(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 May 2013 (10.05.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/064455 A1

(51) International Patent Classification:
A61K 47/48 (2006.01) *C07K 5/06* (2006.01)
C07K 5/02 (2006.01) *C07K 5/08* (2006.01)

(21) International Application Number:
PCT/EP2012/071373

(22) International Filing Date:
29 October 2012 (29.10.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
11187737.9 3 November 2011 (03.11.2011) EP

(71) Applicant: **BAYER INTELLECTUAL PROPERTY GMBH** [DE/DE]; Alfred-Nobel-Str. 10, 40789 Monheim (DE).

(72) Inventors: **FLAMME, Ingo**; Auf den Schmitten 1, 51580 Reichshof (DE). **KÖBBERLING, Johannes**; Azalienstr. 30, 41466 Neuss (DE). **LERCHEN, Hans-Georg**; Sürderstr. 3, 51375 Leverkusen (DE). **GRIEBENOW, Nils**; Kurfürstenstr. 39, 41541 Dormagen (DE). **SCHOHE-LOOP, Rudolf**; Arndtstr. 10 a, 42327 Wuppertal (DE). **WITTROCK, Sven**; Am Sonnenschein 60, 42115 Wuppertal (DE). **KRENZ, Ursula**; Am Kloster 33, 42799 Leichlingen (DE).

(74) Agent: **HANDKE-ERGÜDEN, Gabriele**; Bayer Intellectual Property GmbH, Alfred-Nobel-Straße 10, 40789 Monheim (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

WO 2013/064455 A1

(54) Title: TYROSINE BASED LINKERS FOR THE RELEASABLE CONNECTION OF PEPTIDES

(57) Abstract: The invention relates to novel tyrosine based linkers that allow the releasable connection of peptides or proteins with other molecular entities, e.g. polyethylene glycol, to processes for their preparation and their use for preparing medicaments for the treatment and/or prophylaxis of diseases.

Tyrosine based linkers for the releasable connection of peptides

The invention relates to novel tyrosine based linkers that allow the releasable connection of peptides or proteins with other molecular entities, e.g. polyethylene glycol, to processes for their preparation and their use for preparing medicaments for the treatment and/or prophylaxis of diseases.

Many therapeutically active peptides or proteins suffer from high clearance in vivo. Several approaches to form an injectable depot of such drugs exist that involve the use of macromolecules.

Polymer matrices that contain a drug molecule in a non covalently bound state are well known. These can also be injectable as hydro gels, micro particles or micelles. The release kinetics of such drug products can be quite unreliable with high inter patient variability. Production of such polymers can harm the sensitive drug substance or it can undergo side reactions with the polymer during its degradation (D.H. Lee et al., J. Contr. Rel., 2003, 92, 291-299).

Permanent PEGylation of peptides or proteins to enhance their solubility, reduce immunogenicity and increase half live by reducing renal clearance is a well known concept since early 1980s (Caliceti P.,Veronese F.M., Adv. Drug Deliv. Rev.2003, 55, 1261-1277). For several drugs this has been used with success, but with many examples the PEGylation reduces efficacy of drug substance to an extent that this concept is not suitable any more (T. Peleg-Shulman et al., J. Med. Chem., 2004, 47, 4897-4904).

A suitable alternative are polymer based prodrugs. The current definitions for prodrugs by the IUPAC state the following terms (International Union of Pure and Applied Chemistry and International Union of Biochemistry: GLOSSARY OF TERMS USED IN MEDICINAL CHEMISTRY (Recommendations 1998); in Pure & Appl. Chem. Vol 70, No. 5, 1998, p. 1129-1143):

Prodrug: A prodrug is any compound that undergoes biotransformation before exhibiting its pharmacological effects. Prodrugs can thus be viewed as drugs containing specialized non-toxic protective groups used in a transient manner to alter or to eliminate undesirable properties in the parent molecule.

Carrier-linked prodrug (Carrier prodrug): A carrier-linked prodrug is a prodrug that contains a temporary linkage of a given active substance with a transient carrier group that produces improved physicochemical or pharmacokinetic properties and that can be easily removed in vivo, usually by a hydrolytic cleavage.

Cascade prodrug: A cascade prodrug is a prodrug for which the cleavage of the carrier group becomes effective only after unmasking an activating group.

Several examples of PEG-based carrier prodrugs exist, most of them with the need for enzymatic activation of the linker between the active drug and the carrier, mostly initiated by enzymatic hydrolysis. Since esters are cleaved very readily and unpredictably in vivo, direct ester linkers for carrier pro drug have limitations to their usability (J. Rautio et al., Nature Reviews Drug discovery, 2008, 7 255-270).

Commonly used alternative approaches are cascading linkers attached to an amine functionality in the peptide or protein. In cascading linkers a masking group has to be removed as the rate limiting step in the cascade. This activates the linker to decompose in a second position to release the peptide or protein. Commonly the masking group can be removed by an enzymatic mechanism (R.B.Greenwald et al. in WO2002/089789, Greenwald, et al., J. Med. Chem. 1999, 42, 3657-3667, F.M.H. DeGroot et al. in WO2002/083180 and WO2004/043493, and D. Shabat et al. in WO2004/019993).

An alternative not relying on enzymatic activation is the concept of U. Hersel et al. in WO2005/099768. In their approach the masking group on a phenol is removed in a purely pH dependent manner by the attack of an internal nucleophile. This activates the linker for further decomposition.

As mentioned by U. Hersel et al. in WO2005/099768, "The disadvantage in the abovementioned prodrug systems described by Greenwald, DeGroot and Shabat is the release of potentially toxic aromatic small molecule side products like quinone methides after cleavage of the temporary linkage. The potentially toxic entities are released in a 1:1 stoichiometry with the drug and can assume high in vivo concentrations." The same problem holds true for the system by Hersel et al. as well.

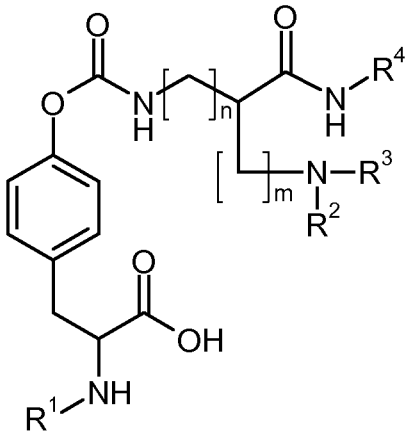
For small organic molecules a plethora of different prodrug approaches exist (J. Rautio et al., Nature Reviews Drug discovery, 2008, 7 255-270). The approach used by U. Hersel et al. as release mechanism for their masking group has been used as a prodrug approach for phenolic groups of small molecules since the late 1980s. (W.S. Saari in EP 0296 811 and W.S. Saari et al., J. Med. Chem. 1990, Vol 33, No 1, p 97-101).

Alternative amine based prodrug system are based on the slow hydrolysis of bis-hydroxyethyl glycine as a cascading prodrug. The hydroxy groups of the bis-hydroxyethyl glycine are masked by esters that are prone to hydrolysis by esterases (R. Greenwald et al., J. Med. Chem. 2004, 47, 726–734 and D. Vetter et al. in WO 2006/136586).

In contrast to the prodrug approaches listed above, which are all based on masking amine functionalities, the current invention is based on masking the phenolic group of a tyrosine in peptides or proteins. A carrier-linked prodrug is used, based on the internal nucleophile assisted cleavage of a carbamate on this phenolic group. The key advantage to other prodrug classes mentioned above is the toxicological harmlessness of the linker decomposition product, a cyclic urea permanently attached to the carrier. Furthermore, the decomposition of the prodrug is not dependent on enzymatic mechanisms that might cause a high inter patient variability of cleavage kinetics. The cleavage mechanism is solely pH dependent as an internal amine that is protonated at acidic pH gets activated at higher (neutral) pH to act as a nucleophile attacking the phenolic carbamate based on the tyrosine.

In the context of the present invention, compounds are now described which encompass tyrosine amino acid based molecular entities that enable the construction of said carrier linker prodrugs of any peptide or protein that contains at least one tyrosine.

The present invention provides compounds of the formula



(I),

in which

n represents the number 0, 1, 2, 3 or 4,

m represents the number 0, 1, 2, 3 or 4,

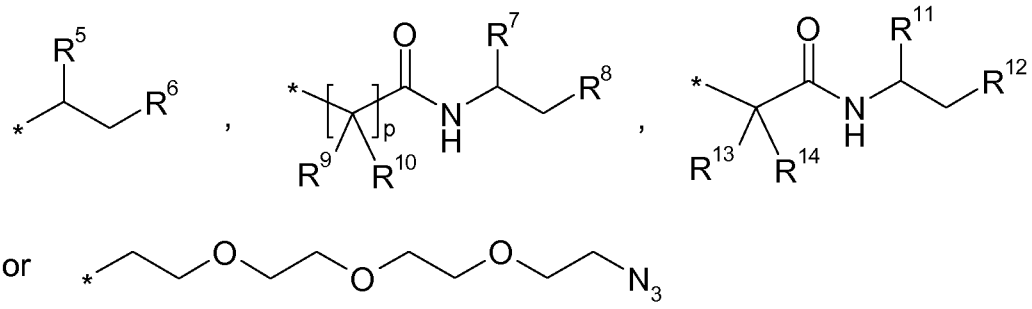
where m and n together are the number 1, 2, 3, 4, 5 or 6,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen, methyl, ethyl, n-propyl, isopropyl or benzyl,

R^4 represents a group of the formula

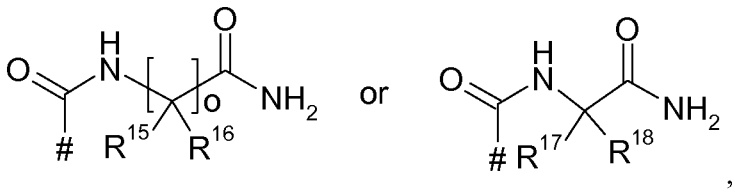


where

* is the point of attachment to the nitrogen,

p represents the number 1, 2, 3, 4 or 5,

R^5 represents hydrogen, aminocarbonyl, $(C_1$-$C_4)$-alkylaminocarbonyl, phenylaminocarbonyl or a group of the formula



where

\# is the point of attachment to the carbon atom,

o represents the number 1, 2, 3, 4 or 5,

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

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

R^{17} represents the side group of a natural α-amino acid or its homologues or isomers,

and

R^{18} represents hydrogen or methyl,

R^6 represents –S-trityl, thiolyl, azidyl, acetylenyl, hydroxycarbonyl or amine,

R^7 represents hydrogen or aminocarbonyl,

R^8 represents -S-trityl, thiolyl, azidyl, acetylenyl, hydroxycarbonyl or amine,

R^9 represents hydrogen or (C_1-C_4)-alkyl,

R^{10} represents hydrogen or (C_1-C_4)-alkyl,

R^{11} represents hydrogen or aminocarbonyl,

R^{12} represents -S-trityl, thiolyl, azidyl, acetylenyl, hydroxycarbonyl or amine,

R^{13} represents the side group of a natural α-amino acid or its homologues or isomers,

and

R^{14} represents hydrogen or methyl,

and salts thereof, solvates thereof and the solvates of salts thereof.

Compounds according to the invention are the compounds of the formula (I) and the salts thereof, solvates thereof and solvates of the salts thereof, the compounds which are embraced by formula (I) and are of the formulae specified below and the salts thereof, solvates thereof and solvates of the salts thereof, and the compounds which are embraced by formula (I) and are specified below as working examples and salts thereof, solvates thereof and solvates of the salts thereof, if the compounds which are embraced by formula (I) and are specified below are not already salts, solvates and solvates of the salts.

Depending on their structure, the compounds according to the invention may exist in stereoisomeric forms (enantiomers, diastereomers). The invention therefore embraces the enantiomers or diastereomers and the particular mixtures thereof. The stereoisomerically homogeneous constituents can be isolated in a known manner from such mixtures of enantiomers and/or diastereomers.

When the compounds according to the invention can occur in tautomeric forms, the present invention embraces all tautomeric forms.

In the context of the present invention, preferred salts are physiologically acceptable salts of the compounds according to the invention. Also included are salts which are not suitable themselves for pharmaceutical applications, but, for example, can be used for the isolation or purification of the compounds according to the invention.

Physiologically acceptable 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, methanesulfonic acid, ethanesulfonic acid, toluenesulfonic acid, benzenesulfonic acid, naphthalenedisulfonic acid, acetic acid, trifluoroacetic acid, propionic acid, lactic acid, tartaric acid, maleic acid, citric acid, fumaric acid, maleic acid and benzoic acid.

Physiologically acceptable salts of the compounds according to the invention also include salts of customary bases, for example and with preference alkali metal salts (e.g. sodium and potassium salts), alkaline earth metal salts (e.g. calcium and magnesium salts) and ammonium salts derived from ammonia or organic amines having 1 to 16 carbon atoms, for example and with preference ethylamine, diethylamine, triethylamine, ethyldiisopropylamine, monoethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, dimethylaminoethanol, procaine, dibenzylamine, *N*-methylmorpholine, arginine, lysine, ethylenediamine and *N*-methylpiperidine.

In the context of the invention, solvates refer to those forms of the compounds according to the invention which, in the solid or liquid state, form a complex by coordination with solvent molecules. Hydrates are a specific form of the solvates, in which the coordination is with water. Preferred solvates in the context of the present invention are hydrates.

In the context of the present invention, the substituents have the following meaning unless otherwise specified:

$(C_1$-$C_4)$-Alkyl are in the context of the invention a straight-chain or branched alkyl radical having respectively 1 to 4 carbon atoms. Examples which may be preferably mentioned are: methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, tert-butyl.

$(C_1$-$C_4)$-Alkylaminocarbonyl in the context of the invention represents an aminocarbonyl group with a straight-chain or branched alkyl substituent which contains 1 to 4 carbon atoms. Examples which may be preferably mentioned are: methylaminocarbonyl, ethylaminocarbonyl, n-propylaminocarbonyl, isopropylaminocarbonyl, n-butylaminocarbonyl, iso-butylaminocarbonyl, sec-butylaminocarbonyl, tert-butylaminocarbonyl.

The side group of an α-amino acid in the meaning of R^{13} and R^{17} encompasses both the side groups of naturally occurring α-amino acids and the side groups of homologs and isomers of these α-amino acids. The α-amino acid may in this connection have both the L and the D configuration or else be a mixture of the L form and D form. Examples of side groups which may be mentioned are: hydrogen

(glycine), methyl (alanine), propan-2-yl (valine), propan-1-yl (norvaline), 2-methylpropan-1-yl (leucine), 1-methylpropan-1-yl (isoleucine), butan-1-yl (norleucine), 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), 3-guanidinopropan-1-yl (arginine), 3-ureidopropan-1-yl (citrulline). Preferred α-amino acid side groups in the meaning of R^2 are hydrogen (glycine), methyl (alanine), propan-2-yl (valine), propan-1-yl (norvaline), imidazol-4-ylmethyl (histidine), hydroxymethyl (serine), 1-hydroxyethyl (threonine), carbamoylmethyl (asparagine), 2-carbamoylethyl (glutamine), 4-aminobutan-1-yl (lysine), 3-aminopropan-1-yl (ornithine), 3-guanidinopropan-1-yl (arginine). The L configuration is preferred in each case.

In the context of the invention modifier means other molecular entities, e.g. polyethylene glycol.

In the formulae of the group which may represent R^4, the end point of the line which is marked by an * is not a carbon atom or a CH_2 group, but is part of the bond to the atom to which R^4 is attached.

In the formulae of the group which may represent R^5, the end point of the line which is marked by an # is not a carbon atom or a CH_2 group, but is part of the bond to the atom to which R^5 is attached.

Preference is given to compounds of the formula (I) in which

n represents the number 0, 1, 2 or 3,

m represents the number 0, 1, 2 or 3,

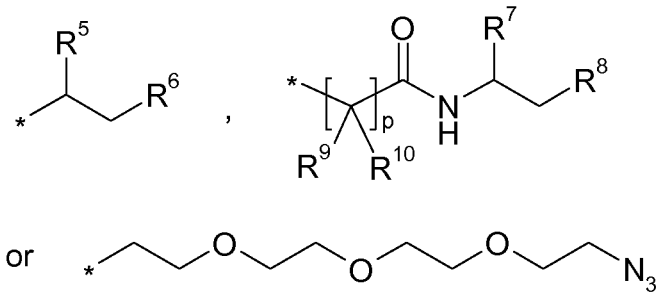
where m and n together are the number 1, 2, 3 or 4,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen, methyl, ethyl, n-propyl, isopropyl or benzyl,

R^4 represents a group of the formula



where

* is the point of attachment to the nitrogen,

p represents the number 1, 2, 3, 4 or 5,

R^5 represents hydrogen, aminocarbonyl or $-(C=O)NHCH_2(C=O)NH_2$,

R^6 represents –S-trityl,

R^7 represents hydrogen or aminocarbonyl,

R^8 represents –S-trityl,

R^9 represents hydrogen,

and

R^{10} represents hydrogen.

Preference is also given to compounds of the formula (I) in which

n represents the number 2 or 3,

and

m represents the number 0,

or

n represents the number 0,

and

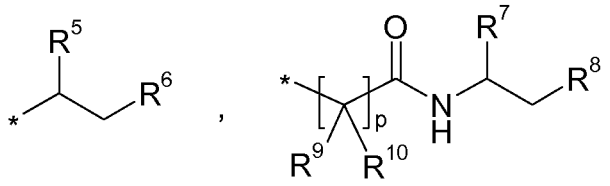
m represents the number 2 or 3,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

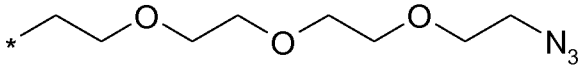
R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen, methyl, ethyl, n-propyl, isopropyl or benzyl,

R^4 represents a group of the formula



,

or 

where

* is the point of attachment to the nitrogen,

p represents the number 1, 2, 3, 4 or 5,

R^5 represents hydrogen, aminocarbonyl or $-(C=O)NHCH_2(C=O)NH_2$,

R^6 represents –S-trityl,

R^7 represents hydrogen or aminocarbonyl,

R^8 represents –S-trityl,

R^9 represents hydrogen,

and

R^{10} represents hydrogen.

Preference is also given to compounds of the formula (I) in which

n represents the number 2 or 3,

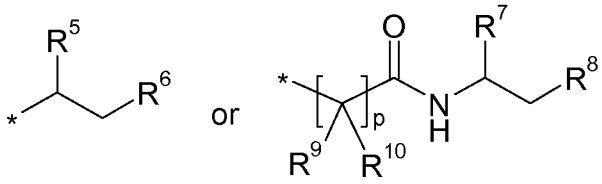
m represents the number 0,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen or methyl,

R^4 represents a group of the formula



where

* is the point of attachment to the nitrogen,

p represents the number 1 or 5,

R^5 represents hydrogen, aminocarbonyl or $-(C=O)NHCH_2(C=O)NH_2$,

R^6 represents –S-trityl,

R^7 represents hydrogen or aminocarbonyl,

R^8 represents –S-trityl,

R^9 represents hydrogen,

and

R^{10} represents hydrogen.

Preference is also given to compounds of the formula (I) in which

n represents the number 0,

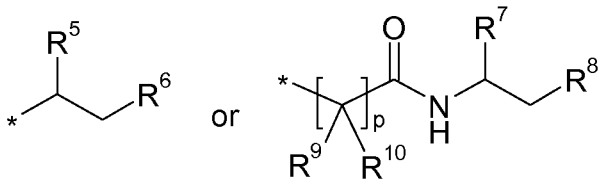
m represents the number 2 or 3,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen or methyl,

R^4 represents a group of the formula



where

* is the point of attachment to the nitrogen,

p represents the number 1 or 5,

R^5 represents hydrogen, aminocarbonyl or $-(C=O)NHCH_2(C=O)NH_2$,

R^6 represents –S-trityl,

R^7 represents hydrogen or aminocarbonyl,

R^8 represents –S-trityl,

R^9 represents hydrogen,

and

R^{10} represents hydrogen.

Preference is also given to compounds of the formula (I) in which

n represents the number 2 or 3,

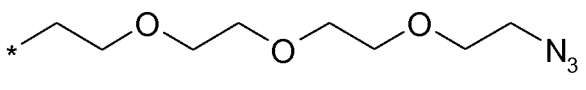
m represents the number 0,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen or methyl,

R^4 represents a group of the formula



where

* is the point of attachment to the nitrogen.

Preference is also given to compounds of the formula (I) in which

n represents the number 0, 1, 2 or 3,

m represents the number 0, 1, 2 or 3,

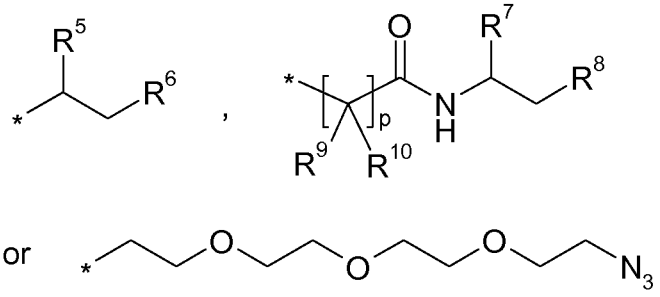
where m and n together are the number 1, 2, 3 or 4,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen, methyl, ethyl, n-propyl, isopropyl or benzyl,

R^4 represents a group of the formula



where

* is the point of attachment to the nitrogen,

p represents the number 1, 2, 3, 4 or 5,

R^5 represents hydrogen, aminocarbonyl, phenylaminocarbonyl or $-(C=O)NHCH_2(C=O)NH_2$,

R^6 represents –S-trityl,

R^7 represents hydrogen or aminocarbonyl,

R^8 represents –S-trityl,

R^9 represents hydrogen,

and

R^{10} represents hydrogen.

Preference is also given to compounds of the formula (I) in which

n represents the number 2 or 3,

and

m represents the number 0,

or

n represents the number 0,

and

m represents the number 2 or 3,

or

n represents the number 0,

and

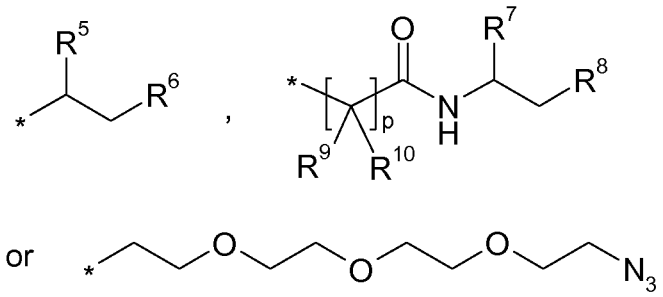
m represents the number 1,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen, methyl, ethyl, n-propyl, isopropyl or benzyl,

R^4 represents a group of the formula



where

* is the point of attachment to the nitrogen,

p represents the number 1, 2, 3, 4 or 5,

R^5 represents hydrogen, aminocarbonyl, phenylaminocarbonyl or -(C=O)NHCH$_2$(C=O)NH$_2$,

R^6 represents –S-trityl,

R^7 represents hydrogen or aminocarbonyl,

R^8 represents –S-trityl,

R^9 represents hydrogen,

and

R^{10} represents hydrogen.

Preference is also given to compounds of the formula (I) in which

n represents the number 2 or 3,

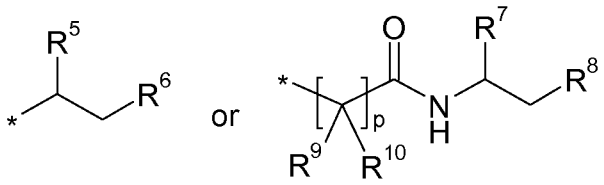
m represents the number 0,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen or methyl,

R^4 represents a group of the formula



where

* is the point of attachment to the nitrogen,

p represents the number 1 or 5,

R^5 represents hydrogen, aminocarbonyl, phenylaminocarbonyl or -(C=O)NHCH$_2$(C=O)NH$_2$,

R^6 represents –S-trityl,

R^7 represents hydrogen or aminocarbonyl,

R^8 represents –S-trityl,

R^9 represents hydrogen,

and

R^{10} represents hydrogen.

Preference is also given to compounds of the formula (I) in which

n represents the number 0,

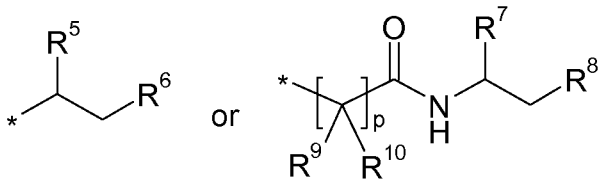
m represents the number 2 or 3,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen or methyl,

R^4 represents a group of the formula



where

* is the point of attachment to the nitrogen,

p represents the number 1 or 5,

R^5 represents hydrogen, aminocarbonyl, phenylaminocarbonyl or -(C=O)NHCH$_2$(C=O)NH$_2$,

R^6 represents –S-trityl,

R^7 represents hydrogen or aminocarbonyl,

R^8 represents –S-trityl,

R^9 represents hydrogen,

and

R^{10} represents hydrogen.

Preference is also given to compounds of the formula (I) in which

n represents the number 0,

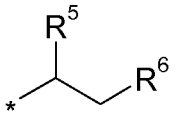
m represents the number 1,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen or methyl,

R^4 represents a group of the formula



where

* is the point of attachment to the nitrogen,

R^5 represents hydrogen, aminocarbonyl, phenylaminocarbonyl or -(C=O)NHCH$_2$(C=O)NH$_2$,

and

R^6 represents –S-trityl.

Preference is also given to compounds of the formula (I) in which n represents the number 2 or 3 and m represents the number 0.

Preference is also given to compounds of the formula (I) in which n represents the number 2 and m represents the number 0.

Preference is also given to compounds of the formula (I) in which n represents the number 3 and m represents the number 0.

Preference is also given to compounds of the formula (I) in which n represents the number 0 and m represents the number 2 or 3.

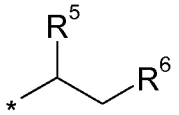
Preference is also given to compounds of the formula (I) in which n represents the number 0 and m represents the number 1.

Preference is also given to compounds of the formula (I) in which R^1 represents tert-butyloxy-carbonyl.

Preference is also given to compounds of the formula (I) in which R^3 represents hydrogen or methyl.

Preference is also given to compounds of the formula (I) in which

R^4 represents a group of the formula



where

* is the point of attachment to the nitrogen,

R^5 represents aminocarbonyl,

and

R^6 represents –S-trityl.

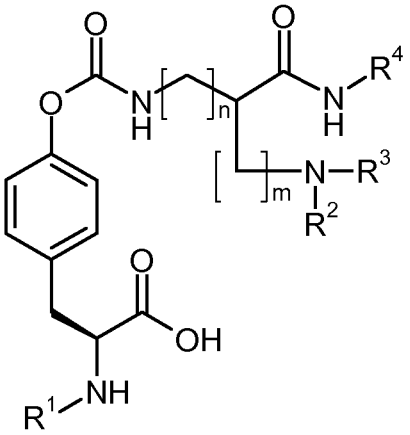
Preference is also given to compounds of the formula (I) in which R^6 represents –S-trityl.

Preference is also given to compounds of the formula (I) in which R^8 represents –S-trityl.

Preference is also given to compounds of the formula (I) in which R^9 represents hydrogen and R^{10} represents hydrogen.

Preference is also given to compounds of the formula (I) in which the carbon atom to which the $-NHR^1$ substituent is bonded has S configuration.

Preference is also given to compounds of the formula (I) which have the structure of the formula (Ia)

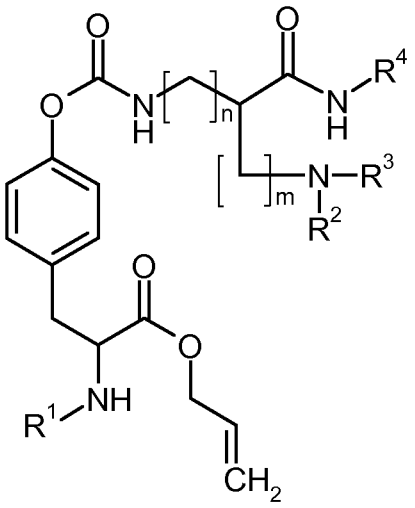


(Ia).

The specific radical definitions given in the particular combinations or preferred combinations of radicals are, irrespective of the particular combination of the radical specified, also replaced by any radical definitions of other combinations.

Very particular preference is given to combinations of two or more of the abovementioned preferred ranges.

The invention further provides a process for preparing the compounds of the formula (I), or salts thereof, solvates thereof or the solvates of salts thereof, wherein the compounds of the formula (II)



(II),

in which

n, m, R^1, R^2, R^3 and R^4 are each as defined above,

are reacted with a Palladium(0) source and a reducing agent.

The reaction is generally effected in inert solvents, optionally in the presents of a weak base, preferably in a temperature range of 0°C to 50°C at standard pressure.

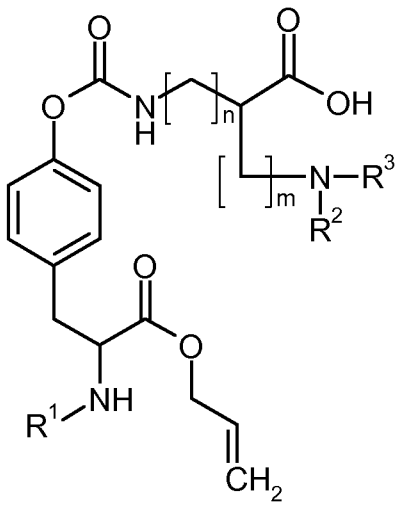
Inert solvents are, for example, halohydrocarbons such as dichloromethane, trichloromethane or 1,2-dichloroethane, ethers such as dioxane, tetrahydrofuran or 1,2-dimethoxyethane, or other solvents such as acetone, dimethylformamide, dimethylacetamide, 2-butanone or acetonitrile. It is equally possible to use mixtures of the solvents. Preference is given to tetrahydrofuran.

Palladium(0) sources are, for example, tetrakis(triphenylphosphin)palladium(0), tris(dibenzylideneacetone)dipalladium(0) or Palladium(II) sources that are reduced in situ to Palladium(0) during the reaction, preference being given to tetrakis(triphenylphosphin)-palladium(0).

Reducing agents are, for example, formic acid or triethyl silan, preference being given to formic acid.

Bases are, for example, triethylamine, N,N-diisopropylethylamine or potassium phosphate solution, preference being given to triethylamine.

The compounds of the formula (II) are known or can be prepared by reacting compounds of the formula (III)

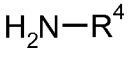


(III),

in which

n, m, R^1, R^2 and R^3 are each as defined above,

with compounds of the formula (IV)



(IV),

in which

R^4 are as defined above.

The reaction is generally effected in inert solvents, in the presence of a dehydrating reagent, optionally in the presence of a base, preferably in a temperature range from room temperature to 70°C at standard pressure.

Inert solvents are, for example, halohydrocarbons such as dichloromethane, trichloromethane or 1,2-dichloroethane, ethers such as dioxane, tetrahydrofuran or 1,2-dimethoxyethane, or other solvents such as acetone, dimethylformamide, dimethylacetamide, 2-butanone or acetonitrile. It is equally possible to use mixtures of the solvents. Preference is given to dichloromethane.

Suitable dehydrating reagents in this context are, for example, carbodiimides, for example *N,N'*-diethyl-, *N,N'*-dipropyl-, *N,N'*-diisopropyl-, *N,N'*-dicyclohexylcarbodiimide, *N*-(3-dimethylaminoisopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), *N*-cyclohexylcarbodiimide-*N'*-propyloxymethyl-polystyrene (PS-carbodiimide), or carbonyl compounds such as carbonyldiimidazole, or 1,2-oxazolium compounds such as 2-ethyl-5-phenyl-1,2-oxazolium 3-sulphate or 2-tert-butyl-5-methylisoxazolium perchlorate, or acylamino compounds such as 2-ethoxy-1-ethoxycarbonyl-1,2-di-

hydroquinoline, or propanephosphonic anhydride, or isobutyl chloroformate, or bis-(2-oxo-3-oxazolidinyl)phosphoryl chloride or benzotriazolyloxytri(dimethylamino)phosphonium hexafluorophosphate, or *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), benzotriazol-1-yl-N-tetramethyl-uronium tetrafluoroborate (TBTU), 2-(2-oxo-1-(2H)-pyridyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TPTU) or *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU), or 1-hydroxybenzotriazole (HOBt), or benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), or benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PYBOP), or *N*-hydroxysuccinimide, or mixtures of these with bases.

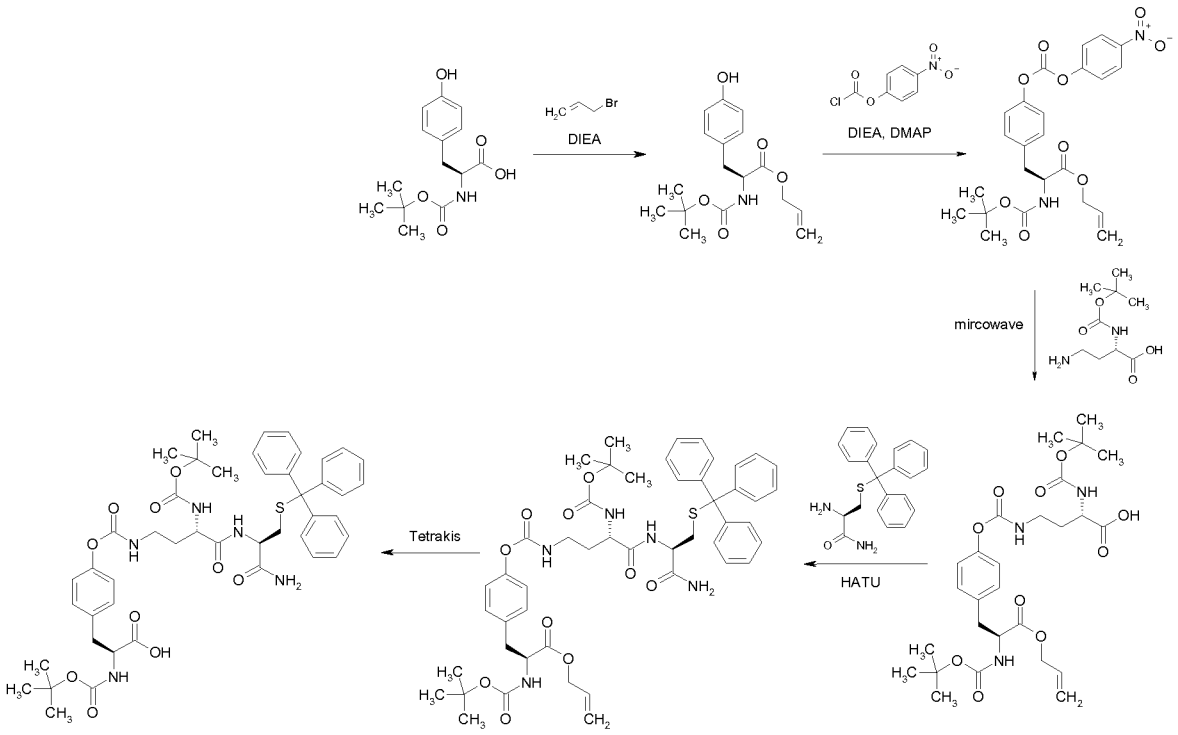
Bases are, for example, alkali metal carbonates, for example sodium carbonate or potassium carbonate, or sodium hydrogencarbonate or potassium hydrogencarbonate, or organic bases such as trialkylamines, for example triethylamine, *N*-methylmorpholine, *N*-methylpiperidine, 4-dimethylaminopyridine or N,N-diisopropylethylamine, preference being given to N,N-diisopropylethylamine.

Preferably, the condensation is carried out with HATU in the presence of N,N-diisopropylethylamine.

The compounds of the formula (III) and (IV) are known or can be synthesized by known processes from the appropriate starting compounds.

The preparation of the compounds according to the invention can be illustrated by the following synthesis scheme:

Scheme 1

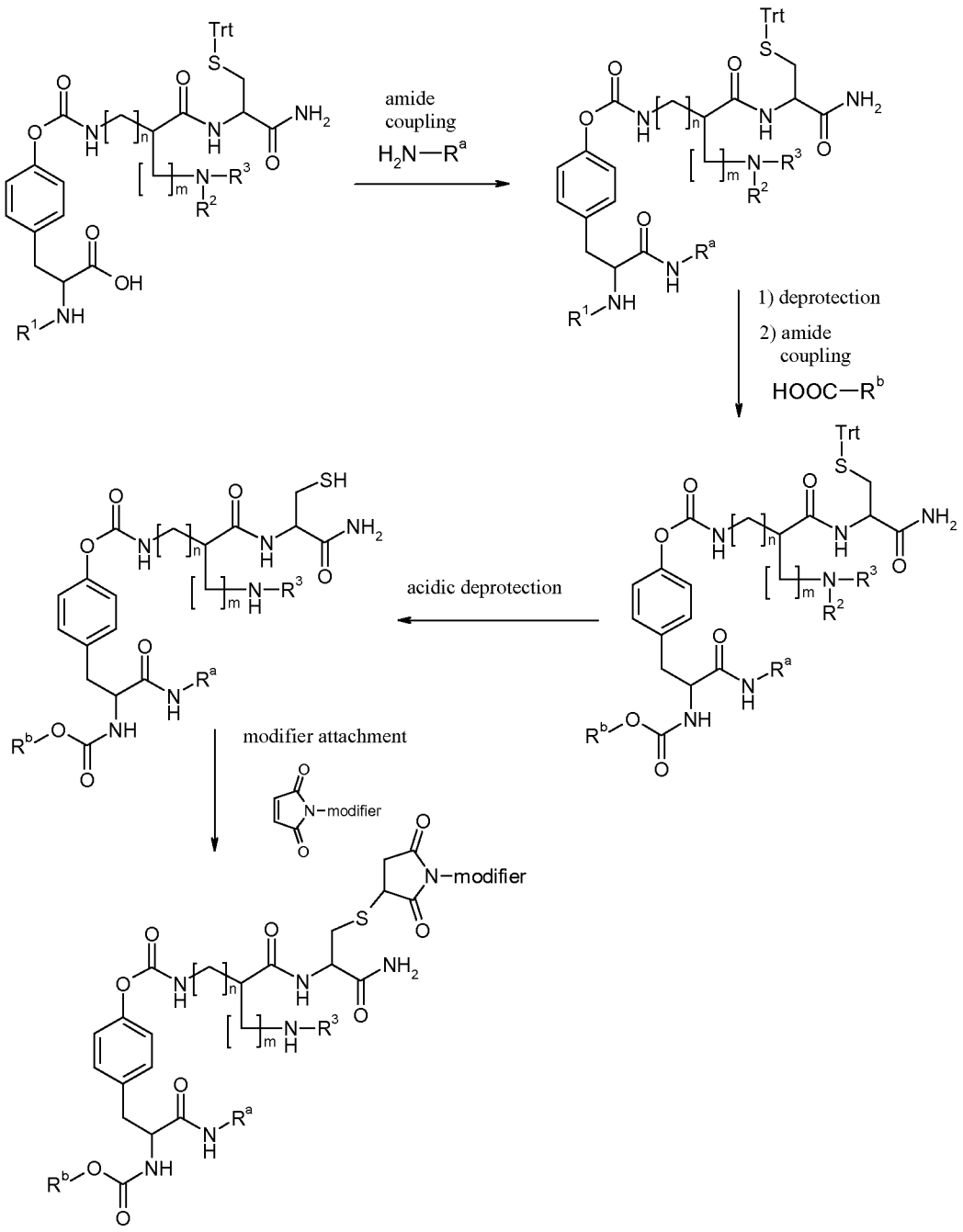


The compounds according to the invention are usable as the releasable connection of peptides or proteins with other molecular entities, e.g. polyethylene glycol or other modifiers, to form a prodrug of said peptides or proteins.

The active principle of these tyrosine derivatives is a carbamate between the penolic OH-group of a tyrosine in a peptide or protein sequence and one amine functionality of a diamino acid. The second amine of the diamino acid is protonated under acidic conditions. But at neutral or basic conditions it acts as a nuleophile attacking the carbamate. This leads to the formation of a cyclic urea and release of the unmodified tyrosine. The acid functionality of the diamino acid is used as the attachment point for a modifier. Many different approaches to attach a modifer at this functionality can be envisioned. A common methodology to attach modifiers such as polyethylene glycol to a peptide is by reacting PEG-maleeimides with cystein residues or other thiols. Therefore a straight foreward way to achive the desired task is the attachment of a cystein residue via its amine functionality to the carboxy group of the diaminoacid. The carboxy terminus of the cystein could be for example a primary amide but many other modifications on its C-terminus are also possible. Between the diamino acid and the cystein or any other thiol functionality, in is easily envisioned that a plethora of spacer groups would be suitable without changing the character of this linking concept since all of this molecular construct remains between the cyclic urea formed from the diamino acid on the one end and the modifier on the other end. The released peptide or protein is not changed in any way. Also the chemistry to attach modifiers to the linker is not limited to the reaction of a thiol functionality with a maleimide. Other well known methods to link modifiers such as PEG to a thiol

are equally suitable. Also many other thiol free linking methodologies such as “click”-chemistry or simple amide bond formations to an amine functinalized modifier are alternatives. Scheme 2 shows an exemplaric attachment of a modifier to a peptide incorporating the tyrosine based amino acid derivative.

Scheme 2



The peptides or proteins are released from said prodrug in a pH dependend manner. The prodrugs are stable around pH 4 but release the active drug at physiological pH. After release of the peptide or protein form the prodrug all that is remaining in the peptide or protein is an unmodified tyrosine at the former attachment point of the linker. Therfore all peptides or proteins containing at least on tyrosine are potentially amenable to such modification.

