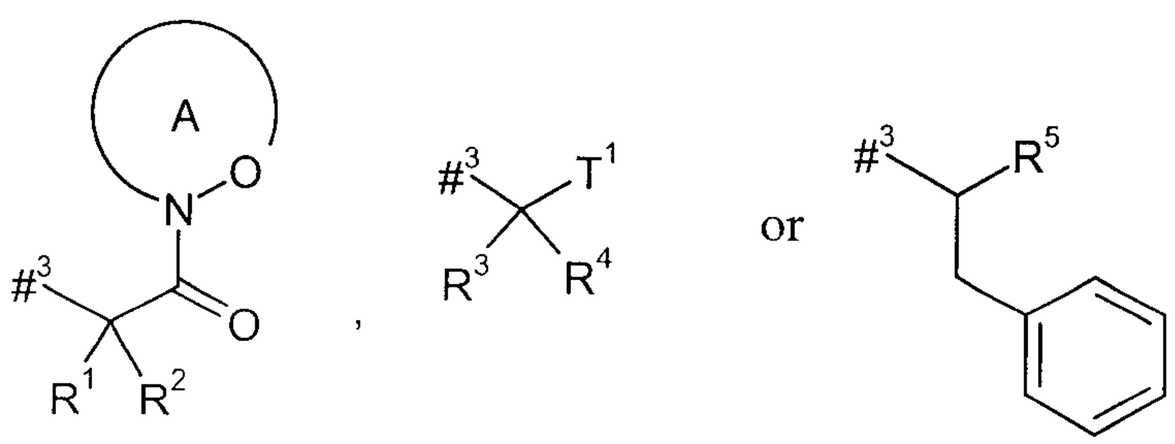
$\#\#^4$ denotes the linkage site to the nitrogen atom,

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,

D stands for a group of the formula



wherein

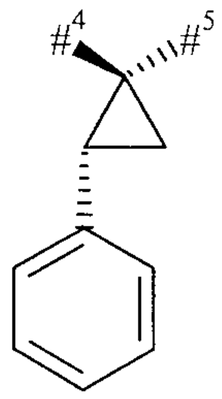
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 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

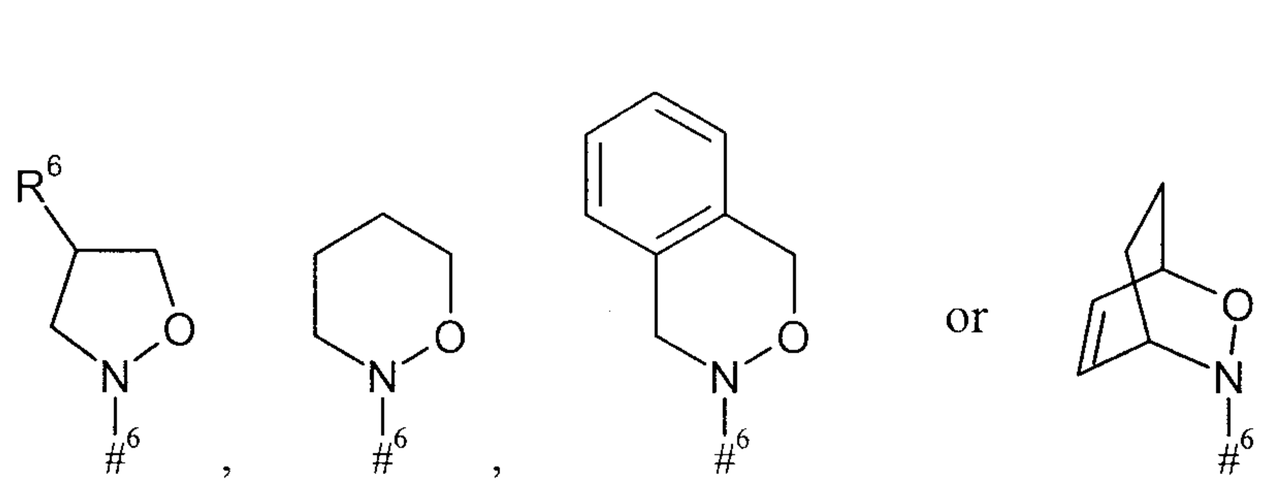


wherein

$\#^4$ 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,

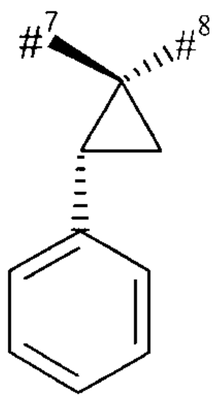
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 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 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,

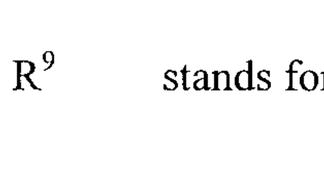
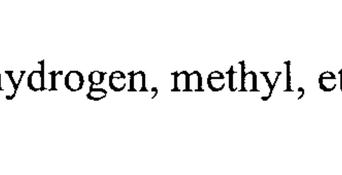
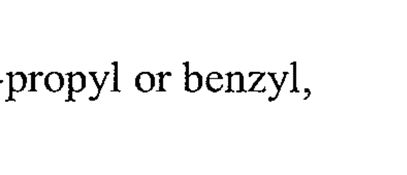
or

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


,  or 

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,

R^{35} stands for methyl or hydroxyl,

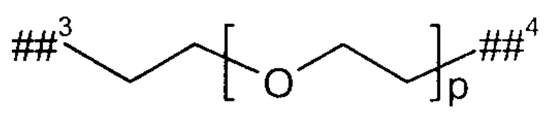
as well as their salts, solvates and solvates of the salts.

12. Compounds of formula (XXXI) according to claims 10 and 11, wherein

L^1 stands for a bond,

B stands for a bond,

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



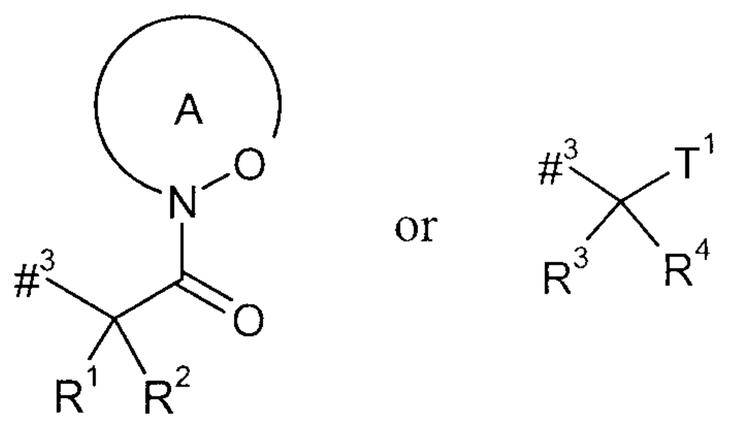
wherein

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



wherein

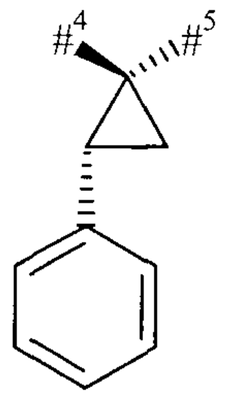
$\#^3$ denotes the linkage site to the nitrogen atom,

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

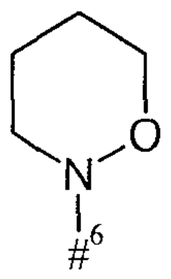


wherein

$\#^4$ 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,

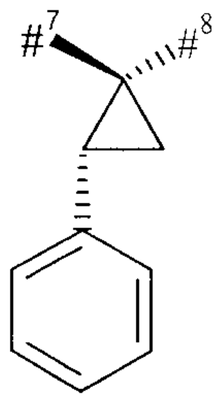
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stand for benzyl 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 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,

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.

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,

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-

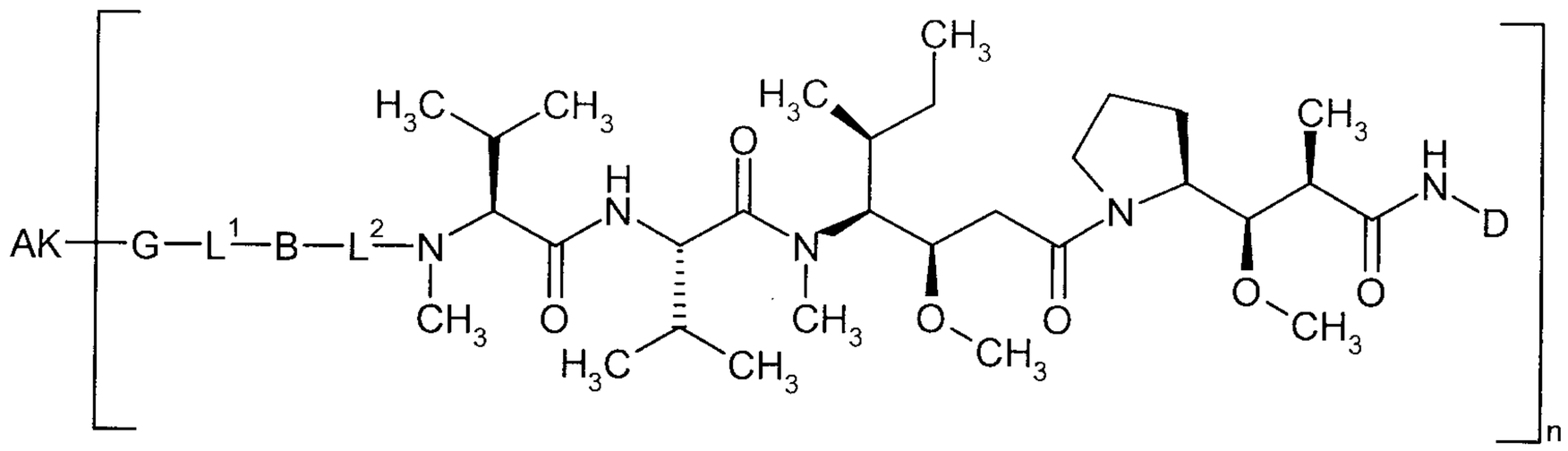
-oxazinan-2-yl)-1-oxopropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin--1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide,

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,

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)



(I),

in which

n stands for a number from 1 to 50,

AK stands for a bond,

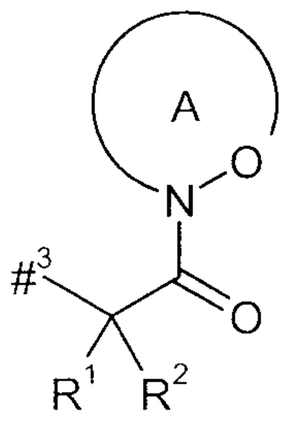
the group §-G-L^1-B-L^2-§§ stands for a linker,

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



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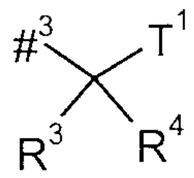

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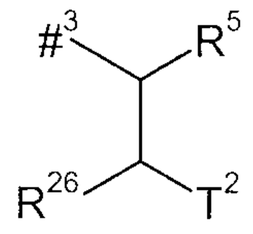


wherein

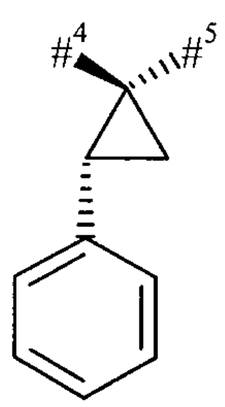
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-yl-methyl,