The pH dependend cleavage of the prodrug to release the peptide or protein helps to design a controlled degradation of such prodrug with predictable pharmaco kinetics.

The compounds according to the invention can be incorporated into a peptide or protein according to solution as well as solid phase peptide synthesis protocols.

Suitable proteins and peptides containing at least one thyrosine amino acid are but are not limited to adenosine deaminase, adiponectin, adrenocorticotropic hormone (ACTH), adrenomedullin (ADM), agalsidase, albumin, alfa-1 proteinase inhibitor (API), alfa-I antitrypsin b (AAT), alteplase, ancrod serine, angiotensin, angiotensinogenangiotensin, anistreplase, antimullerian hormone, antithrombin III, antitrypsins, aprotinin, asparaginases, atriopeptin, biphalin, bradykinin, calcitonin, cholecystokinin, choriogonadotropind, choriomammotropin, collagenase, corticoliberin, corticotropin, DNase, endorphins, enkephalins, enoxacin, erythropoietins, Factor II, Factor IIa, Factor IX, Factor IXa, Factor VII, Factor VIIa, Factor VIII, Factor VIIIa, Factor X, Factor Xa, Factor XI, Factor XIa, fibrinolysin, fibrinolysin, folliberin, follicle-stimulating hormones, follitropin, Fsh, galactosidase, gastrin, ghrelin, glucagon, glucagon-like peptides like (GLP-1), glucocerebrosidase, glumitocin f, gonadoliberin c, gonadotropine, gonadotropin-releasing hormone, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), growth factors, growth hormone-releasing hormone, growth hormones, hemoglobins, hepatitis B vaccines, hirudin, human chorionic gonadotropin, human placental lactogen, hyaluronidases, Idarubicin, idurnonidase, immune globulins, influenza vaccines, inhibin, insulins, interferons, interleukins, isotocin g, kallidin, keratinocyte growth factor (KGF), lactase, leptin, leuprolide, levothyroxine, lipotropin, lisinopril, luliberin, luteinizing hormone, lutropin, melanocyte stimulating hormone, melanoliberin, melanostatin, melanotropinh, natriuretic peptide, orexin, orticotropin-releasing hormone, oxytocin, pancrelipase, pancreozymin, papain, parathyroid hormone, pepsin, phospholipase-activating protein (PLAP), platelet activating factor acetylhydrolase (PAF-AH), proangiotensin, prolactin, prolactoliberin, prolactostatin, proteases, protein C, relaxin, secretin, sennorelin, somatoliberin, somatomedin, somatropins, streptokinase, sucrase, superoxide dismutase (SOD), thrombopoietin, thymopoietinn, thymosin, thyroid stimulating hormone, thyroliberin, thyrotropin, thyrotropin-releasing hormone, tilactase, tissue plasminogen activator (tPA), tumor necrosis factor (TNF), urate oxidase, urogonadotropin k, urokinase, vaccines, vasopressin, vasotocin, α-1 a antitrypsin. Mutant versions of peptides or proteins listed above or all other proteins prepared by recombinant methodologies such as antibodies, antibody fragments, single chain binding proteins and fusion proteins are also included. Also any synthetic peptide or proteins with biological activity are included.

The compounds according to the invention are suitable for use for the preparation of prodrugs which are suitable for use as medicaments for treatment and/or prevention of diseases in humans and animals.

The compounds according to the invention are suitable for use for the preparation of specific adrenomedullin (ADM) releasing prodrugs.

The present invention further provides for the use of the compounds according to the invention for the preparation of prodrugs for treatment and/or prevention of disorders.

For the present invention, the term "treatment" or "treating" includes inhibiting, delaying, relieving, mitigating, arresting, reducing, or causing the regression of a disease, disorder, condition, or state, the development and/or progression thereof, and/or the symptoms thereof. The term "prevention" or "preventing" includes reducing the risk of having, contracting, or experiencing, a disease, disorder, condition, or state, the development and/or progression thereof, and/or the symptoms thereof. The term prevention includes prophylaxis. Treatment or prevention of a disease, disorder, condition, or state may be partial or complete.

On the basis of their pharmacological properties, the prodrugs prepared with the compounds according to the invention can be employed for treatment and/or prevention of cardiovascular diseases, in particular chronic and acute heart failure, diastolic and systolic (congestive) heart failure, acute decompensated heart failure, cardiac insufficiency, coronary heart disease, angina pectoris, myocardial infarction, ischemia reperfusion injury, ischemic and hemorrhagic stroke, arteriosclerosis, atherosclerosis, essential hypertension, malignant essential hypertension, secondary hypertension, renovascular hypertension and hypertension secondary to renal and endocrine disorders, hypertensive heart disease, hypertensive renal disease, secondary pulmonary hypertension, pulmonary hypertension following pulmonary embolism with and without acute cor pulmonale, primary pulmonary hypertension, and peripheral arterial occlusive disease.

The prodrugs prepared with the compounds according to the invention are furthermore suitable for treatment and/or prevention of gestational [pregnancy-induced] edema and proteinuria with and without hypertension (pre-eclampsia).

The prodrugs prepared with the compounds according to the invention are furthermore suitable for treatment and/or prevention of pulmonary disorders, such as chronic obstructive pulmonary disease, asthma, acute and chronic pulmonary edema, allergic alveolitis and pneumonitis due to inhaled organic dust and particles of fungal, actinomycetic or other origin, acute chemical bronchitis, acute and chronic chemical pulmonary edema (e.g. after inhalation of phosgene, nitrogen oxide), neurogenic pulmonary edema, acute and chronic pulmonary manifestations due to radiation, acute and chronic interstitial lung disorders (such as but not restricted to drug-induced interstitial lung disorders, e.g. secondary to Bleomycin treatment), acute lung injury/acute respiratory distress

syndrome (ALI/ARDS) in adult or child including newborn, ALI/ARDS secondary to pneumonia and sepsis, aspiration pneumonia and ALI/ARDS secondary to aspiration (such as but not restricted to aspiration pneumonia due to regurgitated gastric content), ALI/ARDS secondary to smoke gas inhalation, transfusion-related acute lung injury (TRALI), ALI/ARDS or acute pulmonary insufficiency following surgery, trauma or burns, ventilator induced lung injury (VILI), lung injury following meconium aspiration, pulmonary fibrosis, and mountain sickness.

The prodrugs prepared with the compounds according to the invention are furthermore suitable for treatment and/or prevention of chronic kidney diseases (stages 1-5), renal insufficiency, diabetic nephropathy, hypertensive chronic kidney disease, glomerulonephritis, rapidly progressive and chronic nephritic syndrome, unspecific nephritic syndrome, nephrotic syndrome, hereditary nephropathies, acute and chronic tubulo-interstitial nephritis, acute kidney injury, acute kidney failure, posttraumatic kidney failure, traumatic and postprocedural kidney injury, cardiorenal syndrome, and protection and functional improvement of kidney transplants.

The prodrugs prepared with the compounds according to the invention are moreover suitable for treatment and/or prevention of diabetes mellitus and its consecutive symptoms, such as e.g. diabetic macro- and microangiopathy, diabetic nephropathy and neuropathy.

The prodrugs prepared with the compounds according to the invention can moreover be used for treatment and/or prevention of disorders of the central and peripheral nervous system such as viral and bacterial meningitis and encephalitis (e.g. Zoster encephalitis), brain injury, primary or secondary [metastasis] malignant neoplasm of the brain and spinal cord, radiculitis and polyradiculitis, Guillain-Barre syndrome [acute (post-)infective polyneuritis, Miller Fisher Syndrome], amyotrophic lateral sclerosis [progressive spinal muscle atrophy], Parkinson's disease, acute and chronic polyneuropathies, pain, cerebral edema, Alzheimer's disease, degenerative diseases of the nervous system and demyelinating diseases of the central nervous system such as but not restricted to multiple sclerosis.

The prodrugs prepared with the compounds according to the invention are furthermore suitable for treatment and/or prevention of portal hypertension and liver fibrosis [cirrhosis] and its sequelae such as esophageal varices and ascites, for the treatment and/or prevention of pleural effusions secondary to malignancies or inflammations and for the treatment and/or prevention of lymphedema and of edema secondary to varices.

The prodrugs prepared with the compounds according to the invention are furthermore suitable for treatment and/or prevention of inflammatory disorders of the gastrointestinal tract such as

inflammatory bowel disease, Crohn´s disease, ulcerative colitis, and toxic and vascular disorders of the intestine.

The prodrugs prepared with the compounds according to the invention are furthermore suitable for treatment and/or prevention of sepsis, septic shock, systemic inflammatory response syndrome (SIRS) of non-infectious origin, hemorrhagic shock, sepsis or SIRS with organ dysfunction or multi organ failure (MOF), traumatic shock, toxic shock, anaphylactic shock, urticaria, insect sting and bite-related allergies, angioneurotic edema [Giant urticaria, Quincke's edema], acute laryngitis and tracheitis, and acute obstructive laryngitis [croup] and epiglottitis.

The prodrugs prepared with the compounds according to the invention are furthermore suitable for treatment and/or prevention of diseases of the rheumatic type and other disease forms to be counted as autoimmune diseases such as but not restricted to polyarthritis, lupus erythematodes, scleroderma, purpura and vasculitis.

The prodrugs prepared with the compounds according to the invention are furthermore suitable for treatment of ocular hypertension (glaucoma), diabetic retinopathy and macular edema.

The prodrugs prepared with the compounds according to the invention can moreover be used for treatment and/or prevention of operation-related states of ischemia and consecutive symptoms thereof after surgical interventions, in particular interventions on the heart using a heart-lung machine (e.g. bypass operations, heart valve implants), interventions on the carotid arteries, interventions on the aorta and interventions with instrumental opening or penetration of the skull cap.

The prodrugs prepared with the compounds are furthermore suitable for general treatment and/or prevention in the event of surgical interventions with the aim of accelerating wound healing and shortening the reconvalescence time. They are further suited for the promotion of wound healing.

The prodrugs prepared with the compounds are furthermore suitable for treatment and/or prevention of disorders of bone density and structure such as but not restricted to osteoporosis, osteomalacia and hyperparathyroidism-related bone disorders.

The prodrugs prepared with the compounds are furthermore suitable for treatment and/or prevention of sexual dysfunctions, in particular male erectile dysfunction.

Preferable the prodrugs prepared with the compounds are suitable for treatment and/or prevention of heart failure, coronary heart disease, ischemic and/or hemorrhagic stroke, hypertension, pulmonary hypertension, peripheral arterial occlusive disease, pre-eclampsia, chronic obstructive pulmonary

disease, asthma, acute and/or chronic pulmonary edema, allergic alveolitis and/or pneumonitis due to inhaled organic dust and particles of fungal, actinomycetic or other origin, and/or acute chemical bronchitis, acute and/or chronic chemical pulmonary edema, neurogenic pulmonary edema, acute and/or chronic pulmonary manifestations due to radiation, acute and/or chronic interstitial lung disorders, acute lung injury/acute respiratory distress syndrome (ALI/ARDS) in adult or child including newborn, ALI/ARDS secondary to pneumonia and sepsis, aspiration pneumonia and ALI/ARDS secondary to aspiration, ALI/ARDS secondary to smoke gas inhalation, transfusion-related acute lung injury (TRALI), ALI/ARDS and/or acute pulmonary insufficiency following surgery, trauma and/or burns, and/or ventilator induced lung injury (VILI), lung injury following meconium aspiration, pulmonary fibrosis, mountain sickness, chronic kidney diseases, glomerulonephritis, acute kidney injury, cardiorenal syndrome, lymphedema, inflammatory bowel disease, sepsis, septic shock, systemic inflammatory response syndrome (SIRS) of non-infectious origin, anaphylactic shock and/or urticaria.

The present invention further provides for the use of the prodrugs prepared with the compounds according to the invention for treatment and/or prevention of disorders, in particular the disorders mentioned above.

The present invention further provides for the use of the prodrugs prepared with the compounds according to the invention for preparing a medicament for treatment and/or prevention of disorders, in particular the disorders mentioned above.

The present invention further provides a method for treatment and/or prevention of disorders, in particular the disorders mentioned above, using an active amount of the prodrugs prepared with the compounds according to the invention.

The invention further provides medicaments comprising a prodrugs prepared with the compound according to the invention and one or more further active ingredients, in particular for treatment and/or prevention of the disorders mentioned above. Exemplary and preferred active ingredient combinations are:

ACE inhibitors, angiotensin receptor antagonists, beta-2 receptor agonists, phosphodiesterase inhibitors, glucocorticoid receptor agonists, diuretics, or recombinant angiotensin converting enzyme-2 or acetylsalicylic acid (aspirin).

In a preferred embodiment of the invention, the prodrugs prepared with the compounds according to the invention are administered in combination with an ACE inhibitor, such as, by way of example and preferably, enalapril, quinapril, captopril, lisinopril, ramipril, delapril, fosinopril, perindopril,

cilazapril, imidapril, benazepril, moexipril, spirapril or trandopril.

In a preferred embodiment of the invention, the prodrugs prepared with the compounds according to the invention are administered in combination with an angiotensin receptor antagonist, such as, by way of example and preferably, losartan, candesartan, valsartan, telmisartan or embusartan.

In a preferred embodiment of the invention, the prodrugs prepared with the compounds according to the invention are administered in combination with a beta-2 receptor agonist, such as, by way of example and preferably, salbutamol, pirbuterol, salmeterol, terbutalin, fenoterol, tulobuterol, clenbuterol, reproterol or formoterol.

In a preferred embodiment of the invention, the prodrugs prepared with the compounds according to the invention are administered in combination with a phosphodiesterase (PDE) inhibitor, such as, by way of example and preferably, milrinone, amrinone, pimobendan, cilostazol, sildenafil, vardenafil or tadalafil.

In a preferred embodiment of the invention, the prodrugs prepared with the compounds according to the invention are administered in combination with a glucocorticoid receptor agonist, such as, by way of example and preferably, cortiosol, cortisone, hydrocortisone, prednisone, methyl-prednisolone, prednylidene, deflazacort, fluocortolone, triamcinolone, dexamethasone or betamethasone.

In a preferred embodiment of the invention, the prodrugs prepared with the compounds according to the invention are administered in combination with diuretics, such as, by way of example and preferably, furosemide, torasemide and hydrochlorothiazide.

The present invention further relates to medicaments which comprise at least one prodrug prepared with a compound according to the invention, normally together with one or more inert, nontoxic, pharmaceutically suitable excipients, and to the use thereof for the aforementioned purposes.

The prodrugs prepared with the compounds according to the invention can act systemically and/or locally. For this purpose, they can be administered in a suitable way, for example by the parenteral, pulmonary, nasal, sublingual, lingual, buccal, dermal, transdermal, conjunctival, optic route or as implant or stent.

The prodrugs prepared with the compounds according to the invention can be administered in administration forms suitable for these administration routes.

Parenteral administration can take place with avoidance of an absorption step (e.g. intravenous,

intraarterial, intracardiac, intraspinal or intralumbar) or with inclusion of an absorption (e.g. intramuscular, subcutaneous, intracutaneous, percutaneous or intraperitoneal). Administration forms suitable for parenteral administration include preparations for injection and infusion in the form of solutions, suspensions, emulsions, lyophilizates or sterile powders.

Suitable for the other administration routes are, for example, pharmaceutical forms for inhalation (including powder inhalers, nebulizers), nasal drops, eye drops, solutions or sprays; films/wafers or aqueous suspensions (lotions, shaking mixtures), lipophilic suspensions, ointments, creams, transdermal therapeutic systems (e.g. patches), milk, pastes, foams, dusting powders, implants or stents.

Parenteral administration is preferred, especially intravenous administration.

The prodrugs prepared with the compounds according to the invention can be converted into the stated administration forms. This can take place in a manner known per se by mixing with inert, nontoxic, pharmaceutically suitable excipients. These excipients include carriers (for example microcrystalline cellulose, lactose, mannitol), solvents (e.g. liquid polyethylene glycols), emulsifiers and dispersants or wetting agents (for example sodium dodecylsulfate, polyoxysorbitan oleate), binders (for example polyvinylpyrrolidone), synthetic and natural polymers (for example albumin), stabilizers (e.g. antioxidants, for example ascorbic acid), colors (e.g. inorganic pigments, for example iron oxides) and masking flavors and/or odors.

It has generally been found to be advantageous, in the case of parenteral administration, to administer amounts of about 0.001 to 5 mg/kg, preferably about 0.01 to 1 mg/kg, of body weight to achieve effective results.

It may nevertheless be necessary in some cases to deviate from the stated amounts, in particular as a function of the body weight, route of administration, individual response to the active ingredient, nature of the preparation and time or interval over which administration takes place. For instance, less than the aforementioned minimum amount may be sufficient in some cases, whereas in other cases the stated upper limit must be exceeded. In the case of administration of larger amounts, it may be advisable to divide these into a plurality of individual doses over the day.

The following working examples illustrate the invention. The invention is not restricted to the examples.

The percentages in the following tests and examples are, unless stated otherwise, percentages by weight; parts are parts by weight. Solvent ratios, dilution ratios and concentration data for the

liquid/liquid solutions are each based on volume.

A. Examples

Abbreviations

AA	amino acid
Acm	acetamidomethyl
approx.	approximately
Boc	*tert*-butyloxycarbonyl
CDI	carbonyldiimidazole
d	day(s), doublet (in NMR)
TLC	thin-layer chromatography
DCI	direct chemical ionization (in MS)
dd	doublet of doublets (in NMR)
DIEA	N,N-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
of theory	of theory (in yield)
eq.	equivalent(s)
ESI	electrospray ionization (in MS)
Fmoc	(9H-fluoren-9-ylmethoxy)carbonyl
h	hour(s)
HATU	O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
HPLC	high pressure, high performance liquid chromatography
LC-MS	liquid chromatography-coupled mass spectroscopy
m	multiplet (in NMR)
min	minute(s)
MS	mass spectroscopy
NMR	nuclear magnetic resonance spectroscopy
RP	reversed phase (in HPLC)
RT	room temperature
R_t	retention time (in HPLC)
s	singulet (in NMR)
TBTU	benzotriazol-1-yl-N-tetramethyl-uronium tetrafluoroborate
tBu	*tert*-butyl

TFA	trifluoroacetic acid
THF	tetrahydrofuran
Trt	trityl

LC-MS and MS methods

Method 1 (LC-MS): Instrument type: Waters ACQUITY SQD UPLC System; column: Waters Acquity UPLC HSS T3 1.8 µ 50 mm x 1 mm; mobile phase A: 1 l water + 0.25 ml 99% strength formic acid, mobile phase 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: 0.40 ml/min; UV-detection: 210 – 400 nm.

Method 2 (LC-MS): MS instrument: type: Waters (Micromass) Quattro Micro; HPLC instrument type: Agilent 1100 series; column: Thermo Hypersil GOLD 3 µ 20 mm x 4 mm; mobile phase A: 1 l water + 0.5 ml 50% strength formic acid, mobile phase 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; oven: 50°C; flow: 2.0 ml/min; UV-detection: 210 nm.

Method 3 (HPLC): Instrument type: HP 1200 Series; UV DAD; column: Phenomenex Luna 5 µm C5 100Å, 150 mm x 4.6 mm; mobile phase A: 1 l water + 0.5 ml 50% strength formic acid, mobile phase B: 1 l acetonitrile + 0.5 ml 50% strength formic acid; gradient: 0.0 min 95%A → 5 min 5% A; → 5.8 min 95% A → 6.2 min 95% A; flow rate: 2.5 ml/min; oven: RT; UV detection: 210 nm.

Method 4 (HPLC): Instrument type: HP 1200 Series; UV DAD; column: Merck Chromolith Fastgradient RP18 50 mm x 2 mm; mobile phase A: 1 l water + 0.5 ml 50% strength formic acid, mobile phase B: 1 l acetonitrile + 0.5 ml 50% strength formic acid; gradient: 0.0 min 95%A → 2.9 min 5% A → 3.2 min 5% A; flow rate: 3 ml/min; oven: RT; UV detection: 210 nm.

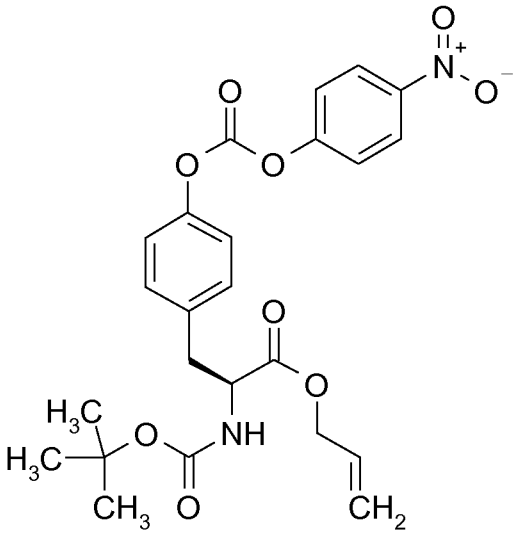
Microwave synthesizer: Biotage Emrys Initiator II synthesizer, with variable vial size up to 20 ml reaction volume and "Robot 60" sample processor

pH 4 citrate buffer: Fluka No 82566; Citrate buffer pH 4, stabilized with sodium azide composition: citric acid, ~0.056 M; sodium azide, ~0.05%; sodium chloride, ~0.044 M; sodium hydroxide, ~0.068 M.

Starting compounds

Example 1A

Allyl-N-(tert-butoxycarbonyl)-O-[(4-nitrophenoxy)carbonyl]-L-tyrosinate

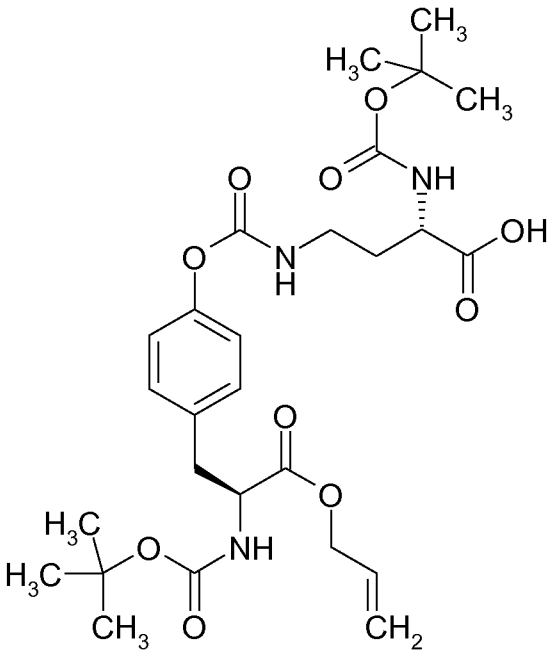


36.7 g (114.3 mmol) N-Boc-L-tyrosine allyl ester, 23.0 g (114.3 mmol) 4-nitrophenyl chloroformate, 17.5 ml (125.7 mmol) triethylamine and 1.40 g (11.4 mmol) 4-dimethylamino pyridine were combined in 1000 ml dichloromethane and stirred at room temperature for 2 h. The reation mixture was extracted with approx. 500 ml water and with approx. 250 ml brine and dried over approx. 100 g sodium sulfate. The solvent was removed by rotary evaporation (approx. 40°C, approx. 200 mbar, approx. 30 min.) and the product was dissolved in warm diethyl ether and crystallized over night at 4°C. The crystals were filtered of, washed with cold diethyl ether and dried in high vacuum (approx. 0.1 mbar, 18 h). The yield was 29.86 g, (59.6 mmol, 52% of theory) of the desired product.

LC-MS (method 1): R_t = 1.23 min., m/z = 487 $(M+H)^+$

Example 2A

(2S)-4-{[(4-{(2S)-3-(Allyloxy)-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)carbonyl]-amino}-2-[(tert-butoxycarbonyl)amino]butanoic acid

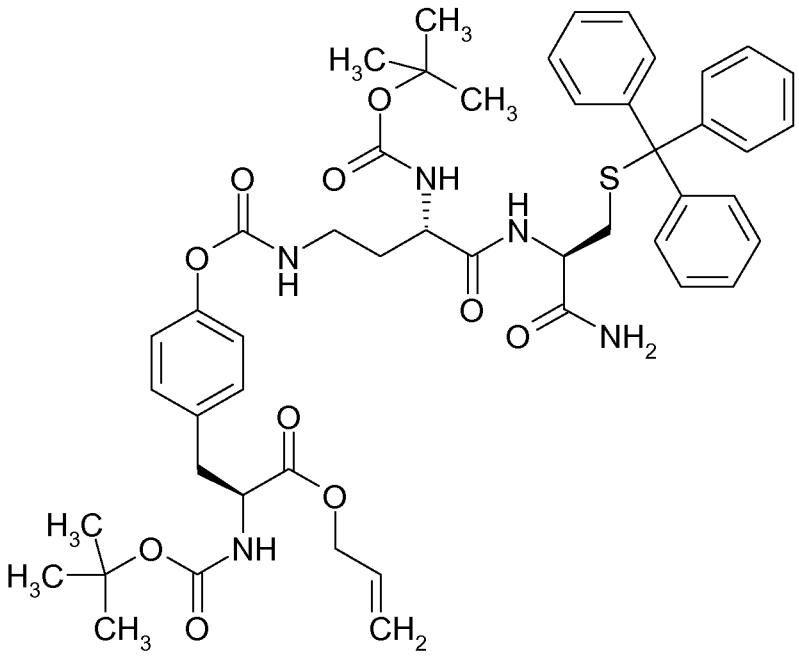


4.0 g (8.22 mmol) of the compound from example 1A was dissolved in 60 ml dichloromethane. 1.795 (8.22 mmol) (2S)-4-Amino-2-[(tert-butoxycarbonyl)amino]butanoic acid and 1.43 ml (8.22 mmol) N,N-diisopropylethylamine were added. The reaction mixture was split into 3 portions. The portions were heated for 30 min in a sealed tube at 75°C in a microwave synthesizer. From the combined reaction mixture the solvent was removed by rotary evaporation (approx. 40°C, approx. 200 mbar, approx. 30 min.). The raw product was dissolved in dichloromethane and chromatographed over approx. 600 ml silica gel. Solvents used were dichloromethane/ethyl acetate 4/1, dichloromethane/ethyl acetate 1/1, dichloromethane/methanol 4/1 and dichloromethane/methanol 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 4.02 g (6.54 mmol, 80% of theory) of the desired product.

LC-MS (method 1): R_t = 1.07 min., m/z = 564 $(M-H)^-$

Example 3A

Allyl O-({(3S)-4-{[(2R)-1-amino-1-oxo-3-(tritylsulfanyl)propan-2-yl]amino}-3-[(tert-butoxycarbonyl)amino]-4-oxobutyl}carbamoyl)-N-(tert-butoxycarbonyl)-L-tyrosinate

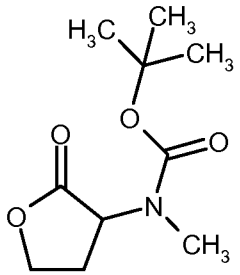


2.50 g (4.42 mmol) of the compound from example 2A was dissolved in 100 ml dichloromethane. 1.602 g (4.42 mmol) S-Trityl-L-cysteinamide, 0.77 ml (4.42 mmol) N,N-diisopropylethylamine and 1.68 g (4.42 mmol) HATU were added. The reaction mixture was split into 5 portions. The portions were heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the combined reaction mixture the solvent was removed by rotary evaporation (approx. 40°C, approx. 200 mbar, approx. 30 min.). The raw product was dissolved in dichloromethane and chromatographed over approx. 600 ml silica gel. Solvents used were dichloromethane/ethyl acetate 2/1, dichloromethane/ethyl acetate 1/1, dichloromethane/methanol 20/1 and dichloromethane/methanol 10/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 4.12 g (3.30 mmol, 75% of theory, 73% purity) of the desired product.

LC-MS (method 1): R_t = 1.36 min., m/z = 911 $(M+H)^+$

Example 4A

tert-Butyl-methyl(2-oxotetrahydrofuran-3-yl)carbamate



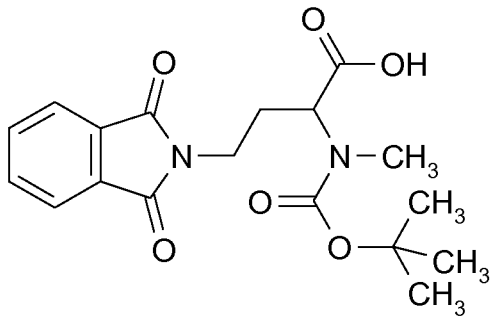
The compound was synthesized according to Alberico, Dino; Paquin, Jean-Francois; Lautens, Mark; Tetrahedron, 2005, vol. 61, p. 6283 - 6297.

5.18 g (25.7 mmol) tert-Butyl(tetrahydro-2-oxo-3-furanyl)carbamate, 4.81 ml (77.2 mmol) iodomethane were dissolced in 100 ml of dry dimethyl fomamide. The solution was cooled to 0°C and 1.34 g (60% in mineral oil, 33.5 mmol) sodium hydride was added. The reaction was warmed to room temperature and stirred over night. The reaction mixture was added to approx. 400 ml water and the mixture was extracted three times with approx. 300 ml ethyl acetate. The combined organic phases were dried over sodium sulfate and concentrated to dryness under reduced pressure. This gave 8.70 g (25.7 mmol, 100% of theory, 63% purity) of the desired product.

The analytic data was in accordance with the literature. The product was used in the next synthetic step without further purification.

Example 5A

2-[(tert-Butoxycarbonyl)(methyl)amino]-4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)butanoic acid



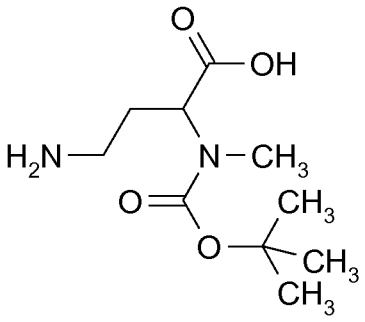
8.70 g (approx. 25 mmol, approx. 63% purity) of the compound from example 4A was dissolved in 560 ml dimethyl formamide. 8.23 g (44.4 mmol) potassium ophtalimide were added and the reaction mixture was heated to 150°C for 7 h. Approx. 400 ml of he solvent was removed by rotary

evaporation (approx. 60°C, approx. 10 mbar, approx. 30 min.). The reaction mixture was poured onto a mixture of approx. 100 ml water, 200 g ice and 15 ml acetic acid. After melting of the remaining ice the reaction mixture was filtered and the filtrate was extracted 3 times with approx. 100 ml dichloromethane. The combined organic phases were dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 70 ml silica gel. Solvents used were dichloromethane/ethyl acetate 9/1 to dichloromethane/ethyl acetate 6/4. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 2.39 g (6.04 mmol, 24% of theory) product.

LC-MS (method 1): R_t = 0.92 min., m/z = 363 $(M+H)^+$

Example 6A

4-Amino-2-[(tert-butoxycarbonyl)(methyl)amino]butanoic acid



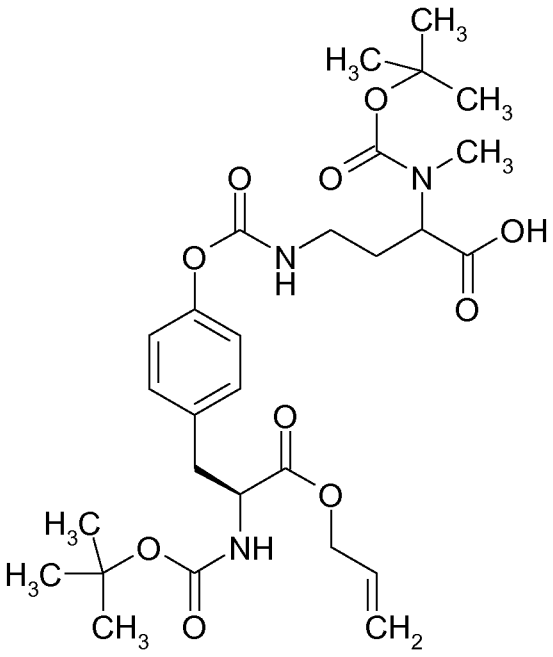
11.8 g (32.6 mmol) of the compound from example 5A was dissolved in approx. 640 ml ethanol and 23.8 ml (488 mmol) hydrazine hydrate was added to the reaction mixture. After stirring over night, the reaction mixture was filtered and the filtrate was concentrated to dryness under reduced pressure. The raw product was dissolved in ethanol and approx. 50 g silica gel was added, the solvent was removed under reduced pressure. The resulting solid was added onto a approx. 500 g silica gel column and chromatographed. Solvents used were dichloromethane/methanol 9/1 to dichloromethane/methanol 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 2.98 g (12.8 mmol, 39% of theory) product.

LC-MS (method 2): R_t = 0.21 min., m/z = 233 $(M+H)^+$

DCI MS (method 5): m/z = 233 $(M+H)^+$

Example 7A

4-{[(4-{(2S)-3-(Allyloxy)-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)carbonyl]-amino}-2-[(tert-butoxycarbonyl)(methyl)amino]butanoic acid

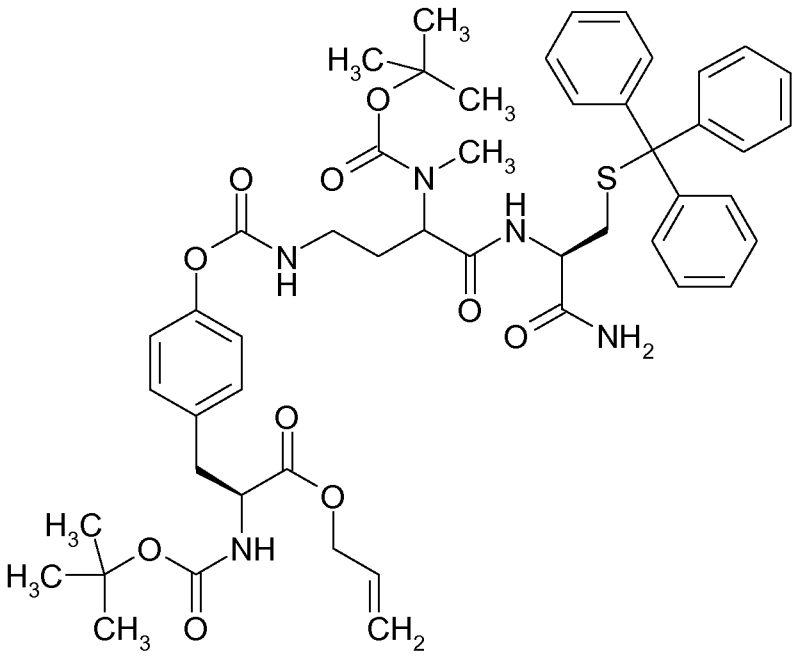


0.931 g (1.92 mmol) of the compound from example 1A was dissolved in 30 ml dichloromethane. 0.455 g (1.92 mmol) of the compound from example 6A was added. The reaction mixture was split into 2 portions. The portions were heated for 30 min in a sealed tube at 80°C in a microwave synthesizer. From the combined reaction mixture the solvent was removed under reduced pressure. The raw product was purified by preparative RP-HPLC on a C18 column with a water methanol gradient from 9/1 to 1/9. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 0.523 g (0.85 mmol, 44% of theory) of the desired product as a mixture of 2 diastereomers.

LC-MS (method 1): R_t = 1.08 and 1.11 min., m/z = 578 $(M-H)^-$

Example 8A

Allyl-O-[(4-{[(2R)-1-amino-1-oxo-3-(tritylsulfanyl)propan-2-yl]amino}-3-[(tert-butoxycarbonyl)-(methyl)amino]-4-oxobutyl)carbamoyl]-N-(tert-butoxycarbonyl)-L-tyrosinate

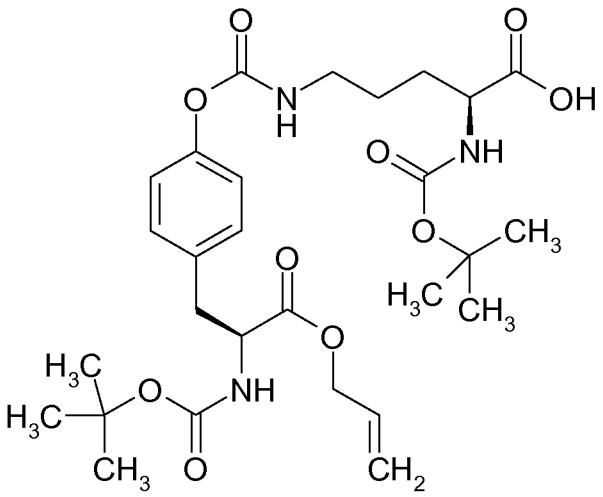


2.24 g (3.86 mmol) of the compound from example 7A was dissolved in 100 ml dichloromethane. 1.401 g (3.86 mmol) S-Trityl-L-cysteinamide, 0.67 ml (3.86 mmol) N,N-diisopropylethylamine and 1.47 g (3.86 mmol) HATU were added. The reaction mixture was split into 5 portions. The portions were heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the combined reaction mixture the solvent was removed by rotary evaporation (approx. 40°C, approx. 200 mbar, approx. 30 min.). The raw product was purified by preparative RP-HPLC on a C18 column with a water methanol gradient from 9/1 to 1/9. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 3.26 g (2.75 mmol, 71% of theory, 78% purity) of the desired product as a mixture of diastereomers.

LC-MS (method 1): R_t = 1.41 and 1.43 min., m/z = 924 $(M+H)^+$

Example 9A

N^5-[(4-{(2S)-3-(Allyloxy)-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)carbonyl]-N^2-(tert-butoxycarbonyl)-L-ornithine

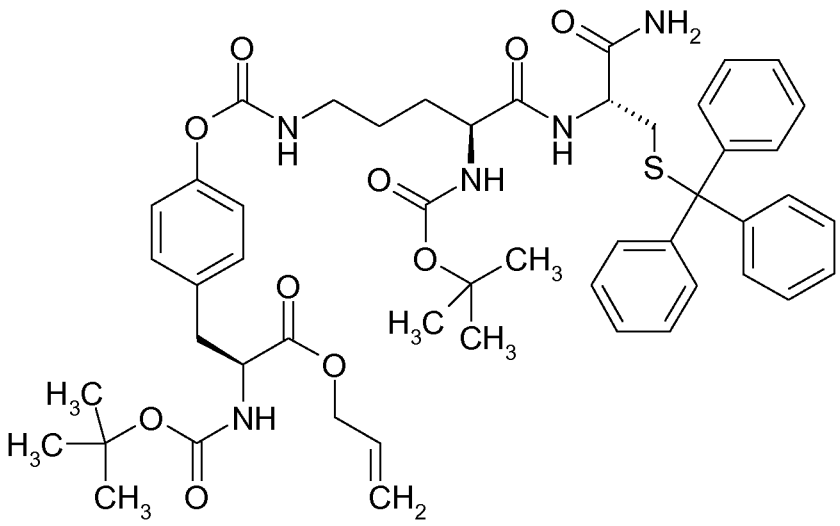


6.00 g (12.33 mmol) of the compound from example 1A was dissolved in 120 ml dichloromethane. 2.57 g (12.33 mmol) N^2-(tert-Butoxycarbonyl)-L-ornithine was added. The reaction mixture was split into 6 portions. The portions were heated for 90 min in a sealed tube at 75°C in a microwave synthesizer. The combined reaction mixture was extracted with approx. 100 ml saturated ammonium chloride solution. The aqueous phase was twice back extracted with approx. 30 ml dichloromethane each. The combined organic phases were extracted with approx. 50 ml brine and dried over sodium sulfate. The solvent was removed under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 600 ml silica gel. Solvents used were dichloromethane, dichloromethane/methanol 40/1 to dichloromethane/methanol 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 2.63 g (4.06 mmol, 33% of theory, 89% purity) of the desired product.

LC-MS (method 1): R_t = 1.03 min., m/z = 578 $(M-H)^-$

Example 10A

N^5-[(4-{(2S)-3-(Allyloxy)-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)carbonyl]-N^2-(tert-butoxycarbonyl)-L-ornithyl-S-trityl-L-cysteinamide

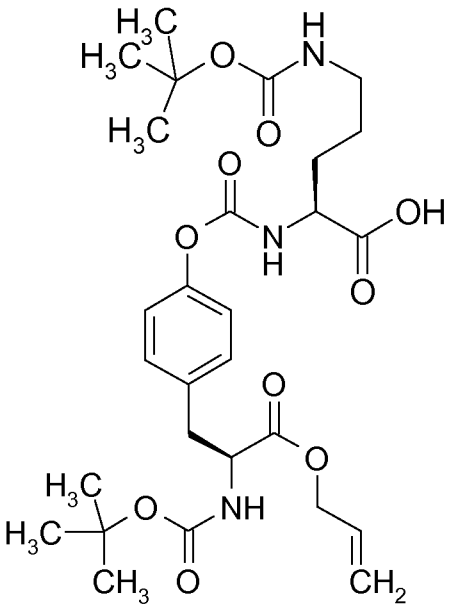


1.20 g (2.07 mmol) of the compound from example 9A was dissolved in 48 ml dichloromethane. 0.750 g (2.07 mmol) S-Trityl-L-cysteinamide, 0.36 ml (2.07 mmol) N,N-diisopropylethylamine and 0.787 g (2.07 mmol) HATU were added. The reaction mixture was split into 3 portions. The portions were heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the combined reaction mixture the solvent was removed by rotary evaporation (approx. 40°C, approx. 200 mbar, approx. 30 min.). The raw product was dissolved in dichloromethane and chromatographed over approx. 400 ml silica gel. Solvents used were dichloromethane/ethyl acetate 2/1, dichloromethane/ethyl acetate 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 1.30 g (1.5 mmol, 56% of theory, 82% purity) of the desired product.

LC-MS (method 1): R_t = 1.35 min., m/z = 924 $(M+H)^+$

Example 11A

N^2-[(4-{(2S)-3-(Allyloxy)-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)carbonyl]-N^5-(tert-butoxycarbonyl)ornithine

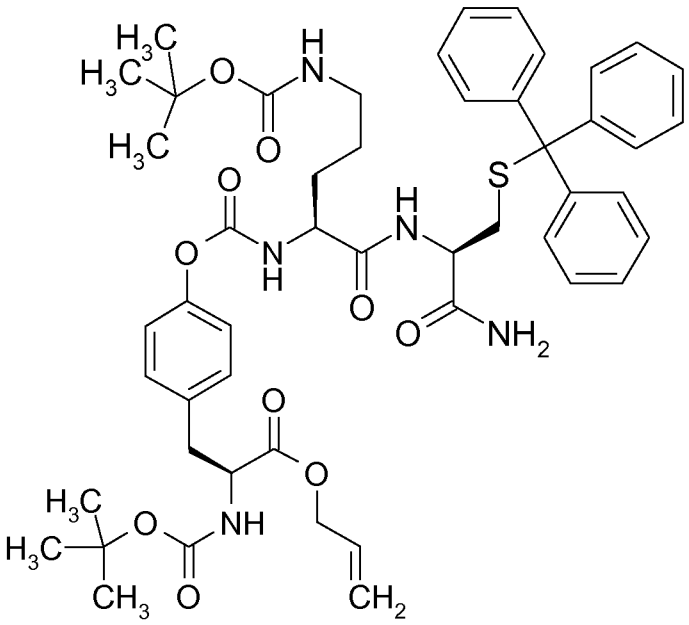


3.00 g (6.16 mmol) of the compound from example 1A was dissolved in 60 ml dichloromethane. 1.43 g (6.16 mmol) N^5-(tert-Butoxycarbonyl)-L-ornithine was added. The reaction mixture was split into 3 portions. The portions were heated for 30 min in a sealed tube at 75°C in a microwave synthesizer. The combined reaction mixture was extracted with approx. 500 ml saturated ammonium chloride solution. The aqueous phase was twice back extracted with approx. 30 ml dichloromethane each. The combined organic phases were extracted with approx. 50 ml brine and dried over sodium sulfate. The solvent was removed under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 500 ml silica gel. Solvents used were dichloromethane, dichloromethane/methanol 20/1 to dichloromethane/methanol 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 2.29 g (3.50 mmol, 57% of theory, 89% purity) of the desired product.

LC-MS (method 1): R_t = 1.07 min., m/z = 578 $(M-H)^-$

Example 12A

N^2-[(4-{(2S)-3-(Allyloxy)-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)carbonyl]-N^5-(tert-butoxycarbonyl)-L-ornithyl-S-trityl-L-cysteinamide

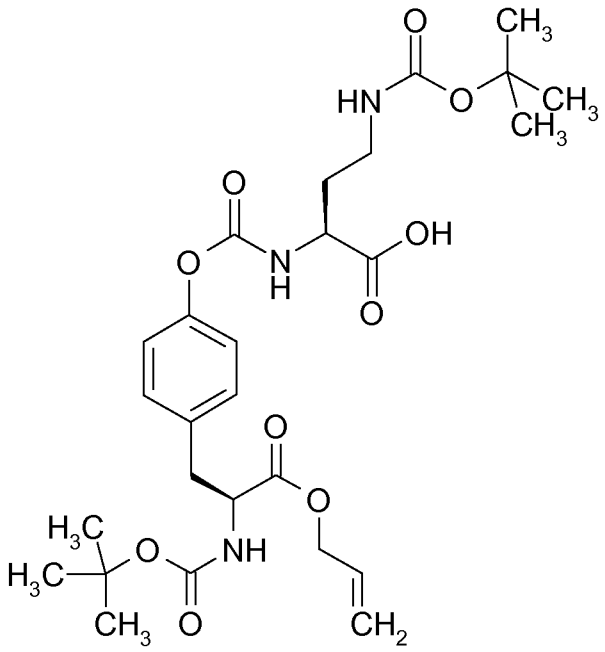


1.50 g (2.59 mmol) of the compound from example 11A was dissolved in 60 ml dichloromethane. 0.940 g (2.59 mmol) S-Trityl-L-cysteinamide, 0.45 ml (2.60 mmol) N,N-diisopropylethylamine and 0.984 g (2.59 mmol) HATU were added. The reaction mixture was split into 3 portions. The portions were heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the combined reaction mixture the solvent was removed by rotary evaporation (approx. 40°C, approx. 200 mbar, approx. 30 min.). The raw product was dissolved in dichloromethane and chromatographed over approx. 400 ml silica gel. Solvents used were dichloromethane/ethyl acetate 2/1, dichloromethane/ethyl acetate 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 1.72 g (1.64 mmol, 63% of theory, 88% purity) of the desired product.

LC-MS (method 1): R_t = 1.35 min., m/z = 924 $(M+H)^+$

Example 13A

(2S)-2-{[(4-{(2S)-3-(Allyloxy)-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)carbonyl]-amino}-4-[(tert-butoxycarbonyl)amino]butanoic acid

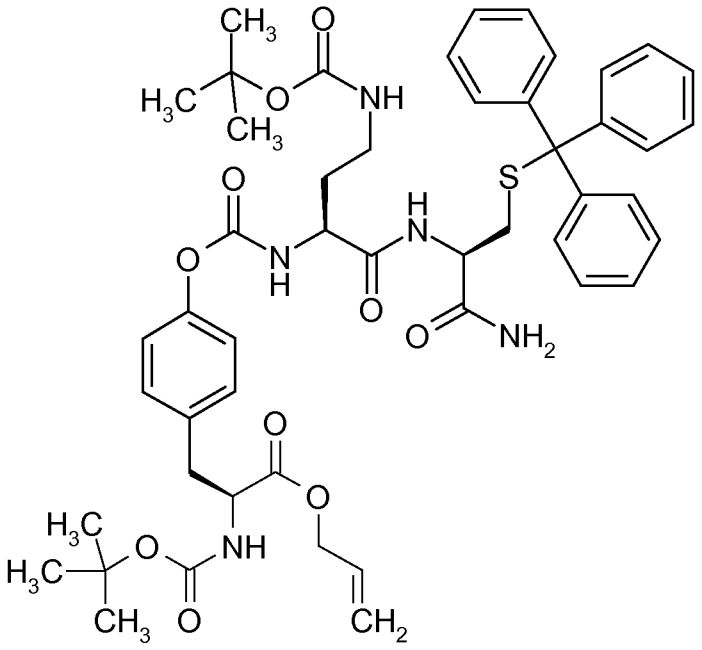


7.50 g (15.4 mmol) of the compound from example 1A was dissolved in 150 ml dichloromethane. 3.36 g (15.4 mmol) (2S)-2-Amino-4-[(tert-butoxycarbonyl)amino]butanoic acid was added. The reaction mixture was split into 10 portions. The portions were heated for 30 min in a sealed tube at 75°C in a microwave synthesizer. The combined reaction mixture was extracted with approx. 100 ml saturated ammonium chloride solution. The aqueous phase was twice back extracted with approx. 50 ml dichloromethane each. The combined organic phases were extracted with approx. 50 ml brine and dried over sodium sulfate. The solvent was removed under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 1 l silica gel. Solvents used were diclorometh ane/ethyl acetate 4/1, dichloromethane/methanol 10/1 to dichloromethane/methanol 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 8.70 g (10.8 mmol, 70% of theory) of the desired product.

LC-MS (method 1): R_t = 1.06 min., m/z = 564 $(M-H)^-$

Example 14A

Allyl-N-(tert-butoxycarbonyl)-O-{[(4R,7S)-4-carbamoyl-13,13-dimethyl-6,11-dioxo-1,1,1-triphenyl-12-oxa-2-thia-5,10-diazatetradecan-7-yl]carbamoyl}-L-tyrosinate

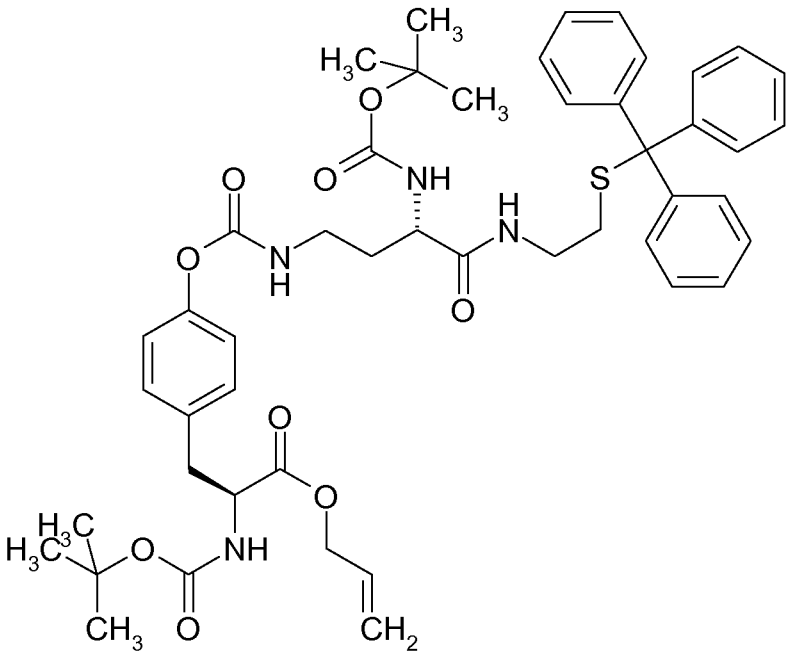


3.00 g (5.30 mmol) of the compound from example 13A was dissolved in 120 ml dichloromethane. 1.92 g (5.30 mmol) S-Trityl-L-cysteinamide, 0.92 ml (5.30 mmol) N,N-diisopropylethylamine and 2.02 g (5.30 mmol) HATU were added. The reaction mixture was split into 6 portions. The portions were heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the combined reaction mixture the solvent was removed by rotary evaporation (approx. 40°C, approx. 200 mbar, approx. 30 min.). The raw product was dissolved in dichloromethane and chromatographed over approx. 800 ml silica gel. Solvents used were dichloromethane/ethyl acetate 2/1, dichloromethane/ethyl acetate 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 4.91 g (3.73 mmol, 70% of theory, 69% purity) of the desired product.

LC-MS (method 1): R_t = 1.35 min., m/z = 910 $(M+H)^+$

Example 15A

Allyl-N-(tert-butoxycarbonyl)-O-{[(3S)-3-[(tert-butoxycarbonyl)amino]-4-oxo-4-{[2-(tritylsulfanyl)ethyl]amino}butyl]carbamoyl}-L-tyrosinate

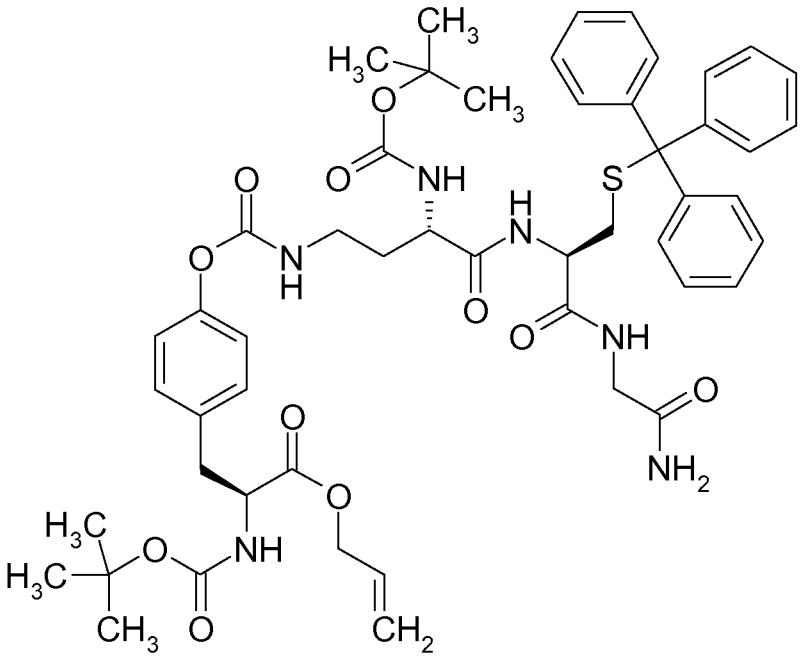


351 mg (0.63 mmol) of the compound from example 2A was dissolved in 15 ml dichloromethane. 200 mg (0.63 mmol) 2-(Tritylsulfanyl)ethanamine, 0.11 ml (0.63 mmol) N,N-diisopropylethyl-amine and 238 mg (0.63 mmol) HATU were added. The reaction mixture was heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the reaction mixture the solvent was removed under reduced pressure. The raw product was purified by preparative RP-HPLC on a C18 column with a water methanol gradient from 9/1 to 1/9. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 98 mg (0.110 mmol, 16% of theory) of the desired product.

LC-MS (method 1): R_t = 1.45 min., m/z = 867 $(M+H)^+$

Example 16A

N-{(2S)-4-{[(4-{(2S)-3-(Allyloxy)-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)-carbonyl]amino}-2-[(tert-butoxycarbonyl)amino]butanoyl}-S-trityl-L-cysteinylglycinamide

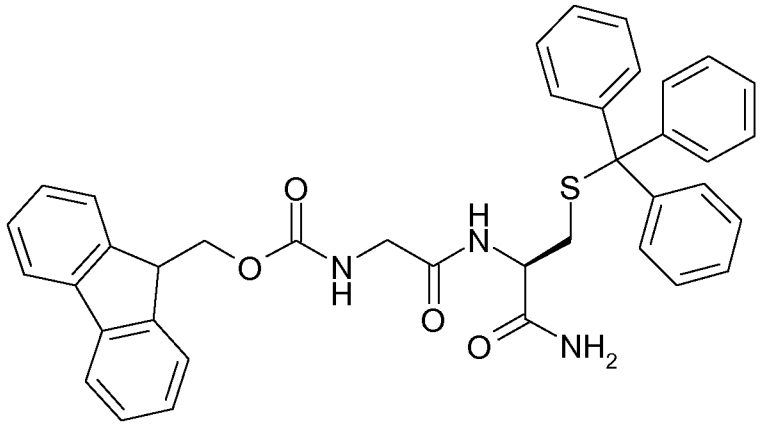


173 mg (0.31mmol) of the compound from example 2A was dissolved in 10 ml dichloromethane. 128 mg (0.31 mmol) S-Trityl-L-cysteinylglycinamide, 53 µl (0.31 mmol) N,N-diisopropylethylamine and 116 mg (0.31 mmol) HATU were added. The reaction mixture was heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the reaction mixture the solvent was removed under reduced pressure. The raw product was purified by preparative RP-HPLC on a C18 column with a water methanol gradient from 9/1 to 1/9. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 57 mg (0.02 mmol, 18% of theory) of the desired product.

LC-MS (method 1): R_t = 1.31 min., m/z = 968 $(M+H)^+$

Example 17A

N-[(9H-Fluoren-9-ylmethoxy)carbonyl]glycyl-S-trityl-L-cysteinamide

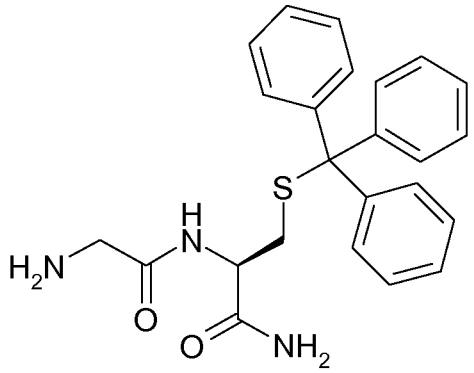


1.00 g (3.36 mmol) of N-[(9H-Fluoren-9-ylmethoxy)carbonyl]glycine was dissolved in 30 ml dichloromethane. 1.41 g (3.36 mmol) S-Trityl-L-cysteinylglycinamide, 0.59 ml (3.36 mmol) N,N-diisopropylethylamine and 1.28 g (3.36 mmol) HATU were added. The reaction mixture was heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the reaction mixture the solvent was removed under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 300 ml silica gel. Solvents used were dichloromethane, dichloromethane/methanol 20/1, dichloromethane/methanol 10/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 1.63 g (2.06 mmol, 81% of theory) of the desired product.

LC-MS (method 1): R_t = 1.31 min., m/z = 642 $(M+H)^+$

Example 18A

Glycyl-S-trityl-L-cysteinamide

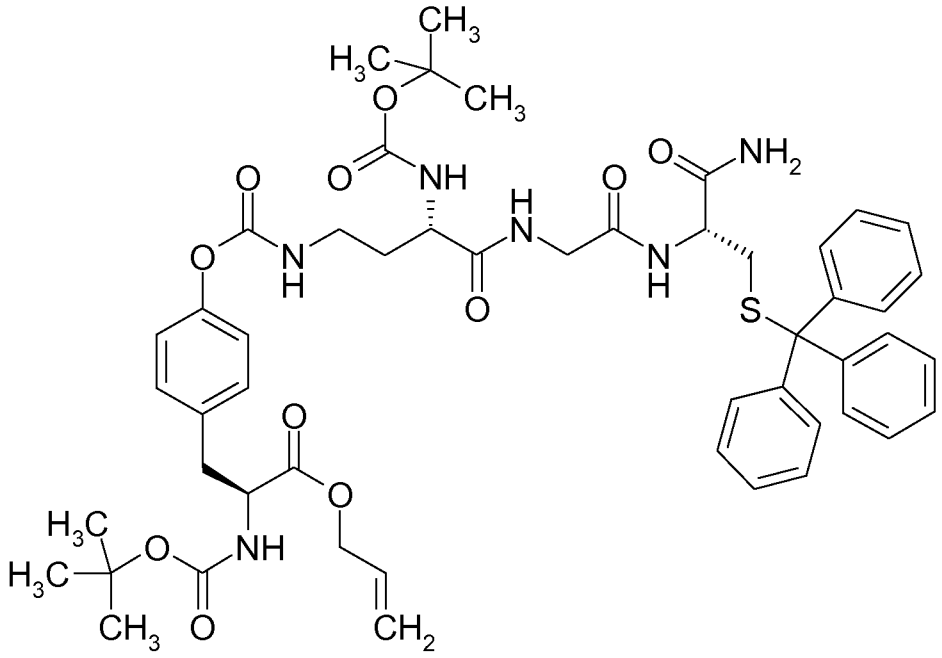


1.53 g (2.38 mmol) of the compound from example 17A was dissolved in 18 ml dimethyl formamide and 0.47 ml (4.79 mmol) DIEA was added. After one hour reaction time, the raw product was purified by preparative RP-HPLC on a C18 column with a water methanol gradient from 9/1 to 1/9. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 416 mg (0.97 mmol, 40% of theory) of the desired product.

LC-MS (method 1): R_t = 0.76 min., m/z = 418 $(M-H)^-$

Example 19A

N-{(2S)-4-{[(4-{(2S)-3-(Allyloxy)-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)-carbonyl]amino}-2-[(tert-butoxycarbonyl)amino]butanoyl}glycyl-S-trityl-L-cysteinamide

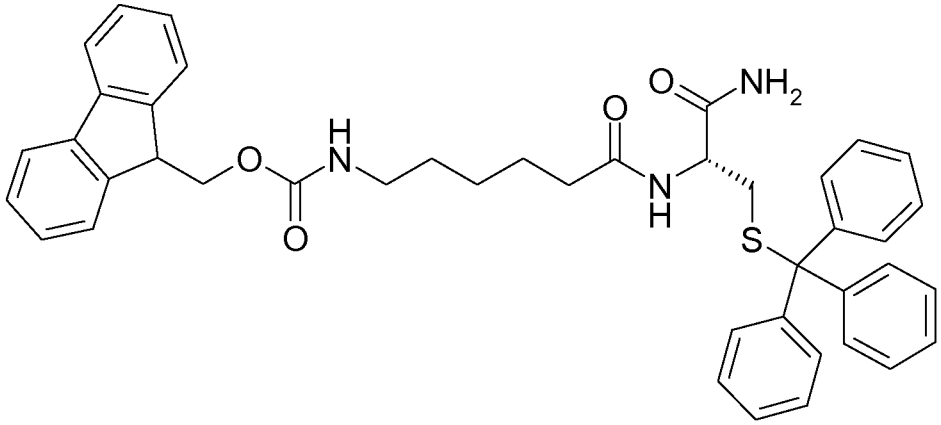


559 mg (0.99 mmol) of the compound from example 2A was dissolved in 15 ml dichloromethane. 415 mg (0.99 mmol) of the compound from example 18A, 173 µl (0.99 mmol) N,N-diisopropylethylamine and 376 mg (0.99 mmol) HATU were added. The reaction mixture was heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the reaction mixture the solvent was removed under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 70 ml silica gel. Solvents used were dichloromethane, dichloromethane/methanol 20/1 to dichloromethane/methanol 5/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 860 mg (0.69 mmol, 70% of theory) of the desired product.

LC-MS (method 1): R_t = 1.30 min., m/z = 968 $(M+H)^+$

Example 20A

9H-Fluoren-9-ylmethyl-(6-{[(2R)-1-amino-1-oxo-3-(tritylsulfanyl)propan-2-yl]amino}-6-oxohexyl)carbamate

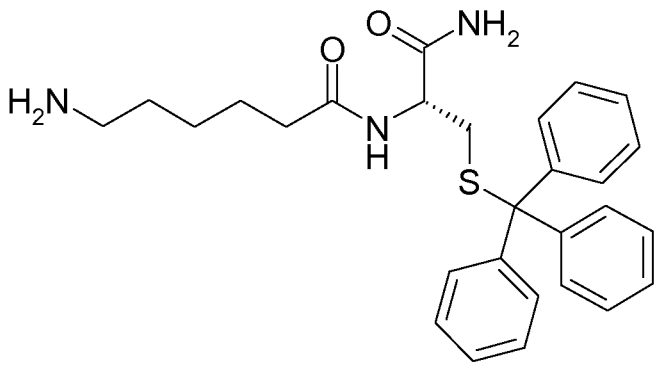


500 mg (1.42 mmol) 6-{[(9H-Fluoren-9-ylmethoxy)carbonyl]amino}hexanoic acid was dissolved in 18 ml dichloromethane. 513 mg (1.42 mmol) S-Trityl-L-cysteinylglycinamide, 246 µl (1.42 mmol) N,N-diisopropylethylamine and 537 m g (1.42 mmol) HATU were added. The reaction mixture was heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the reaction mixture the solvent was removed under reduced pressure. The raw product was purified by preparative RP-HPLC on a C18 column with a water methanol gradient from 9/1 to 1/9. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 678 mg (0.70 mmol, 49% of theory) of the desired product.

LC-MS (method 1): R_t = 1.38 min., m/z = 698 $(M+H)^+$

Example 21A

6-Amino-N-[(2R)-1-amino-1-oxo-3-(tritylsulfanyl)propan-2-yl]hexanamide

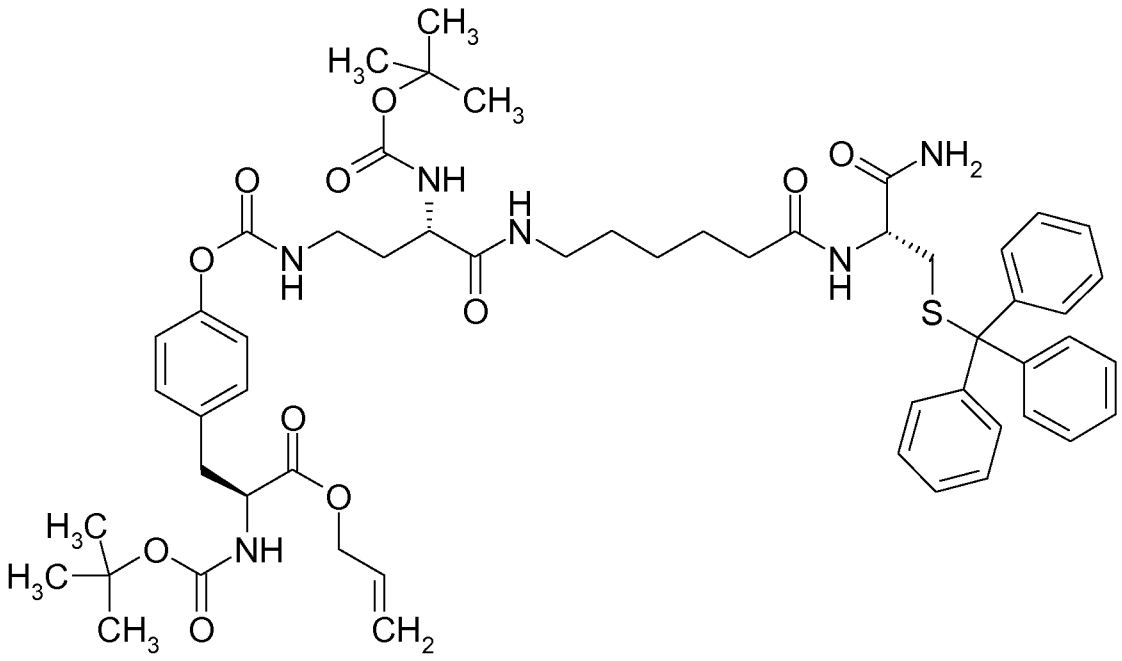


678 mg (0.97 mmol) of the compound of example 20A was dissolved in 7 ml dimethyl formamide and 0.19 ml (1.94 mmol) DIEA was added. After one hour reaction time, the raw product was purified by preparative RP-HPLC on a C18 column with a water methanol gradient from 9/1 to 1/9. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 457 mg (0.93 mmol, 95% of theory) of the desired product.

LC-MS (method 1): R_t = 0.85 min., m/z = 476 $(M+H)^+$

Example 22A

Allyl-O-({(3S)-4-[(6-{[(2R)-1-amino-1-oxo-3-(tritylsulfanyl)propan-2-yl]amino}-6-oxohexyl)-amino]-3-[(tert-butoxycarbonyl)amino]-4-oxobutyl}carbamoyl)-N-(tert-butoxycarbonyl)-L-tyrosinate

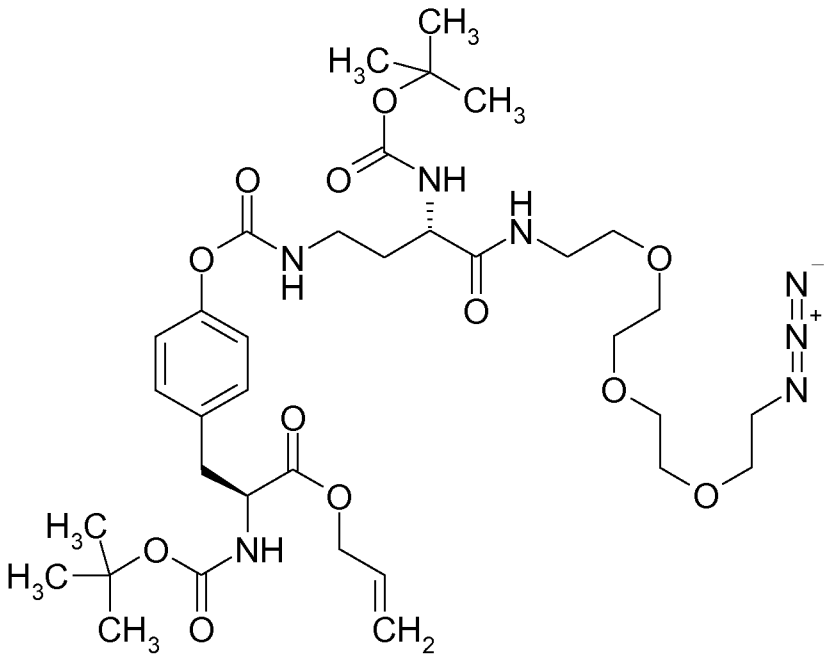


457 mg (0.81 mmol) of the compound from example 2A was dissolved in 15 ml dichloromethane. 384 mg (0.81 mmol) of the compound from example 21A, 141 µl (0.81 mmol) N,N-diisopropylethylamine and 307 mg (0.81 mmol) HATU were added. The reaction mixture was heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the reaction mixture the solvent was removed under reduced pressure. The raw product was purified in two portions by preparative RP-HPLC on a C18 column with a water methanol gradient from 9/1 to 1/9. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 255 mg (0.22 mmol, 28% of theory) of the desired product.

LC-MS (method 1): R_t = 1.31 min., m/z = 1032 $(M+H)^+$

Example 23A

Allyl-O-({(14S)-1-azido-14-[(tert-butoxycarbonyl)amino]-13-oxo-3,6,9-trioxa-12-azahexadecan-16-yl}carbamoyl)-N-(tert-butoxycarbonyl)-L-tyrosinate

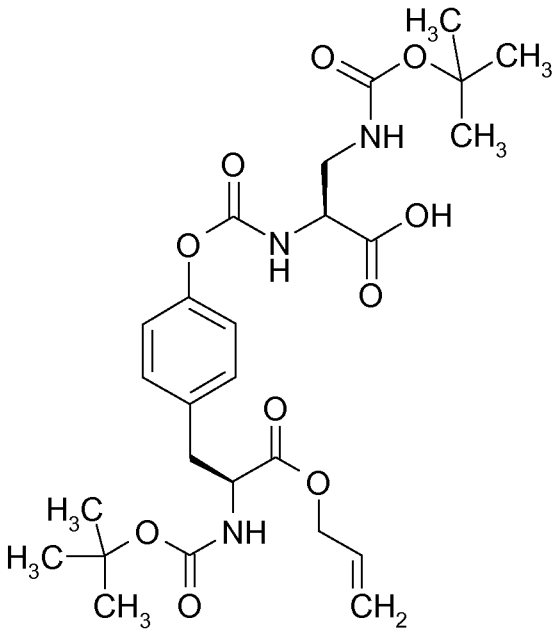


518 mg (0.92 mmol) of the compound from example 2A was dissolved in 15 ml dichloromethane. 200 mg (0.92 mmol) 2-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}ethanamine, 160 µl (0.92 mmol) N,N-diisopropylethylamine and 348 mg (0.92 mmol) HATU were added. The reaction mixture was heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. From the reaction mixture the solvent was removed under reduced pressure. The raw product was purified by preparative RP-HPLC on a C18 column with a water methanol gradient from 9/1 to 1/9. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 276 mg (0.34 mmol, 37% of theory) of the desired product.

LC-MS (method 1): R_t = 1.15 min., m/z = 766 $(M+H)^+$

Example 24A

N-[(4-{(2S)-3-(Allyloxy)-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)carbonyl]-3-[(tert-butoxycarbonyl)amino]-L-alanine

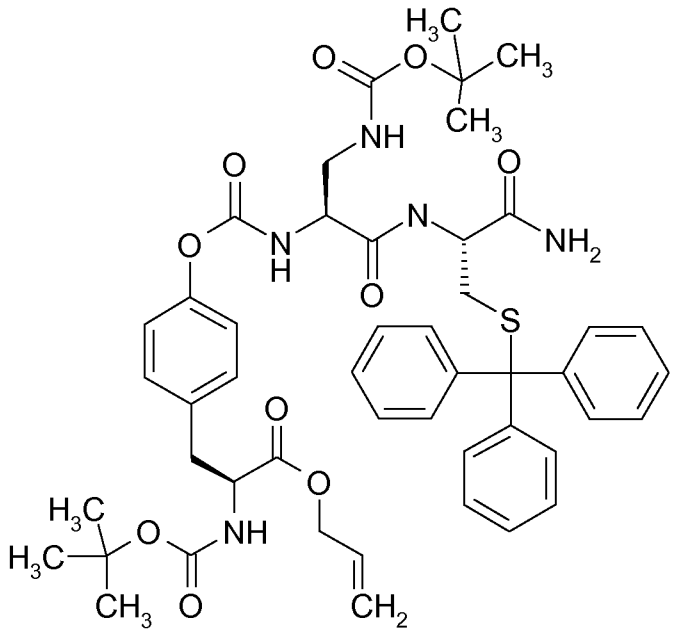


2.45 g (5.0 mmol) of the compound from example 1A was dissolved in 40 ml dichloroethane. 1.03 g (5.0 mmol) 3-[(tert-Butoxycarbonyl)amino]-L-alanine was added. The reaction mixture was heated to 85°C for 2 h. The solvent was removed under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 150 ml silica gel. Solvents used were dicloromethane/methanol 20/1 to dichloromethane/methanol 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 1.23 g (2.2 mmol, 44% of theory) of the desired product.

LC-MS (method 1): R_t = 1.06 min., m/z = 550 $(M-H)^-$

Example 25A

N-[(4-{(2S)-3-(Allyloxy)-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)carbonyl]-3-[(tert-butoxycarbonyl)amino]-L-alanyl-S-trityl-L-cysteinamide

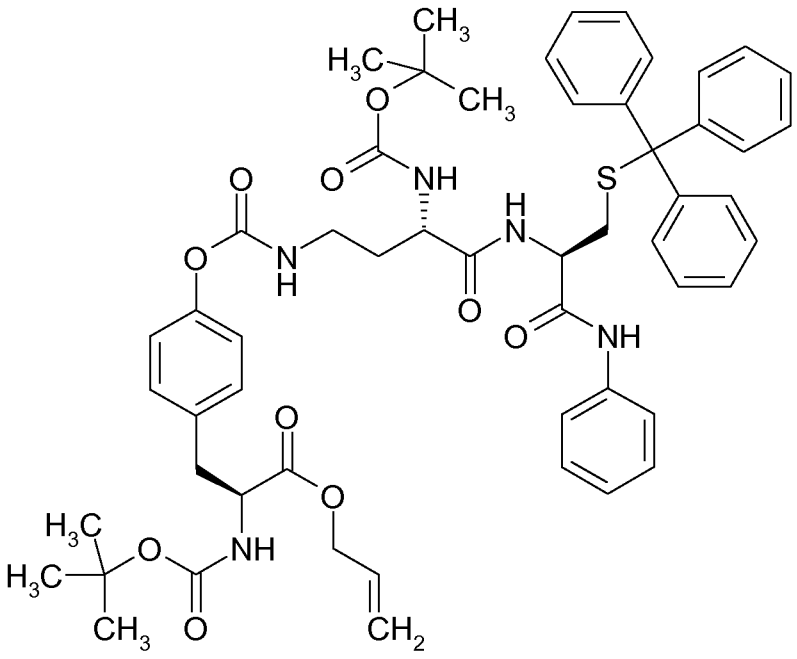


1.23 g (2.23 mmol) of the compound from example 24A was dissolved in 25 ml dichloromethane. 0.81 g (2.23 mmol) S-Trityl-L-cysteinamide, 0.39 ml (2.23 mmol) N,N-diisopropylethylamine and 0.85 g (2.23 mmol) HATU were added. The reaction mixture was stirred at room temperature for 3 h. From the reaction mixture the solvent was removed by rotary evaporation (approx. 40°C, approx. 200 mbar, approx. 30 min.). The raw product was dissolved in dichloromethane and chromatographed over approx. 70 ml silica gel. Solvents used were dicloromethane/methanol 20/1 to dichloromethane/methanol 5/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 2.38 g (2.03 mmol, 91% of theory, 76% purity) of the desired product.

LC-MS (method 1): R_t = 1.37 min., m/z = 897 $(M+H)^+$

Example 26A

Allyl-O-({(3S)-4-{[(2R)-1-anilino-1-oxo-3-(tritylsulfanyl)propan-2-yl]amino}-3-[(tert-butoxycarbonyl)amino]-4-oxobutyl}carbamoyl)-N-(tert-butoxycarbonyl)-L-tyrosinate

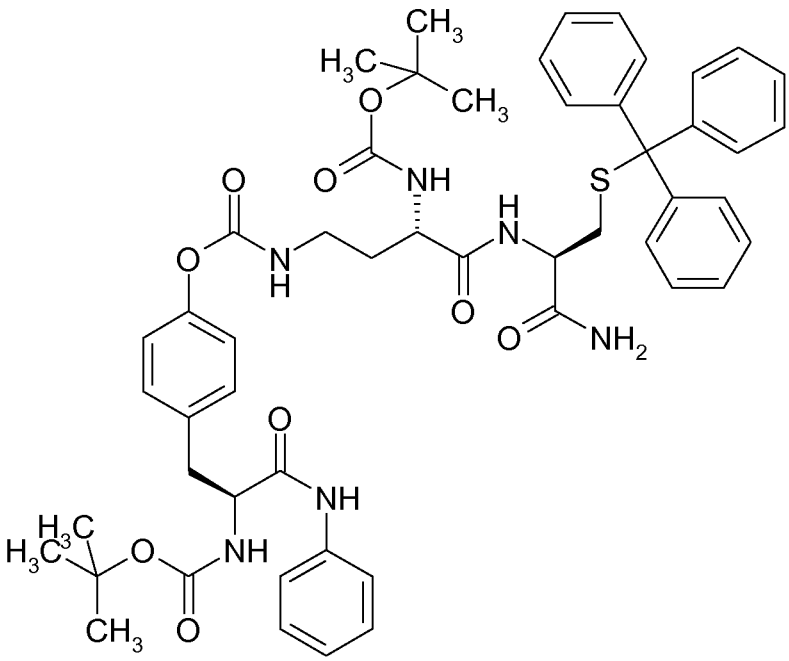


456 mg (0.68 mmol) of the compound from example 2A was dissolved in 8 ml dichloromethane. 300 mg (0.68 mmol) N-Phenyl-S-trityl-L-cysteinamide, 0.12 ml (0.68 mmol) N,N-diisopropylethylamine and 260 mg (0.68 mmol) HATU were added. The reaction mixture was stirred at romm temperature for 4 h. From the reaction mixture the solvent was removed under reduced pressure. The raw product was purified in two portions by preparative RP-HPLC on a C18 column with a water methanol gradient from 9/1 to 1/9. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 361 mg (0.37 mmol, 53% of theory) of the desired product.