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

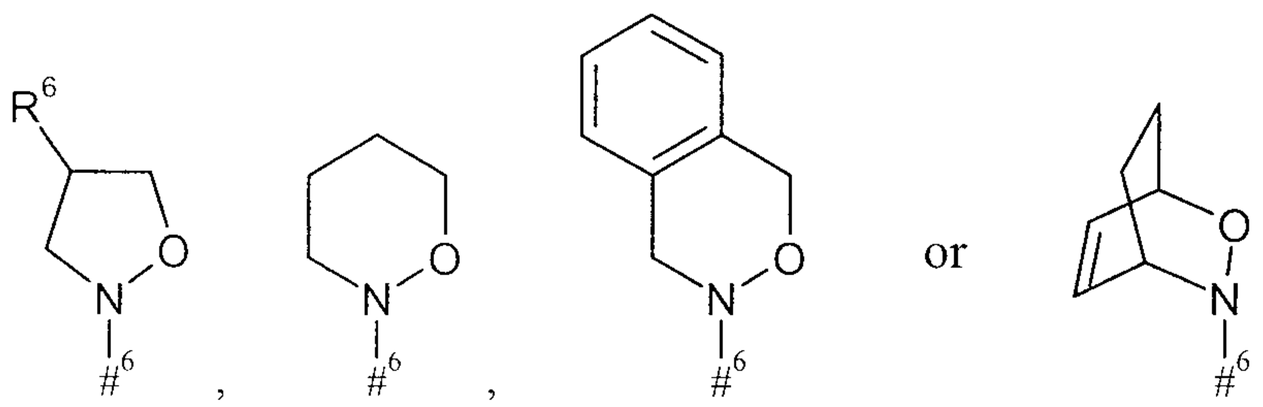


wherein

$\#^4$ 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,

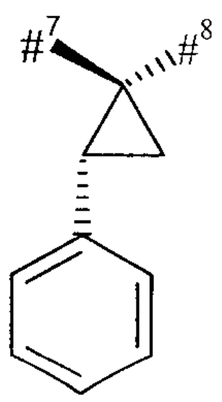
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-yl-methyl,

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 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,

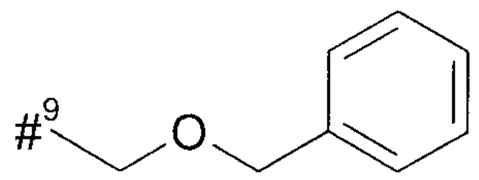
or

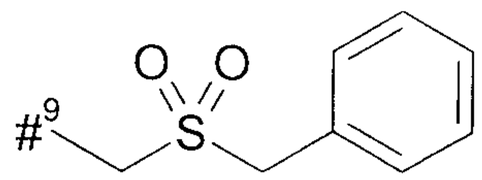
R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

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





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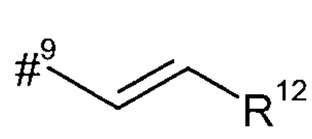

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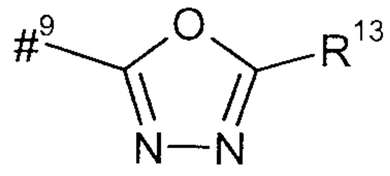


wherein

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

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^{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.

15. Binder-drug conjugates according to claim 14 of general formula (I),

in which

n stands for a number from 1 to 50,

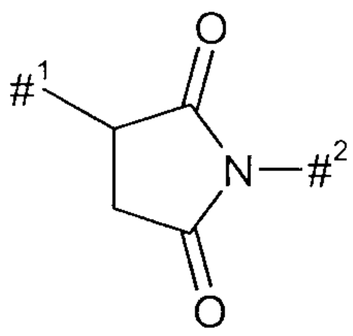
AK stands for AK_1 or AK_2

wherein

AK_1 stands for a binder bound to the group G via a sulfur atom of the binder,

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



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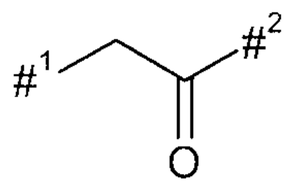


wherein

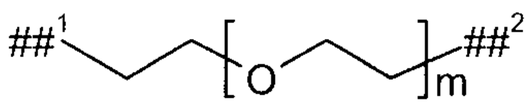
$\#^1$ denotes the linkage site to the sulfur atom of the binder,

$\#^2$ 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_1-C_{10})-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,

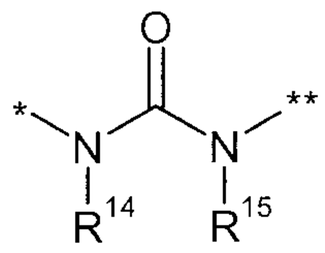
$\#\#^2$ denotes the linkage site to the group B,

wherein $(C_1\text{-}C_{10})$-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 $(C_3\text{-}C_6)$-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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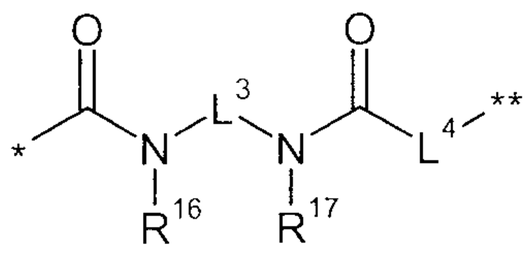
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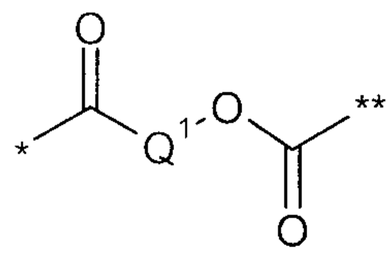
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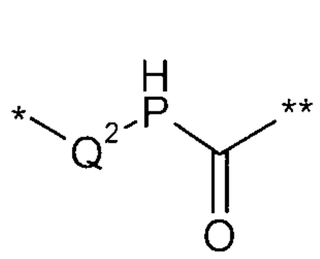
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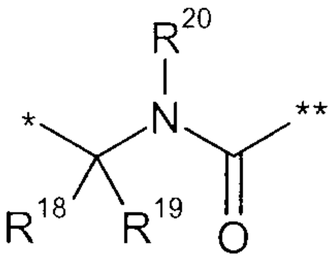
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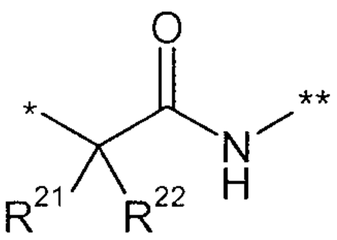
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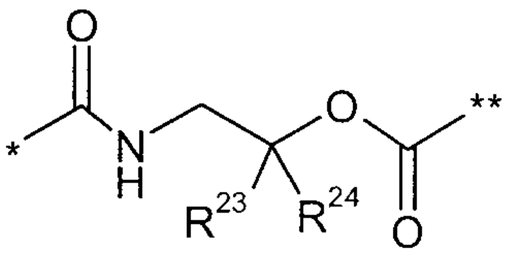
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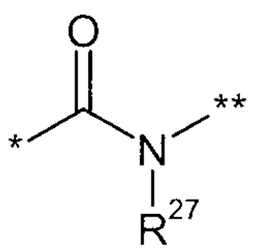


wherein

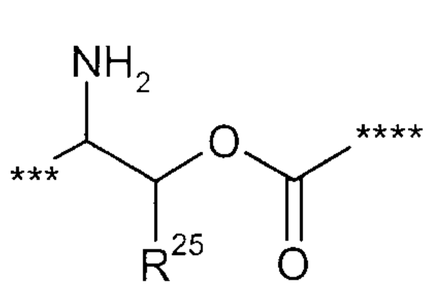
* denotes the linkage site to L^1,

** denotes the linkage site to L^2,

P stands for O or NH,

L^3 for a bond or $(C_2\text{-}C_4)$-alkanediyl,

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



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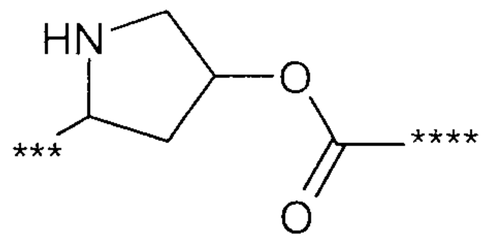


wherein

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

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

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 (C_1-C_4)-alkyl,

R^{15} stands for hydrogen or (C_1-C_4)-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 (C_1-C_4)-alkyl,

R^{17} stands for hydrogen or (C_1-C_4)-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 (C_1-C_4)-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 (C_1-C_4)-alkyl,

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 (C_1-C_4)-alkyl,

R^{22} stands for hydrogen or (C_1-C_4)-alkyl,

or

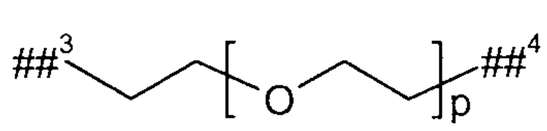
R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{23} stands for (C_1-C_4)-alkyl,

R^{24} stands for hydrogen or (C_1-C_4)-alkyl,

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



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 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 (C_3-C_6) 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,

AK stands for AK_1 or AK_2

wherein

AK_1 stands for an antibody or an antigen-binding antibody fragment and is bound to the group G via a sulfur atom,

AK_2 stands for an antibody or an antigen-binding antibody fragment and is bound to the group G via a nitrogen atom,

n, G, L^1, B, L^2 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

n stands for a number from 1 to 20,

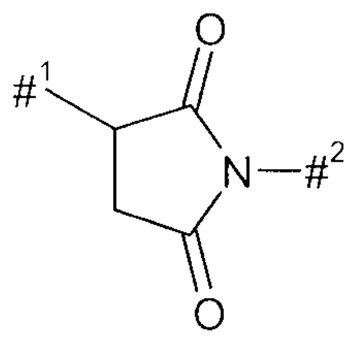
AK stands for AK_1 or AK_2

wherein

AK_1 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,

AK_2 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 = AK_1 stands for a group of the formula



wherein

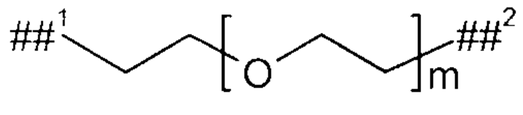
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

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

or

for the case when AK = AK_2, G stands for carbonyl,

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



wherein

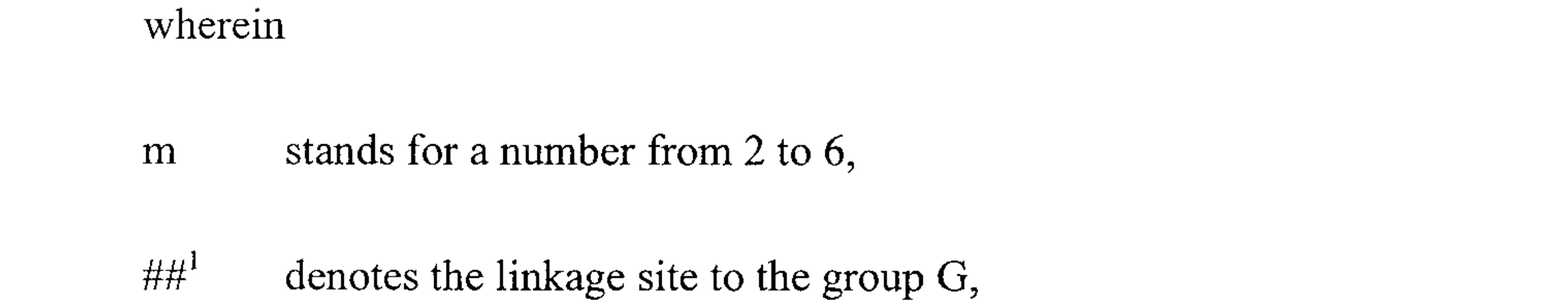
m stands for a number from 2 to 6,

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

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

wherein (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



wherein

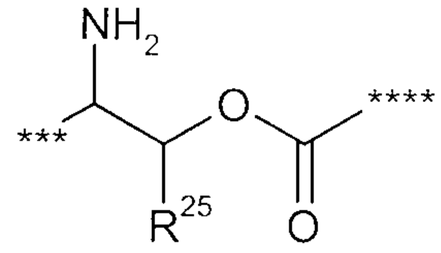
* denotes the linkage site to L^1,

** 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



wherein

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

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

R^{25} stands for methyl,

Q^2 stands for cyclopentyl or cyclohexyl,

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^{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,