LC-MS (method 1): R_t = 1.48 min., m/z = 987 $(M+H)^+$

Example 1B

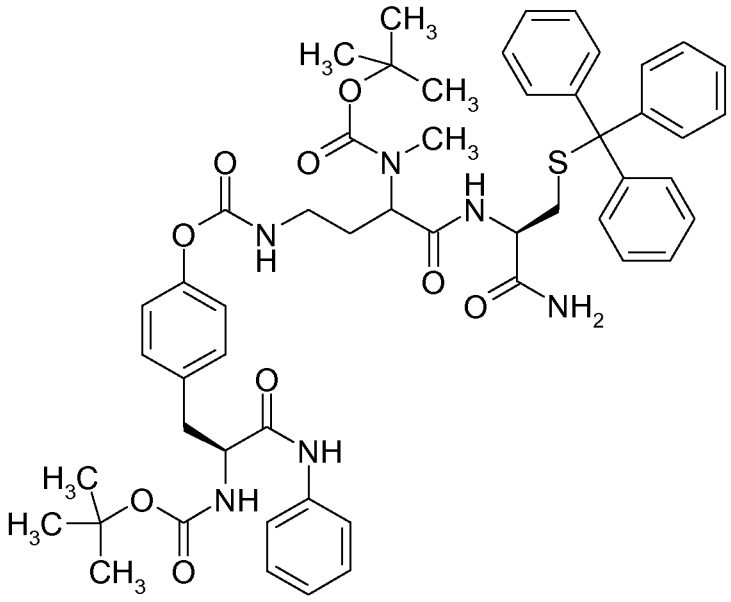
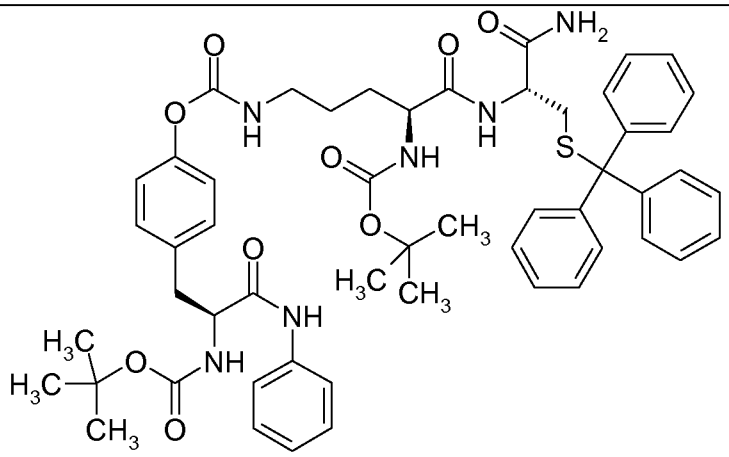
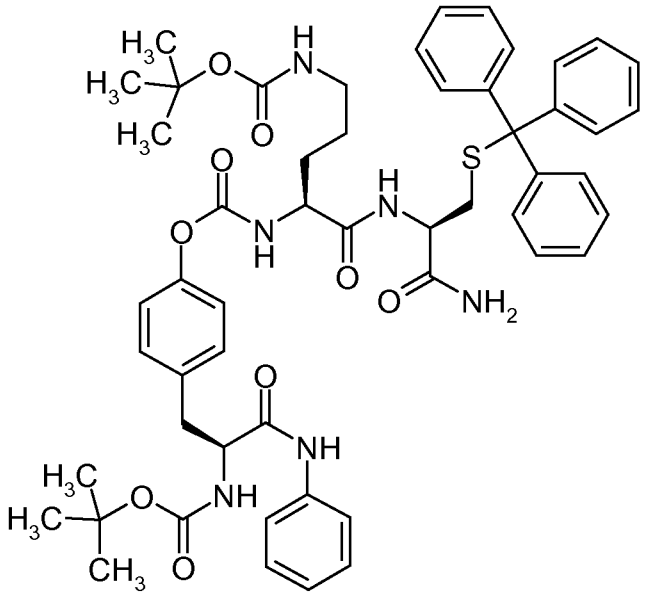
tert-Butyl-[(2S)-1-{[(2R)-1-amino-1-oxo-3-(tritylsulfanyl)propan-2-yl]amino}-4-{[(4-{(2S)-3-anilino-2-[(tert-butoxycarbonyl)amino]-3-oxopropyl}phenoxy)carbonyl]amino}-1-oxobutan-2-yl]carbamate

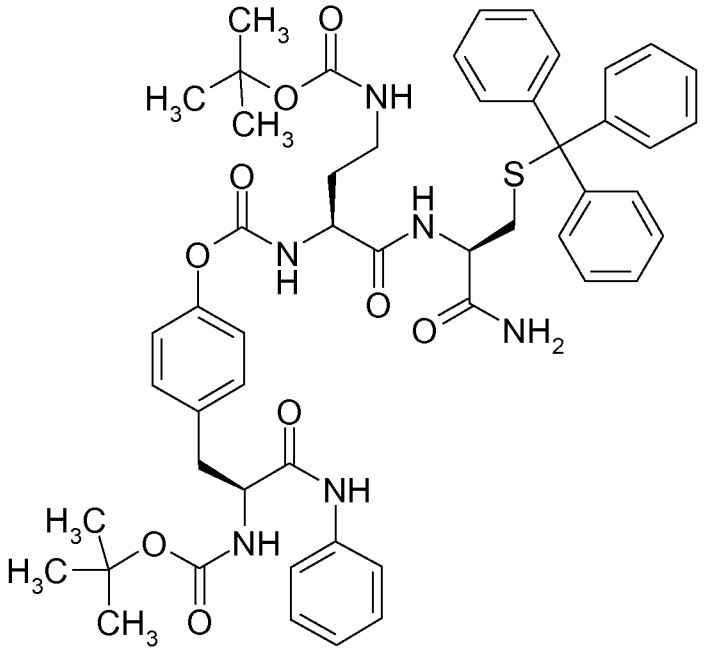
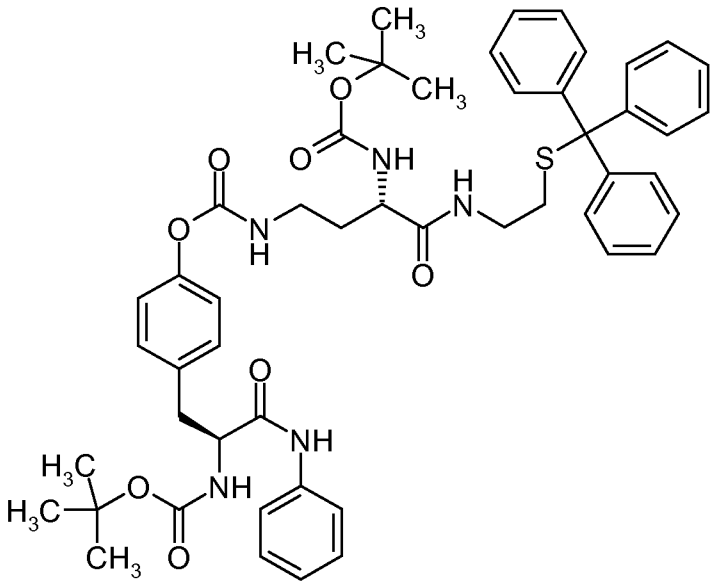


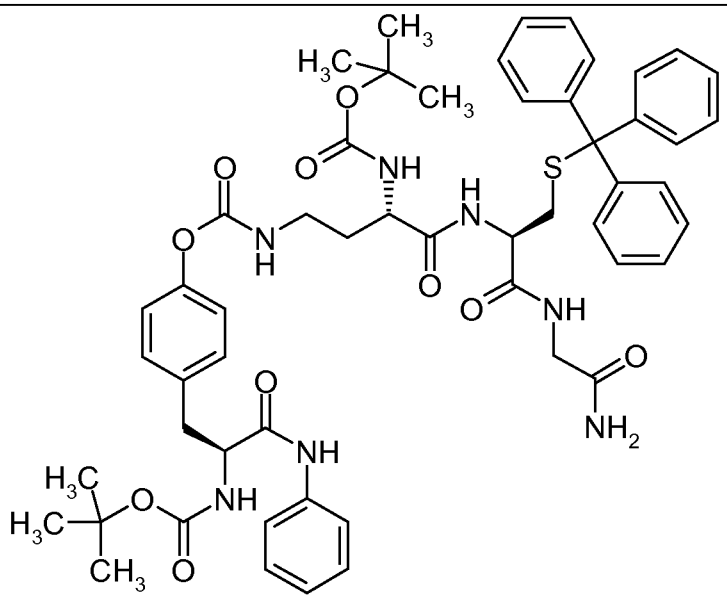
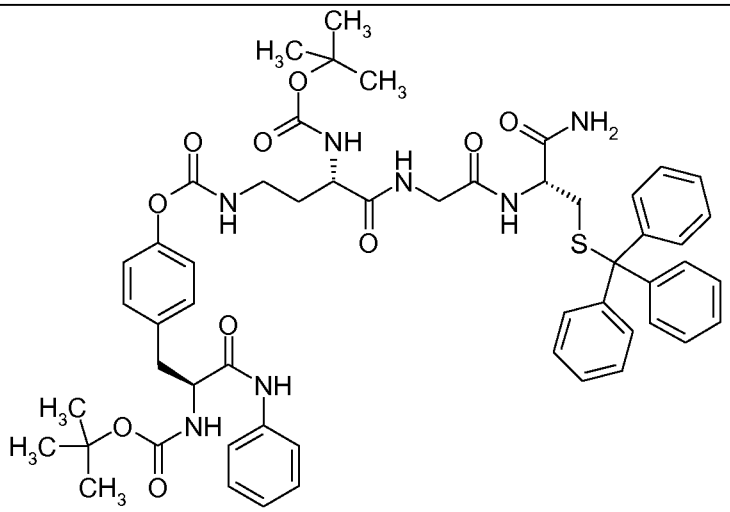
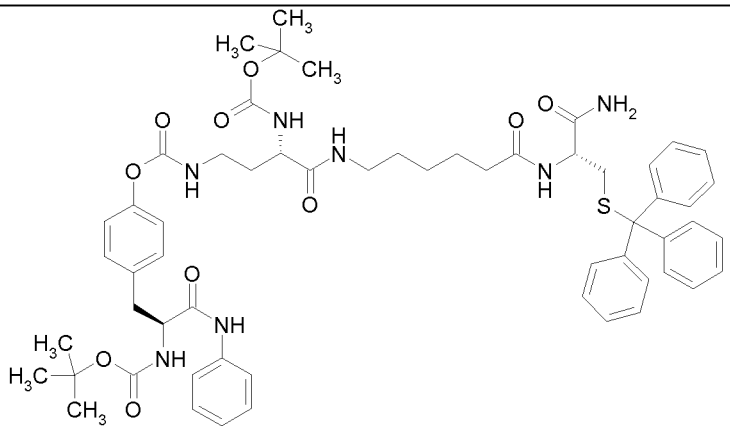
250 mg (0.29 mmol) of the compound of example 1 was dissolved in 10 ml dichloromethane. 40 mg (0.43 mmol) Aniline, 164 mg (0.43 mmol) HATU and 75 µl (0.43 mmol) DIEA were added. The reaction mixture wase heated for 30 min in a sealed tube at 60°C in a microwave synthesizer. The raw product was concentrated to dryness under reduced pressure. The raw product was dissolved in methanol and purified by preparative RP-HPLC on a C18 column with a water/methanol gradient to yield 271 mg product (88% of theory).

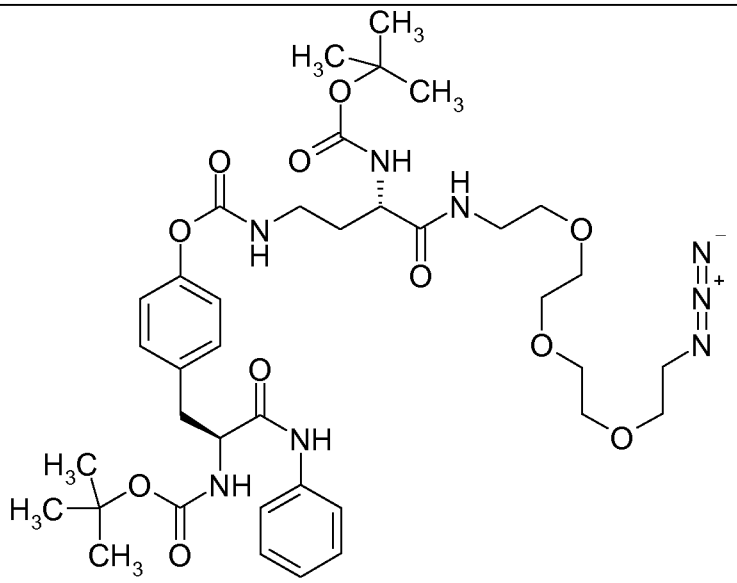
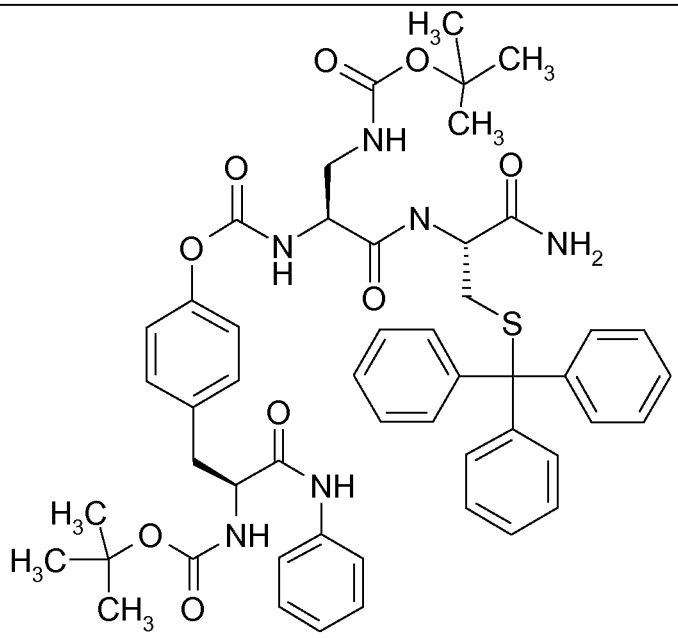
LC-MS (method 1): R_t = 1.31 min., m/z = 945 $(M+H)^+$

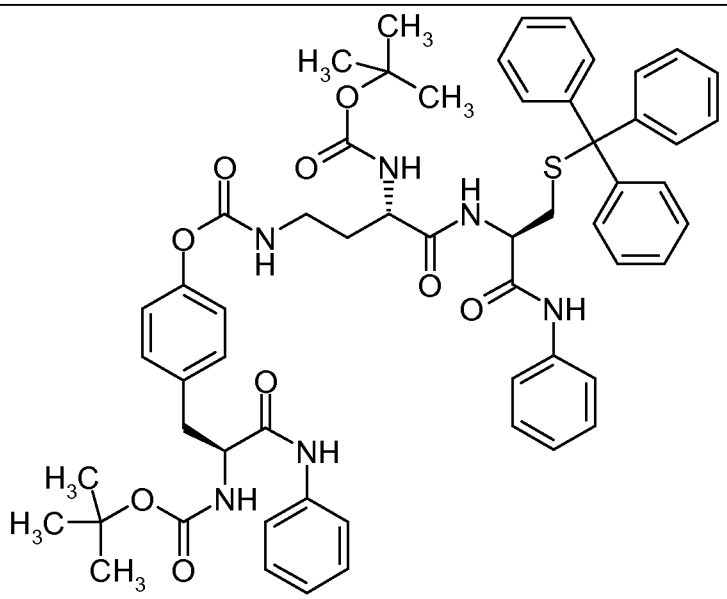
Using the appropriate carboxylic acids (working examples 2 to 12), the examples of the table below are prepared analogously to Example 1B.

Example	Structure	Characterization
2B		LC-MS (method 1): R_t = 1.34 and 1.37 min., m/z = 959 $(M+H)^+$
3B		LC-MS (method 1): R_t = 1.32 min., m/z = 959 $(M+H)^+$
4B		LC-MS (method 1): R_t = 1.32 min., m/z = 959 $(M+H)^+$

Example	Structure	Characterization
5B		LC-MS (method 1): R_t = 1.30 min, m/z = 945 $(M+H)^+$
6B		LC-MS (method 1): R_t = 1.43 min, m/z = 903 $(M+H)^+$

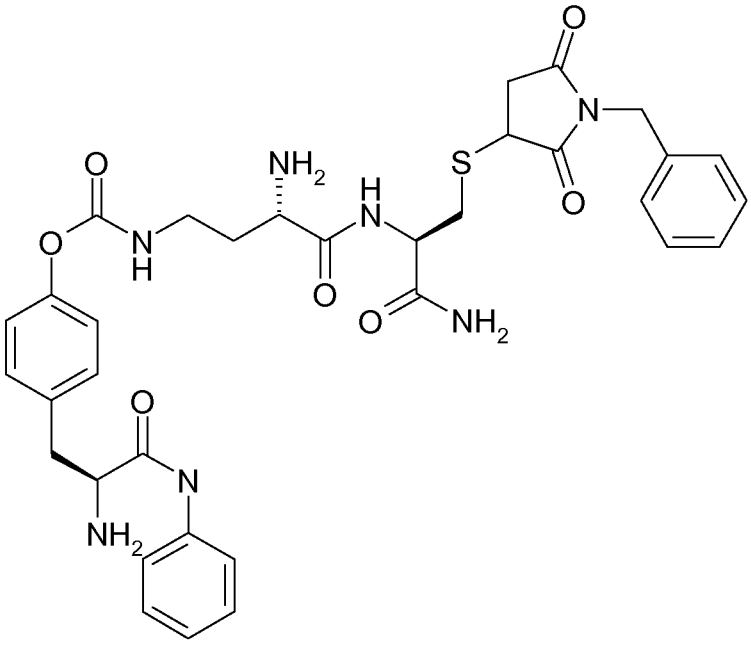
Example	Structure	Characterization
7B		LC-MS (method 1): R_t = 1.29 min, m/z = 1002 $(M+H)^+$
8B		LC-MS (method 1): R_t = 1.27 min, m/z = 1002 $(M+H)^+$
9B		LC-MS (method 1): R_t = 1.29 min, m/z = 1058 $(M+H)^+$

Example	Structure	Characterization
10B		LC-MS (method 2): R_t = 2.42 min, m/z = 801 $(M+H)^+$
11B		LC-MS (method 1): R_t = 1.36 min, m/z = 931 $(M+H)^+$

Example	Structure	Characterization
12B		LC-MS (method 1): R_t = 1.48 min, m/z = 1022 $(M+H)^+$

Example 1C

O-{[(3S)-3-Amino-4-({(2R)-1-amino-3-[(1-benzyl-2,5-dioxopyrrolidin-3-yl)sulfanyl]-1-oxopropan-2-yl}amino)-4-oxobutyl]carbamoyl}-N-phenyl-L-tyrosinamide

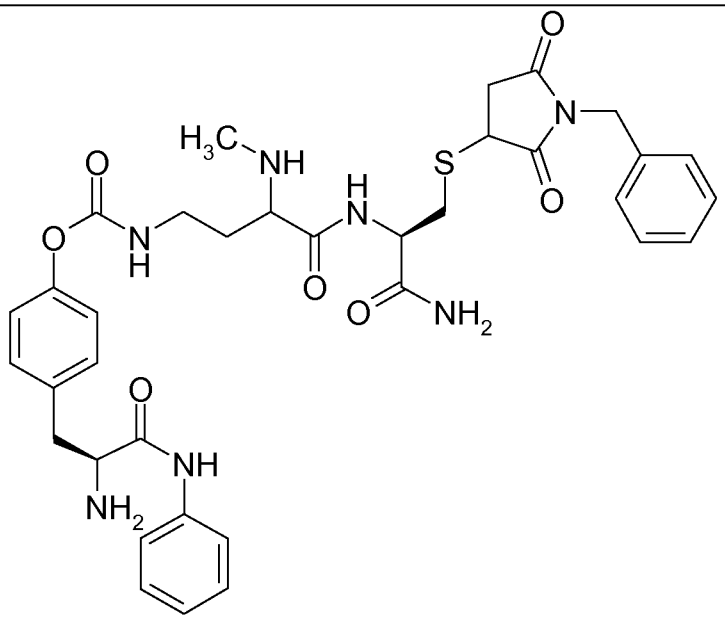


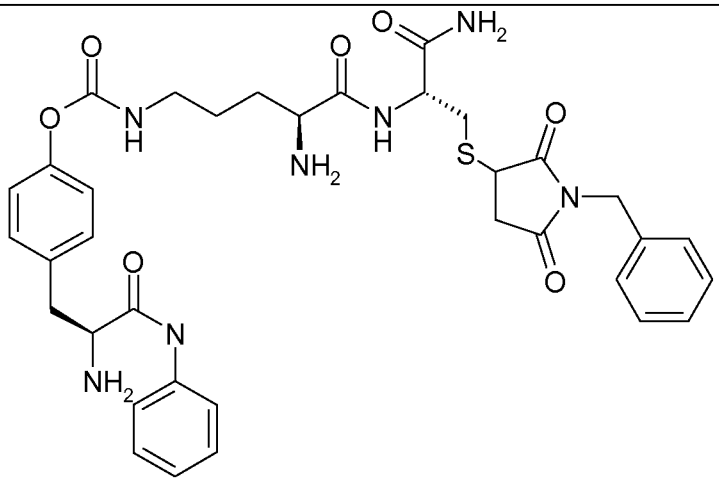
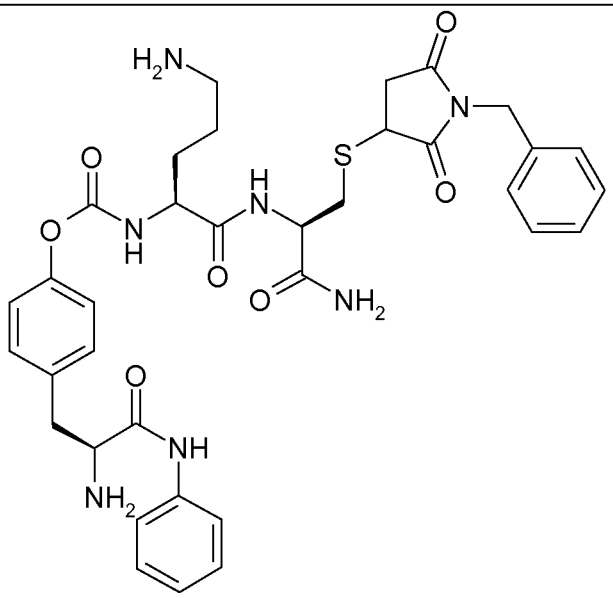
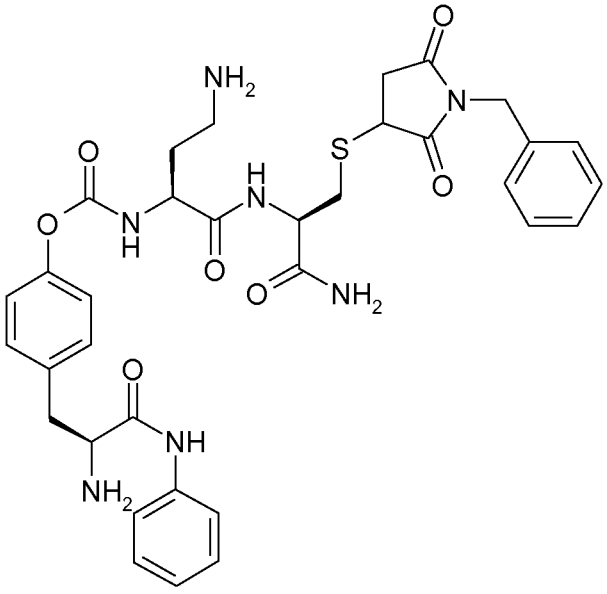
238 mg (0.25 mmol) of the compound of example 1B was dissolved in 10 ml dichloroethane. 0.12 ml Triethylsilane, approx. 10 ml trifluoroacetic acid and approx. 0.5 ml water was added. The reaction mixture was stirred for approx. 30 min at room temperature. 100 ml dichloroethane were added and

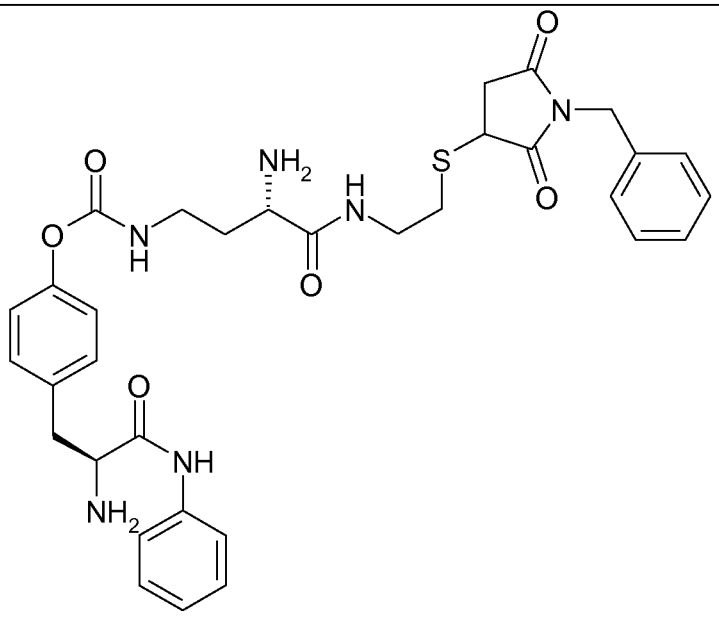
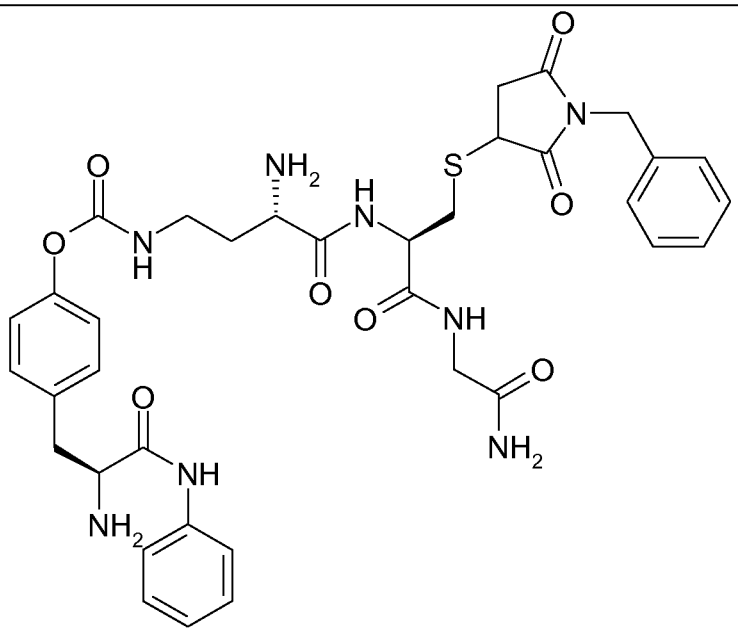
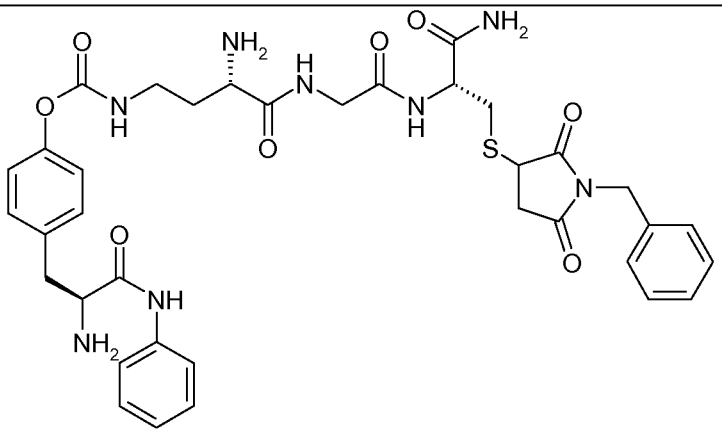
the reaction mixture was evaporated under reduced pressure to approx. 1 ml of solvent volume. Approx. 100 ml water was added and the reaction mixture was extracted three times with approx. 50 ml dichloromethane. To the aqueous phase 15 ml of acetic acid was added. The aqueous phase was frozen and lyophilized. The lyophylistae was dissolved in approx. 50 ml methanol and 0.183 mg (0.98 mmol) N-benzylmaleimide was added. The reaction mixture was stirred over night at room temperature. The reaction mixture was evaporated to dryness and redissolved in approx. 5 ml methanol and purified by preparative RP-HPLC on a C18 with a water/methanol gradient. The fractions were collected in test tubes of 20 ml on an automated fraction collector. To ensure sufficient acidity each vial was filled with 0.5 ml acedic acid prior to collection. All fractions containing the compound of example 1C were combined. Acetonitrile was partially removed on a rotary evaporator at 30°C water bath temperature and approx. 50 mbar for approx. 30 min. After addition of 0.5 ml acedic acid, the remaining solution was lyophilized. The total yield was 168 mg (0.24 mmol, 98% of theory) of the desired product.

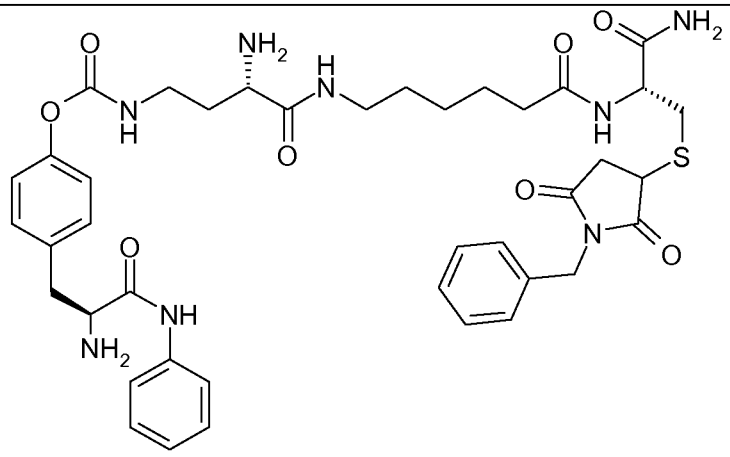
LC-MS (method 1): R_t = 0.55 min., m/z = 690 $(M+H)^+$

Using the appropriate precursors (examples 2B to 9B), the examples of the table below are prepared analogously to example 1C.

Example	Structure	Characterization
2C		LC-MS (method 1): R_t = 0.64 min., m/z = 704 $(M+H)^+$

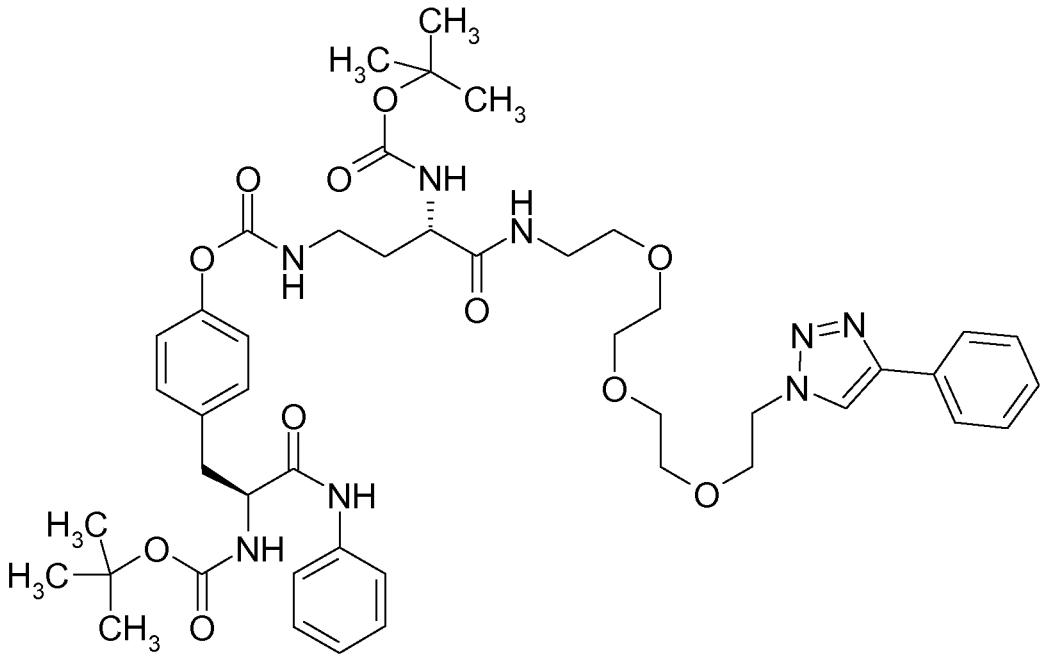
Example	Structure	Characterization
3C		LC-MS (method 1): R_t = 0.63 min., m/z = 704 $(M+H)^+$
4C		LC-MS (method 1): R_t = 0.61 min., m/z = 704 $(M+H)^+$
5C		LC-MS (method 1): R_t = 0.55 min., m/z = 690 $(M+H)^+$

Example	Structure	Characterization
6C		LC-MS (method 1): R_t = 0.67 min., m/z = 647 $(M+H)^+$
7C		LC-MS (method 1): R_t = 0.61 min., m/z = 747 $(M+H)^+$
8C		LC-MS (method 1): R_t = 0.61 min., m/z = 747 $(M+H)^+$

Example	Structure	Characterization
9C		LC-MS (method 2): R_t = 1.53 min., m/z = 803 $(M+H)^+$

Example 10Ca

Nalpha-(tert-butoxycarbonyl)-O-{[(14S)-14-[(tert-butoxycarbonyl)amino]-13-oxo-1-(4-phenyl-1H-1,2,3-triazol-1-yl)-3,6,9-trioxa-12-azahexadecan-16-yl]carbamoyl}-N-phenyl-L-tyrosinamide



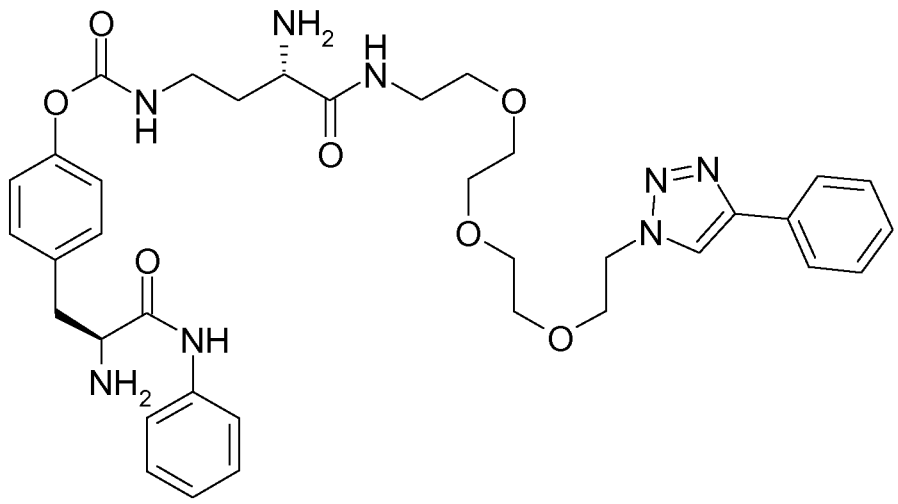
40 mg (0.05 mmol) of the compound of example 10B was dissolved in a mixture of 4 ml DMSO and 1 ml water. 10 mg (0.10 mmol) Phenylacetylene, 0.8 mg copper(II)sulfate (0.005 mmol), 445 mg (2.25 mmol) sodium ascorbate and 1.8 mg (0.01 mmol) 1,10-phenantroline were added. The pH of the reaction mixture was adjusted to 4 by addition of 3 to 4 drops of 10% sulfuric acid and the reaction mixture was stirred over night. The reaction mixture was diluted with approx. 10 ml water and extracted twice with approx. 10 ml ethyl actetate. The combined organic phases were evaporated

to dryness and redissolved in approx. 5 ml methanol and purified by preparative RP-HPLC on a C18 with a water/methanol gradient. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 36 mg (0.04 mmol, 79% of theory) of the desired product.

LC-MS (method 1): R_t = 1.12 min., m/z = 903 $(M+H)^+$

Example 10Cb

O-{[(14S)-14-Amino-13-oxo-1-(4-phenyl-1H-1,2,3-triazol-1-yl)-3,6,9-trioxa-12-azahexadecan-16-yl]carbamoyl}-N-phenyl-L-tyrosinamide

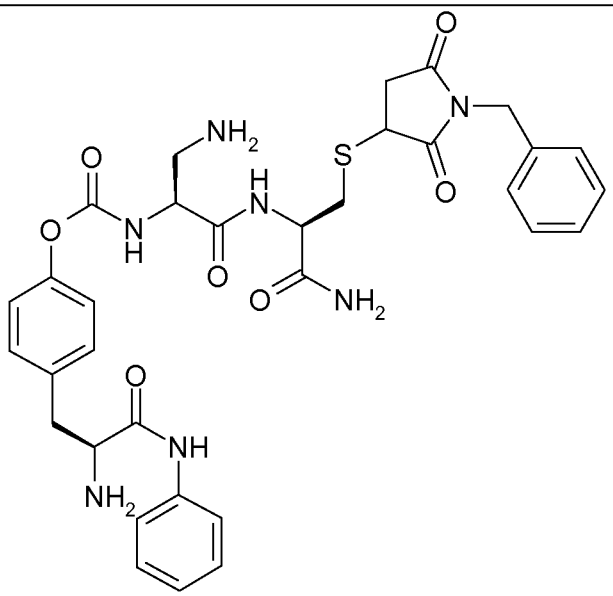
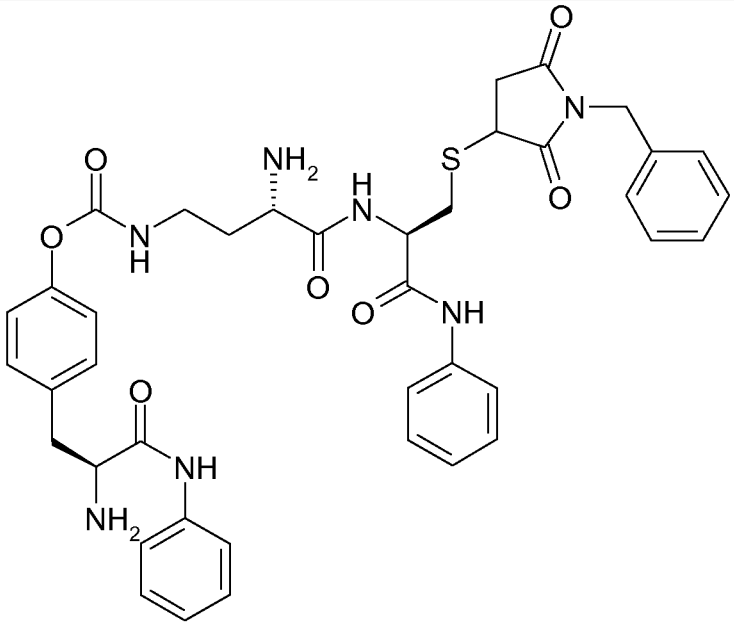


36 mg (0.04 mmol) of the compound of example 10Ca was dissolved in 2.5 ml dichloroethane. 0.02 ml Triethylsilane, approx. 2.5 ml trifluoroacetic acid and approx. 0.1 ml water was added. The reaction mixture was stirred for approx. 30 min at room temperature. The reaction mixrture was evaporated to dryness, and redissolved in approx. 15 ml water. The reaction mixture was extracted three times with approx. 10 ml of dichloromethane. After adition of approx. 0.5 ml acetic acid, the aqueous phase was lyophilized. The lyophylisate was redissolved in approx. 5 ml methanol and purified by preparative RP-HPLC on a C18 with a water/methanol gradient. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 13 mg (0.02 mmol, 45% of theory) of the desired product.

LC-MS (method 1): R_t = 0.57 min., m/z = 703 $(M+H)^+$

Using the appropriate precursors (examples 11B and 12B), the examples of the table below are

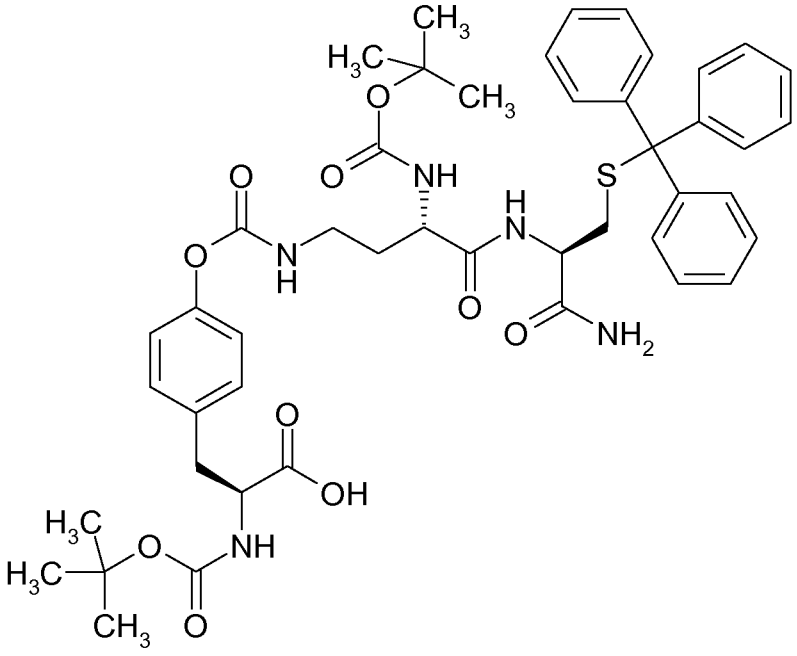
prepared analogously to example 1C.

Example	Structure	Characterization
11C		LC-MS (method 1): R_t = 0.60 min., m/z = 676 $(M+H)^+$
12C		LC-MS (method 2): R_t = 1.69 min., m/z = 767 $(M+H)^+$

Working examples

Example 1

O-({(3S)-4-{[(2R)-1-Amino-1-oxo-3-(tritylsulfanyl)propan-2-yl]amino}-3-[(tert-butoxycarbonyl)-amino]-4-oxobutyl}carbamoyl)-N-(tert-butoxycarbonyl)-L-tyrosine



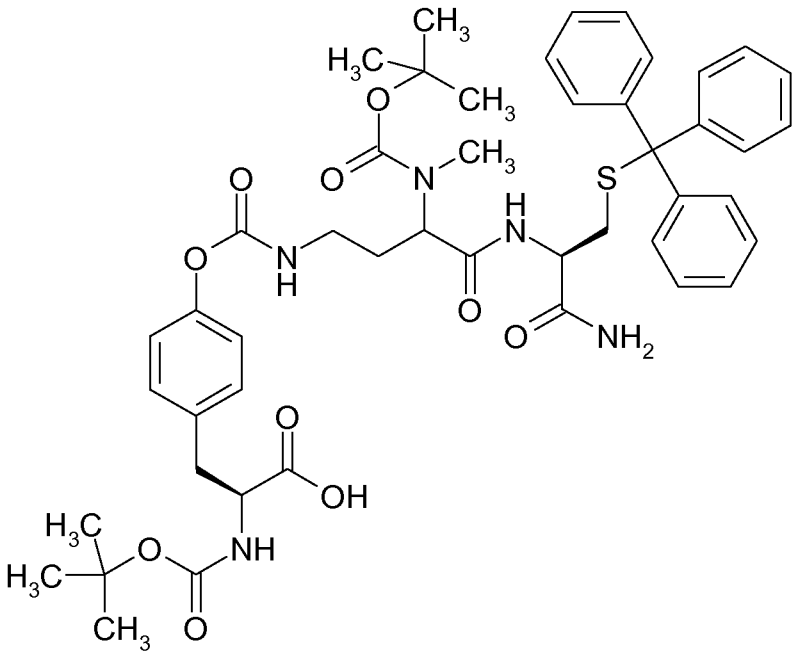
4.14 g (4.55 mmol) of the compound from example 3A was dissolved in 90 ml tetrahydrofuran. 3.17 ml (22.8 mmol) triethylamine, 0.86 ml (22.8 mmol) formic acid and 0.526 g (0.455 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 100 ml water, and twice extracted with approx. 100 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 500 ml silica gel. Solvents used were dichloromethane, dichloromethane/methanol 20/1 and dichloromethane/methanol 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 2.62 g raw product of 94.5% purity. The product was further purified by preparative RP-HPLC on a C18 with a water/methanol gradient to yield 2.35 g (2.70 mmol, 59% of theory) pure product.

LC-MS (method 1): R_t = 1.22 min., m/z = 871 $(M+H)^+$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ = 7.92 (d, 1H), 7.65 (t, 1H), 7.28-7.35 (m, 12H), 7.25-7.28 (t, 3H), 7.15-7.20 (m, 4H), 6.95 (d, 2H), 4.29 (q, 1H), 4.00 (m, 1H), 3.92 (m, 1H), 3.11 (m, 3H), 2.90 (m, 1H), 2.36 (m, 2H), 1.84 (m, 1H), 1.68 (m, 1H), 1.34 (d, 18H).

Example 2

O-[(4-{[(2R)-1-Amino-1-oxo-3-(tritylsulfanyl)propan-2-yl]amino}-3-[(tert-butoxycarbonyl)-(methyl)amino]-4-oxobutyl)carbamoyl]-N-(tert-butoxycarbonyl)-L-tyrosine



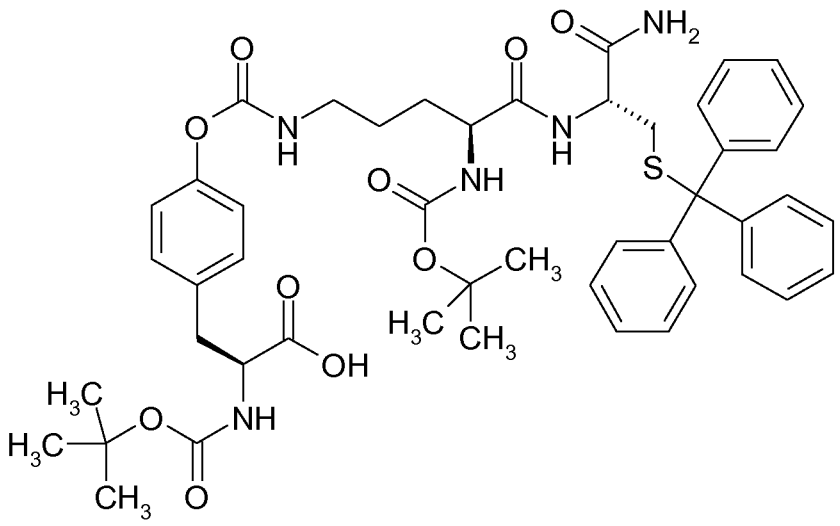
2.2 g (2.38 mmol) of the compound from example 8A was dissolved in 48 ml tetrahydrofuran. 1.66 ml (11.9 mmol) triethylamine, 0.45 ml (11.9 mmol) formic acid and 0.275 g (0.238 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 50 ml water and twice extracted with approx. 50 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 100 g silica gel. Solvents used were dichloromethane, dichloromethane/methanol 50/1 and dichloromethane/methanol 4/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 1.44 g (1.61 mmol, 68% of theory) product as a mixture of diastereomers.

LC-MS (method 1): R_t = 1.20 and 1.24 min., m/z = 884 $(M+H)^+$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ = 8.00 (m, 1H), 7.65-7.90 (m, 4H), 7.18-7.35 (m, 18H), 7.10 (m, 2H), 6.96 (m, 4H), 4.60 (m, 1H), 4.46 (m, 1H), 4.30 (m, 2H), 4.05 (m, 2H), 3.00 (m, 4H), 2.75 (m, 6H), 2.36 (m, 3H), 2.00 (m, 2H), 1.82 (m, 2H), 1.40 (m, 3H), 1.35 (s, 18H).

Example 3

N^2-(tert-Butoxycarbonyl)-N^5-[(4-{(2S)-2-[(tert-butoxycarbonyl)amino]-2-carboxyethyl}phenoxy)-carbonyl]-L-ornithyl-S-trityl-L-cysteinamide



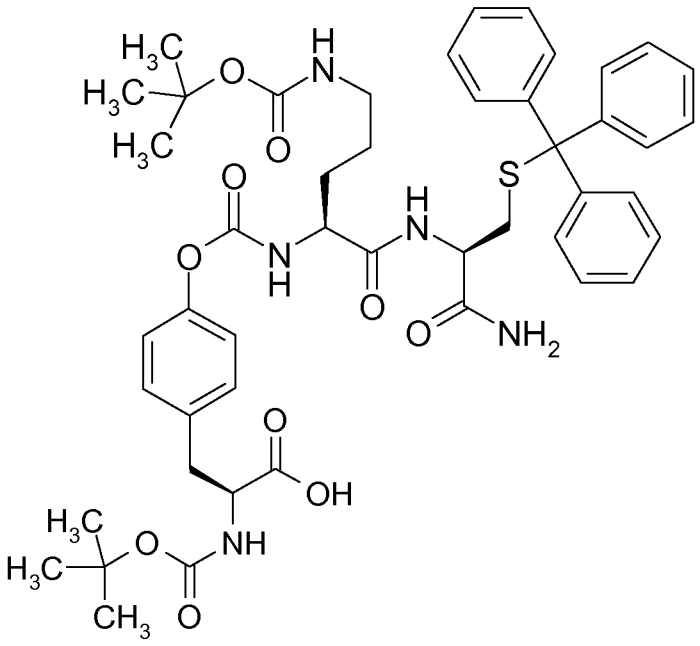
3.06 g (2.33 mmol) of the compound from example 10A was dissolved in 46 ml tetrahydrofuran. 1.63 ml (11.6 mmol) triethylamine, 0.44 ml (11.6 mmol) formic acid and 0.265 g (0.233 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 50 ml water and twice extracted with approx. 50 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 500 ml silica gel. Solvents used were dichloromethane, dichloromethane/methanol 40/1 and dichloromethane/methanol 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. This gave 1.40 g raw product of 86% purity. The product was further purified by preparative RP-HPLC on a C18 column with a water/methanol gradient to yield 2 fractions: 0.93 g product (45% of theory).

LC-MS (method 1): R_t = 1.18 min., m/z = 885 $(M+H)^+$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ = 7.89 (d, 1H), 7.65 (t, 1H), 7.25-7.35 (m, 12H), 7.20-7.25 (m, 6H), 7.10-7.20 (m, 3H), 6.95 (d, 2H), 4.29 (m, 1H), 4.05 (m, 1H), 3.88 (m, 1H), 3.11 (d, 1H), 3.00 (m, 4H), 2.75 (m, 2H), 2.36 (m, 3H), 1.64 (m, 1H), 1.51 (m, 3H), 1.36 (s, 9H), 1.32 (s, 9H).

Example 4

N^5-(tert-Butoxycarbonyl)-N^2-[(4-{(2S)-2-[(tert-butoxycarbonyl)amino]-2-carboxyethyl}phenoxy)-carbonyl]-L-ornithyl-S-trityl-L-cysteinamide



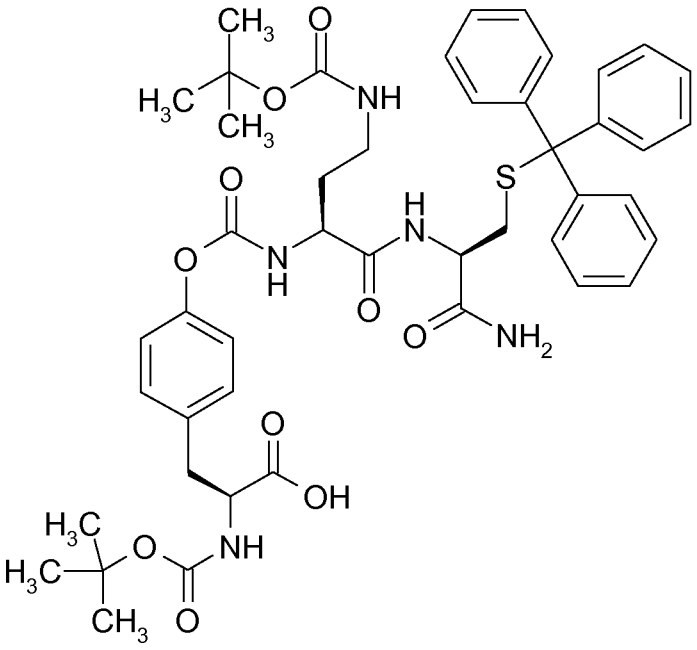
5.27 g (5.65 mmol) of the compound from example 12A was dissolved in approx. 60 ml tetrahydrofuran. 2.1 ml (15.2 mmol) Triethylamine, 0.57 ml (15.2 mmol) formic acid and 0.35 g (0.30 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 60 ml water and twice extracted with approx. 50 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 500 ml silica gel. Solvents used were dichloromethane, dichloromethane/methanol 20/1 and dichloromethane/ methanol 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. The raw product was further purified by preparative RP-HPLC on a C18 column with a water/methanol gradient to yield 1.37 g (24% of theory) product.

LC-MS (method 1): R_t = 1.17 min., m/z = 885 $(M+H)^+$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ = 12.6 (bs, 1H), 8.05 (d, 1H), 7.97 (d, 1H), 7.06 - 7.39 (m, 20H), 6.97 (d, 2H), 6.79 (t, 1H), 4.30 (dd, 1H), 4.07 (m, 1H), 4.00 (m, 1H), 2.85 – 3.04 (m, 3H), 2.30 – 2.40 (m, 2H), 1.65 (m, 1H), 1.41 – 1.60 (m, 4H), 1.37 (s, 9H), 1.32 (s, 9H).

Example 5

N-(tert-Butoxycarbonyl)-O-{[(4R,7S)-4-carbamoyl-13,13-dimethyl-6,11-dioxo-1,1,1-triphenyl-12-oxa-2-thia-5,10-diazatetradecan-7-yl]carbamoyl}-L-tyrosine



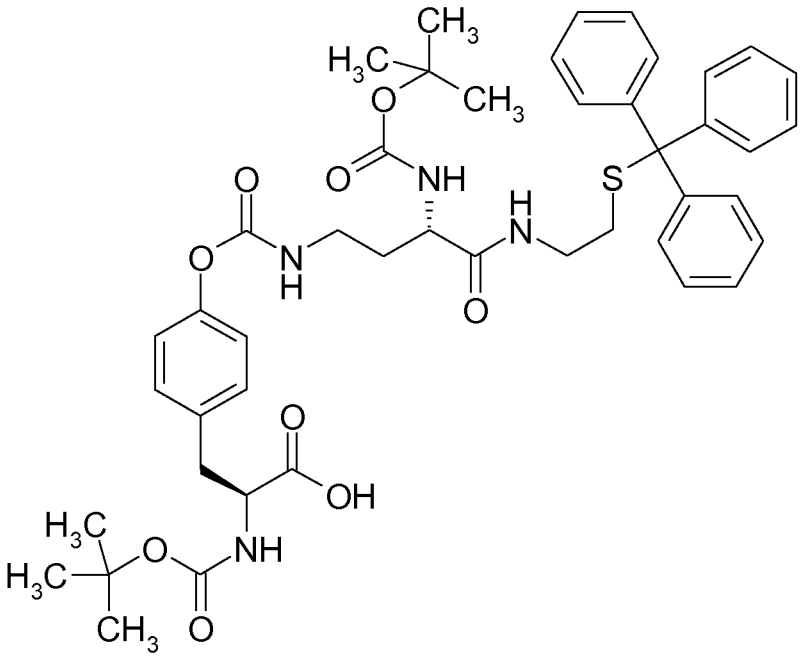
4.91 g (5.40 mmol) of the compound from example 14A was dissolved in approx. 110 ml tetrahydrofuran. 3.8 ml (27 mmol) Triethylamine, 1.02 ml (27 mmol) formic acid and 0.62 g (0.54 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 60 ml water and twice extracted with approx. 50 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 500 ml silica gel. Solvents used were dichloromethane, dichloromethane/methanol 40/1 and dichloromethane/ methanol 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure. The raw product was further purified by preparative RP-HPLC on a C18 column with a water/methanol gradient to yield 1.96 g (42% of theory) product.

LC-MS (method 1): R_t = 1.20 min., m/z = 871 $(M+H)^+$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ = 12.6 (bs, 1H), 8.05 (t, 2H), 7.16 – 7.39 (m, 19H), 7.12 (d, 1H), 6.98 (d, 2H), 6.83 (t, 1H), 4.32 (dd, 1H), 4.00 – 4.11 (m, 2H), 2.92 – 3.12 (m, 3H), 2.81 (m, 1H), 2.30 – 2.40 (m, 2H), 1.82 (m, 1H), 1.67 (m, 1H), 1.38 (s, 9H), 1.32 (s, 9H).

Example 6

N-(tert-Butoxycarbonyl)-O-{[(3S)-3-[(tert-butoxycarbonyl)amino]-4-oxo-4-{[2-(tritylsulfanyl)-ethyl]amino}butyl]carbamoyl}-L-tyrosine



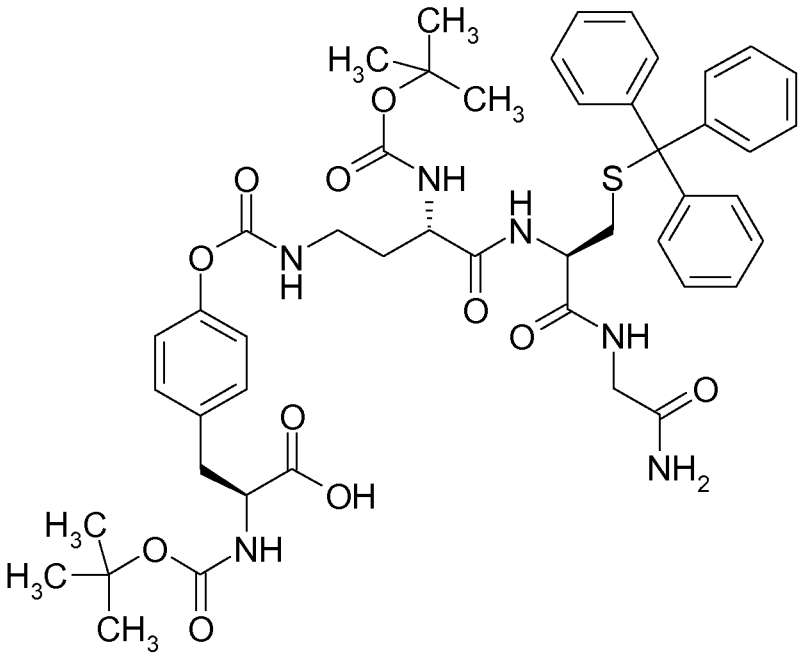
98 mg (0.1 mmol) of the compound from example 15A was dissolved in approx. 4 ml tetrahydrofuran. 70 µl (0.5 mmol) Triethylamine, 19 µl (0.5 mmol) formic acid and 11 mg (0.01 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 5 ml water and twice extracted with approx. 5 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was purified by preparative RP-HPLC on a C18 column with a water/methanol gradient to yield 67 mg (79% of theory) product.

LC-MS (method 1): R_t = 1.32 min., m/z = 827 $(M+H)^+$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ = 12.6 (bs, 1H), 7.85 (t, 1H), 7.59 (m, 1H), 7.29 – 7.37 (m, 12H), 7.18 - 7.27 (m, 5H), 7.07 (bs, 1H), 6.98 (d, 2H), 6.88 (d, 1H), 4.07 (m, 1H), 3.90 (m, 1H), 2.93 – 3.09 (m, 5H), 2.81 (m, 1H), 2.20 (t, 2H), 1.78 (m, 1H), 1.64 (m, 1H), 1.36 (s, 9H), 1.32 (s, 9H).

Example 7

N-[(2S)-2-[(tert-Butoxycarbonyl)amino]-4-{[(4-{(2S)-2-[(tert-butoxycarbonyl)amino]-2-carboxyethyl}phenoxy)carbonyl]amino}butanoyl]-S-trityl-L-cysteinylglycinamide



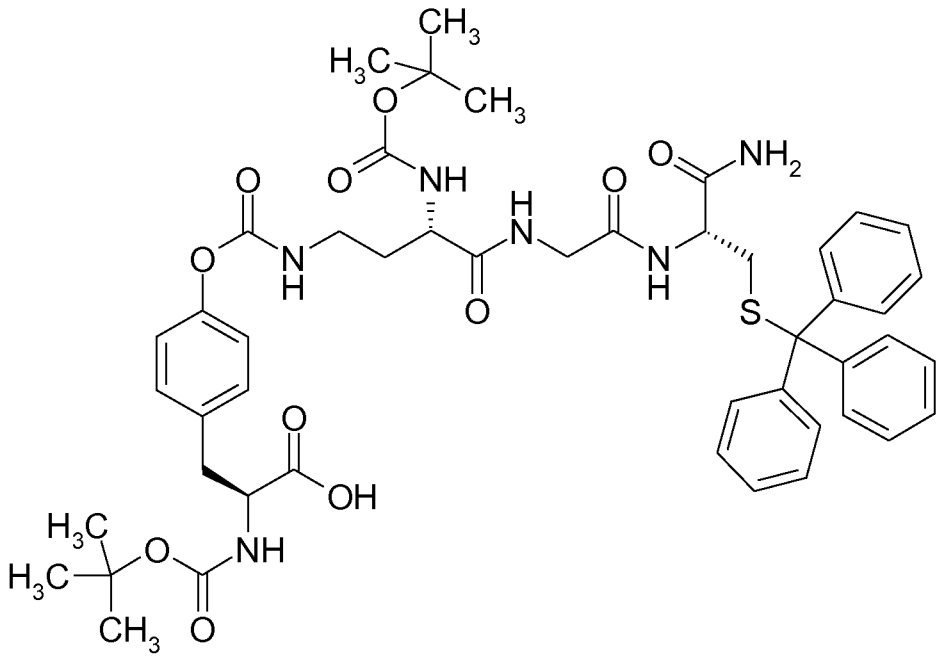
60 mg (0.031 mmol) of the compound from example 16A was dissolved in approx. 3 ml tetrahydrofuran. 22 µl (0.16 mmol) Triethylamine, 6 µl (0.16 mmol) formic acid and 4 mg (0.003 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 5 ml water and twice extracted with approx. 5 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was purified by preparative RP-HPLC on a C18 column with a water/methanol gradient to yield 26 mg (86% of theory) product.

LC-MS (method 2): R_t = 2.55 min., m/z = 927 $(M+H)^+$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): *d* = 12.6 (bs, 1H), 8.08 (m, 2H), 7.63 (t, 1H), 7.18 – 7.38 (m, 18H), 7.03 – 7.15 (m, 3H), 6.99 (d, 2H), 4.28 (dd, 1H), 3.95 – 4.10 (m, 2H), 3.64 (dd, 1H), 3.51 (m, 1H), 3.04 – 3.13 (m, 2H), 3.00 (dd, 1H), 2.81 (m, 1H), 2.42 (d, 2H), 1.84 (m, 1H), 1.67 (m, 1H), 1.36 (s, 9H), 1.32 (s, 9H).

Example 8

N-[(2S)-2-[(tert-Butoxycarbonyl)amino]-4-{[(4-{(2S)-2-[(tert-butoxycarbonyl)amino]-2-carboxyethyl}phenoxy)carbonyl]amino}butanoyl]glycyl-S-trityl-L-cysteinamide



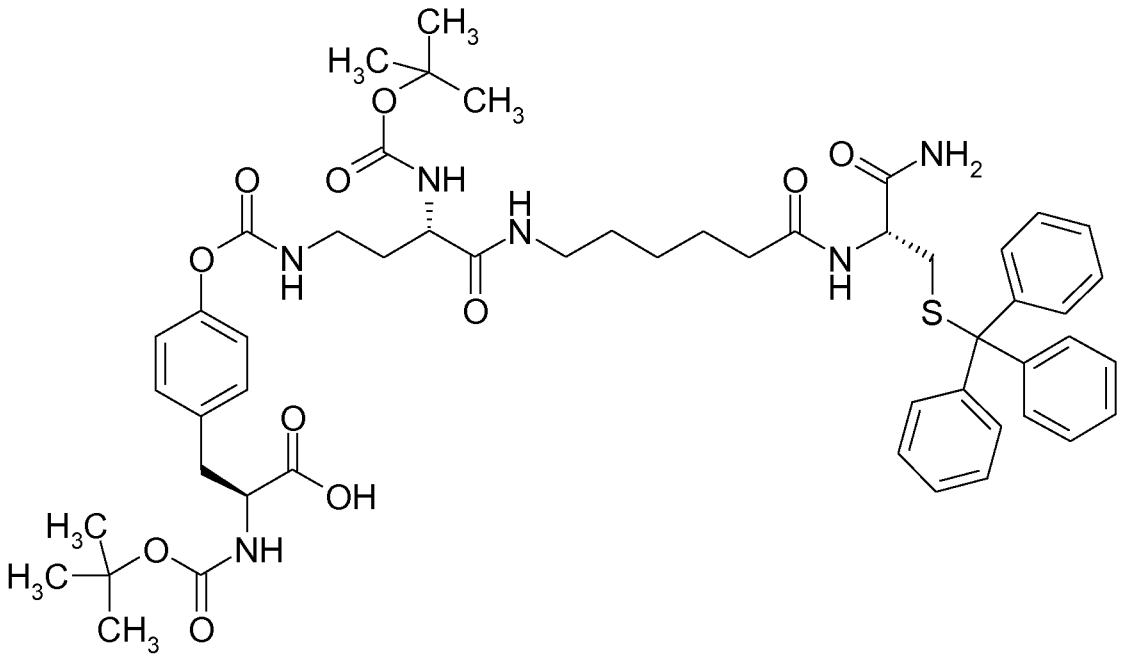
860 mg (0.89 mmol) of the compound from example 19A was dissolved in approx. 20 ml tetrahydrofuran. 620 µl (4.45 mmol) Triethylamine, 168 µl (4.45 mmol) formic acid and 103 mg (0.089 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 50 ml water and twice extracted with approx. 50 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was purified by preparative RP-HPLC on a C18 column with a water/methanol gradient to yield 329 mg (38% of theory) product.

LC-MS (method 1): R_t = 1.16 min., m/z = 927 $(M+H)^+$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): *d* = 8.16 (d, 1H), 8.04 (t, 1H), 7.64 (t, 1H), 7.20 – 7.39 (m, 15H), 7.15 (d, 3H), 7.07 (d, 1H), 6.95 (d, 2H), 4.28 (dd, 1H), 4.02 (dd, 1H), 3.91 (m, 1H), 3.76 (m, 2H), 2.99 – 3.15 (m, 3H), 2.88 (m, 1H), 2.29 – 2.42 (m, 2H), 1.86 (m, 1H), 1.68 (m, 1H), 1.37 (s, 9H), 1.33 (s, 9H).

Example 9

O-({(3S)-4-[(6-{[(2R)-1-Amino-1-oxo-3-(tritylsulfanyl)propan-2-yl]amino}-6-oxohexyl)amino]-3-[(tert-butoxycarbonyl)amino]-4-oxobutyl}carbamoyl)-N-(tert-butoxycarbonyl)-L-tyrosine



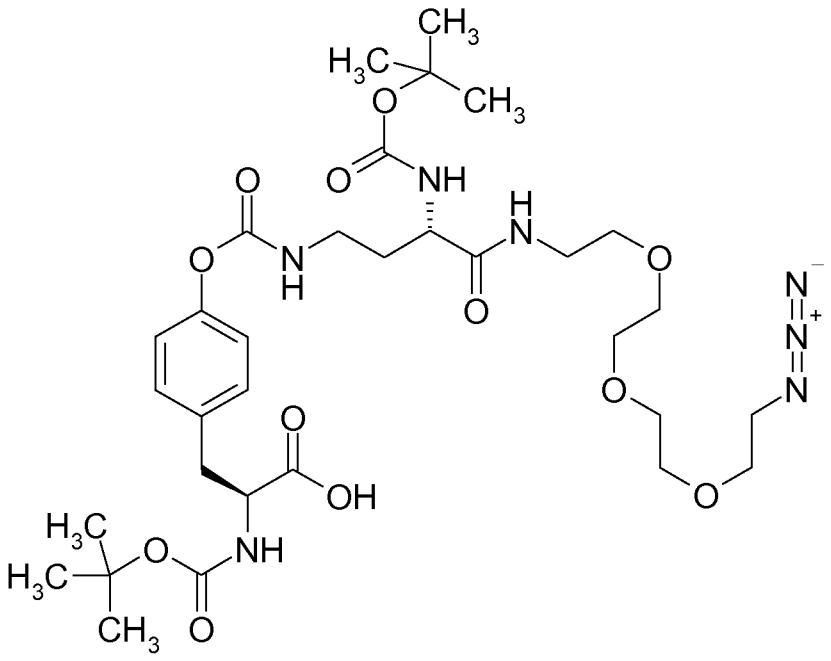
250 mg (0.24 mmol) of the compound from example 22A was dissolved in approx. 5 ml tetrahydrofuran. 170 µl (1.22 mmol) Triethylamine, 48 µl (1.22 mmol) formic acid and 28 mg (0.024 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 20 ml water and twice extracted with approx. 20 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was purified by preparative RP-HPLC on a C18 column with a water/methanol gradient to yield 167 mg (65% of theory) product.

LC-MS (method 1): R_t = 1.19 min., m/z = 983 $(M+H)^+$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): *d* = 12.6 (bs, 1H), 8.00 (d, 1H), 7.75 (t, 1H), 7.63 (t, 1H), 7.18 – 7.37 (m, 19H), 7.12 (d, 1H), 7.08 (s, 1H), 7.00 (d, 2H), 4.31 (m, 1H), 4.06 (m, 1H), 3.93 (m, 1H), 2.92 – 3.11 (m, 6H), 2.81 (dd, 1H), 2.30 (m, 2H), 2.10 (t, 2H), 1.79 (m, 1H), 1.66 (m, 1H), 1.40 – 1.54 (m, 3H), 1.37 (s, 9H), 1.32 (s, 9H), 1.23 (m, 2H).

Example 10

O-({(14S)-1-Azido-14-[(tert-butoxycarbonyl)amino]-13-oxo-3,6,9-trioxa-12-azahexadecan-16-yl}carbamoyl)-N-(tert-butoxycarbonyl)-L-tyrosine



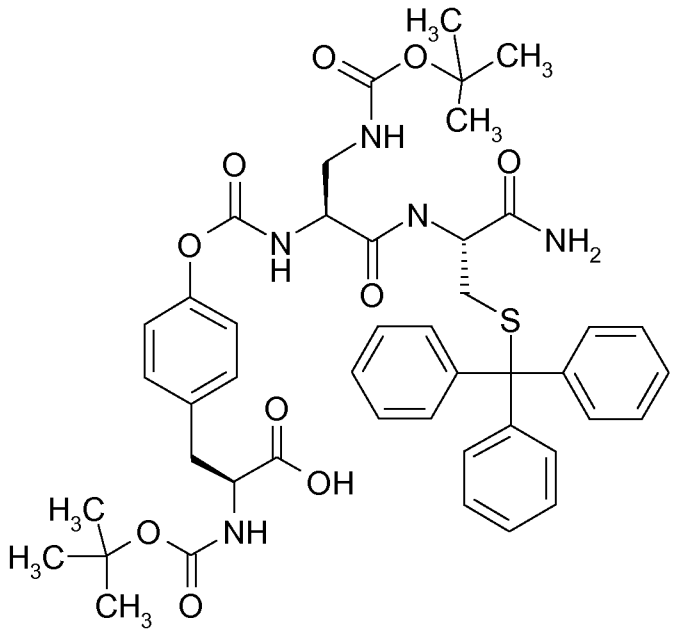
276 mg (0.344 mmol) of the compound from example 23A was dissolved in approx. 15 ml tetrahydrofuran. 235 µl (1.68 mmol) Triethylamine, 63 µl (1.68 mmol) formic acid and 39 mg (0.034 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 20 ml water and twice extracted with approx. 20 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was purified by preparative RP-HPLC on a C18 column with a water/methanol gradient to yield 167 mg (65% of theory) product.

LC-MS (method 1): R_t = 0.98 min., m/z = 726 $(M+H)^+$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): *d* = 7.85 (t, 1H), 7.61 (t, 1H), 7.15 (d, 2H), 6.91 – 6.99 (m, 3H), 3.96 (m, 1H), 3.86 (bs, 1H), 3.59 (dd, 2H), 3.46 – 3.57 (m, 9H), 3.40 (m, 4H), 3.13 – 3.29 (m, 2H), 2.98 – 3.11 (m, 3H), 2.82 – 2.92 (m, 1H), 1.79 (m, 1H), 1.66 (m, 1H), 1.38 (s, 9H), 1.34 (s, 9H).

Example 11

3-[(tert-Butoxycarbonyl)amino]-N-[(4-{(2S)-2-[(tert-butoxycarbonyl)amino]-2-carboxyethyl}-phenoxy)carbonyl]-L-alanyl-S-trityl-L-cysteinamide



2.38 g (2.03 mmol) of the compound from example 25A was dissolved in approx. 35 ml tetrahydrofuran. 1.42 ml (10 mmol) Triethylamine, 0.38 ml (10 mmol) formic acid and 0.24 g (0.20 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 20 ml water and twice extracted with approx. 30 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was dissolved in dichloromethane and chromatographed over approx. 70 ml silica gel. Solvents used were dichloromethane/methanol 10/1 to dichloromethane/methanol 1/1. The product-containing fractions were combined and concentrated to dryness under reduced pressure to yield 0.72 g (41% of theory) product.

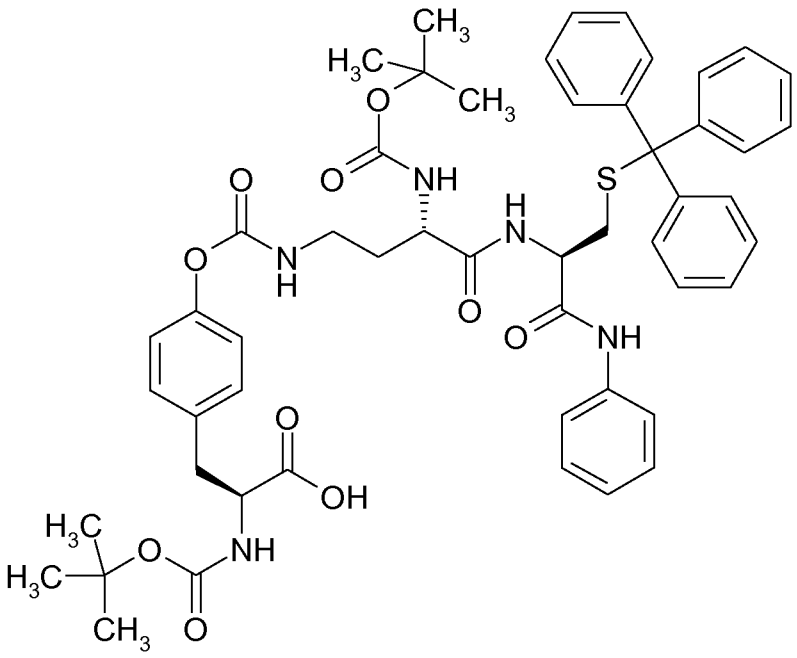
LC-MS (method 1): R_t = 1.18 min., m/z = 855 $(M-H)^-$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ = 8.15 (m, 1H), 7.75 (m, 1H), 7.16 – 7.39 (m, 19H), 6.99 (d, 2H), 6.80 (m, 1H), 4.25 (m, 2H),), 4.13 (m, 2H),), 4.00 (m, 2H), 2.92 – 3.12 (m, 3H), 2.81 (m, 1H), 2.40 (m, 2H), 1.38 (s, 9H), 1.32 (s, 9H), 1.10 (m, 4H).

Example 12

O-({(3S)-4-{[(2R)-1-Anilino-1-oxo-3-(tritylsulfanyl)propan-2-yl]amino}-3-[(tert-butoxycarbonyl)-

amino]-4-oxobutyl}carbamoyl)-N-(tert-butoxycarbonyl)-L-tyrosine



405 mg (0.41 mmol) of the compound from example 26A was dissolved in 10 ml tetrahydrofuran. 0.29 ml (2.05 mmol) triethylamine, 78 µl (2.05 mmol) formic acid and 47 mg (0.04 mmol) tetrakis(triphenylphosphin)palladium(0) were added. The reaction mixture was stirred over night at room temperature. The reaction was diluted with approx. 10 ml water, and twice extracted with approx. 10 ml dichloromethane. The combined organic phases were extracted with brine, dried over sodium sulfate and concentrated to dryness under reduced pressure. The raw product was purified by preparative RP-HPLC on a C18 column with a water/methanol gradient to yield 306 mg (79% of theory) product.

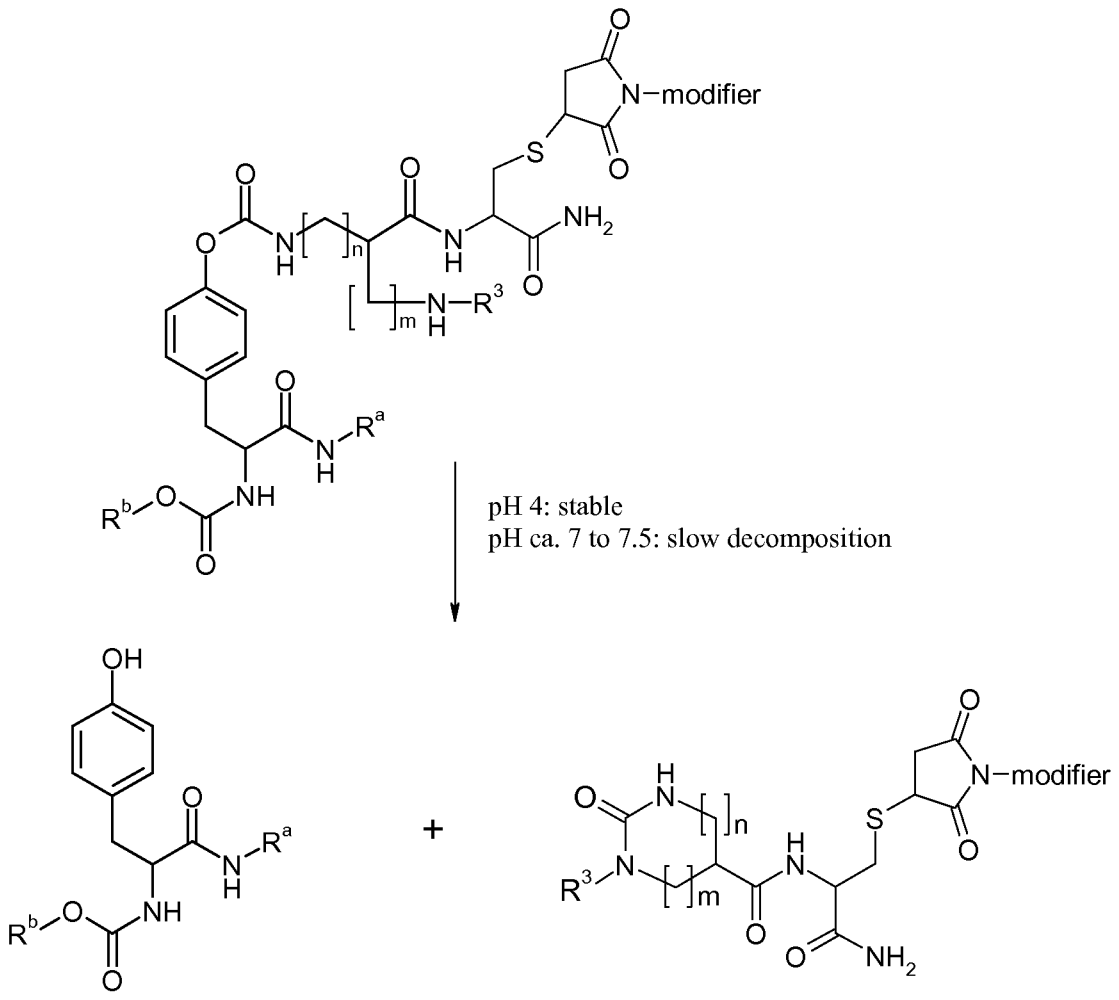
LC-MS (method 1): R_t = 1.39 min., m/z = 947 $(M+H)^+$

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ = 8.08 (d, 1H), 7.60 (m, 1H), 7.55 (m, 1H), 7.28-7.35 (m, 16H), 7.22-7.6 (m, 4H), 7.07 (m, 2H), 6.92 (m, 2H), 4.60(m, 1H), 4.05 (m, 4H), 2.85-3.20 (m, 4H), 2.80 (m, 1H), 2.45 (m, 2H), 1.85 (m, 1H), 1.66 (m, 1H), 1.35 (d, 18H) , 1.28 (m, 2H).

B. Assessment of the carrier linker activity

The suitability of the compounds according to the invention for use as carrier linker can be demonstrated using the following assay systems. To illustrate the different kinetics of different linkers, simple derivatives of the tyrosine based molecule were synthesized and the cleavage at different time points in buffer at pH 4 and pH 7.4 were monitored. Based on the exact composition of the tyrosine based linker structure the formation of the cyclic urea with concomittant release of the free tyrosine OH group has different cleavage kinetics. These can be easily measured in vitro and used as predictors for the in vivo kinetics. Scheme 3 shows exemplaric the decomposition of a prodrug releasing the tyrosine containing peptide and a cyclic urea derivative based on the former linker with the modifier attached.

Scheme 3

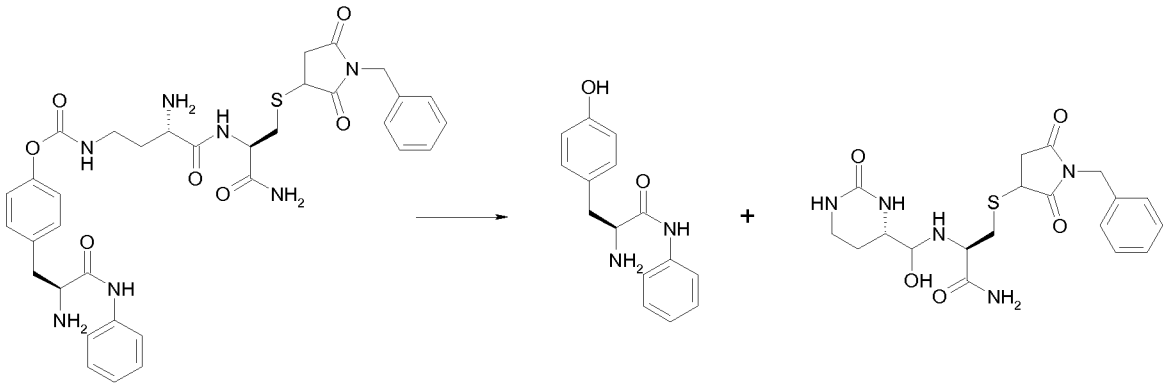


free peptide or protein incorporating a tyrosin at the former attachment site to the linker/modifier

cyclic urea derivative based on former linker with modifier attached

With example 1C, O-{[(3S)-3-Amino-4-({(2R)-1-amino-3-[(1-benzyl-2,5-dioxopyrrolidin-3-yl)sulfanyl]-1-oxopropan-2-yl}amino)-4-oxobutyl]carbamoyl}-N-phenyl-L-tyrosinamide, the cleavage reaction is as follows:

Scheme 4



1) Test description (*in vitro*)

For the kinetic studies with regard to the stability of the different linkers 0.3 mg of the dry test compound are dissolved in 0.5 ml acetonitrile. For a better dilution the sample is sonified for about 10 seconds. Then 1.0 ml of the buffer solutions are added and the samples are sonified again.

Chemical composition of the solution/buffer which are used:

pH 4: 1 litre of deionized water was adjusted to pH 4 with 1N hydrochloric acid

pH 7.4: 90 g sodium chloride, 13.61 g potassium dihydrogene phosphate and 83.35 g 1 M sodium hydroxide solution were dissolved in 1 litre of deionized water. This solution was diluted with water at the rate of 1:10.

The test compound concentration is analysed by HPLC every hour during 24 hours at room temperature. The quanitiy of the test compound is determined by the peak areas.

HPLC method: Agilent 1100 with DAD (G1315B), binary pump (g1312A), autosampler (G1329A), column thermostat (G1330B), Column: Kromasil 100 C18 / 250 mm x 4 mm / 5 µm, Column temperature: 30°C, Eluent A: water + 5 ml perchloric acid/l, Eluent B: acetonitrile, Gradient: 0-1.0 min 90% A, 10% B; 1.0-20.0 min 10% A, 90% B; 20.0-21.0 min 10% A, 90% B; 21.0-23.0 min 90% A, 10% B; 23.0-25.0 min 90% A, 10% B; Flow rate: 1.5 ml/min, Detection: 210 nm, Injection volume: 10 µl.

The results of the cleavage of the test compounds are shown in Table 1.

Table 1:

Example No	% cleaved pH 4, 0 h	% cleaved pH 4, 24 h	% cleaved pH 7.4, 0 h	% cleaved pH 7.4, 6 h	% cleaved pH 7.4, 24 h
1C	**0**	**1**	**0**	**7**	**21**
2C	**0**	**0**	**0**	**6**	**21**
3C	**0**	**0**	**0**	**2**	**11**
4C	**0**	**0**	**0**	**23**	**65**
5C	**0**	**0**	**0**	**75**	**100**
6C	**0**	**0**	**0**	**6**	**20**
7C	**0**	**0**	**0**	**6**	**23**
8C	**0**	**0**	**0**	**6**	**23**
9C	**0**	**0**	**0**	**7**	**25**
10Cb	**0**	**0**	**0**	**4**	**14**
11C	**0**	**19**	**0**	**100**	**100**
12C	**0**	**0**	**0**	**29**	**76**

The data show that example 11C is cleaved very quickly, even at pH4. Example 4C, example 5C and example 12C are cleaved quickly whereas example 3C and example 10Cb are cleaved slowly. All others have a moderate cleavage kinetic.

C. Exemplary embodiments of pharmaceutical compositions

The compounds according to the invention can be converted into pharmaceutical preparations in the following ways:

i.v. solution:

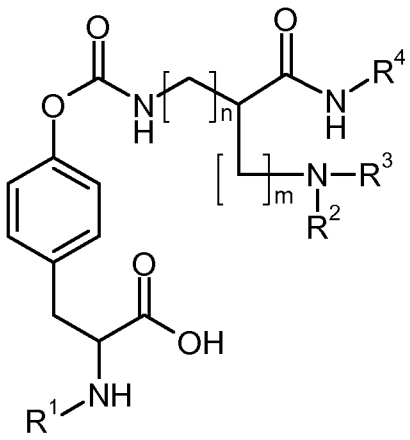
A compound according to the invention is dissolved at a concentration below saturation solubility in a physiologically acceptable solvent (for example buffers of pH 4 to pH 7, isotonic sodium chloride solution, glucose solution 5% and/or PEG 400 solution 30%). The solution is sterilized by filtration and filled into sterile and pyrogen-free injection containers.

s.c. solution:

A compound according to the invention is dissolved at a concentration below saturation solubility in a physiologically acceptable solvent (for example for example buffers of pH 4 to pH 7, isotonic sodium chloride solution, glucose solution 5% and/or PEG 400 solution 30%). The solution is sterilized by filtration and filled into sterile and pyrogen-free injection containers.