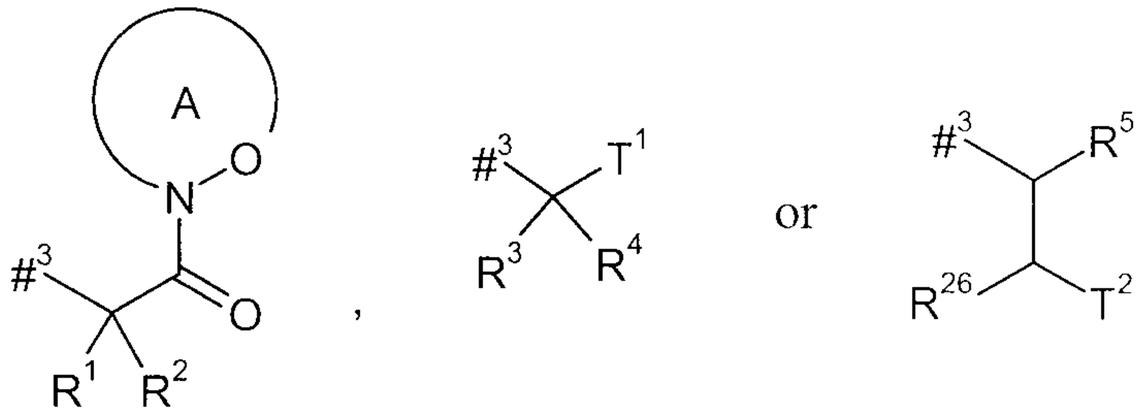
or

R^{19} and R^{20} together with the atoms to which they are bound 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

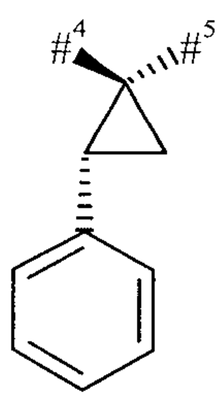
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-yl-methyl,

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

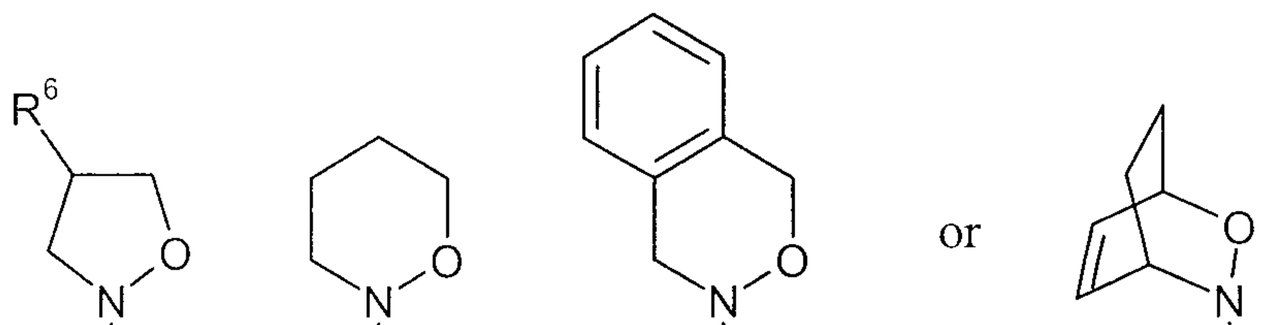


wherein

$\#^4$ 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,

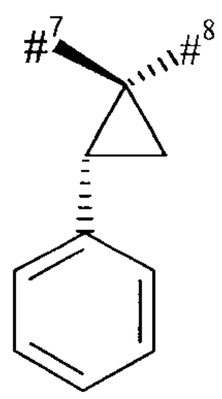
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-yl-methyl,

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 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,

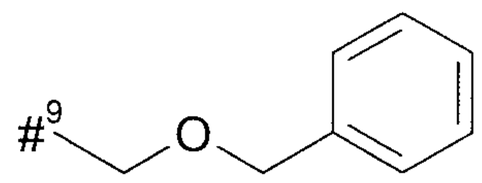
or

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



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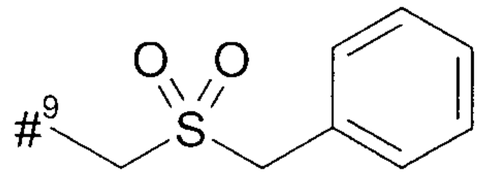

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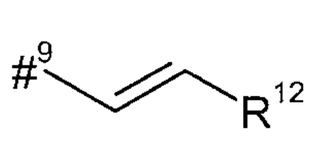

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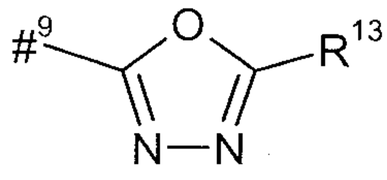


wherein

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

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^{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,

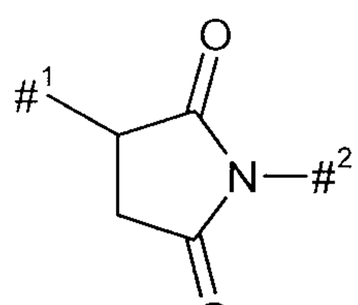
AK stands for AK_1 or AK_2

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,

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,

$\#^2$ 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 or stands for a group of the formula

wherein

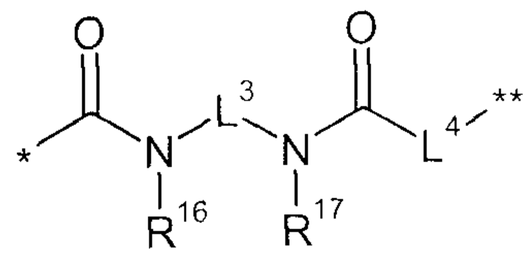
m stands for a number of 2 or 3,

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

##[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



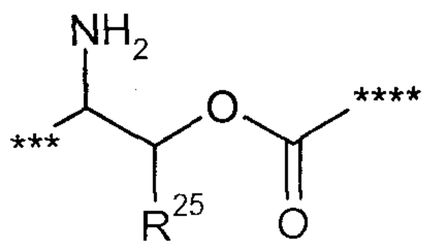
wherein

* 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



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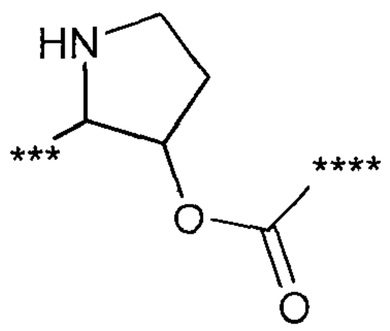


wherein

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

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

R^{25} stands for methyl,

R^{16} stands for hydrogen or methyl,

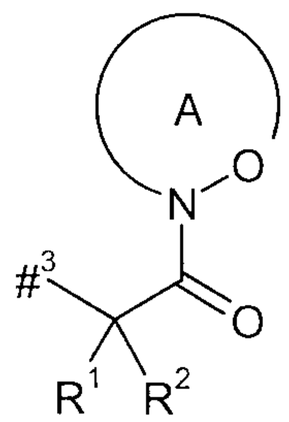
R^{17} stands for hydrogen or methyl,

or

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

L^2 stands for linear $(C_2$-$C_6)$-alkanediyl,

D stands for a group of the formula



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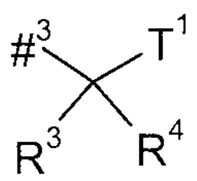

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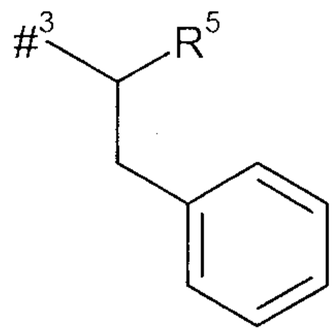


wherein

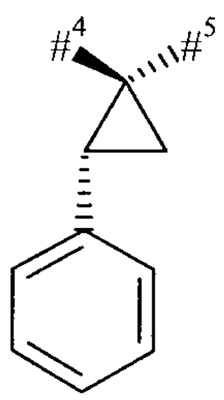
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-yl-methyl,

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

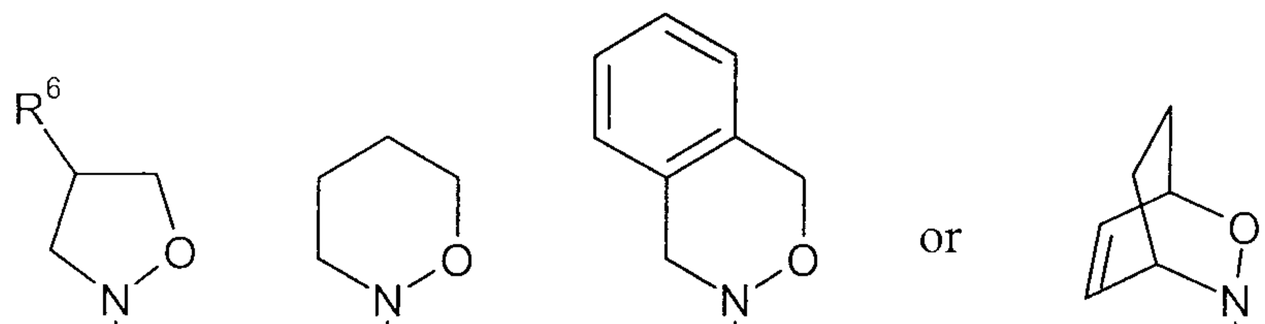


wherein

$\#^4$ 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,

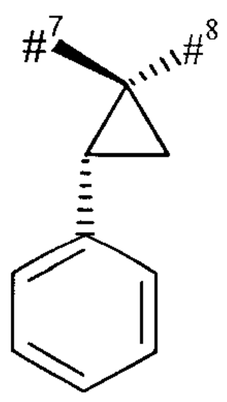
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 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

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

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

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

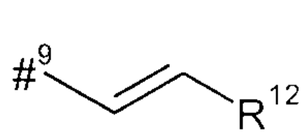
R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

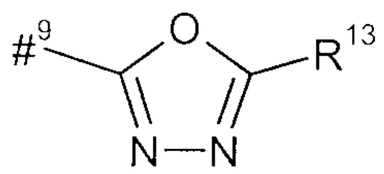
R^9 stands for hydrogen, methyl, ethyl, *n*-propyl or benzyl,

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

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



or



wherein

$\#^9$ denotes the linkage site to –CHC(R^{26})phenyl,

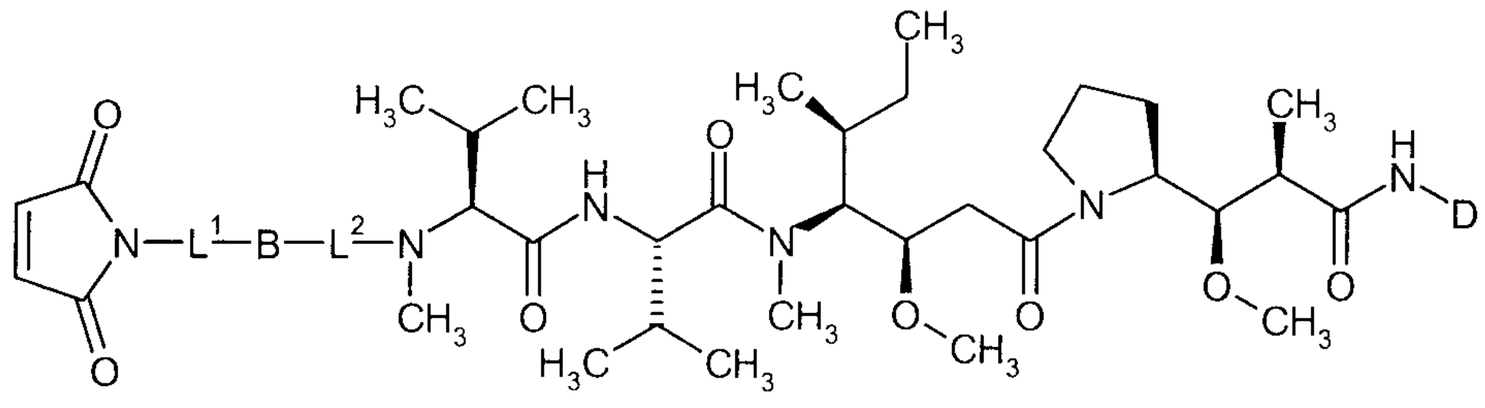
R^{12} stands for phenyl, which may be substituted with methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