Claims

1. A compound of the formula



(I)

in which

n represents the number 0, 1, 2, 3 or 4,

m represents the number 0, 1, 2, 3 or 4,

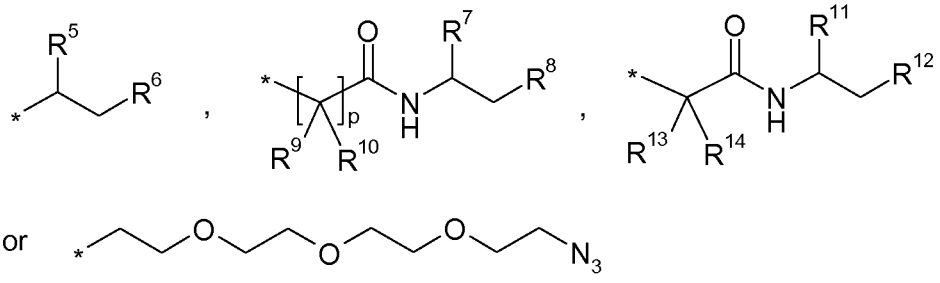
where m and n together are the number 1, 2, 3, 4, 5 or 6,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen, methyl, ethyl, n-propyl, isopropyl or benzyl,

R^4 represents a group of the formula

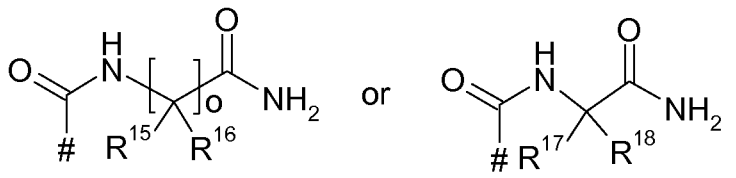


where

* is the point of attachment to the nitrogen,

p represents the number 1, 2, 3, 4 or 5,

R^5 represents hydrogen, aminocarbonyl, $(C_1$-$C_4)$-alkylaminocarbonyl, phenylaminocarbonyl or a group of the formula



,

where

\# is the point of attachment to the carbon atom,

o represents the number 1, 2, 3, 4 or 5,

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

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

R^{17} represents the side group of a natural α-amino acid or its homologues or isomers,

and

R^{18} represents hydrogen or methyl,

R^6 represents –S-trityl, thiolyl, azidyl, acetylenyl, hydroxycarbonyl or amine,

R^7 represents hydrogen or aminocarbonyl,

R^8 represents -S-trityl, thiolyl, azidyl, acetylenyl, hydroxycarbonyl or amine,

R^9 represents hydrogen or $(C_1$-$C_4)$-alkyl,

R^{10} represents hydrogen or $(C_1$-$C_4)$-alkyl,

R^{11} represents hydrogen or aminocarbonyl,

R^{12} represents -S-trityl, thiolyl, azidyl, acetylenyl, hydroxycarbonyl or amine,

R^{13} represents the side group of a natural α-amino acid or its homologues or

isomers,

and

R^{14} represents hydrogen or methyl,

or one of the salts thereof, solvates thereof or the solvates of salts thereof.

2. A compound as claimed in claim 1, characterized in that

n represents the number 0, 1, 2 or 3,

m represents the number 0, 1, 2 or 3,

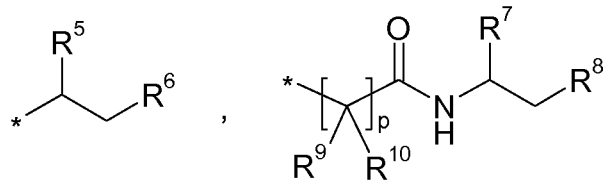
where m and n together are the number 1, 2, 3 or 4,

R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen, methyl, ethyl, n-propyl, isopropyl or benzyl,

R^4 represents a group of the formula



where

* is the point of attachment to the nitrogen,

p represents the number 1, 2, 3, 4 or 5,

R^5 represents hydrogen, aminocarbonyl, phenylaminocarbonyl or $-(C=O)NHCH_2(C=O)NH_2$,

R^6 represents –S-trityl,

R^7 represents hydrogen or aminocarbonyl,

R^8 represents –S-trityl,

R^9 represents hydrogen,

and

R^{10} represents hydrogen.

3. A compound as claimed in either of claims 1 and 2, characterized in that

n represents the number 2 or 3,

and

m represents the number 0,

or

n represents the number 0,

and

m represents the number 2 or 3,

or

n represents the number 0,

and

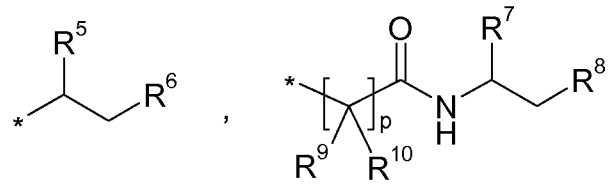
m represents the number 1,

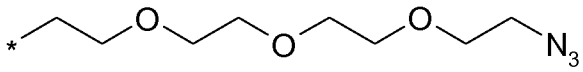
R^1 represents tert-butyloxycarbonyl or (9H-fluoren-9-ylmethoxy)carbonyl,

R^2 represents tert-butyloxycarbonyl,

R^3 represents hydrogen, methyl, ethyl, n-propyl, isopropyl or benzyl,

R^4 represents a group of the formula



or 

where

* is the point of attachment to the nitrogen,

p represents the number 1, 2, 3, 4 or 5,

R^5 represents hydrogen, aminocarbonyl, phenylaminocarbonyl or -(C=O)NHCH$_2$(C=O)NH$_2$,

R^6 represents –S-trityl,

R^7 represents hydrogen or aminocarbonyl,

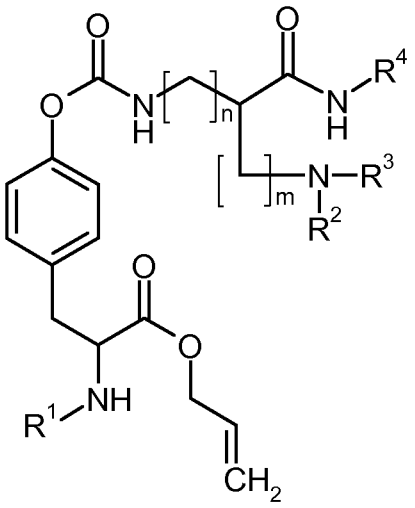
R^8 represents –S-trityl,

R^9 represents hydrogen,

and

R^{10} represents hydrogen.

4. A process for preparing a compound of the formula (I) or one of the salts thereof, solvates thereof or the solvates of salts thereof as claimed in claim 1, characterized in that a compound of the formula (II)



(II),

in which

n, m, R^1, R^2, R^3 and R^4 are each as defined in claim 1,

is reacted with a Palladium(0) source and a reducing agent.

5. A compound as claimed in any of claims 1 to 3 for the preparation of prodrugs for treatment and/or prevention of diseases.

6. The use of a prodrug prepared with a compound as claimed in any of claims 1 to 3 for producing a medicament for treatment and/or prevention of diseases.

7. The use of a prodrug prepared with a compound as claimed in any of claims 1 to 3 for producing a medicament for treatment and/or prevention of of cardiovascular, edematous and/or inflammatory disorders.

8. A medicament comprising a prodrug prepared with a compound as claimed in any of claims 1 to 3 in combination with an inert nontoxic pharmaceutically suitable excipient.

9. A medicament comprising a prodrug prepared with a compound as claimed in any of claims 1 to 3 in combination with a further active ingredient.

10. The medicament as claimed in claim 8 or 9 for treatment and/or prevention of cardiovascular, edematous and/or inflammatory disorders.

11. The use of a prodrug prepared with a compound as claimed in any of claims 1 to 3 for producing a medicament for treatment and/or prevention of heart failure, coronary heart disease, ischemic and/or hemorrhagic stroke, hypertension, pulmonary hypertension,

peripheral arterial occlusive disease, pre-eclampsia, chronic obstructive pulmonary disease, asthma, acute and/or chronic pulmonary edema, allergic alveolitis and/or pneumonitis due to inhaled organic dust and particles of fungal, actinomycetic or other origin, and/or acute chemical bronchitis, acute and/or chronic chemical pulmonary edema, neurogenic pulmonary edema, acute and/or chronic pulmonary manifestations due to radiation, acute and/or chronic interstitial lung disorders, acute lung injury/acute respiratory distress syndrome (ALI/ARDS) in adult or child including newborn, ALI/ARDS secondary to pneumonia and sepsis, aspiration pneumonia and ALI/ARDS secondary to aspiration, ALI/ARDS secondary to smoke gas inhalation, transfusion-related acute lung injury (TRALI), ALI/ARDS and/or acute pulmonary insufficiency following surgery, trauma and/or burns, and/or ventilator induced lung injury (VILI), lung injury following meconium aspiration, pulmonary fibrosis, mountain sickness, chronic kidney diseases, glomerulonephritis, acute kidney injury, cardiorenal syndrome, lymphedema, inflammatory bowel disease, sepsis, septic shock, systemic inflammatory response syndrome (SIRS) of non-infectious origin, anaphylactic shock and/or urticaria.

12. A compound as defined in any of claims 1 to 3 for use in a process for treatment and/or prevention of cardiovascular, edematous and/or inflammatory disorders.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/071373

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/48 C07K5/02 C07K5/06 C07K5/08
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/186832 A1 (FRANKLIN RICHARD [GB] ET AL) 23 July 2009 (2009-07-23)	6-12
A	claim 5; example 9; table 2	1-5

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 2013	18/01/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schleifenbaum, A

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/071373

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2009186832 A1	23-07-2009	US 2009186832 A1	23-07-2009
		US 2009192095 A1	30-07-2009
		WO 2009092071 A2	23-07-2009
		WO 2009092073 A2	23-07-2009

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 104144705 A
(43)申请公布日 2014.11.12

(21)申请号 201280065842.4
(22)申请日 2012.10.29
(30)优先权数据
11187737.9 2011.11.03 EP
(85)PCT国际申请进入国家阶段日
2014.07.02
(86)PCT国际申请的申请数据
PCT/EP2012/071373 2012.10.29
(87)PCT国际申请的公布数据
WO2013/064455 EN 2013.05.10
(71)申请人 拜耳知识产权有限责任公司
地址 德国蒙海姆
申请人 拜耳药业股份公司
(72)发明人 I·法莱姆 J·库伯林
汉斯-乔治·勒珍 N·格里布诺
R·舒赫-路普 S·威特罗克
U·克兰兹
(74)专利代理机构 北京北翔知识产权代理有限公司 11285
代理人 孙占华 张广育
(51)Int.Cl.
A61K 47/48(2006.01)
C07K 5/02(2006.01)
C07K 5/06(2006.01)
C07K 5/08(2006.01)

权利要求书4页 说明书64页

(54)发明名称

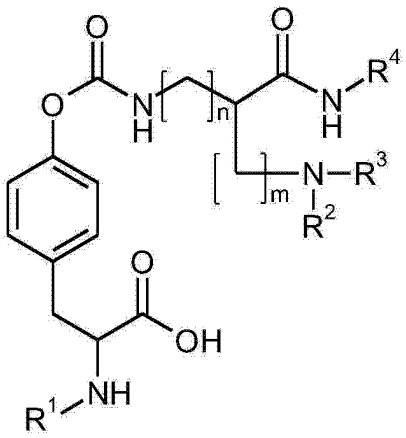
用于肽的可释放性连接的基于酪氨酸的连接体

(57)摘要

本发明涉及新的基于酪氨酸的连接体，所述连接体使得肽或蛋白质与其他分子实体例如聚乙二醇形成可释放性的连接，涉及用于它们的制备的方法以及它们用于制备用于治疗和/或预防疾病的药物的用途。

CN 104144705 A

1. 一种式 (I) 的化合物，或者其盐、其溶剂合物或其盐的溶剂合物中的一种



(I)

其中

n 代表数值 0、1、2、3 或 4，

m 代表数值 0、1、2、3 或 4，

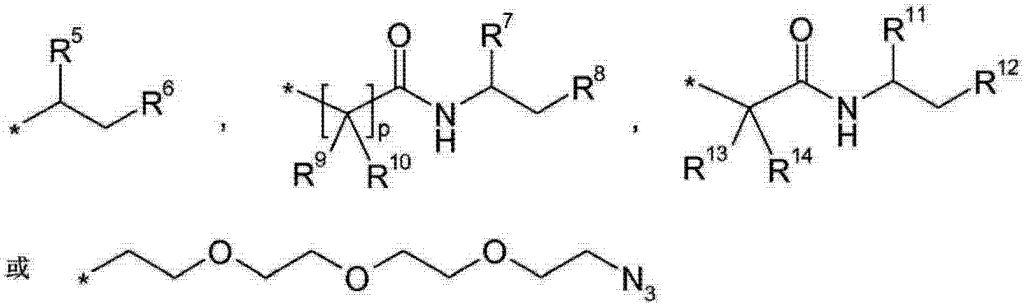
其中 m 和 n 一起为数值 1、2、3、4、5 或 6，

R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

R^2 代表叔丁氧羰基，

R^3 代表氢、甲基、乙基、正丙基、异丙基或苯甲基，

R^4 代表下式的基团

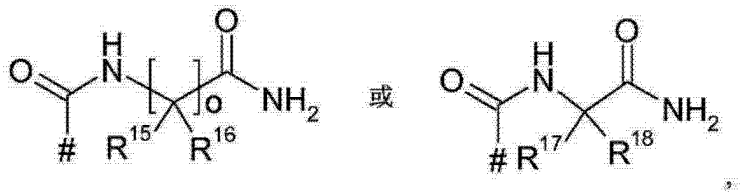


其中

* 为与氮的连接位点，

p 代表数值 1、2、3、4 或 5，

R^5 代表氢、氨基羰基、(C_1-C_4) 烷基氨基羰基、苯基氨基羰基或下式的基团，



其中

为与碳原子的连接位点，

o 代表数值 1、2、3、4 或 5，

R^{15} 代表氢或 (C_1-C_4) 烷基，

R^{16} 代表氢或 (C_1-C_4) 烷基，

R^{17} 代表天然 α - 氨基酸或者其同系物或同分异构体的侧基，

和

R^{18} 代表氢或甲基，

R^6 代表 -S- 三苯甲基、硫羟基、叠氮基、乙炔基、羟基羰基或胺，

R^7 代表氢或氨基羰基，

R^8 代表 -S- 三苯甲基、硫羟基、叠氮基、乙炔基、羟基羰基或胺，

R^9 代表氢或 (C_1-C_4) 烷基，

R^{10} 代表氢或 (C_1-C_4) 烷基，

R^{11} 代表氢或氨基羰基，

R^{12} 代表 -S- 三苯甲基、硫羟基、叠氮基、乙炔基、羟基羰基或胺，

R^{13} 代表天然 α - 氨基酸或者其同系物或同分异构体的侧基，

和

R^{14} 代表氢或甲基。

2. 权利要求 1 要求保护的化合物，其特征在于

n 代表数值 0、1、2 或 3，

m 代表数值 0、1、2 或 3，

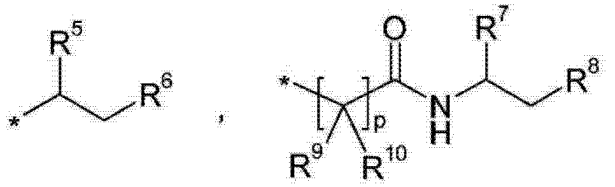
其中 m 和 n 一起为数值 1、2、3 或 4，

R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

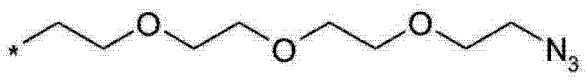
R^2 代表叔丁氧羰基，

R^3 代表氢、甲基、乙基、正丙基、异丙基或苯甲基，

R^4 代表下式的基团，



或



其中

* 为与氮的连接位点，

p 代表数值 1、2、3、4 或 5，

R^5 代表氢、氨基羰基、苯基氨基羰基或 $-(C=O)NHCH_2(C=O)NH_2$，

R^6 代表 -S- 三苯甲基，

R^7 代表氢或氨基羰基，

R^8 代表 -S- 三苯甲基，

R^9 代表氢，

和

R^{10} 代表氢。

3. 权利要求 1 和 2 任一项要求保护的化合物，其特征在于

n 代表数值 2 或 3，

和

m 代表数值 0，

或

n 代表数值 0，

和

m 代表数值 2 或 3，

或

n 代表数值 0，

和

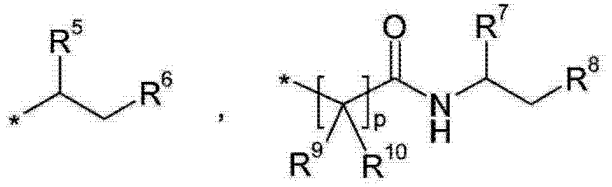
m 代表数值 1，

R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

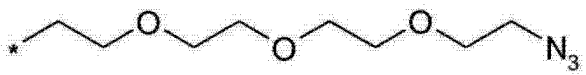
R^2 代表叔丁氧羰基，

R^3 代表氢、甲基、乙基、正丙基、异丙基或苯甲基，

R^4 代表下式的基团，



或



其中

* 为与氮的连接位点，

p 代表数值 1、2、3、4 或 5，

R^5 代表氢、氨基羰基、苯基氨基羰基或 $-(C=O)NHCH_2(C=O)NH_2$，

R^6 代表 -S- 三苯甲基，

R^7 代表氢或氨基羰基，

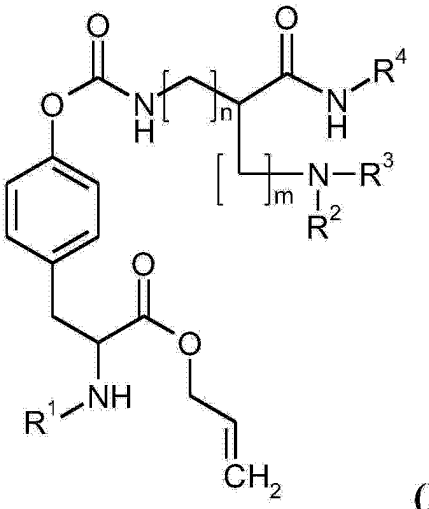
R^8 代表 -S- 三苯甲基，

R^9 代表氢，

和

R^{10} 代表氢。

4. 一种用于制备权利要求 1 要求保护的式 (I) 的化合物或者其盐、其溶剂合物或其盐的溶剂合物中的一种的方法，其特征在于式 (II) 的化合物与钯 (0) 源和还原剂反应，



(II)

其中

n、m、R^1、R^2、R^3 和 R^4 各自如权利要求 1 中所定义的。

5. 权利要求 1-3 任一项要求保护的化合物，用于制备用于治疗和 / 或预防疾病的前药。

6. 用权利要求 1-3 任一项要求保护的化合物制备的前药用于制备治疗和 / 或预防疾病的药物的用途。

7. 用权利要求 1-3 任一项要求保护的化合物制备的前药用于制备治疗和 / 或预防心血管性、水肿性和 / 或炎症性病症的药物的用途。

8. 一种药物，包含用权利要求 1-3 任一项要求保护的化合物制备的前药以及惰性、无毒、药学上合适的赋形剂。

9. 一种药物，包含用权利要求 1-3 任一项要求保护的化合物制备的前药以及另外的活性成分。

10. 权利要求 8 或 9 要求保护的药物，用于治疗和 / 或预防心血管性、水肿性和 / 或炎症性病症。

11. 用权利要求 1-3 任一项要求保护的化合物制备的前药用于制备用于治疗和 / 或预防以下病症的药物的用途 :心力衰竭、冠状动脉心脏病、缺血性中风和 / 或出血性中风、高血压、肺动脉高压、周围动脉闭塞疾病、先兆子痫、慢性阻塞性肺病、哮喘、急性和 / 或慢性肺水肿、由吸入的有机粉尘以及真菌、放线菌或其他来源的颗粒引起的变应性肺泡炎和 / 或变应性肺炎、和 / 或急性化学性支气管炎、急性和 / 或慢性化学性肺水肿、神经性肺水肿、由辐射引起的急性和 / 或慢性肺表现、急性和 / 或慢性间质性肺病症、成人或包括新生儿的儿童中的急性肺损伤 / 急性呼吸窘迫综合征 (ALI/ARDS)、继发于肺炎和脓毒症的 ALI/ARDS、继发于误吸 (aspiration) 的吸入性肺炎和 ALI/ARDS、继发于烟气吸入的 ALI/ARDS、输血相关的急性肺损伤 (TRALI)，手术、外伤和 / 或烧伤后的 ALI/ARDS 和 / 或急性肺功能不全，和 / 或呼吸机诱发的肺损伤 (VILI)、胎粪吸入后的肺损伤、肺纤维化、高山症、慢性肾疾病、肾小球肾炎、急性肾损伤、心肾综合征、淋巴水肿、炎性肠病、脓毒症、感染性休克、非传染性来源的全身性炎性反应综合征 (SIRS)、过敏性休克和 / 或荨麻疹。

12. 权利要求 1-3 任一项定义的化合物，用在治疗和 / 或预防心血管性、水肿性和 / 或炎症性障碍的方法中。

用于肽的可释放性连接的基于酪氨酸的连接体

[0001] 本发明涉及新的基于酪氨酸的连接体，所述连接体使得肽或蛋白质能够与其他分子实体（例如聚乙二醇）形成可释放性的连接，涉及它们的制备方法和它们用于制备治疗和/或预防疾病的药物的用途。

[0002] 许多有治疗活性的肽或蛋白质遭受高的体内清除率。存在一些形成这类药物的可注射储库的方法，所述方法包括使用大分子。

[0003] 以非共价连接的状态含有药物分子的聚合物基质是众所周知的。这些还可以作为水凝胶、微粒或微团进行注射。这类药物产品的释放动力学可以非常不可靠，具有高度的患者间差异性。生成这类聚合物可对敏感的原料药(drug substance)产生危害，或者原料药可在其降解过程中与所述聚合物产生副作用(D.H.Lee et al.,J.Contr.Rel.,2003,92,291-299)。

[0004] 自从20世纪80年代早期以来，众所周知的观点是对肽或蛋白质进行永久性的PEG化以增强其溶解性、降低免疫原性并通过降低肾清除率来延长半衰期(Caliceti P.,Veronese F.M.,Adv.Drug Deliv.Rev.2003,55,1261-1277)。该观点已经成功用于一些药物，但在许多实例中，PEG化会将原料药的功效降低至这种观点不再适用的程度(T.Peleg-Shulman et al.,J.Med.Chem.,2004,47,4897-4904)。

[0005] 合适的替代物是基于聚合物的前药。目前IUPAC对前药的定义记载了以下术语(International Union of Pure and Applied Chemistry and International Union of Biochemistry:GLOSSARY OF TERMS USED IN MEDICINAL CHEMISTRY(Recommendations1998);in Pure & Appl.Chem.Vol70,No.5,1998,p.1129-1143)：

[0006] 前药：前药是经过生物转化之后展示其药理学效应的任何化合物。因此前药被视为含有特定的无毒性的保护性基团的药物，所述保护性基团以暂时性方式使用以改变或消除亲本分子中不合需要的性质。

[0007] 载体连接的前药（载体前药）：载体连接的前药是含有给定的活性物质与暂时性的载体基团之间的暂时性连接的前药，所述载体基团产生改善的物理化学性质或药物代谢动力学性质，并且在体内可以通常通过水解断裂而被轻易地除去。

[0008] 级联前药：级联前药是一种前药，其中载体基团的裂解仅在暴露活化基团之后才变得有效。

[0009] 存在一些基于PEG的载体前药的实例，它们中的大多数需要对活性药(active drug)与载体之间的连接体进行酶的活化作用，这主要通过酶法水解起始。由于酯在体内可以被非常容易且不可预知地裂解，因此用于载体前药的直接酯连接体的可用性具有局限性(J.Rautio et al.,Nature Reviews Drug discovery,2008,7255-270)。

[0010] 通常使用的替代性方法是将连接至肽或蛋白质的胺官能度(functionality)的级联连接体。在级联连接体中，必须在级联的限速步骤时除去掩蔽基团。这活化连接体以在二级位置中分解，以释放肽或蛋白质。通常所述掩蔽基团可以通过酶法机制除去(R.B.Greenwald et al.in WO2002/089789,Greenwald,et al.,J.Med.

Chem. 1999, 42, 3657-3667, F. M. H. DeGroot et al. in WO2002/083180and WO2004/043493和 D. Shabat et al. in WO2004/019993)。

[0011] 不依赖于酶法活化的替代物为 WO2005/099768 中 U. Hersel 等人的观点。在他们的方法中，以纯粹的 pH 依赖的方式通过攻击内部亲核基团来去除苯酚上的掩蔽基团。这活化了所述连接体，用于进一步的分解。

[0012] U. Hersel 等人在 WO2005/099768 中提到，“由 Greenwald、DeGroot 和 Shabat 描述的上述前药系统中的缺点是暂时性连接断裂之后释放可能有毒性的芳香族小分子副产物，例如醌甲基化物。释放的可能有毒性的实体与所述药物的化学计量比为 1:1，并可呈现高的体内浓度”。相同的问题也适用于 Hersel et al 的系统。

[0013] 对于小的有机分子，存在许多不同的前药方法 (J. Rautio et al., Nature Reviews Drug discovery, 2008, 7255-270)。对于小分子的酚基来说，自从 20 世纪 90 年代末期开始，U. Hersel 等人使用的作为其掩蔽基团的释放机制的方法，已经被用作前药方法 (W. S. Saari in EP0296811 和 W. S. Saari et al., J. Med. Chem. 1990, Vol33, No1, p97-101)。

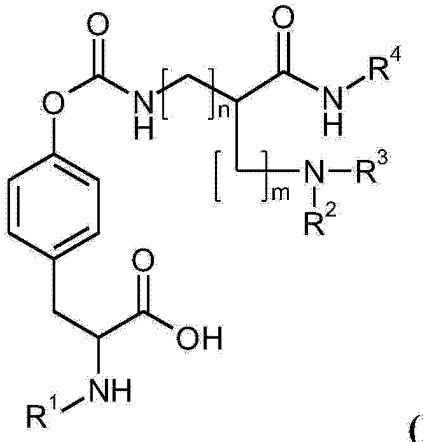
[0014] 替代性的基于胺的前药系统是基于作为级联前药的二羟乙基甘氨酸的缓慢水解。所述二羟乙基甘氨酸的羟基被酯掩蔽，所述酯易于被酯酶水解 (R. Greenwald et al., J. Med. Chem. 2004, 47, 726 - 734and D. Vetter et al. in WO2006/136586)。

[0015] 与上文所列前药方法——都基于掩蔽胺官能度——相比，本发明是基于掩蔽肽或蛋白质中酪氨酸的酚基。使用的载体连接的前药是基于对该酚基上氨基甲酸酯的内部亲核基团协助的断裂。相对于上文提及的其他前药类型的关键优势是连接体分解产物的毒理学无害性，所述连接体分解产物是一种与载体永久性连接的环脲。此外，所述前药的分解不依赖于可能导致高的患者间裂解动力学差异的酶法机制。断裂机制为仅 pH 依赖性的，这是因为在酸性 pH 下被质子化的内胺在更高的 (中性)pH 下被活化而充当亲核基团，攻击基于酪氨酸的酚的氨基甲酸酯。

[0016] 在本发明的上下文中，现在描述的化合物包含基于酪氨酸氨基酸的分子实体，所述分子实体使得能够构建任何含有至少 1 个酪氨酸的肽或蛋白质的所述载体连接体前药。

[0017] 本发明提供了下式的化合物，及其盐、其溶剂合物和其盐的溶剂合物

[0018]



(I)

[0019] 其中

[0020] n 代表数值 0、1、2、3 或 4，

[0021] m 代表数值 0、1、2、3 或 4，

[0022] 其中 m 和 n 一起为数值 1、2、3、4、5 或 6，

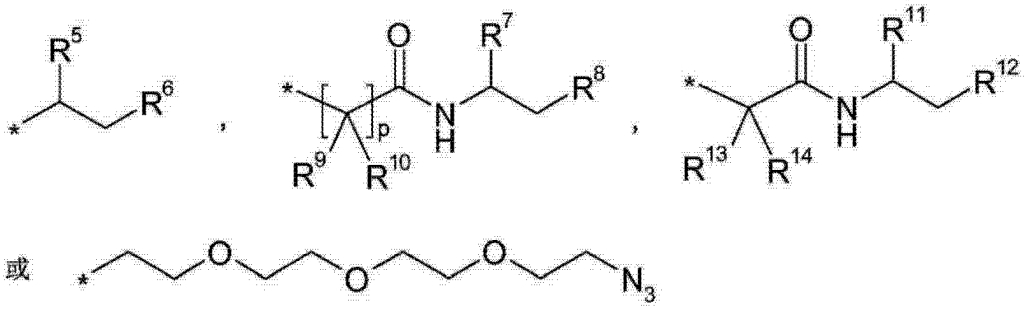
[0023] R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

[0024] R^2 代表叔丁氧羰基，

[0025] R^3 代表氢、甲基、乙基、正丙基、异丙基或苯甲基，

[0026] R^4 代表下式的基团

[0027]



或

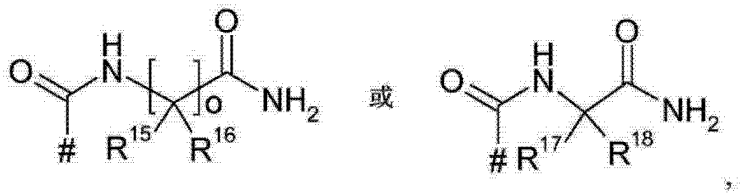
[0028] 其中

[0029] * 为与氮的连接位点，

[0030] p 代表数值 1、2、3、4 或 5，

[0031] R^5 代表氢、氨基羰基、(C_1-C_4) 烷基氨基羰基、苯基氨基羰基或下式的基团，

[0032]



[0033] 其中

[0034] # 为与碳原子的连接位点，

[0035] o 代表数值 1、2、3、4 或 5，

[0036] R^{15} 代表氢或 (C_1-C_4) 烷基，

[0037] R^{16} 代表氢或 (C_1-C_4) 烷基，

[0038] R^{17} 代表天然 α - 氨基酸或者其同系物或同分异构体的侧基，

[0039] 并且

[0040] R^{18} 代表氢或甲基，

[0041] R^6 代表 -S- 三苯甲基、硫羟基 (thiolyl)、叠氮基 (azidyl)、乙炔基 (acetylenyl)、羟基羰基或胺，

[0042] R^7 代表氢或氨基羰基，

[0043] R^8 代表 -S- 三苯甲基、硫羟基、叠氮基、乙炔基、羟基羰基或胺，

[0044] R^9 代表氢或 (C_1-C_4) 烷基，

[0045] R^{10} 代表氢或 (C_1-C_4) 烷基，

[0046] R^{11} 代表氢或氨基羰基，

[0047] R^{12} 代表 -S- 三苯甲基、硫羟基、叠氮基、乙炔基、羟基羰基或胺，

[0048] R^{13} 代表天然 α－氨基酸或者其同系物或同分异构体的侧基，

[0049] 并且

[0050] R^{14} 代表氢或甲基。

[0051] 本发明化合物为式 (I) 的化合物及其盐、其溶剂合物和其盐的溶剂合物，式 (I) 所包括的并且具有下文具体说明的式的化合物及其盐、其溶剂合物和其盐的溶剂合物，和式 (I) 所包括的并且下文具体说明作为工作实施例的化合物及其盐、其溶剂合物和其盐的溶剂合物，如果式 (I) 所包括的并且下文具体说明的化合物并非已经是盐、溶剂合物和盐的溶剂合物。

[0052] 根据它们的结构，本发明化合物可以以立体异构的形式（对映异构体、非对映异构体）存在。因此，本发明包括对映异构体或非对映异构体及其特定的混合物。可通过已知的方法将立体异构上均一的组分从这类对映异构体和 / 或非对映异构体的混合物中分离。

[0053] 当本发明化合物可以以互变异构的形式存在时，本发明包括所有互变异构的形式。

[0054] 在本发明的上下文中，优选的盐为生理上可接受的本发明化合物的盐。还包括本身不适合药物应用但例如可用来分离或提纯本发明化合物的盐。

[0055] 本发明化合物的生理上可接受的盐包括无机酸、羧酸和磺酸的酸加成盐，例如以下酸的盐：氢氯酸、氢溴酸、硫酸、磷酸、甲磺酸、乙磺酸、甲苯磺酸、苯磺酸、萘二磺酸、乙酸、三氟乙酸、丙酸、乳酸、酒石酸、马来酸、柠檬酸、富马酸、马来酸和苯甲酸。

[0056] 本发明化合物的生理上可接受的盐还包括常规碱的盐，例如且优选碱金属盐（例如钠盐和钾盐）、碱土金属盐（例如钙盐和镁盐）和衍生自氨或具有 1-16 个碳原子的有机胺的铵盐，所述有机胺例如且优选乙胺、二乙胺、三乙胺、乙基二异丙胺、单乙醇胺、二乙醇胺、三乙醇胺、二环己基胺、二甲基氨基乙醇、普鲁卡因、二苄胺、N- 甲基吗啉、精氨酸、赖氨酸、乙二胺和 N- 甲基哌啶。

[0057] 在本发明的上下文中，溶剂合物是指通过以固态或液态形式与溶剂分子发生配位作用而形成复合物的那些本发明化合物的形式。水合物是一种具体形式的溶剂合物，其中所述配位作用是与水进行的。本发明的上下文中优选的溶剂合物为水合物。

[0058] 在本发明的上下文中，除非另有具体说明，所述取代基具有以下含义：

[0059] (C_1-C_4) 烷基在本发明的上下文中是分别具有 1-4 个碳原子的直链或支链的烷基。可优选提及的实例为：甲基、乙基、正丙基、异丙基、正丁基、异丁基、仲丁基、叔丁基。

[0060] (C_1-C_4) 烷基氨基羰基在本发明的上下文中代表具有含 1-4 个碳原子的直链或支链的烷基取代基的氨基羰基基团。可优选提及的实例为甲基氨基羰基、乙基氨基羰基、正丙基氨基羰基、异丙基氨基羰基、正丁基氨基羰基、异丁基氨基羰基、仲丁基氨基羰基、叔丁基氨基羰基。

[0061] 在 R^{13} 和 R^{17} 的含义中 α－氨基酸的侧基包括天然存在的 α－氨基酸的侧基和这些 α－氨基酸的同系物和同分异构体的侧基。此时 α－氨基酸可具有 L 和 D 构型或者为 L 型和 D 型的混合物。可提及的侧基的实例为：氢（甘氨酸）、甲基（丙氨酸）、丙 -2- 基（缬氨酸）、丙 -1- 基（正缬氨酸）、2- 甲基丙 -1- 基（亮氨酸）、1- 甲基丙 -1- 基（异亮氨酸）、丁 -1- 基（正亮氨酸）、苯基 (2- 苯基甘氨酸)、苯甲基（苯丙氨酸）、对羟基苯甲基（酪氨酸）、吲哚 -3- 基甲基（色氨酸）、咪唑 -4- 基甲基（组氨酸）、羟甲基（丝氨酸）、2- 羟乙基

（高丝氨酸）、1- 羟乙基（苏氨酸）、巯基甲基（半胱氨酸）、甲基硫代甲基 (S- 甲基半胱氨酸）、2- 巯基乙基（高半胱氨酸）、2- 甲基硫代乙基（甲硫氨酸）、氨基甲酰基甲基（天冬酰胺）、2- 氨基甲酰基乙基（谷氨酰胺）、羧甲基（天冬氨酸）、2- 羧乙基（谷氨酸）、4- 氨基丁 -1- 基（赖氨酸）、4- 氨基 -3- 羟基丁 -1- 基（羟基赖氨酸）、3- 氨基丙 -1- 基（鸟氨酸）、3- 胍基丙 -1- 基（精氨酸）、3- 脲基丙 -1- 基（瓜氨酸）。在 R^2 的含义中优选的 α - 氨基酸侧基为氢（甘氨酸）、甲基（丙氨酸）、丙 -2- 基（缬氨酸）、丙 -1- 基（正缬氨酸）、咪唑 -4- 基甲基（组氨酸）、羟甲基（丝氨酸）、1- 羟乙基（苏氨酸）、氨基甲酰基甲基（天冬酰胺）、2- 氨基甲酰基乙基（谷氨酰胺）、4- 氨基丁 -1- 基（赖氨酸）、3- 氨基丙 -1- 基（鸟氨酸）、3- 胍基丙 -1- 基（精氨酸）。在每种情况下优选 L 构型。

[0062] 在本发明的上下文中，改性剂意指其他分子实体，例如聚乙二醇。

[0063] 在可代表 R^4 的基团的式中，以 * 标记的线的末端并非碳原子或 CH_2 基团，而是至 R^4 所连接的原子的键的一部分。

[0064] 在可代表 R^5 的基团的式中，以 # 标记的线的末端并非碳原子或 CH_2 基团，而是至 R^5 所连接的原子的键的一部分。

[0065] 优选式 (I) 的化合物，其中

[0066] n 代表数值 0、1、2 或 3，

[0067] m 代表数值 0、1、2 或 3，

[0068] 其中 m 和 n 一起为数值 1、2、3 或 4，

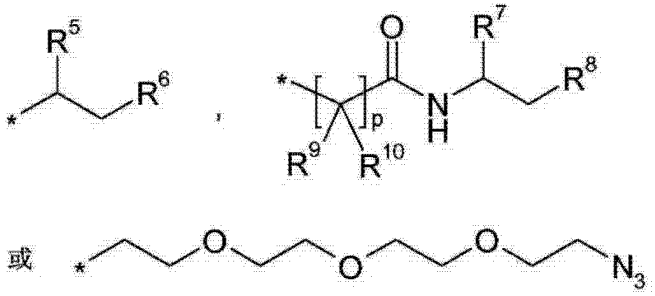
[0069] R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基）羰基，

[0070] R^2 代表叔丁氧羰基，

[0071] R^3 代表氢、甲基、乙基、正丙基、异丙基或苯甲基，

[0072] R^4 代表下式的基团，

[0073]



[0074] 其中

[0075] * 为与氮的连接位点，

[0076] p 代表数值 1、2、3、4 或 5，

[0077] R^5 代表氢、氨基羰基或 $-(C=O)NHCH_2(C=O)NH_2$，

[0078] R^6 代表 -S- 三苯甲基，

[0079] R^7 代表氢或氨基羰基，

[0080] R^8 代表 -S- 三苯甲基，

[0081] R^9 代表氢，

[0082] 并且

[0083] R^{10} 代表氢。

[0084] 还优选式 (I) 的化合物，其中

[0085] n 代表数值 2 或 3，

[0086] 并且

[0087] m 代表数值 0，

[0088] 或者

[0089] n 代表数值 0，

[0090] 并且

[0091] m 代表数值 2 或 3，

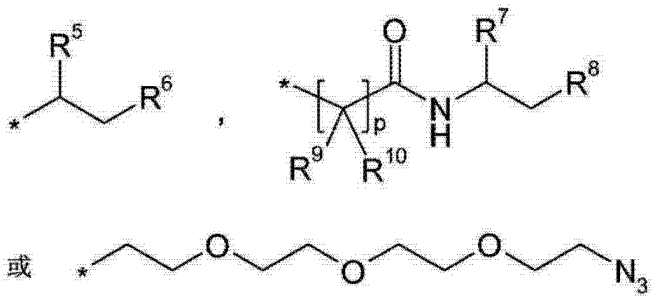
[0092] R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

[0093] R^2 代表叔丁氧羰基，

[0094] R^3 代表氢、甲基、乙基、正丙基、异丙基或苯甲基，

[0095] R^4 代表下式的基团，

[0096]



[0097] 其中

[0098] * 为与氮的连接位点，

[0099] p 代表数值 1、2、3、4 或 5，

[0100] R^5 代表氢、氨基羰基或 $-(C=O)NHCH_2(C=O)NH_2$，

[0101] R^6 代表 -S- 三苯甲基，

[0102] R^7 代表氢或氨基羰基，

[0103] R^8 代表 -S- 三苯甲基，

[0104] R^9 代表氢，

[0105] 并且

[0106] R^{10} 代表氢。

[0107] 还优选式 (I) 的化合物，其中

[0108] n 代表数值 2 或 3，

[0109] m 代表数值 0，

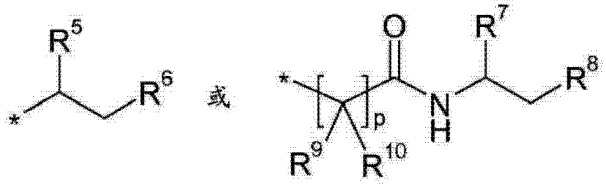
[0110] R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

[0111] R^2 代表叔丁氧羰基，

[0112] R^3 代表氢或甲基，

[0113] R^4 代表下式的基团，

[0114]



[0115] 其中

[0116] * 为与氮的连接位点，

[0117] p 代表数值 1 或 5，

[0118] R^5 代表氢、氨基羰基或 -(C = O)$NHCH_2$(C = O)NH_2，

[0119] R^6 代表 -S- 三苯甲基，

[0120] R^7 代表氢或氨基羰基，

[0121] R^8 代表 -S- 三苯甲基，

[0122] R^9 代表氢，

[0123] 并且

[0124] R^{10} 代表氢。

[0125] 还优选式 (I) 的化合物，其中

[0126] n 代表数值 0，

[0127] m 代表数值 2 或 3，

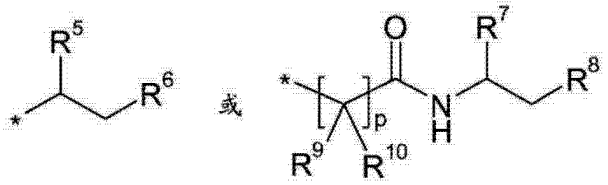
[0128] R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