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

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 14 to 18, characterized in that a solution of the binder in a buffer

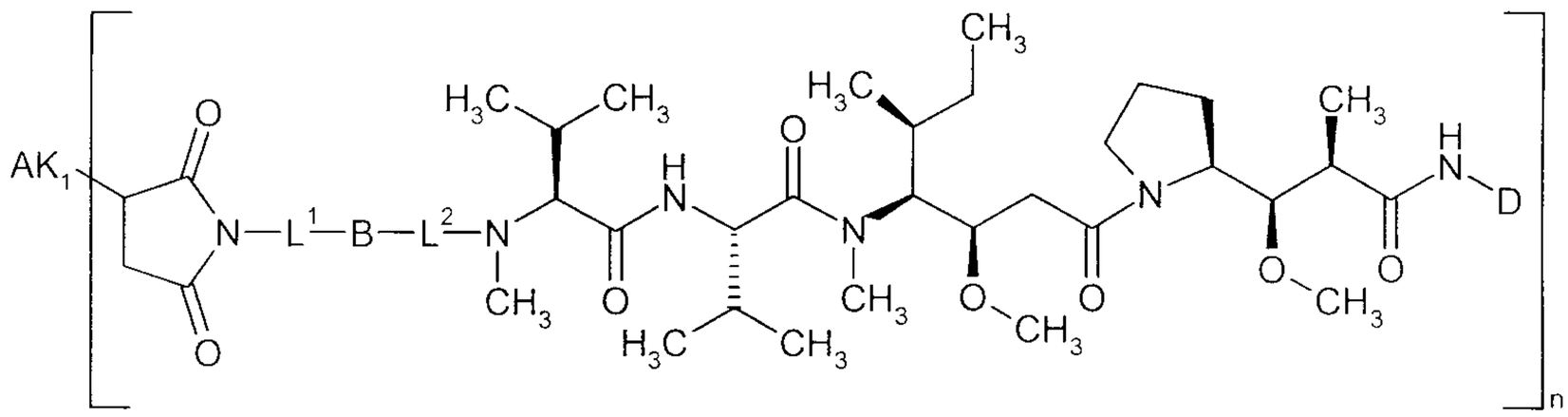
[A] is mixed with a suitable reducing agent such as, for example, dithiothreitol or *tris*-(2-carboxyethyl)phosphine hydrochloride and then is reacted with a compound of formula (II)



(II),

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)

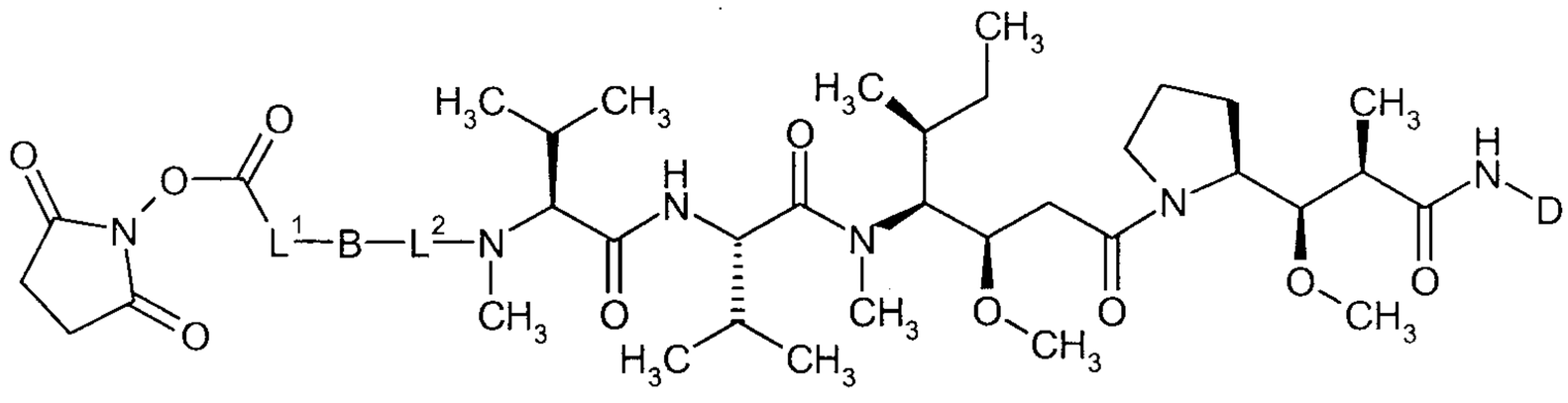


(I-A),

in which n, AK_1, D, L^1, B and L^2 each have the meanings given in claims 14 through 18,

or

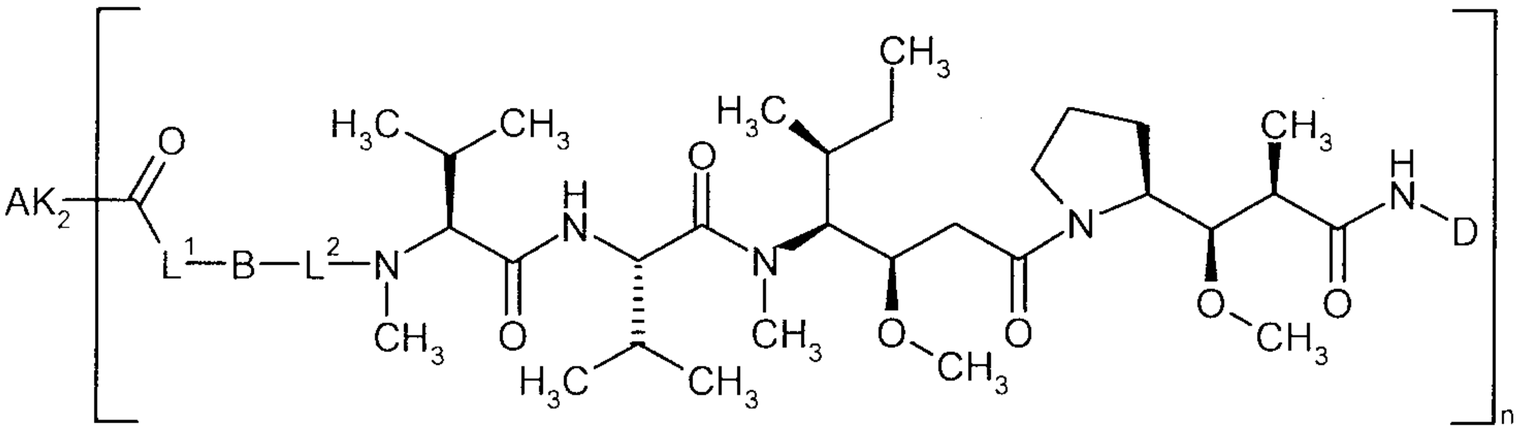
[B] reacting it with a compound of formula (III)



(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)

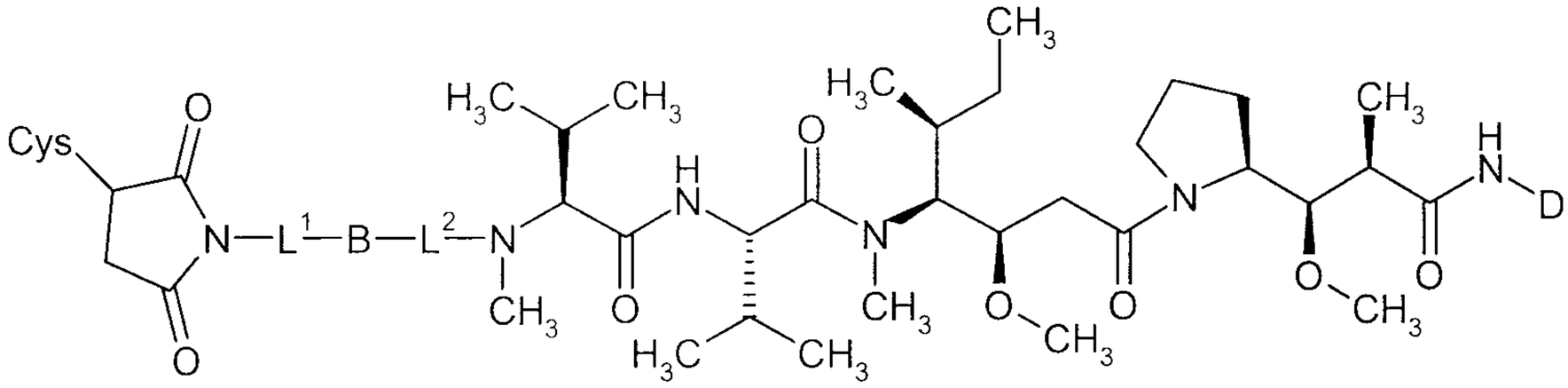


(I-B),

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

21. Compounds of formula (XXX)

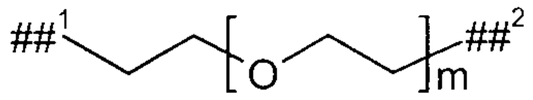


(XXX),

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,

L^1 stands for a bond, linear (C_1-C_{10})-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,

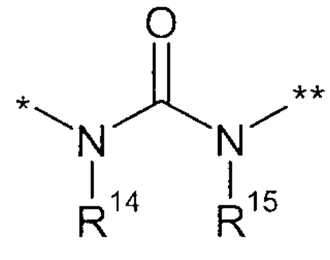
$##^2$ denotes the linkage site to the group B,

wherein (C_1-C_{10})-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 (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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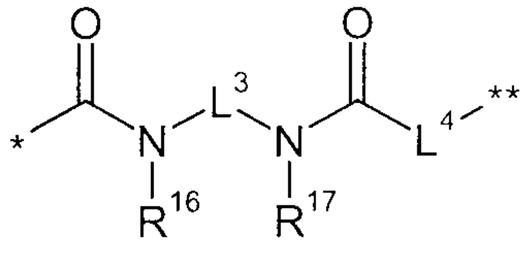
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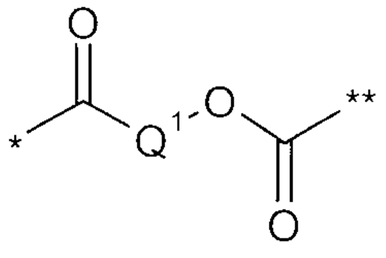
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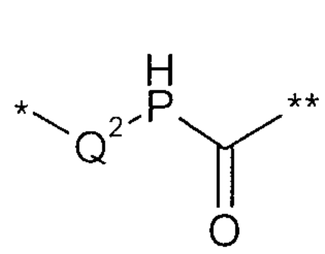
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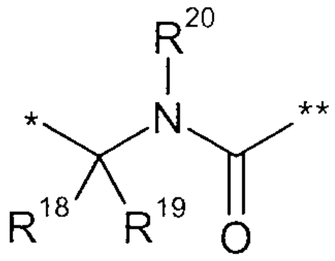
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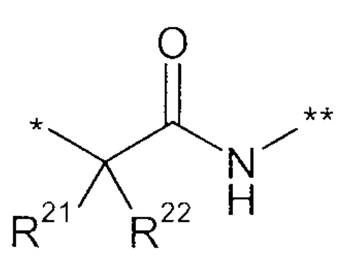
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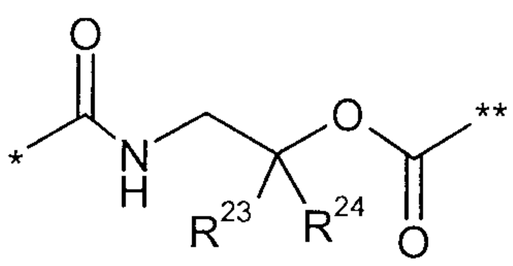
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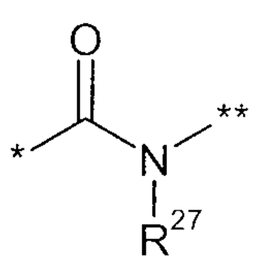


wherein

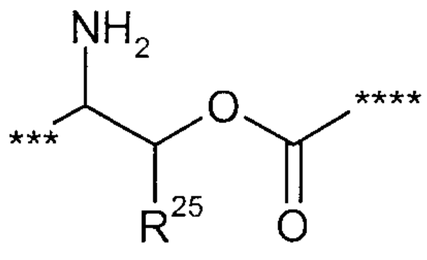
* denotes the linkage site to L^1,

** 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 or a group of the formula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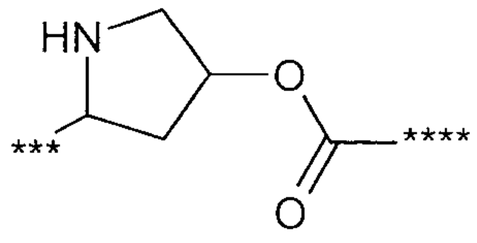


wherein

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

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

R^{25} stands for hydrogen or methyl,

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 $(C_1$-$C_4)$-alkyl,

R^{15} stands for hydrogen or $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-alkyl,

R^{17} stands for hydrogen or $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-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 $(C_1$-$C_4)$-alkyl,

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 $(C_1$-$C_4)$-alkyl,

R^{22} stands for hydrogen or $(C_1$-$C_4)$-alkyl,

or

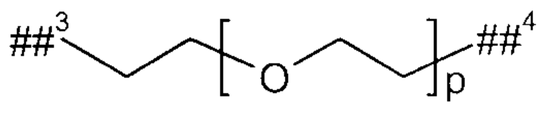
R^{21} and R^{22} together with the atoms to which they are bound form a three- to seven-membered carbocycle,

R^{23} stands for $(C_1\text{-}C_4)$-alkyl,

R^{24} stands for hydrogen or $(C_1\text{-}C_4)$-alkyl,

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

L^2 stands for linear $(C_2\text{-}C_{10})$-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,

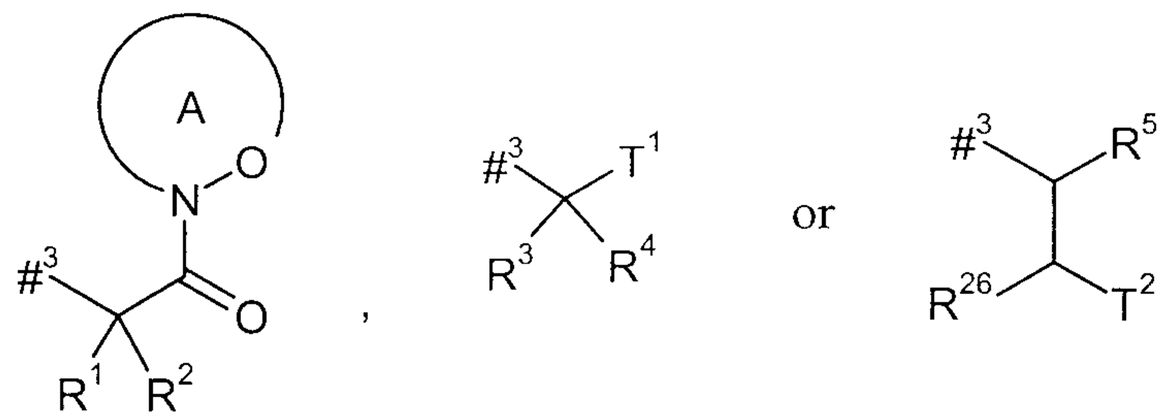
$##^4$ denotes the linkage site to the nitrogen atom,

wherein $(C_2\text{-}C_{10})$-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 $(C_3\text{-}C_6)$ cycloalkyl ring or a phenyl ring.

D stands for a group of the formula



wherein

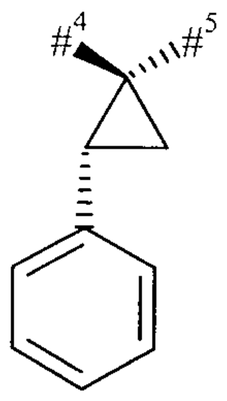
$#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-yl-methyl,

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

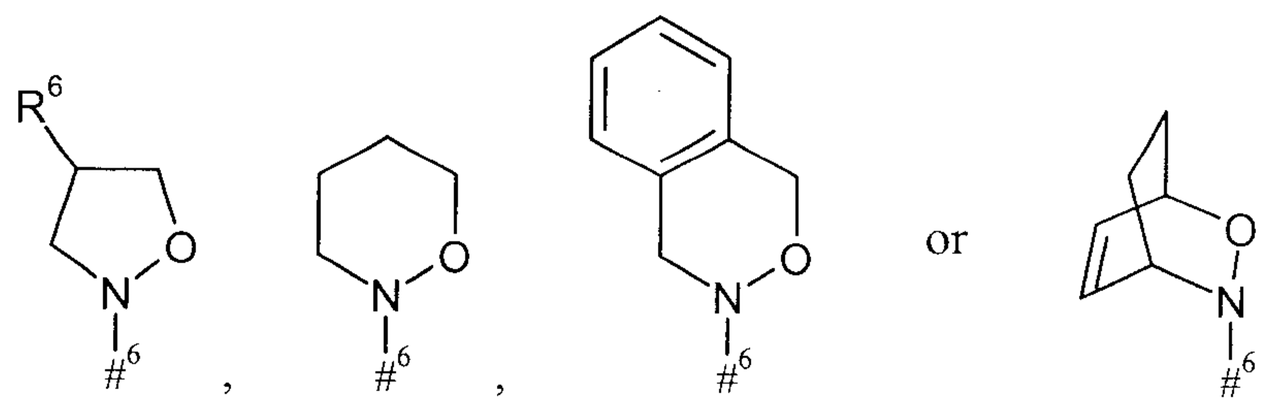


wherein

$\#^4$ 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,

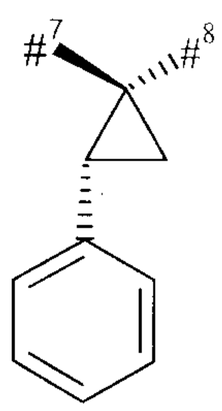
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-yl-methyl,

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 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,

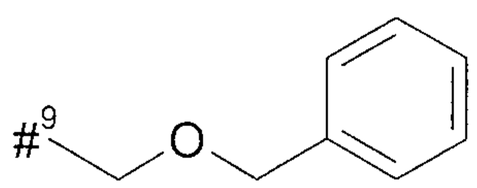
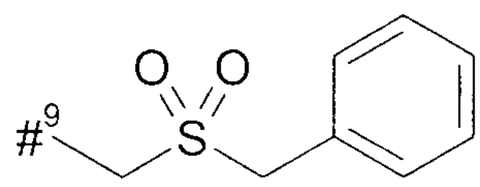
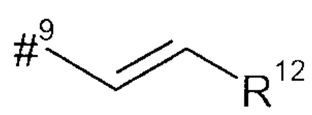
or

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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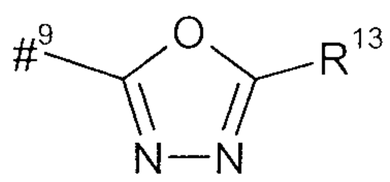


wherein

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

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^{26} stands for hydrogen or hydroxyl,

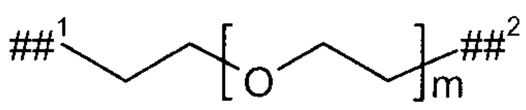
T^2 stands for phenyl, benzyl, 1H-indol-3-yl or 1H-indol-3-ylmethyl,

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

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 (C_2-C_6)-alkanediyl or stands for a group of the formula



wherein

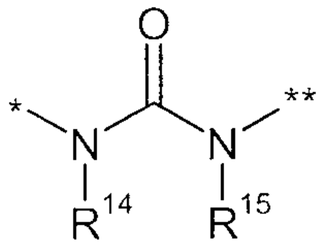
m stands for a number from 2 to 6,

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

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

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

B stands for a bond or a group of the formula



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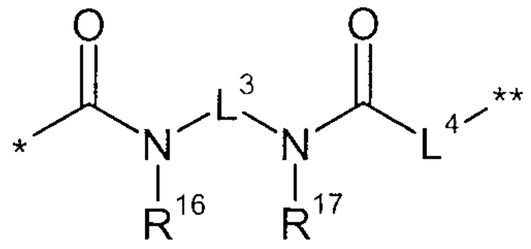

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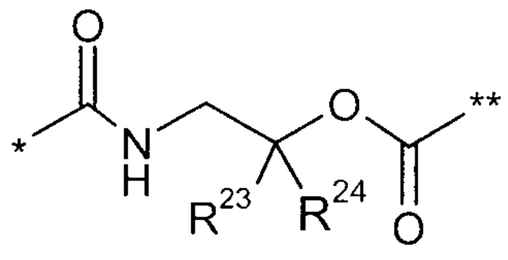


wherein

* 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,

R^{14} stands for hydrogen,

R^{15} stands for hydrogen,

R^{16} stands for hydrogen or methyl,

R^{17} stands for hydrogen or methyl,

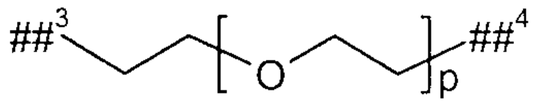
or

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,

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



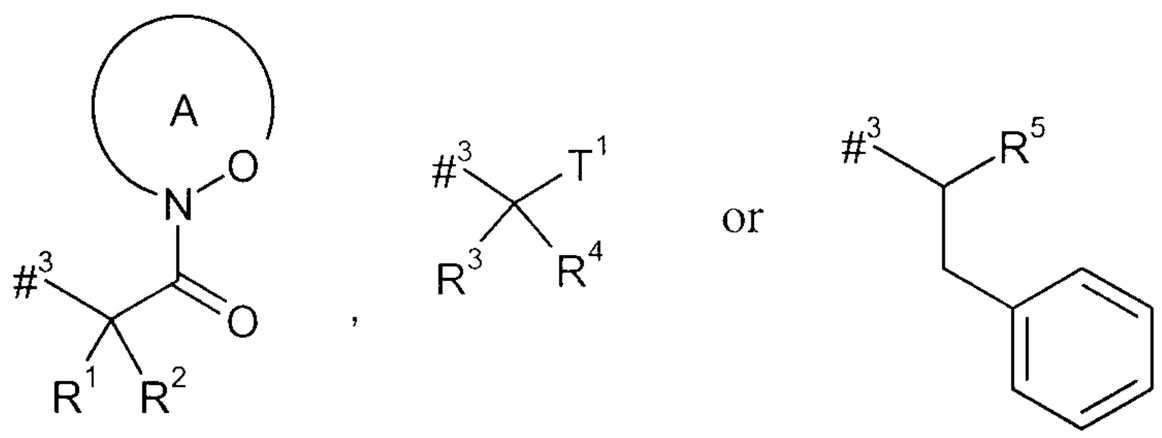
wherein

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



wherein

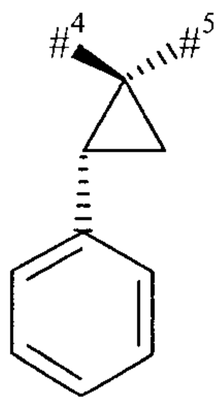
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R2 stands for 1-hydroxyethyl, benzyl, 1-phenylethyl or 1H-indol-3-yl-methyl,

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

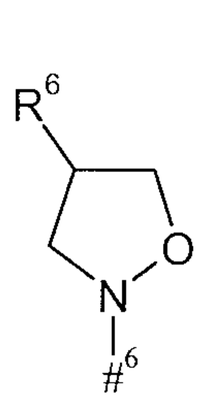


wherein

$\#^4$ 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



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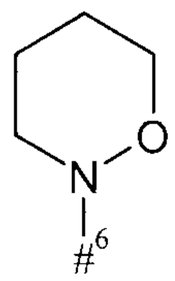

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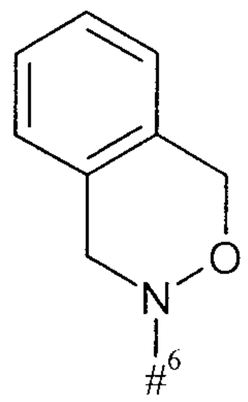

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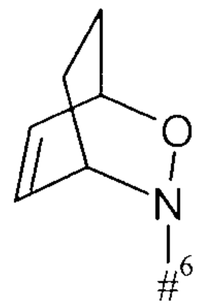


wherein

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

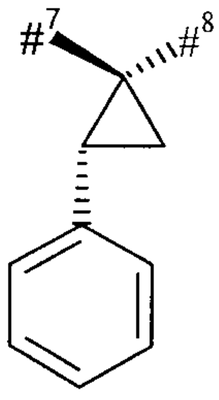
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 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

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

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

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, benzyl or adamantylmethyl,

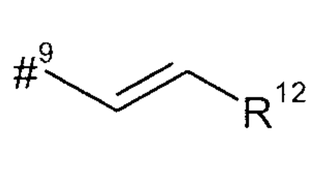
R^8 stands for hydrogen or methyl,

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

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 or a group of the formula



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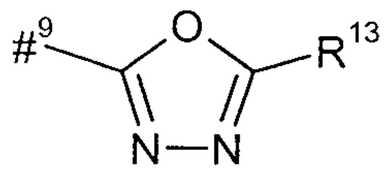


wherein

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

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), carboanhydrase 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), carboanhydrase IX (CA IX; NP_001207.2)), glypican-3, TYRP1, 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, 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, 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 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 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.