[0129] R^2 代表叔丁氧羰基，

[0130] R^3 代表氢或甲基，

[0131] R^4 代表下式的基团，

[0132]



[0133] 其中

[0134] * 为与氮的连接位点，

[0135] p 代表数值 1 或 5，

[0136] R^5 代表氢、氨基羰基或 -(C = O)$NHCH_2$(C = O)NH_2，

[0137] R^6 代表 -S- 三苯甲基，

[0138] R^7 代表氢或氨基羰基，

[0139] R^8 代表 -S- 三苯甲基，

[0140] R^9 代表氢，

[0141] 并且

[0142] R^{10} 代表氢。

[0143] 还优选式 (I) 的化合物，其中

[0144] n 代表数值 2 或 3，

[0145] m 代表数值 0，

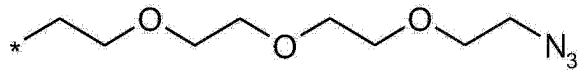
[0146] R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

[0147] R^2 代表叔丁氧羰基，

[0148] R^3 代表氢或甲基，

[0149] R^4 代表下式的基团

[0150]



[0151] 其中

[0152] * 为与氮的连接位点。

[0153] 还优选式 (I) 的化合物，其中

[0154] n 代表数值 0、1、2 或 3，

[0155] m 代表数值 0、1、2 或 3，

[0156] 其中 m 和 n 一起为数值 1、2、3 或 4，

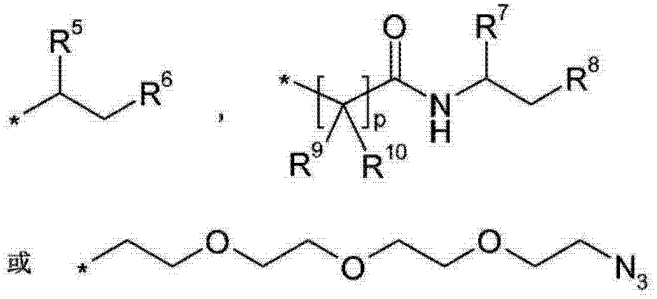
[0157] R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

[0158] R^2 代表叔丁氧羰基，

[0159] R^3 代表氢、甲基、乙基、正丙基、异丙基或苯甲基，

[0160] R^4 代表下式的基团，

[0161]



[0162] 其中

[0163] * 为与氮的连接位点，

[0164] p 代表数值 1、2、3、4 或 5，

[0165] R^5 代表氢、氨基羰基、苯基氨基羰基或 $-(C=O)NHCH_2(C=O)NH_2$，

[0166] R^6 代表 -S- 三苯甲基，

[0167] R^7 代表氢或氨基羰基，

[0168] R^8 代表 -S- 三苯甲基，

[0169] R^9 代表氢，

[0170] 并且

[0171] R^{10} 代表氢。

[0172] 还优选式 (I) 的化合物，其中

[0173] n 代表数值 2 或 3，

[0174] 并且

[0175] m 代表数值 0，

[0176] 或者

[0177] n 代表数值 0，

[0178] 并且

[0179] m 代表数值 2 或 3，

[0180] 或者

[0181] n 代表数值 0，

[0182] 并且

[0183] m 代表数值 1，

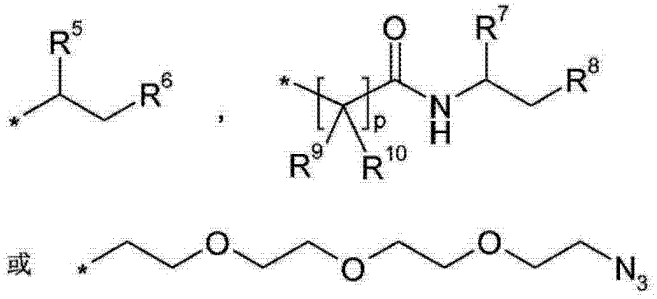
[0184] R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

[0185] R^2 代表叔丁氧羰基，

[0186] R^3 代表氢、甲基、乙基、正丙基、异丙基或苯甲基，

[0187] R^4 代表下式的基团，

[0188]



[0189] 其中

[0190] * 为与氮的连接位点，

[0191] p 代表数值 1、2、3、4 或 5，

[0192] R^5 代表氢、氨基羰基、苯基氨基羰基或 $-(C=O)NHCH_2(C=O)NH_2$，

[0193] R^6 代表 -S- 三苯甲基，

[0194] R^7 代表氢或氨基羰基，

[0195] R^8 代表 -S- 三苯甲基，

[0196] R^9 代表氢，

[0197] 并且

[0198] R^{10} 代表氢。

[0199] 还优选式 (I) 的化合物，其中

[0200] n 代表数值 2 或 3，

[0201] m 代表数值 0，

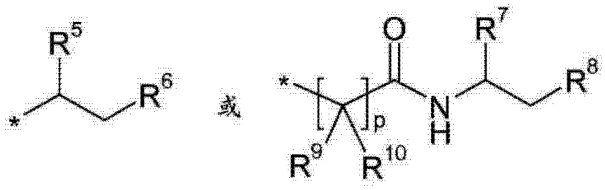
[0202] R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

[0203] R^2 代表叔丁氧羰基，

[0204] R^3 代表氢或甲基，

[0205] R^4 代表下式的基团，

[0206]



[0207] 其中

[0208] * 为与氮的连接位点，

[0209] p 代表数值 1 或 5，

[0210] R^5 代表氢、氨基羰基、苯基氨基羰基或 -(C = O)$NHCH_2$(C = O)NH_2，

[0211] R^6 代表 -S- 三苯甲基，

[0212] R^7 代表氢或氨基羰基，

[0213] R^8 代表 -S- 三苯甲基，

[0214] R^9 代表氢，

[0215] 并且

[0216] R^{10} 代表氢。

[0217] 还优选式 (I) 的化合物，其中

[0218] n 代表数值 0，

[0219] m 代表数值 2 或 3，

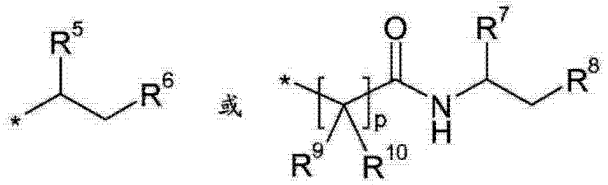
[0220] R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

[0221] R^2 代表叔丁氧羰基，

[0222] R^3 代表氢或甲基，

[0223] R^4 代表下式的基团

[0224]



[0225] 其中

[0226] * 为与氮的连接位点，

[0227] p 代表数值 1 或 5，

[0228] R^5 代表氢、氨基羰基、苯基氨基羰基或 -(C = O)$NHCH_2$(C = O)NH_2，

[0229] R^6 代表 -S- 三苯甲基，

[0230] R^7 代表氢或氨基羰基，

[0231] R^8 代表 -S- 三苯甲基，

[0232] R^9 代表氢，

[0233] 并且

[0234] R^{10} 代表氢。

[0235] 还优选式 (I) 的化合物，其中

[0236] n 代表数值 0，

[0237] m 代表数值 1，

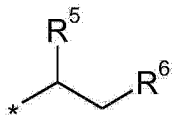
[0238] R^1 代表叔丁氧羰基或 (9H- 芴 -9- 基甲氧基) 羰基，

[0239] R^2 代表叔丁氧羰基，

[0240] R^3 代表氢或甲基，

[0241] R^4 代表下式的基团，

[0242]



[0243] 其中

[0244] * 为与氮的连接位点，

[0245] R^5 代表氢、氨基羰基、苯基氨基羰基或 $-(C=O)NHCH_2(C=O)NH_2$，

[0246] 并且

[0247] R^6 代表 -S- 三苯甲基。

[0248] 还优选式 (I) 的化合物，其中 n 代表数值 2 或 3 并且 m 代表数值 0。

[0249] 还优选式 (I) 的化合物，其中 n 代表数值 2 并且 m 代表数值 0。

[0250] 还优选式 (I) 的化合物，其中 n 代表数值 3 并且 m 代表数值 0。

[0251] 还优选式 (I) 的化合物，其中 n 代表数值 0 并且 m 代表数值 2 或 3。

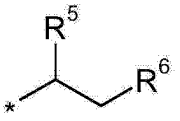
[0252] 还优选式 (I) 的化合物，其中 n 代表数值 0 并且 m 代表数值 1。

[0253] 还优选式 (I) 的化合物，其中 R^1 代表叔丁氧羰基。

[0254] 还优选式 (I) 的化合物，其中 R^3 代表氢或甲基。

[0255] 还优选式 (I) 的化合物，其中 R^4 代表下式的基团

[0256]



[0257] 其中

[0258] * 为与氮的连接位点，

[0259] R^5 代表氨基羰基，

[0260] 并且

[0261] R^6 代表 -S- 三苯甲基。

[0262] 还优选式 (I) 的化合物，其中 R^6 代表 -S- 三苯甲基。

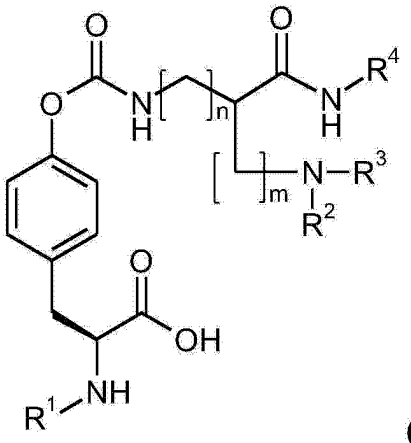
[0263] 还优选式 (I) 的化合物，其中 R^8 代表 -S- 三苯甲基。

[0264] 还优选式 (I) 的化合物，其中 R^9 代表氢并且 R^{10} 代表氢。

[0265] 还优选式 (I) 的化合物，其中与 $-NHR^1$ 取代基连接的碳原子具有 S 构型。

[0266] 还优选式 (I) 的化合物，其具有式 (Ia) 的结构。

[0267]



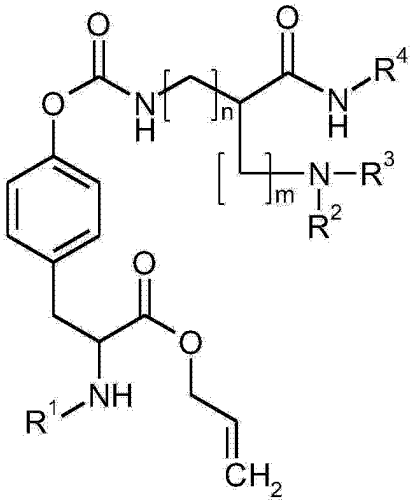
(Ia)

[0268] 在基团的具体组合或优选组合中给出的特定基团定义，不考虑具体说明的基团的特定组合，还可以被其他组合的任何基团定义所代替。

[0269] 非常特别优选上述优选范围的两个或更多个的组合。

[0270] 本发明还提供了用于制备式 (I) 的化合物或者其盐、其溶剂合物或其盐的溶剂合物的方法，其中使式 (II) 的化合物与钯 (0) 源和还原剂反应

[0271]



(II)，

[0272] 其中

[0273] n、m、R^1、R^2、R^3 和 R^4 各自如上文所定义。

[0274] 所述反应通常在惰性溶剂中，任选地在弱碱的存在下，优选在 0℃ -50℃的温度范围内在标准压力下实现。

[0275] 惰性溶剂为，例如卤代烃例如二氯甲烷、三氯甲烷或 1,2- 二氯乙烷，醚例如二噁烷、四氢呋喃或 1,2- 二甲氧基乙烷，或其他溶剂例如丙酮、二甲基甲酰胺、二甲基乙酰胺、2- 丁酮或乙腈。同样可能使用所述溶剂的混合物。优选四氢呋喃。

[0276] 钯 (0) 源为例如四 (三苯基膦) 钯 (0)、三 (二亚苄基丙酮) 二钯 (0) 或在反应过程中被原位还原为钯 (0) 的钯 (II) 源，优选四 (三苯基膦) 钯 (0)。

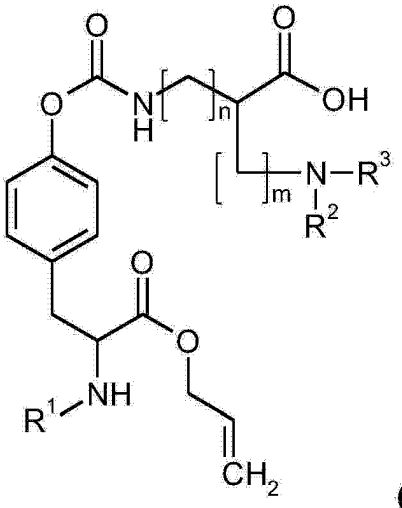
[0277] 还原剂为例如甲酸或三乙基硅烷，优选甲酸。

[0278] 碱为例如三乙胺、N,N- 二异丙基乙胺或磷酸钾溶液，优选三乙胺。

[0279] 式 (II) 的化合物是已知的或可通过使式 (III) 的化合物与式 (IV) 的化合物反应

而制备，

[0280]



(III)

[0281] 其中

[0282] n、m、R^1、R^2 和 R^3 各自如上文所定义，

[0283] H_2N-R^4 (IV)

[0284] 其中

[0285] R^4 如上文所定义。

[0286] 所述反应通常在惰性溶剂中，在脱水试剂的存在下，任选地在碱的存在下，优选地在从室温至 70℃的温度范围内在标准压力下实现。

[0287] 惰性溶剂为，例如卤代烃例如二氯甲烷、三氯甲烷或 1,2- 二氯乙烷，醚例如二噁烷、四氢呋喃或 1,2- 二甲氧基乙烷，或其他溶剂例如丙酮、二甲基甲酰胺、二甲基乙酰胺、2- 丁酮或乙腈。同样可能使用所述溶剂的混合物。优选二氯甲烷。

[0288] 在该上下文中，合适的脱水试剂为，例如碳二亚胺例如 N,N'- 二乙基 -、N,N'- 二丙基 -、N,N'- 二异丙基 -、N,N'- 二环己基碳二亚胺，N-(3- 二甲基氨基异丙基)-N'- 乙基碳二亚胺盐酸盐 (EDC)，N- 环己基碳二亚胺 -N'- 丙基氧基甲基 - 聚苯乙烯 (PS- 碳二亚胺)，或羰基化合物例如羰基二咪唑，或 1,2- 噁唑鎓化合物，例如 2- 乙基 -5- 苯基 -1,2- 噁唑鎓 3- 硫酸盐或 2- 叔丁基 -5- 甲基异噁唑鎓高氯酸盐，或酰基氨基化合物例如 2- 乙氧基 -1- 乙氧基羰基 -1,2- 二氢喹啉，或丙基膦酸酐，或氯甲酸异丁酯，或双 -(2- 氧代 -3- 噁唑烷基) 膦酰氯或苯并三唑基氧基三 (二甲基氨基) 磷鎓六氟磷酸盐，或 O-(苯并三唑 -1- 基)-N,N,N',N'- 四甲基脲六氟磷酸盐 (HBTU)，苯并三唑 -1- 基 -N- 四甲基 - 脲四氟硼酸盐 (TBTU)，2-(2- 氧代 -1-(2H)- 吡啶基)-1,1,3,3- 四甲基脲四氟硼酸盐 (TPTU) 或 O-(7- 氮杂苯并三唑 -1- 基)-N,N,N',N'- 四甲基脲六氟磷酸盐 (HATU)，或 1- 羟基苯三氮唑 (HOBt)，或苯并三唑 -1- 基氧基三 (二甲基氨基) 膦六氟磷酸盐 (BOP)，或苯并三唑 -1- 基氧基三 (吡咯烷基) 膦六氟磷酸盐 (PYBOP)，或 N- 羟基琥珀酰亚胺，或这些与碱的混合物。

[0289] 碱为例如碱金属碳酸盐如碳酸钠或碳酸钾、或碳酸氢钠或碳酸氢钾，或有机碱例如三烷基胺，例如三乙胺、N- 甲基吗啉、N- 甲基哌啶、4- 二甲基氨基吡啶或 N,N- 二异丙基乙胺，优选 N,N- 二异丙基乙胺。

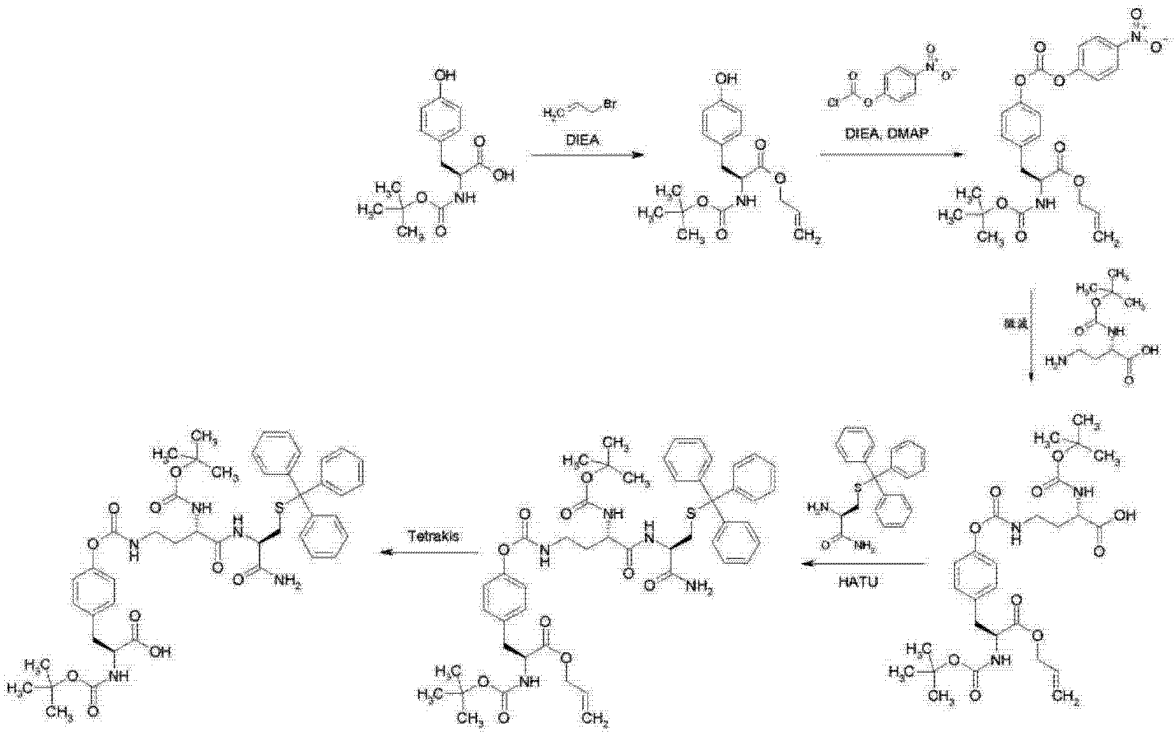
[0290] 优选地，在 N,N- 二异丙基乙胺的存在下，使用 HATU 进行缩合反应。

[0291] 式(III)和(IV)的化合物是已知的,或可通过已知的方法从合适的起始化合物来合成。

[0292] 本发明化合物的制备可通过以下合成方案进行说明:

[0293] 方案1

[0294]

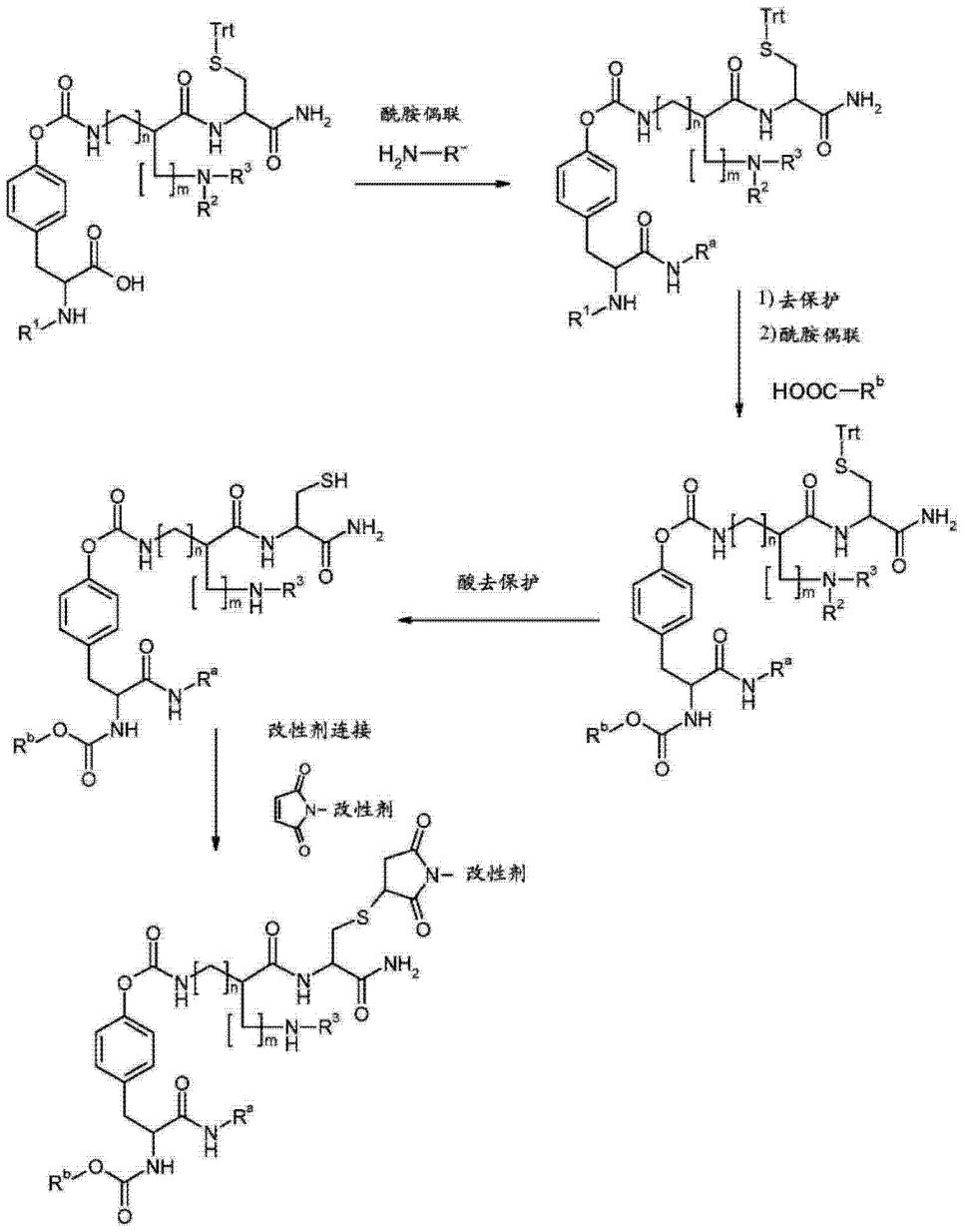


[0295] 本发明化合物可用作肽或蛋白质与其他分子实体(例如聚乙二醇或其他改性剂)之间的可释放的连接体,以形成所述肽或蛋白质的前药。

[0296] 这些酪氨酸衍生物的活性成分是肽或蛋白质序列中酪氨酸的酚OH-基与二氨基酸的一个胺官能度之间的氨基甲酸酯。在酸性条件下,所述二氨基酸的第二个胺为质子化的。但是在中性或碱性条件下,其作为亲核基团攻击所述氨基甲酸酯。这导致形成环脲并释放未修饰的酪氨酸。所述二氨基酸的酸官能度被用作改性剂的连接位点。可以想象有许多不同的在该官能度上连接改性剂的方法。将改性剂(例如聚乙二醇)连接至肽的通常方法是通过使PEG-马来酰亚胺与半胱氨酸残基或其他巯基(thiol)进行反应。因此,完成所需任务的直接方式是将所述半胱氨酸残基通过其胺官能度连接至所述二氨基酸的羧基。半胱氨酸的羧基末端可为例如伯酰胺,但其C末端上也可能有许多其他的修饰。很容易想象,许多间隔基基团(spacer group)适合在所述二氨基酸和半胱氨酸或其他巯基官能度之间,而不会改变该连接概念的性质,这是因为该分子构造全部维持在一端的环脲(由二氨基酸形成的)与另一端的改性剂之间。释放的肽或蛋白质未以任何方式被改变。并且将改性剂连接至所述连接体的化学作用不限于巯基官能度与马来酰亚胺的反应。其他熟知的将改性剂(例如PEG)连接至巯基的方法同样合适。还有许多其他的不含巯基的连接方法也是替代方案,例如"点击"化学或与胺官能化的改性剂形成简单的酰胺键。方案2示出了将改性剂连接至含有基于酪氨酸的氨基酸衍生物的肽的示例性连接。

[0297] 方案2

[0298]



[0299] 肽或蛋白质以 pH 依赖的方式从所述前药释放。所述前药在约 pH4 是稳定的，但在生理 pH 下释放活性药。肽或蛋白质从所述前药释放后，仍保留在所述肽或蛋白质中的只有在之前的所述连接体的连接位点上的未修饰的酪氨酸。因此，所有含有至少 1 个酪氨酸的肽或蛋白质都可以进行这类修饰。

[0300] 所述前药的用于释放所述肽或蛋白质的 pH 依赖性断裂，有助于设计这类前药的具有可预测的药物动力学的受控降解。

[0301] 根据溶液和固相肽合成方案，可将本发明化合物纳入肽或蛋白质中。

[0302] 合适的包含至少一个酪氨酸氨基酸的蛋白质和肽为，但不限于，腺苷脱氨酶、脂联素 (adiponectin)、促肾上腺皮质激素 (ACTH)、肾上腺髓质素 (ADM)、半乳糖苷酶 (agalsidase)、白蛋白、α-1 蛋白酶抑制剂 (API)、α-1 抗胰蛋白酶 b(AAT)、阿替普酶、安克洛酶丝氨酸、血管紧张素、血管紧张素原血管紧张素 (angiotensinogenangiotensin)、阿尼普酶、抗苗勒氏管激素 (antimullerian hormone)、抗凝血酶 III、抗胰蛋白酶、抑肽酶、

天冬酰胺酶、心房肽、阿片肽 (biphalin)、缓激肽、降钙素、胆囊收缩素、绒毛膜促性腺激素 d(choriogonadotropind)、绒毛膜催乳激素、胶原酶、促皮质激素释放激素、促肾上腺皮质激素、DNA 酶、内啡肽、脑啡肽、依诺沙星、促红细胞生成素、因子 II、因子 IIa、因子 IX、因子 IXa、因子 VII、因子 VIIa、因子 VIII、因子 VIIIa、因子 X、因子 Xa、因子 XI、因子 XIa、溶纤维蛋白溶酶 (fibrinolysin)、溶纤维蛋白溶酶、促卵泡素释放激素、促卵泡激素、促滤泡素、卵泡刺激素、Fsh、半乳糖苷酶、促胃液素、脑肠肽 (ghrelin)、胰高血糖素、胰高血糖素样肽类似物 (GLP-1)、葡糖脑苷脂酶、谷催产素 f、促性腺素释放素 c、促性腺激素、促性腺激素释放激素、粒细胞集落刺激因子 (G-CSF)、粒细胞巨噬细胞集落刺激因子 (GM-CSF)、生长因子、生长激素释放激素、生长激素、血红蛋白、乙型肝炎疫苗、水蛭素、人绒毛膜促性腺激素、人胎盘催乳素、透明质酸酶、伊达比星、艾杜糖苷酸酶、免疫球蛋白、流感疫苗、抑制素、胰岛素、干扰素、白细胞介素、硬骨鱼催产素 g(isotocin g)、胰激肽、角质形成细胞生长因子 (KGF)、乳糖酶、瘦素、亮丙瑞林 (leuprolide)、左甲状腺素、促脂解素、赖诺普利、促黄体素释放素 (luliberin)、黄体化激素 (luteinizing hormone)、促黄体激素、促黑色素细胞激素、促黑素释放素、促黑素抑制素、促黑激素 h(melanotropinh)、钠尿肽、阿立新 (orexin)、促肾上腺皮质激素释放激素 (orticotropin-releasing hormone)、催产素、胰脂肪酶、促胰酶素、木瓜蛋白酶、甲状旁腺激素、胃蛋白酶、磷脂酶活化蛋白 (PLAP)、血小板活化因子乙酰水解酶 (PAF-AH)、血管紧张素原、促乳素、促乳素释放素、促乳素抑制素、蛋白酶、蛋白 C、松弛素、分泌素 (secretin)、sennorelin、促生长素释放素 (somatoliberin)、生长调节素、生长激素 (somatropin)、链激酶、蔗糖酶、超氧化物歧化酶 (SOD)、血小板生成素、胸腺生成素 n(thymopoiethinn)、胸腺素、促甲状腺激素、促甲状腺素释放素、促甲状腺素、促甲状腺激素释放激素、半乳糖苷酶 (tilactase)、组织型纤溶酶原激活剂 (tPA)、肿瘤坏死因子 (TNF)、尿酸氧化酶、尿促性腺激素 k、尿激酶、疫苗、加压素、加压催产素、α -1a 抗胰蛋白酶。也包括以上所列的肽或蛋白质的突变体形式或所有通过重组方法制备的其他蛋白，例如抗体、抗体片段、单链结合蛋白和融合蛋白。还包括具有生物活性的任何合成的肽或蛋白质。

[0303] 本发明化合物适用于制备前药，所述前药适合用作用于治疗和 / 或预防人和动物中的疾病的药物。

[0304] 本发明化合物适用于制备特定的肾上腺髓质素 (ADM) 释放性前药。

[0305] 本发明还提供了本发明化合物用于制备用于治疗和 / 或预防病症的前药的用途。

[0306] 对于本发明，术语“治疗”包括抑制、延迟、减轻、缓解、阻止、减少疾病、障碍、病症或病征 (state)、其发展和 / 或进展、和 / 或其症状，或引起疾病、障碍、病症或病征、其发展和 / 或进展、和 / 或其症状的消退。术语“预防”包括降低患有、感染或经历疾病、障碍、病症或病征、其发展和 / 或进展、和 / 或其症状的风险。术语预防 (prevention) 包括预防 (prophylaxis)。疾病、障碍、病症或病征的治疗或预防可为部分的或完全的。

[0307] 基于它们的药理学性质，用本发明化合物制备的前药可用于治疗和 / 或预防心血管疾病，尤其是慢性和急性心力衰竭、舒张性和收缩性 (充血性) 心力衰竭、急性失代偿性心力衰竭、心机能不全、冠状动脉心脏病、心绞痛、心肌梗死、缺血再灌注损伤、缺血性中风和出血性中风、动脉硬化、动脉粥样硬化、原发性高血压、恶性原发性高血压、继发性高血压、肾血管性高血压和继发于肾脏和内分泌障碍的高血压、高血压性心脏病、高血压性肾

病、继发性肺动脉高压、伴随和不伴随急性肺源性心脏病的继肺栓塞之后的肺动脉高压、原发性肺动脉高压和周围动脉闭塞疾病。

[0308] 此外，用本发明化合物制备的前药适用于治疗和/或预防伴随和不伴随高血压的妊娠期［妊娠引发的］水肿和蛋白尿（先兆子痫(pre-eclampsia)）。

[0309] 此外，用本发明化合物制备的前药适用于治疗和/或预防肺病症，例如慢性阻塞性肺病、哮喘、急性和慢性肺水肿，由吸入的有机粉尘和真菌、放线菌或其他来源的颗粒引起的变应性肺泡炎和肺炎，急性化学性支气管炎、急性和慢性化学性肺水肿（例如吸入光气、一氧化氮之后）、神经性肺水肿、由辐射引起的急性和慢性肺表现(pulmonary manifestation)、急性和慢性间质性肺病症（例如，但不限于，药物诱发的间质性肺病症，例如继发于博来霉素治疗）、成人或儿童（包括新生儿）中的急性肺损伤/急性呼吸窘迫综合征(ALI/ARDS)、继发于肺炎和脓毒症的ALI/ARDS、继发于误吸的吸入性肺炎和ALI/ARDS（例如，但不限于，由反流的胃内容物引起的吸入性肺炎）、继发于烟气吸入的ALI/ARDS、输血相关的急性肺损伤(TRALI)，手术、外伤或烧伤后的ALI/ARDS或急性肺功能不全，呼吸机诱发的肺损伤(ventilator induced lung injury，VILI)、胎粪吸入后的肺损伤、肺纤维化和高山症。

[0310] 此外，用本发明化合物制备的前药适用于治疗和/或预防慢性肾疾病(1-5期)、肾功能不全、糖尿病性肾病、高血压性慢性肾脏疾病、肾小球肾炎、急进性和慢性肾炎综合征、非特异性(unspecific)肾炎综合征、肾病综合征、遗传性肾病、急性和慢性肾小管间质性肾炎、急性肾损伤、急性肾功能衰竭、创伤后肾功能衰竭、外伤性和手术后肾损伤、心肾综合征，以及肾移植物的保护和功能性改善。

[0311] 此外，用本发明化合物制备的前药适用于治疗和/或预防糖尿病及其连续症状，例如糖尿病性大血管病变和微血管病变、糖尿病性肾病和神经病。

[0312] 此外，用本发明化合物制备的前药可用于治疗和/或预防中枢神经系统和周围神经系统障碍，例如病毒性和细菌性脑膜炎和脑炎（例如带状疱疹性脑炎）、脑损伤、脑和脊髓的原发性或继发性［转移性］恶性肿瘤、脊神经根炎和多神经根炎、格林-巴利综合征［急性感染性（后）多神经炎、米勒〃费希尔综合征］、肌萎缩侧索硬化［进行性脊髓性肌萎缩］、帕金森病、急性和慢性多发神经病、疼痛、脑水肿、阿尔茨海默病、神经系统的退行性疾病和中枢神经系统的脱髓鞘疾病，例如但不限于多发性硬化。

[0313] 此外，用本发明化合物制备的前药适用于治疗和/或预防门静脉高血压和肝纤维化［肝硬化］及其后遗症，例如食管静脉曲张和腹水，用于治疗和/预防继发于恶性肿瘤或炎症的胸腔积液，以及用于治疗和/预防淋巴水肿和继发于静脉曲张(varices)的水肿。

[0314] 此外，用本发明化合物制备的前药适用于治疗和/或预防胃肠道的炎症性障碍例如炎性肠病、克罗恩病、溃疡性结肠炎，以及肠的中毒性和血管性障碍。

[0315] 此外，用本发明化合物制备的前药适用于治疗和/或预防脓毒症、感染性休克、非传染性来源的全身性炎性反应综合征(SIRS)、出血性休克、伴随器官功能障碍或多器官功能衰竭(MOF)的脓毒症或SIRS、创伤性休克、中毒性休克、过敏性休克、荨麻疹、虫螫和虫咬相关的过敏、血管神经性水肿［巨大荨麻疹、昆克水肿］、急性喉炎和气管炎，以及急性阻塞性喉炎［哮吼］和会厌炎

[0316] 此外，用本发明化合物制备的前药适用于治疗和/或预防被视为自身免疫性疾病

的风湿性类型的疾病和其他疾病形式，例如但不限于多发性关节炎、红斑狼疮、硬皮病、紫癜和血管炎。

[0317] 此外，用本发明化合物制备的前药适用于治疗高眼压症（青光眼）、糖尿病性视网膜病变和黄斑水肿。

[0318] 此外，用本发明化合物制备的前药可用于治疗和/或预防手术干预之后与手术相关的缺血状态及其连续症状，尤其是使用心肺机在心脏上进行的干预（例如搭桥手术(bypass operation)、心脏瓣膜移植物）、在颈动脉上进行的干预、在大动脉上进行的干预和使用仪器打开或穿透颅盖的干预。

[0319] 此外，用所述化合物制备的前药适用于在进行外科手术的情况下进行一般性治疗和/预防，以期加速伤口愈合和缩短再康复时间(reconvalescence time)。其还适用于促进伤口愈合。

[0320] 此外，用所述化合物制备的前药适用于治疗和/或预防骨密度和结构的障碍，例如但不限于骨质疏松症、骨软化症和甲状旁腺功能亢进相关的骨障碍。

[0321] 此外，用所述化合物制备的前药适用于治疗和/或预防性功能障碍，尤其是男性勃起功能障碍。

[0322] 此外，用所述化合物制备的前药适用于治疗和/或预防心力衰竭、冠状动脉心脏病、缺血性和/或出血性中风、高血压、肺动脉高压、周围动脉闭塞疾病、先兆子痫、慢性阻塞性肺病、哮喘、急性和/或慢性肺水肿，由吸入的有机粉尘和真菌、放线菌或其他来源的颗粒引起的变应性肺泡炎和/或肺炎，和/或急性化学性支气管炎、急性和/或慢性化学性肺水肿、神经性肺水肿、由辐射引起的急性和/或慢性肺表现、急性和/或慢性间质性肺病症、成人或儿童（包括新生儿）中的急性肺损伤/急性呼吸窘迫综合征(ALI/ARDS)、继发于肺炎和脓毒症的ALI/ARDS、继发于误吸的吸入性肺炎和ALI/ARDS、继发于烟气吸入的ALI/ARDS、输血相关的急性肺损伤(TRALI)，继发于手术、外伤和/或烧伤的ALI/ARDS和/或急性肺功能不全，和/或呼吸机诱发的肺损伤(VILI)、胎粪吸入后的肺损伤、肺纤维化、高山症、慢性肾疾病、肾小球肾炎、急性肾损伤、心肾综合征、淋巴水肿、炎性肠病、脓毒症、感染性休克、非传染性来源的全身性炎性反应综合征(SIRS)、过敏性休克和/或荨麻疹。

[0323] 本发明还提供了用本发明化合物制备的前药用于治疗和/或预防病症（尤其是上文提及的病症）的用途。

[0324] 本发明还提供了用本发明化合物制备的前药用于制备治疗和/或预防病症（尤其是上文提及的病症）的药物的用途。

[0325] 本发明还提供了用于治疗和/或预防病症（尤其是上文提及的病症）的方法，所述方法使用有效量的用本发明化合物制备的前药。

[0326] 本发明还提供了包含用本发明化合物制备的前药和一种或多种另外的活性成分的药物，尤其是用于治疗和/或预防上文提及的病症。示例性和优选的活性成分结合物为：

[0327] ACE抑制剂、血管紧张素受体拮抗剂、β-2受体激动剂、磷酸二酯酶抑制剂、糖皮质激素受体激动剂、利尿剂，或重组血管收缩素转换酶-2或乙酰水杨酸（阿司匹林）。

[0328] 在本发明的一个优选的实施方案中，用本发明化合物制备的前药与ACE抑制剂相结合进行给药，所述ACE抑制剂例如并优选依那普利(enalapril)、喹那普利(,quinapril)、卡托普利(,captopril)、赖诺普利(lisinopril)、雷米普利(ramipril)、

地拉普利 (delapril)、福辛普利 (fosinopril)、培哚普利 (perindopril)、西拉普利 (cilazapril)、咪达普利 (imidapril)、贝那普利 (benazepril)、莫昔普利 (moexipril)、螺普利 (spirapril) 或川多普利 (trandopril)。

[0329] 在本发明的一个优选的实施方案中，用本发明化合物制备的前药与血管紧张素受体拮抗剂相结合进行给药，所述血管紧张素受体拮抗剂例如并优选氯沙坦 (losartan)、坎地沙坦 (candesartan)、缬沙坦 (valsartan)、替米沙坦 (telmisartan) 或恩布沙坦 (embusartan)。

[0330] 在本发明的一个优选的实施方案中，用本发明化合物制备的前药与 β-2 受体激动剂相结合进行给药，所述 β-2 受体激动剂例如并优选沙丁胺醇 (salbutamol)、吡布特罗 (pirbuterol)、沙美特罗 (salmeterol)、特布他林 (terbutalin)、非诺特罗 (fenoterol)、妥洛特罗 (tulobuterol)、克仑特罗 (clenbuterol)、瑞普特罗 (reproterol) 或福莫特罗 (formoterol)。

[0331] 在本发明的一个优选的实施方案中，用本发明化合物制备的前药与磷酸二酯酶 (PDE) 抑制剂相结合进行给药，所述磷酸二酯酶抑制剂例如并优选米力农 (milrinone)、氨力农 (amrinone)、匹莫苯旦 (pimobendan)、西洛他唑 (cilostazol)、西地那非 (sildenafil)、伐地那非 (vardenafil) 或他达拉非 (tadalafil)。

[0332] 在本发明的一个优选的实施方案中，用本发明化合物制备的前药与糖皮质激素受体激动剂相结合进行给药，所述糖皮质激素受体激动剂例如并优选皮质醇 (cortiosol)、可的松 (cortisone)、氢化可的松 (hydrocortisone)、泼尼松 (prednisone)、甲泼尼龙 (methyl-prednisolone)、泼尼立定 (prednylidene)、地夫可特 (deflazacort)、氟可龙 (fluocortolone)、曲安西龙 (triamcinolone)、地塞米松 (dexamethasone) 或倍他米松 (betamethasone)。

[0333] 在本发明的一个优选的实施方案中，用本发明化合物制备的前药与利尿剂相结合进行给药，所述利尿剂例如并优选呋塞米 (furosemide)、托拉塞米 (torasemide) 和氢氯噻嗪 (hydrochlorothiazide)。