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,

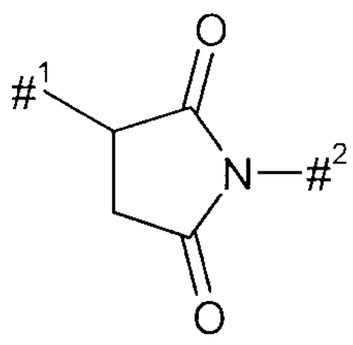
AK stands for AK_1 or AK_2

wherein

AK_1 stands for a binder bound to the group G via a sulfur atom of the binder,

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

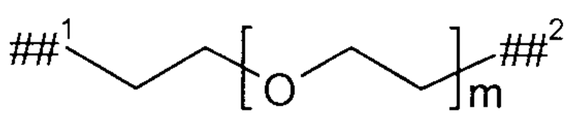
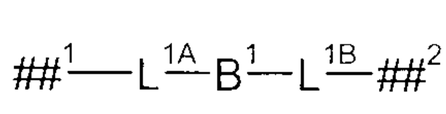
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ 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

 or 

wherein

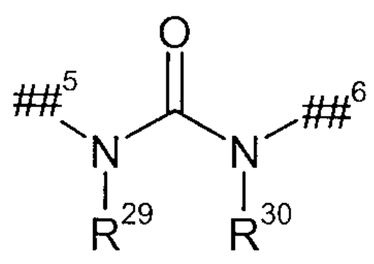
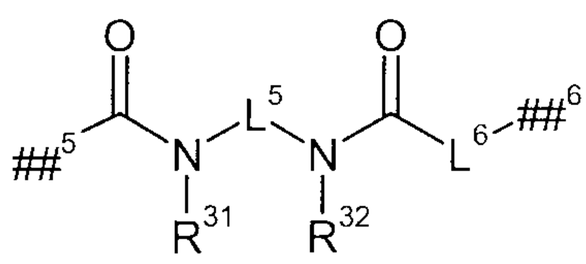
m stands for a number from 2 to 6,

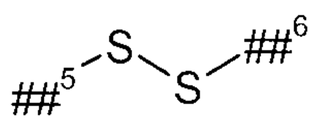
$\#\#^1$ denotes the linkage site to the group G,

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

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

B1 stands for a group of the formula

 , 

or 

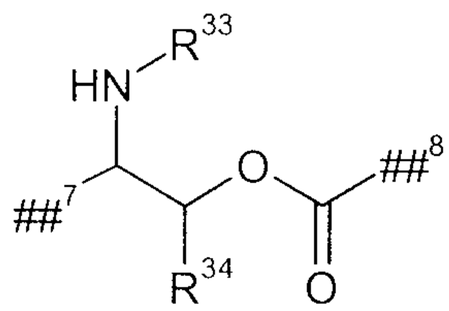
wherein

$##^5$ 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,

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



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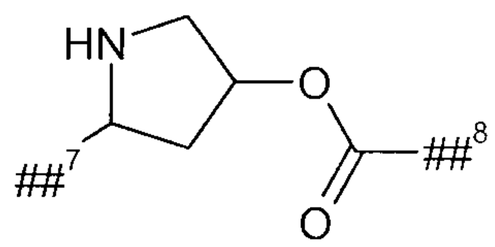


wherein

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

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

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

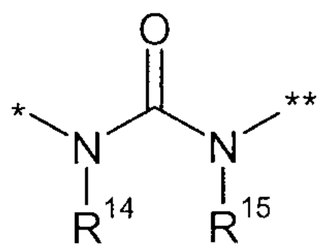
R^{32} stands for hydrogen or methyl,

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

and

wherein $(C_2\text{-}C_6)$-alkanediyl may be substituted with one to two methyl substituents,

B stands for a bond or a group of the formula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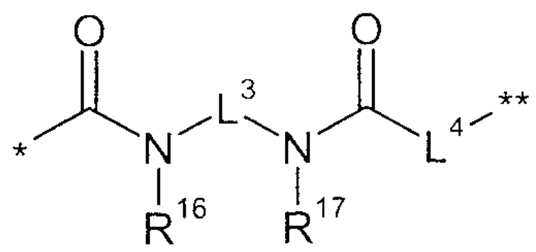
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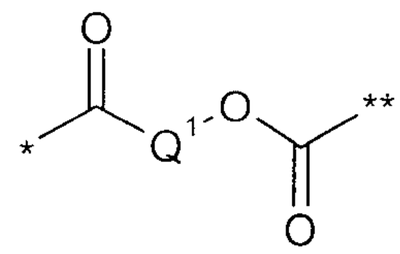
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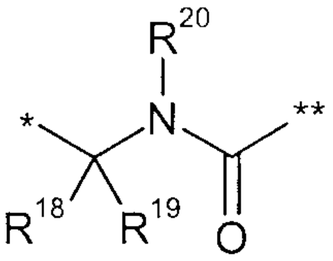
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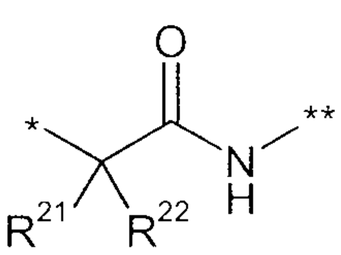
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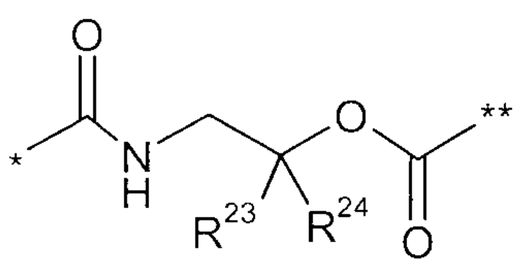
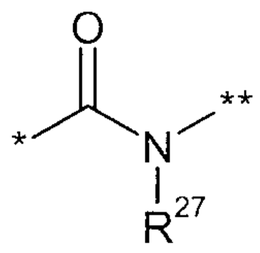


or 

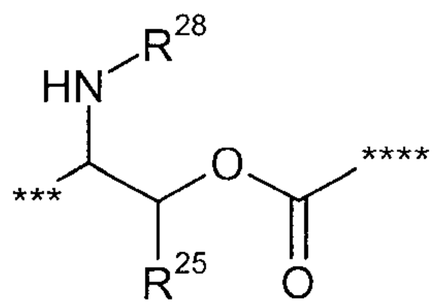
wherein

* 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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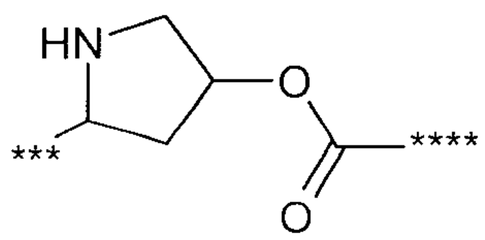


wherein

*** 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,

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,

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 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,

R^{22} stands for hydrogen or methyl,

or

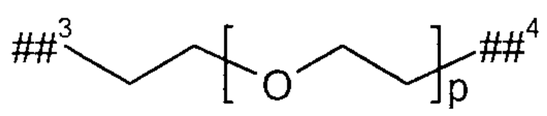
R^{21} and R^{22} together with the atoms to which they are bound form a cyclopropyl ring,

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



wherein

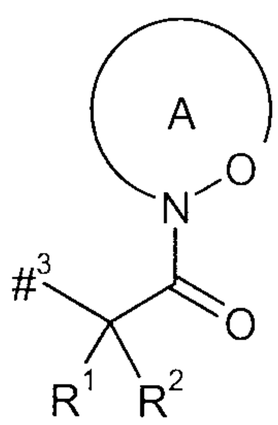
p stands for a number from 2 to 6,

$\#^3$ 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,

D stands for a group of the formula



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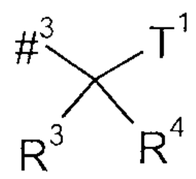

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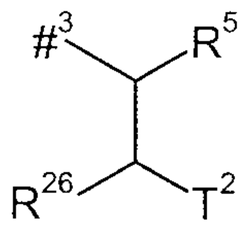


wherein

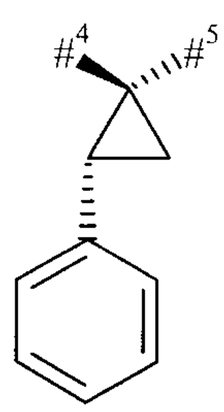
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

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

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

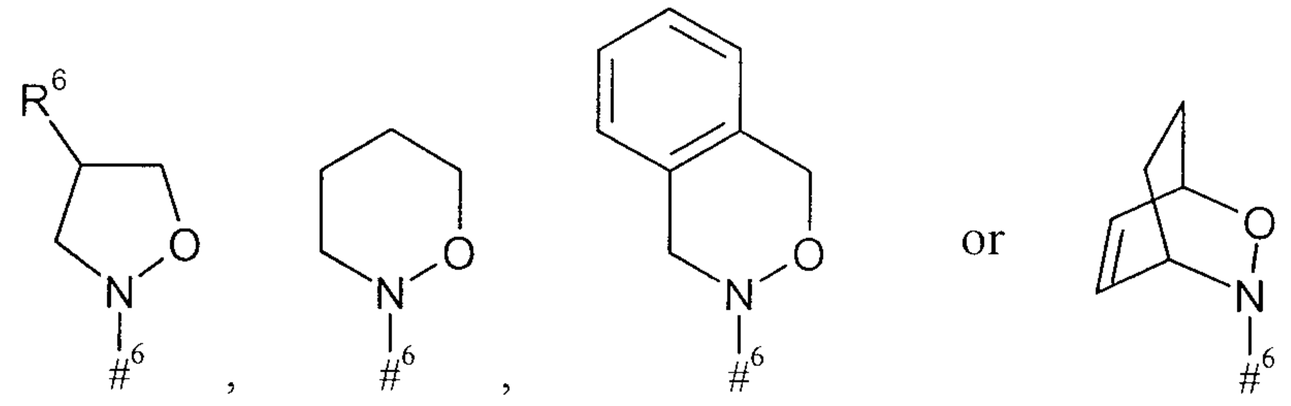


wherein

$\#^4$ 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,

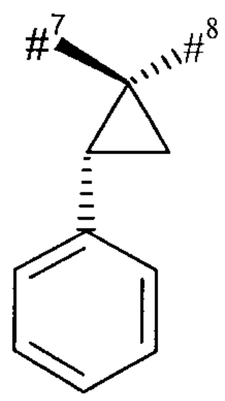
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 1-hydroxyethyl, benzyl, 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 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,

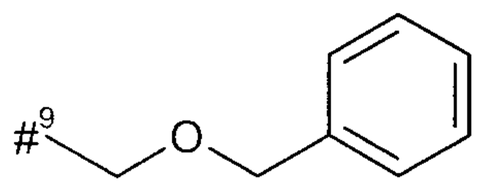
or

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



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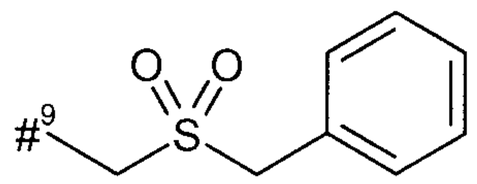

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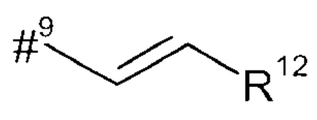

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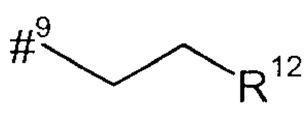

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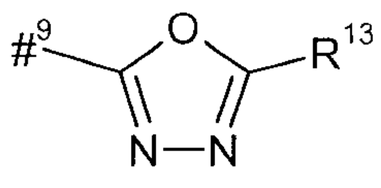


,

wherein

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

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^{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.

50. Binder-drug conjugates of general formula (Ia) according to claim 1, wherein

n stands for a number from 1 to 10,

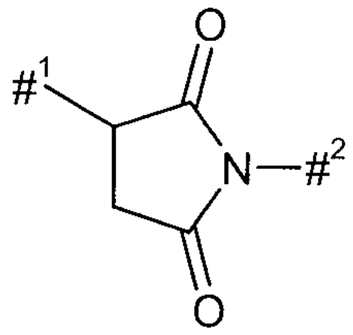
AK stands for AK_1 or AK_2

wherein

AK_1 stands for a binder bound to the group G via a sulfur atom of the binder,

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

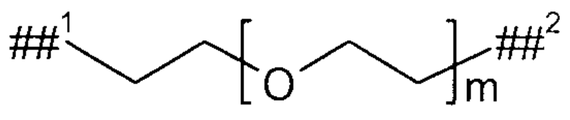
$\#^1$ denotes the linkage site to the cysteine radical of the binder,

$\#^2$ 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 $(C_2\text{-}C_6)$-alkanediyl, a group of the formula



wherein

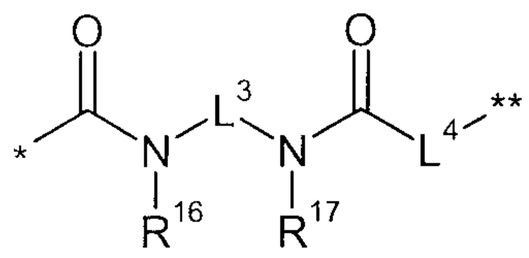
m stands for a number of 2 or 3,

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

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

wherein (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



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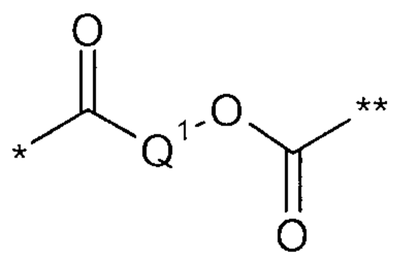

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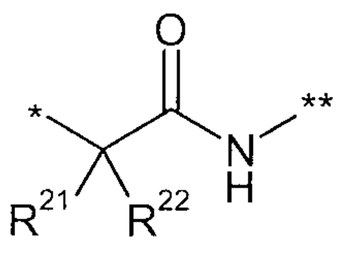

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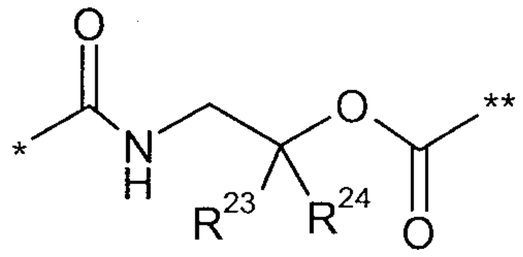


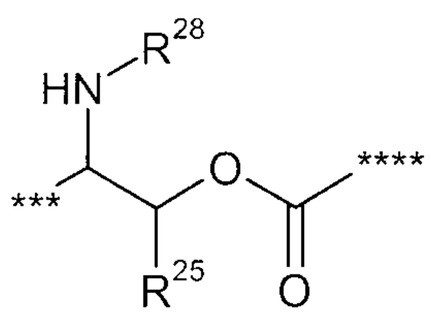
wherein

* 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



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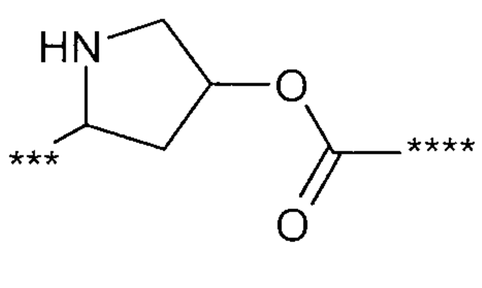


wherein

*** 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,

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,

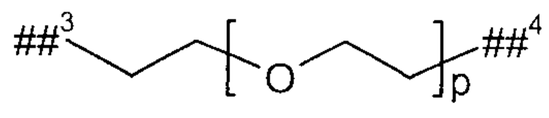
or

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



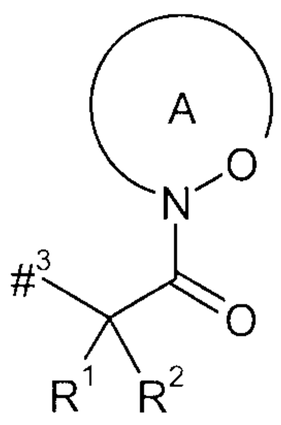
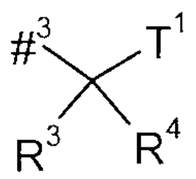
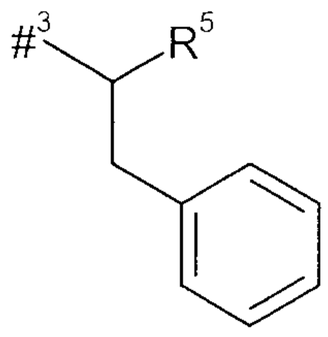
wherein

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

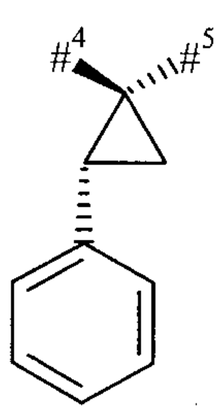
$\#^3$ denotes the linkage site to the nitrogen atom,

R^1 stands for hydrogen,

R^2 stands for 1-hydroxyethyl, benzyl, 1-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

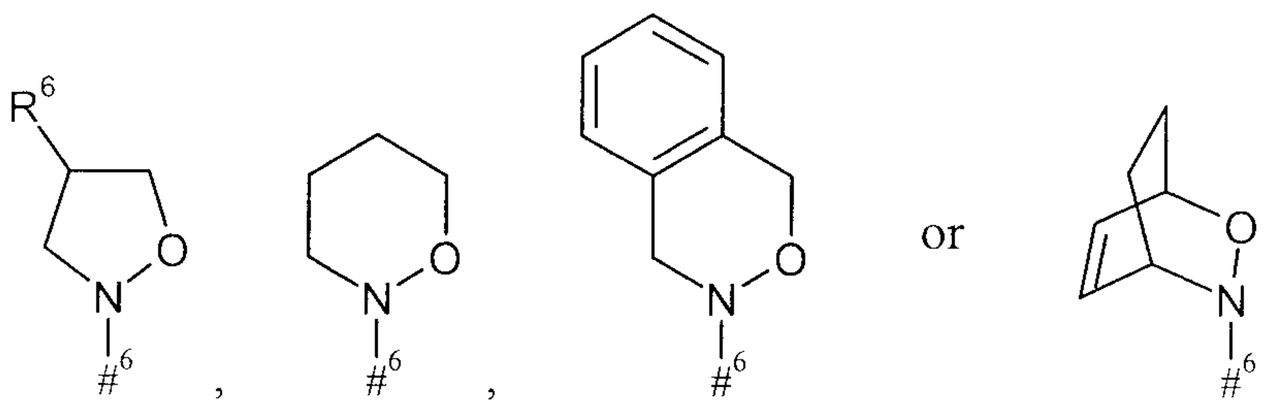


wherein

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

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

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,

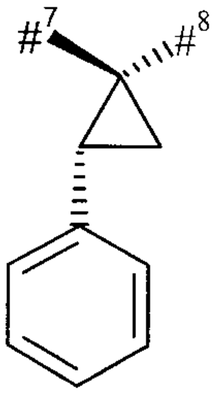
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen,

R^4 stands for 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



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

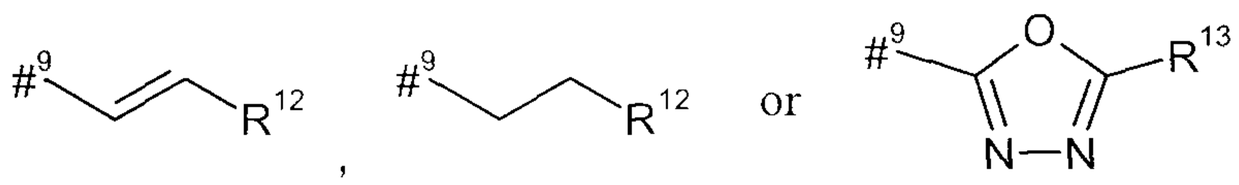
R^7 stands for hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 stands for hydrogen or methyl,

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

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 $-S(O)_2OH$,

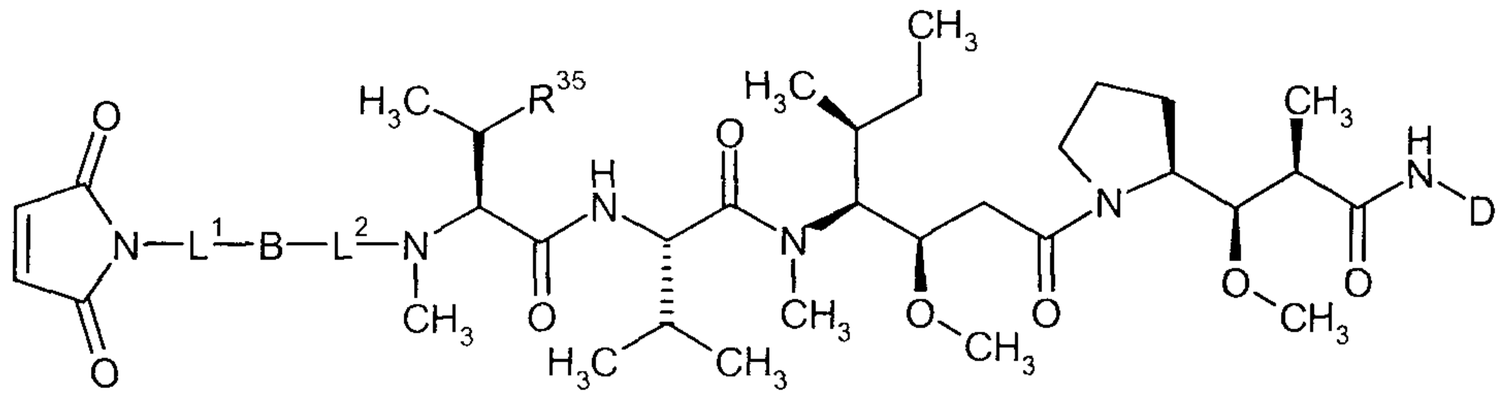
R^{13} stands for phenyl, which may be substituted with methoxycarbonyl or carboxyl,

R^{35} stands for methyl or hydroxyl,

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

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

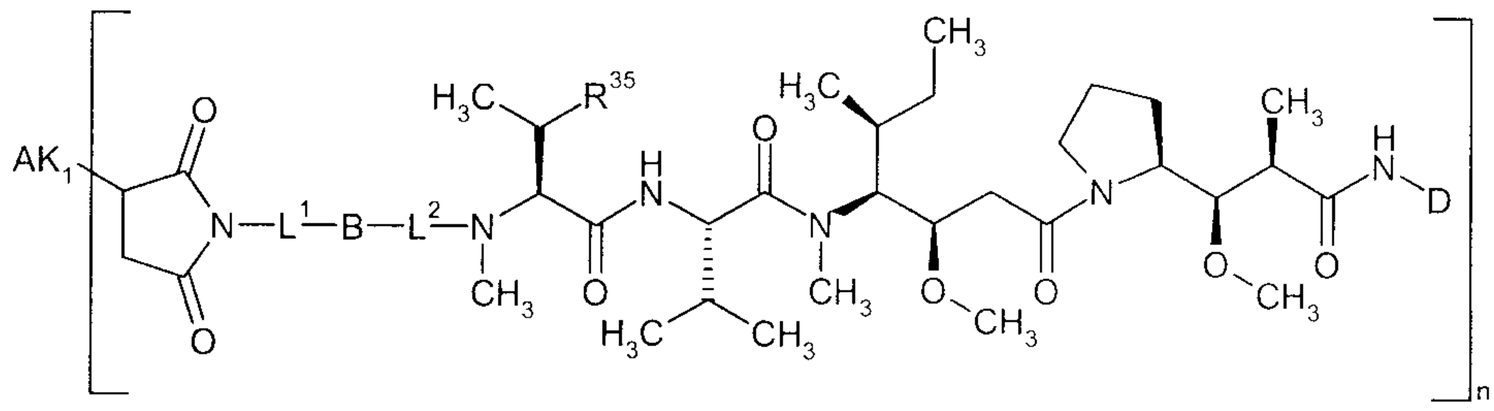
[A] is mixed with a suitable reducing agent such as, for example, dithiothreitol or *tris*-(2-carboxyethyl)phosphine hydrochloride and then is reacted with a compound of formula (IIa)



(IIa),

in which D, L^1, B, L^2 and R^{35} each have the meanings given in claims 1 to 6,

to form a compound of formula (Ia-A)

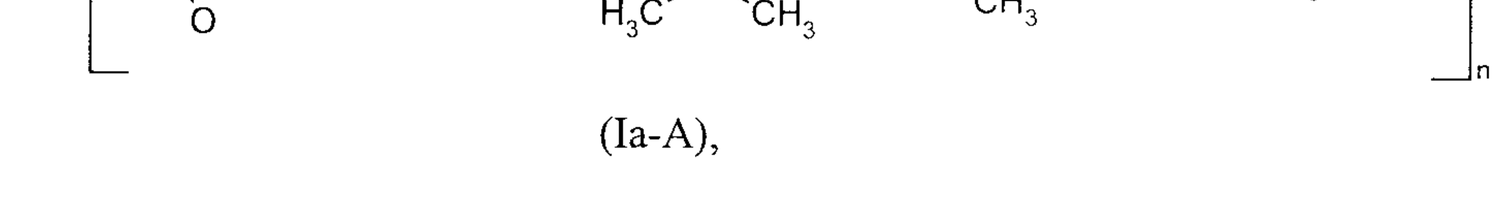


(Ia-A),

in which n, AK_1, D, L^1, B, L^2 and R^{35} each have the meanings given in claims 1 to 6,

or

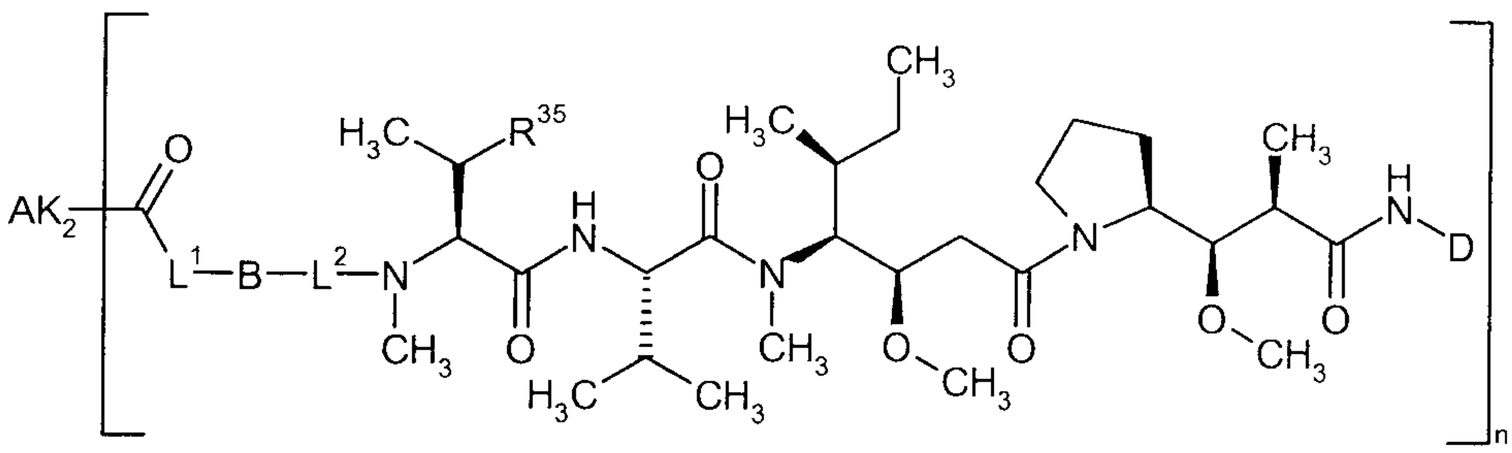
[B] is reacted with a compound of formula (IIIa)



(IIIa),

in which D, L^1, B, L^2 and R^{35} each have the meanings given in claims 1 to 6,

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

53. Binder-drug conjugates of general formula (Ia) according to claim 1, wherein

n stands for a number from 1 to 50,

AK stands for a bond,

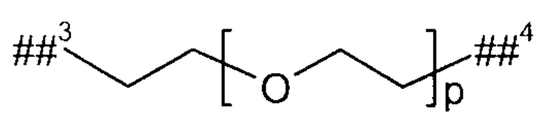
the group §-G-L^1-B-§§ stands for a linker,

wherein

§ denotes the linkage site to the group AK and

§§ 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,

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

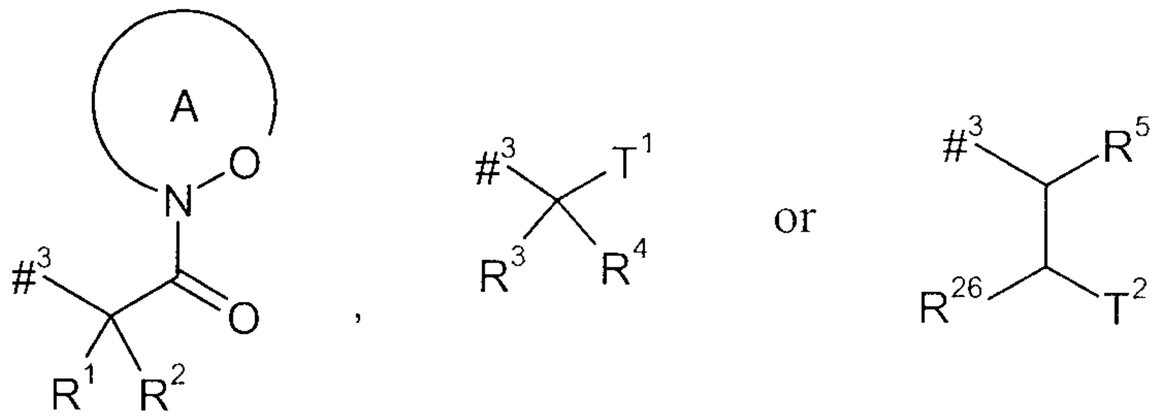
$##^4$ denotes the linkage site to the nitrogen atom,

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,

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

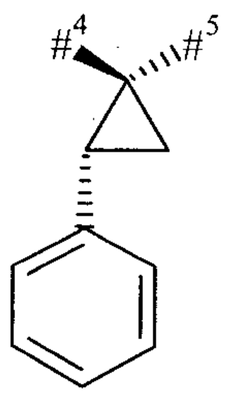
$\#^3$ denotes the linkage site to the nitrogen atom,

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 (1S,2R)-2-phenylcyclopropane-1,1-diyl group of the formula

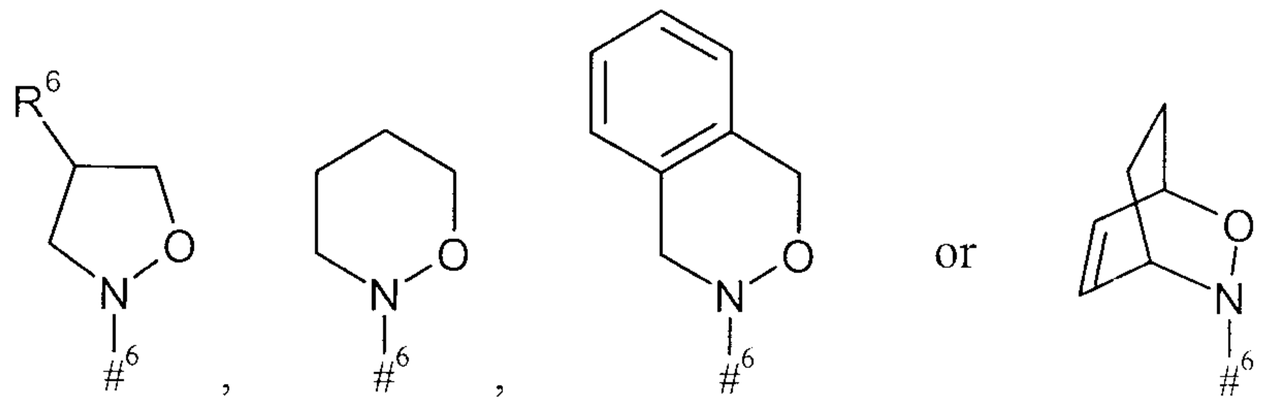


wherein

$\#^4$ 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,

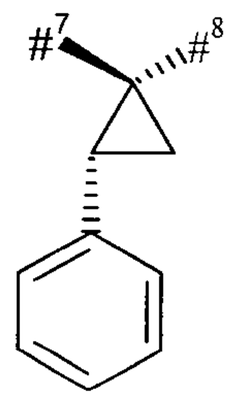
R^6 stands for hydrogen, hydroxyl or benzyloxy,

R^3 stands for hydrogen 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 (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 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,

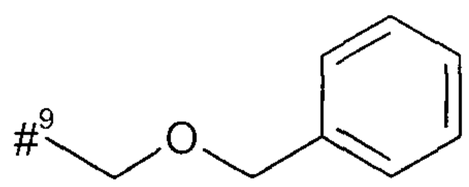
or

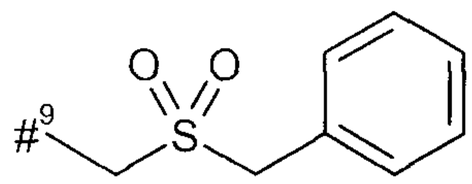
R^8 and R^9 together with the nitrogen atom to which they are bound form a four- to seven-membered heterocycle,

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





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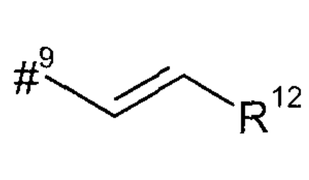

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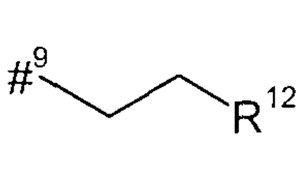

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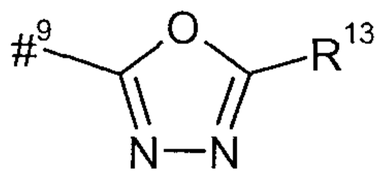

wherein

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

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^{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.