[0334] 本发明还涉及包含至少一种用本发明化合物制备的前药，以及通常一种或多种惰性、无毒、可药用的赋形剂的药物，还涉及其用于前述目的的用途。

[0335] 用本发明化合物制备的前药可在全身和 / 或局部起作用。为此，可以合适的方式给予所述前药，例如通过肠胃外的、肺的、鼻的、舌下的、舌的、颊的、真皮的、透皮的、结膜的、眼的途径或作为植入物或支架。

[0336] 用本发明化合物制备的前药可以适合这些给药途径的给药形式进行给药。

[0337] 进行胃肠外给药可以避免吸收步骤 (例如静脉内的、动脉内的、心内的、脊柱内的或腰髓内的) 或同时包括吸收步骤 (例如肌内的、皮下的、皮内的、经皮的或腹膜内的)。适合胃肠外给药的给药形式包括用于以溶液剂、悬液剂、乳剂 (emulsion)、冻干物 (lyophilizate) 或无菌粉剂的形式注射或输注的制剂。

[0338] 适合于其他给药途径的是，例如用于吸入的药物形式 (包括粉末吸入剂、喷雾剂)、滴鼻剂、滴眼剂、溶液剂或喷雾剂 ; 薄膜剂 / 薄片剂 (wafer) 或水性悬液剂 (洗剂、振荡合剂 (shaking mixture))、亲脂性悬液剂、软膏剂、乳膏剂、透皮治疗系统 (例如贴剂)、乳剂 (milk)、糊剂、泡沫剂、撒布粉 (dusting powder)、植入物或支架。

[0339] 优选胃肠外给药，特别是静脉内给药。

[0340] 用本发明化合物制备的前药可转化成所述的给药形式。这可按照本身已知的方法，通过与惰性、无毒、药物学上合适的赋形剂混合来进行。这些赋形剂包括载体（例如微晶纤维素、乳糖、甘露醇）、溶剂（例如液体聚乙二醇）、乳化剂和分散剂或润湿剂（例如十二烷基硫酸钠、聚氧脱水山梨糖醇油酸酯）、粘合剂（例如聚乙烯吡咯烷酮），合成和天然聚合物（例如白蛋白）、稳定剂（例如抗氧化剂，如抗坏血酸）、色料（例如无机颜料，如铁氧化物）和矫香剂和 / 或矫臭剂。

[0341] 通常发现有利的是，在肠胃外给药的情况下，给予约 0.001-5mg/kg，优选约 0.01-1mg/kg 体重的量以获得有效结果。

[0342] 尽管如此，在某些情况下可能必须偏离所述用量，尤其是根据体重、给药途径、对活性成分的个体响应、制剂的性质以及给药进行的时间或间隔。例如，在某些情况下，比上述的最低量更少的量可能是足够的，而在其他情况下必须超过所述上限。在给予更大量的情况下，可建议将这些量分成在一天内的多个单个剂量。

[0343] 以下工作实施例举例说明本发明。本发明不限于所述实施例。

[0344] 除非另有说明，否则以下试验和实施例中的百分数是重量百分数；份是重量份。溶剂比率、稀释比率和液体 / 液体溶液的浓度数据均是基于体积计。

[0345] A. 实施例

[0346] 缩写

[0347]	AA	氨基酸
[0348]	Acm	乙酰氨基甲基
[0349]	approx.	约
[0350]	Boc	叔丁氧羰基
[0351]	CDI	羰基二咪唑
[0352]	d	天、双重峰（在 NMR 中）
[0353]	TLC	薄层色谱法
[0354]	DCI	直接化学电离（在 MS 中）
[0355]	dd	双二重峰（在 NMR 中）
[0356]	DIEA	N,N- 二异丙基乙胺
[0357]	DMAP	4- 二甲基氨基吡啶
[0358]	DMF	N,N- 二甲基甲酰胺
[0359]	DMSO	二甲基亚砜
[0360]	of theory	理论值的（在产率中）
[0361]	eq.	当量
[0362]	ESI	电喷雾离子化（在 MS 中）
[0363]	Fmoc	(9H- 芴 -9- 基甲氧基）羰基
[0364]	h	小时
[0365]	HATU	O-(7- 氮杂苯并三唑 -1- 基）-N,N,N',N'- 四甲基脲六氟磷酸盐
[0366]	HPLC	高压、高效液相色谱

[0367] LC-MS 与液相色谱法偶联的质谱法

[0368] m 多重峰（在 NMR 中）

[0369] min 分钟

[0370] MS 质谱法

[0371] NMR 核磁共振色谱法

[0372] RP 反相（在 HPLC 中）

[0373] RT 室温

[0374] R_t 保留时间（在 HPLC 中）

[0375] s 单峰（在 NMR 中）

[0376] TBTU 苯并三唑 -1- 基 -N- 四甲基 - 脲四氟硼酸盐

[0377] tBu 叔丁基

[0378] TFA 三氟乙酸

[0379] THF 四氢呋喃

[0380] Trt 三苯甲基

[0381] LC-MS 和 MS 方法

[0382] 方法 1(LC-MS) :仪器类型 :Waters ACQUITY SQD UPLC 系统 ;柱 :Waters Acquity UPLC HSS T31.8μ 50mm×1mm ;流动相 A :11 的水 +0.25ml99％浓度的甲酸，流动相 B :11 的乙腈 +0.25ml99％浓度的甲酸 ;梯度 :0.0 分钟 90％ A → 1.2 分钟 5％ A → 2.0 分钟 5％ A ;烘箱 :50℃ ;流速 :0.40ml/min ;UV 检测 :210-400nm。

[0383] 方法 2(LC-MS) :MS 仪器 : 类型 :Waters(Micromass)Quattro Micro ;HPLC 仪器类型 :Agilent1100 系列 ; 柱 :Thermo Hypersil GOLD3μ 20mm×4mm ; 流动相 A :11 的水 +0.5ml50％浓度的甲酸，流动相 B :11 的乙腈 +0.5ml50％浓度的甲酸 ;梯度 :0.0 分钟 100％ A → 3.0 分钟 10％ A → 4.0 分钟 10％ A ;烘箱 :50℃ ;流速 :2.0ml/min ;UV 检测 :210nm。

[0384] 方法 3(HPLC) : 仪器类型 :HP1200 系列 ;UV DAD ; 柱 :Phenomenex Luna5μm C5 **100Å,** 150mm×4.6mm ;流动相 A :11 的水 +0.5ml50％浓度的甲酸，流动相 B :11 的乙腈 +0.5ml50％浓度的甲酸 ;梯度 :0.0 分钟 95％ A → 5 分钟 5％ A ;→ 5.8 分钟 95％ A → 6.2 分钟 95％ A ;流速 :2.5ml/min ;烘箱 :RT ;UV 检测 :210nm。

[0385] 方法 4(HPLC) : 仪器类型 :HP1200 系列 ;UV DAD ; 柱 :Merck Chromolith Fastgradient RP1850mm×2mm ;流动相 A :11 的水 +0.5ml50％浓度的甲酸，流动相 B :11 的乙腈 +0.5ml50％浓度的甲酸 ;梯度 :0.0 分钟 95％ A → 2.9 分钟 5％ A → 3.2 分钟 5％ A ;流速 :3ml/min ;烘箱 :RT ;UV 检测 :210nm。

[0386] 微波合成器 :带有容量最高达 20ml 反应体积的可变量瓶和“Robot60”样品处理器的 Biotage Emrys Initiator II 合成器。

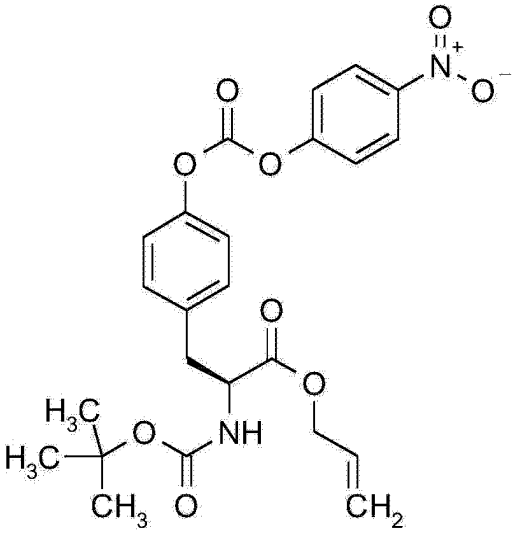
[0387] pH4 柠檬酸缓冲液 :Fluka No82566 ;用叠氮化钠组合物稳定的 pH4 的柠檬酸缓冲液 :柠檬酸，～ 0.056M ;叠氮化钠，～ 0.05％ ;氯化钠，～ 0.044M ;氢氧化钠，～ 0.068M。

[0388] 起始化合物

[0389] 实施例 1A

[0390] 烯丙基 -N-(叔丁氧羰基)-O-[(4- 硝基苯氧基) 羰基]-L- 酪氨酸酯

[0391]



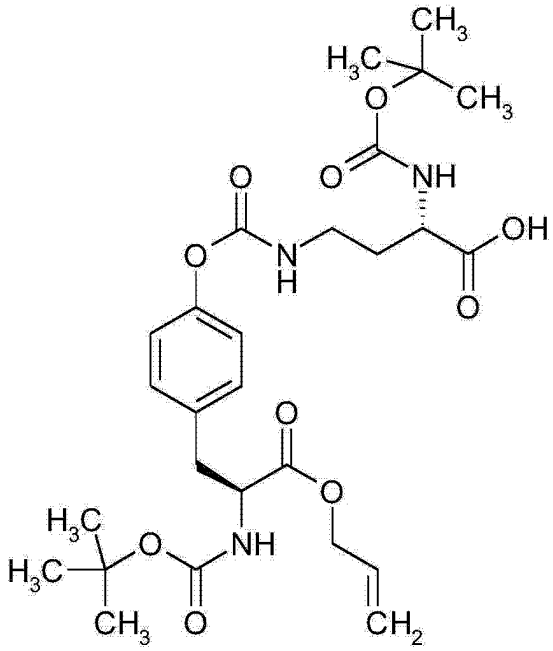
[0392] 将 36.7g(114.3mmol)N-Boc-L- 酪氨酸烯丙基酯、23.0g(114.3mmol)4- 硝基苯基氯甲酸酯、17.5ml(125.7mmol) 三乙胺和 1.40g(11.4mmol)4- 二甲基氨基吡啶合并在 1000ml 的二氯甲烷中，室温下搅拌 2h。该反应混合物用约 500ml 的水和用约 250ml 的盐水萃取，并用约 100g 的硫酸钠干燥。溶剂是通过旋转蒸发（约 40℃，约 200mbar，约 30 分钟）被除去，将该产物溶于温的乙醚中，4℃下结晶过夜。将该晶体滤出，用冷的乙醚洗涤，在高真空中干燥（约 0.1mbar，18h）。产率为 29.86g(59.6mmol，理论值的 52％）的所需产物。

[0393] LC-MS（方法 1）：R_t = 1.23min，m/z = 487(M+H)$^+$

[0394] 实施例 2A

[0395] (2S)-4-{[(4-{(2S)-3-（烯丙氧基）-2-[（叔丁氧羰基）氨基]-3- 氧代丙基} 苯氧基）羰基] 氨基}-2-[（叔丁氧羰基）氨基] 丁酸

[0396]



[0397] 将 4.0g(8.22mmol) 来自实施例 1A 的化合物溶于 60ml 二氯甲烷中。添加 1.795(8.22mmol)(2S)-4- 氨基 -2-[（叔丁氧羰基）氨基] 丁酸和 1.43ml(8.22mmol)N,N- 二异丙基乙胺。将反应混合物分成 3 部分。在微波合成器中将这些部分在密封管中在 75℃下

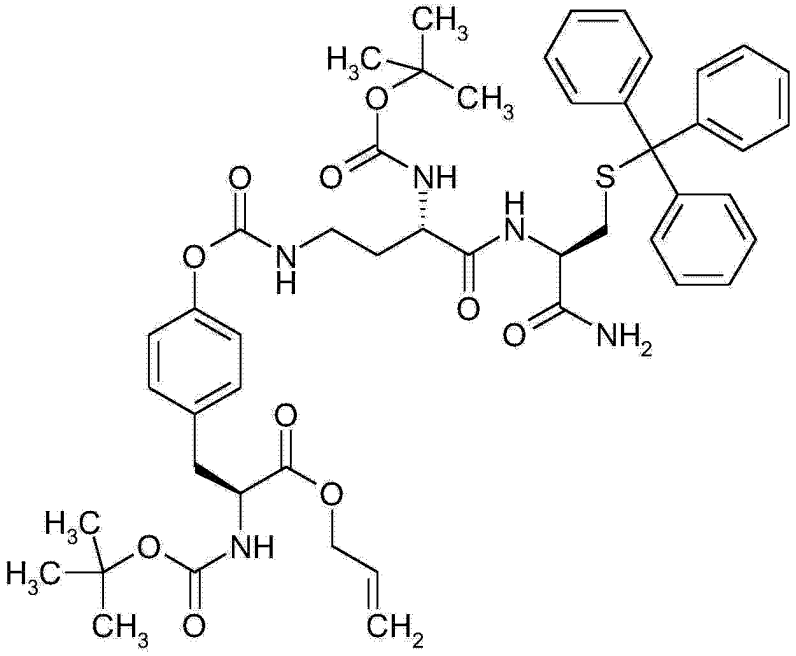
加热 30 分钟。通过旋转蒸发（约 40℃，约 200mbar，约 30 分钟）将溶剂从合并的反应混合物中除去。将粗产物溶于二氯甲烷，在约 600ml 硅胶中进行色谱分析。使用的溶剂为 4/1 的二氯甲烷 / 乙酸乙酯、1/1 的二氯甲烷 / 乙酸乙酯、4/1 的二氯甲烷 / 甲醇和 1/1 的二氯甲烷 / 甲醇。将含有产物的级分合并，在减压下浓缩至干燥。这得到 4.02g(6.54mmol，理论值的 80％）的所需产物。

[0398] LC-MS（方法 1）：R_t = 1.07min，m/z = 564 $(M-H)^-$

[0399] 实施例 3A

[0400] O-({(3S)-4-{[(2R)-1- 氨基 -1- 氧代 -3-（三苯甲基硫烷基）丙 -2- 基］氨基}-3-[（叔丁氧羰基）氨基］-4- 氧代丁基｝氨基甲酰基）-N-（叔丁氧羰基）-L- 酪氨酸烯丙基酯

[0401]



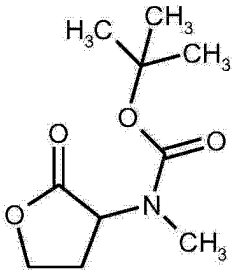
[0402] 将 2.50g(4.42mmol) 来自实施例 2A 的化合物溶于 100ml 的二氯甲烷。添加 1.602g(4.42mmol)S- 三苯甲基 -L- 半胱氨酰胺 (cysteinamide)、0.77ml(4.42mmol)N,N- 二异丙基乙胺和 1.68g(4.42mmol)HATU。将反应混合物分成 5 部分。在微波合成器中将这些部分在密封管中在 60℃下加热 30 分钟。通过旋转蒸发（约 40℃，约 200mbar，约 30 分钟）将溶剂从合并的反应混合物中除去。将粗产物溶于二氯甲烷，在约 600ml 硅胶中进行色谱分析。使用的溶剂为 2/1 的二氯甲烷 / 乙酸乙酯、1/1 的二氯甲烷 / 乙酸乙酯、20/1 的二氯甲烷 / 甲醇和 10/1 的二氯甲烷 / 甲醇。将含有产物的级分合并，在减压下浓缩至干燥。这得到 4.12g(3.30mmol，理论值的 75％，73％纯度）的所需产物。

[0403] LC-MS（方法 1）：R_t = 1.36min，m/z = 911 $(M+H)^+$

[0404] 实施例 4A

[0405] 叔丁基甲基 (2- 氧代四氢呋喃 -3- 基）氨基甲酸酯

[0406]



[0407] 所述化合物根据 Alberico, Dino ; Paquin, Jean-Francois ; Lautens, Mark ; Tetrahedron, 2005, vol. 61, p. 6283 - 6297 来合成。

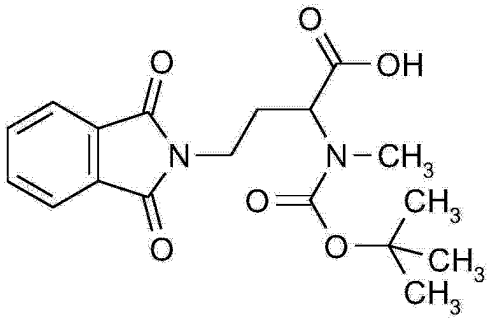
[0408] 将 5.18g(25.7mmol) 叔丁基(四氢-2-氧代-3-呋喃基)氨基甲酸酯、4.81ml(77.2mmol) 碘甲烷溶于 100ml 的干燥的二甲基甲酰胺。该溶液冷却至 0℃,添加 1.34g(60%,在矿物油中,33.5mmol) 氢化钠。反应升温至室温并搅拌过夜。将反应混合物加入到约 400ml 水中,混合物用约 300ml 乙酸乙酯萃取三次。合并的有机相经硫酸钠干燥,在减压下浓缩至干燥。这得到 8.70g(25.7mmol,理论值的 100%,63%纯度)的所需产物。

[0409] 分析数据与文献一致。产物无需进一步纯化就用于下一个合成步骤。

[0410] 实施例 5A

[0411] 2-[(叔丁氧羰基)(甲基)氨基]-4-(1,3-二氧代-1,3-二氢-2H-异吲哚-2-基)丁酸

[0412]



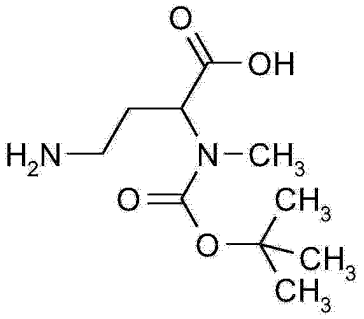
[0413] 将 8.70g(约 25mmol,约 63%纯度)来自实施例 4A 的化合物溶于 560ml 二甲基甲酰胺。添加 8.23g(44.4mmol) 邻苯二甲酰亚胺钾 (potassium ophtalimide),反应混合物加热至 150℃持续 7h。约 400ml 的溶剂通过旋转蒸发(约 60℃,约 10mbar,约 30 分钟)被除去。将反应混合物倾倒入约 100ml 水、200g 冰和 15ml 乙酸的混合物中。剩余的冰融化后,过滤反应混合物,滤液用约 100ml 的二氯甲烷萃取 3 次。合并的有机相经硫酸钠干燥,在减压下浓缩至干燥。将粗产物溶于二氯甲烷中,在约 70ml 硅胶中进行色谱分析。使用的溶剂为 9/1 的二氯甲烷 / 乙酸乙酯至 6/4 的二氯甲烷 / 乙酸乙酯。将含有产物的级分合并、在减压下浓缩至干燥。这得到 2.39g(6.04mmol,理论值的 24%)的产物。

[0414] LC-MS(方法 1) : R_t = 0.92min, m/z = 363 $(M+H)^+$

[0415] 实施例 6A

[0416] 4-氨基-2-[(叔丁氧羰基)(甲基)氨基]丁酸

[0417]



[0418] 将 11.8g(32.6mmol) 来自实施例 5A 的化合物溶于约 640ml 的乙醇中，将 23.8ml(488mmol) 水合肼加入反应混合物中。搅拌过夜后，过滤反应混合物，滤液在减压下浓缩至干燥。将粗产物溶于乙醇中并添加约 50g 的硅胶，溶剂在减压下被除去。将所获得的固体添加到约 500g 硅胶柱上并进行色谱分析。使用的溶剂为 9/1 的二氯甲烷 / 甲醇至 1/1 的二氯甲烷 / 甲醇。将含有产物的级分合并，在减压下浓缩至干燥。这得到 2.98g(12.8mmol，理论值的 39％) 的产物。

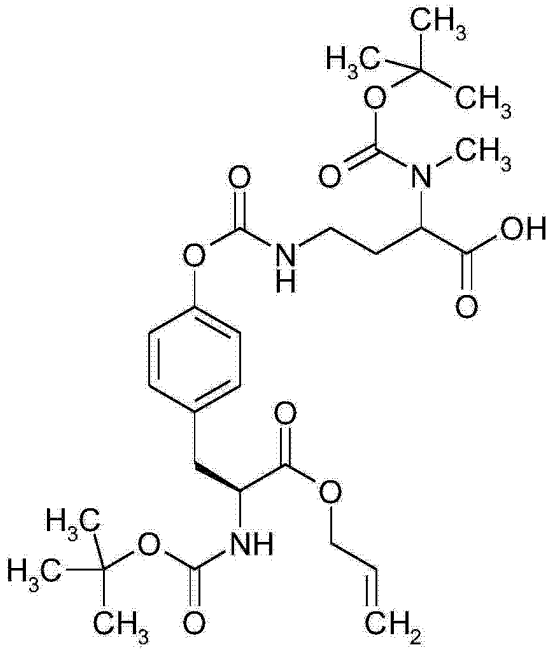
[0419] LC-MS(方法 2)R_t = 0.21min，m/z = 233$(M+H)^+$

[0420] DCI MS(方法 5) :m/z = 233$(M+H)^+$

[0421] 实施例 7A

[0422] 4-{[(4-{(2S)-3-(烯丙基氧基)-2-[(叔丁氧羰基) 氨基]-3- 氧代丙基 } 苯氧基) 羰基]- 氨基 }-2-[(叔丁氧羰基)(甲基) 氨基] 丁酸

[0423]



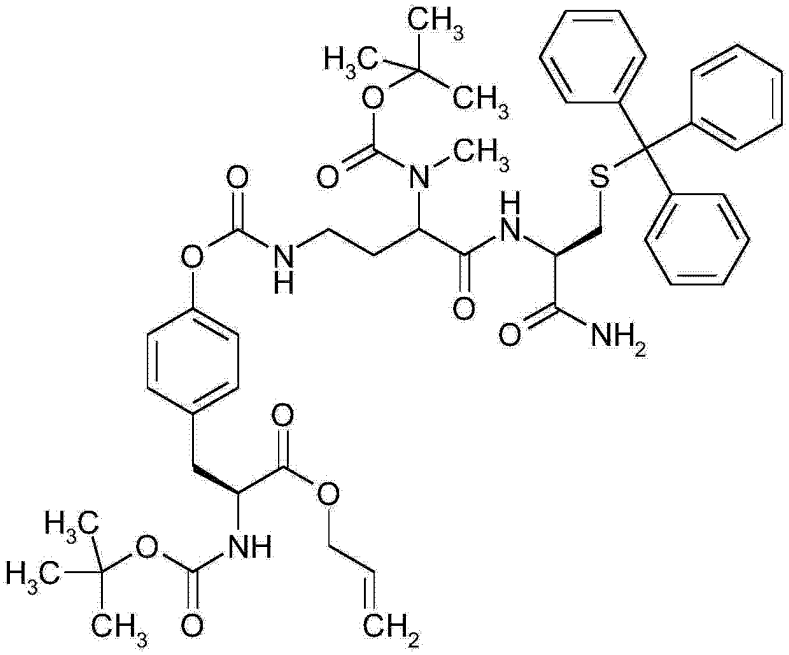
[0424] 将 0.931g(1.92mmol) 来自实施例 1A 的化合物溶于 30ml 二氯甲烷。添加 0.455g(1.92mmol) 来自实施例 6A 的化合物。将反应混合物分成 2 部分。在微波合成器中将这些部分在密封管中在 80℃下加热 30 分钟。在减压下将溶剂从合并的反应混合物中除去。粗产物通过制备型 RP-HPLC 在 C18 柱上用 9/1-1/9 的水甲醇梯度提纯。将含有产物的级分合并，在减压下浓缩至干燥。这得到 0.523g(0.85mmol，理论值的 44％) 的所需产物，产物为 2 种非对映异构体的混合物。

[0425] LC-MS(方法 1):R_t = 1.08 和 1.11min,m/z = 578$(M-H)^-$

[0426] 实施例 8A

[0427] 烯丙基 -O-[(4-{[(2R)-1- 氨基 -1- 氧代 -3-(三苯甲基硫烷基)丙 -2- 基]氨基 }-3-[(叔丁氧羰基)-(甲基)氨基]-4- 氧代丁基)氨基甲酰基]-N-(叔丁氧羰基)-L- 酪氨酸酯

[0428]



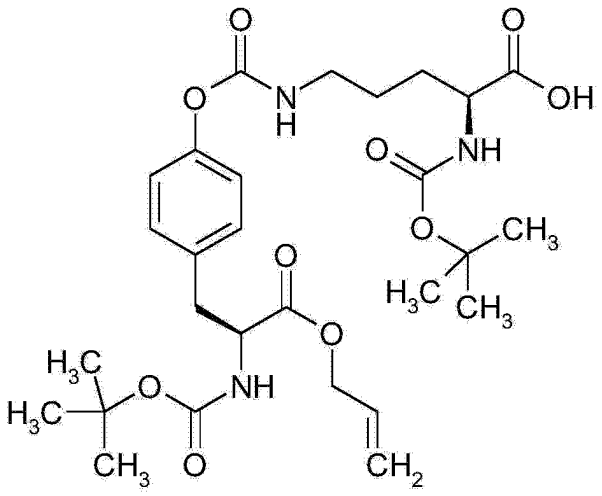
[0429] 将 2.24g(3.86mmol) 的来自实施例 7A 的化合物溶于 100ml 二氯甲烷。添加 1.401g(3.86mmol)S- 三苯甲基 -L- 半胱氨酰胺、0.67ml(3.86mmol)N,N- 二异丙基乙胺和 1.47g(3.86mmol)HATU。将反应混合物分成 5 部分。在微波合成器中将这些部分在密封管中在 60℃下加热 30 分钟。通过旋转蒸发(约 40℃,约 200mbar,约 30 分钟)将溶剂从合并的反应混合物中除去。粗产物通过制备型 RP-HPLC 在 C18 柱上使用 9/1 至 1/9 的水甲醇梯度提纯。将含有产物的级分合并,在减压下浓缩至干燥。这得到 3.26g(2.75mmol,理论值的 71%,78% 纯度)的所需产物,产物为非对映异构体的混合物。

[0430] LC-MS(方法 1):R_t = 1.41 和 1.43min.,m/z = 924$(M+H)^+$

[0431] 实施例 9A

[0432] N^5-[(4-{(2S)-3-(烯丙基氧基)-2-[(叔丁氧羰基)氨基]-3- 氧代丙基 } 苯氧基)羰基]-N^2-(叔丁氧羰基)-L- 鸟氨酸

[0433]



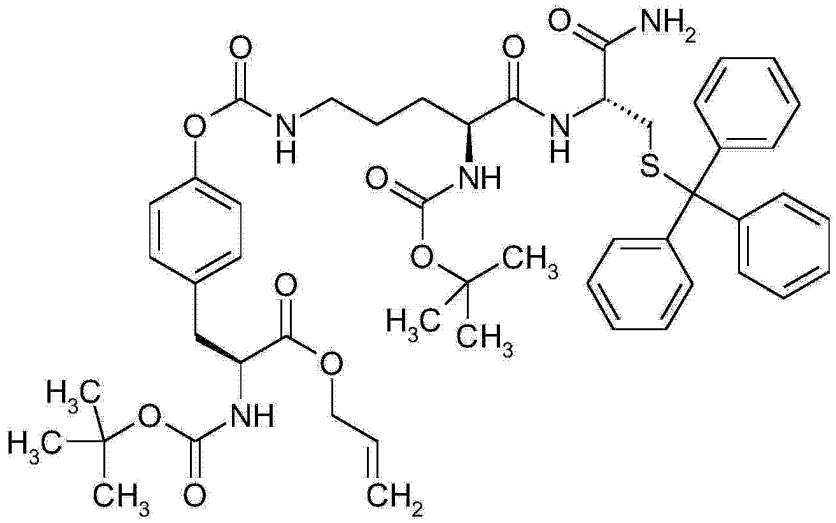
[0434] 将 6.00g(12.33mmol) 来自实施例 1A 的化合物溶于 120ml 二氯甲烷。添加 2.57g(12.33mmol)N^2-(叔丁氧羰基)-L- 鸟氨酸。将反应混合物分成 6 部分。在微波合成器中将这些部分在密封管中在 75℃下加热 90 分钟。合并的反应混合物用约 100ml 饱和氯化铵溶液萃取。水相每次用约 30ml 二氯甲烷各反萃取两次。合并的有机相用约 50ml 盐水萃取,经硫酸钠干燥。溶剂在减压下被除去。将粗产物溶于二氯甲烷,在约 600ml 硅胶中进行色谱分析。使用的溶剂为二氯甲烷,40/1 的二氯甲烷 / 甲醇至 1/1 的二氯甲烷 / 甲醇。将含有产物的级分合并,在减压下浓缩至干燥。这得到 2.63g(4.06mmol,理论值的 33%,89% 纯度) 的所需产物。

[0435] LC-MS(方法 1) :R_t = 1.03min, m/z = 578$(M-H)^-$

[0436] 实施例 10A

[0437] N^5-[(4-{(2S)-3-(烯丙基氧基)-2-[(叔丁氧羰基) 氨基]-3- 氧代丙基 } 苯氧基) 羰基]-N^2-(叔丁氧羰基)-L- 鸟氨酰基 -S- 三苯甲基 -L- 半胱氨酰胺

[0438]



[0439] 将 1.20g(2.07mmol) 来自实施例 9A 的化合物溶于 48ml 二氯甲烷。添加 0.750g(2.07mmol)S- 三苯甲基 -L- 半胱氨酰胺、0.36ml(2.07mmol)N,N- 二异丙基乙胺和 0.787g(2.07mmol)HATU。将反应混合物分成 3 部分。在微波合成器中将这些部分在密封管中在 60℃下加热 30 分钟。通过旋转蒸发 (约 40℃,约 200mbar,约 30 分钟) 将溶剂从合并

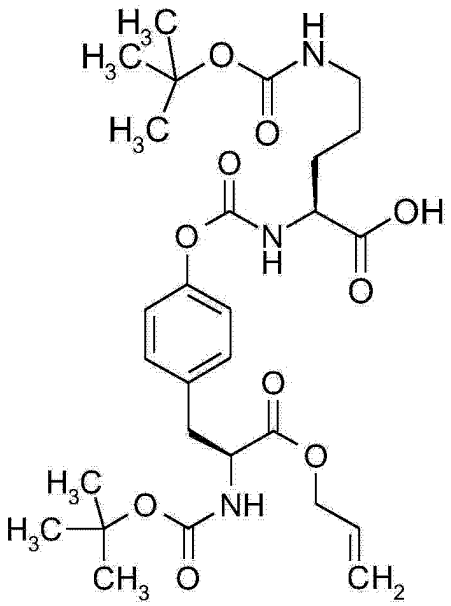
的反应混合物中除去。将粗产物溶于二氯甲烷，在约 400ml 硅胶中进行色谱分析。使用的溶剂为 2/1 的二氯甲烷 / 乙酸乙酯，1/1 的二氯甲烷 / 乙酸乙酯。将含有产物的级分合并，在减压下浓缩至干燥。这得到 1.30g(1.5mmol，理论值的 56％，82％纯度）的所需产物。

[0440] LC-MS（方法 1）：R_t = 1.35min，m/z = 924$(M+H)^+$

[0441] 实施例 11A

[0442] N^2-[(4-{(2S)-3-（烯丙基氧基）-2-[（叔丁氧羰基）氨基]-3- 氧代丙基} 苯氧基）羰基]-N^5-（叔丁氧羰基）鸟氨酸

[0443]



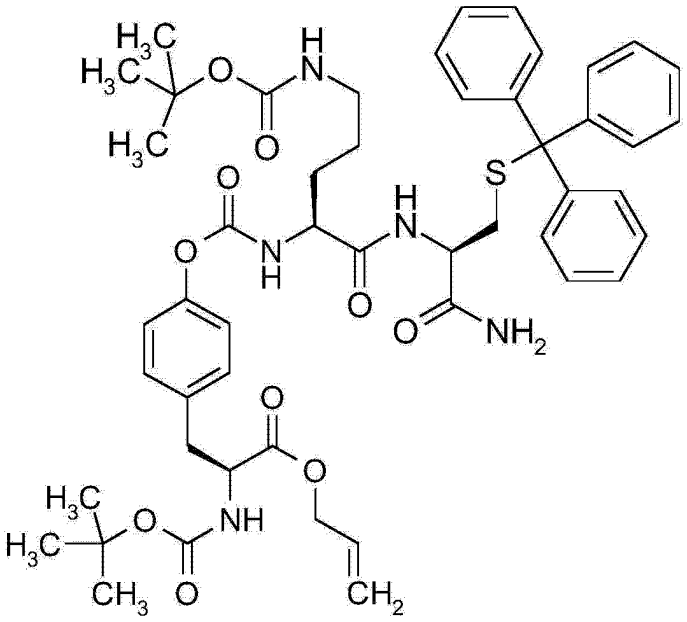
[0444] 将 3.00g(6.16mmol) 来自实施例 1A 的化合物溶于 60ml 二氯甲烷。添加 1.43g(6.16mmol)N^5-（叔丁氧羰基）-L- 鸟氨酸。将反应混合物分成 3 部分。在微波合成器中将这些部分在密封管中在 75℃下加热 30 分钟。合并的反应混合物用约 500ml 饱和氯化铵溶液萃取。水相每次用约 30ml 二氯甲烷各反萃取两次。合并的有机相用约 50ml 盐水萃取，经硫酸钠干燥。溶剂在减压下被除去。将粗产物溶于二氯甲烷，在约 500ml 硅胶中进行色谱分析。使用的溶剂为二氯甲烷，20/1 的二氯甲烷 / 甲醇至 1/1 的二氯甲烷 / 甲醇。将含有产物的级分合并，在减压下浓缩至干燥。这得到 2.29g(3.50mmol，理论值的 57％，89％纯度）的所需产物。

[0445] LC-MS（方法 1）：R_t = 1.07min，m/z = 578$(M-H)^-$

[0446] 实施例 12A

[0447] N^2-[(4-{(2S)-3-（烯丙基氧基）-2-[（叔丁氧羰基）氨基]-3- 氧代丙基} 苯氧基）羰基]-N^5-（叔丁氧羰基）-L- 鸟氨酰基 -S- 三苯甲基 -L- 半胱氨酰胺

[0448]



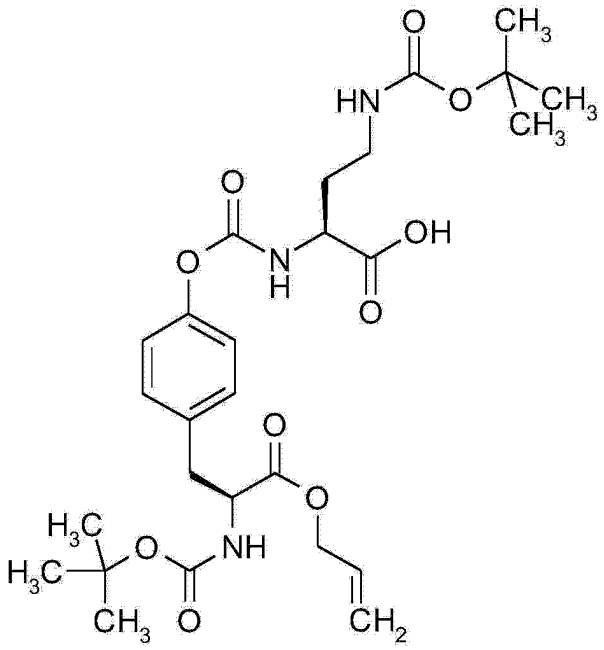
[0449] 将 1.50g(2.59mmol) 来自实施例 11A 的化合物溶于 60ml 二氯甲烷。添加 0.940g(2.59mmol)S- 三苯甲基 -L- 半胱氨酰胺、0.45ml(2.60mmol)N,N- 二异丙基乙胺和 0.984g(2.59mmol)HATU。将反应混合物分成 3 部分。在微波合成器中将这些部分在密封管中在 60℃下加热 30 分钟。通过旋转蒸发（约 40℃，约 200mbar，约 30 分钟）将溶剂从合并的反应混合物中除去。将粗产物溶于二氯甲烷，在约 400ml 硅胶中进行色谱分析。使用的溶剂为 2/1 的二氯甲烷 / 乙酸乙酯，1/1 的二氯甲烷 / 乙酸乙酯。将含有产物的级分合并，在减压下浓缩至干燥。这得到 1.72g(1.64mmol，理论值的 63%，88% 纯度）的所需产物。

[0450] LC-MS（方法 1）：R_t = 1.35min，m/z = 924 $(M+H)^+$

[0451] 实施例 13A

[0452] (2S)-2-{[(4-{(2S)-3-（烯丙基氧基）-2-[（叔丁氧羰基）氨基]-3- 氧代丙基} 苯氧基）羰基] 氨基}-4-[（叔丁氧羰基）氨基] 丁酸

[0453]



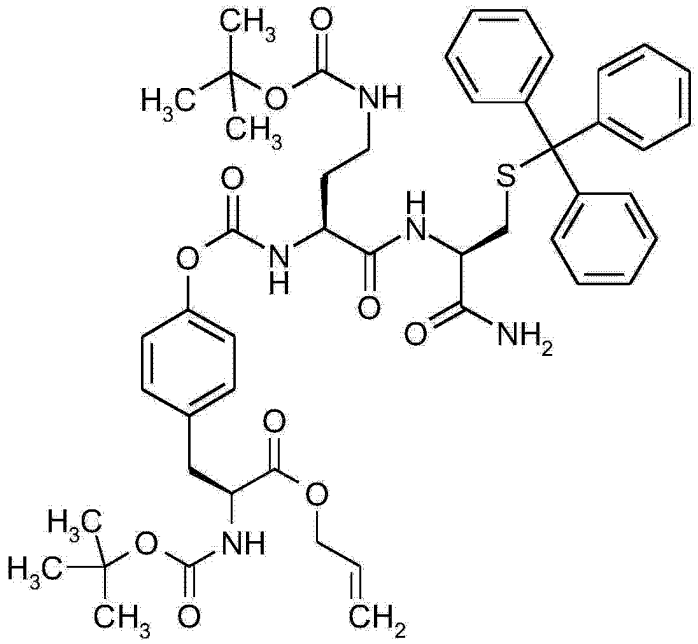
[0454] 将 7.50g(15.4mmol) 来自实施例 1A 的化合物溶于 150ml 二氯甲烷。添加 3.36g(15.4mmol)(2S)-2- 氨基 -4-[(叔丁氧羰基) 氨基] 丁酸。将反应混合物分成 10 部分。在微波合成器中将这些部分在密封管中在 75℃下加热 30 分钟。合并的反应混合物用约 100ml 饱和氯化铵溶液萃取。水相每次用约 50ml 二氯甲烷各反萃取两次。合并的有机相用约 50ml 盐水萃取,经硫酸钠干燥。溶剂在减压下被除去。将粗产物溶于二氯甲烷,在约 1l 硅胶中进行色谱分析。使用的溶剂为 4/1 的二氯甲烷 / 乙酸乙酯,10/1 的二氯甲烷 / 甲醇至 1/1 的二氯甲烷 / 甲醇。将含有产物的级分合并,在减压下浓缩至干燥。这得到 8.70g(10.8mmol,理论值的 70%) 的所需产物。

[0455] LC-MS(方法 1) :R_t = 1.06min, m/z = 564$(M-H)^-$

[0456] 实施例 14A

[0457] 烯丙基 -N-(叔丁氧羰基)-O-{[(4R,7S)-4- 氨基甲酰基 -13,13- 二甲基 -6,11- 二氧代 -1,1,1- 三苯基 -12- 氧杂 -2- 硫杂 -5,10- 二氮杂十四烷 -7- 基] 氨基甲酰基 }-L- 酪氨酸酯

[0458]



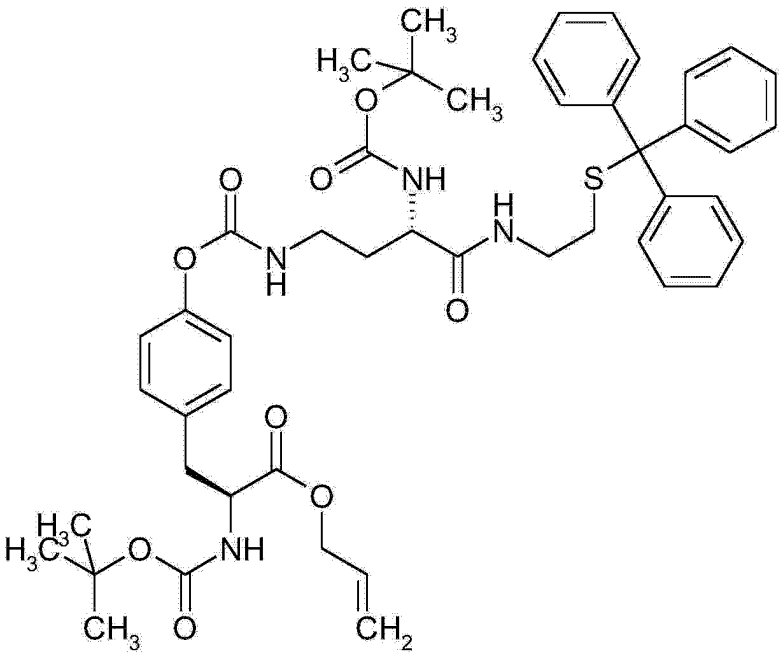
[0459] 将 3.00g(5.30mmol) 来自实施例 13A 的化合物溶于 120ml 二氯甲烷。添加 1.92g(5.30mmol)S- 三苯甲基 -L- 半胱氨酰胺、0.92ml(5.30mmol)N,N- 二异丙基乙胺和 2.02g(5.30mmol)HATU。将反应混合物分成 6 部分。在微波合成器中将这些部分在密封管中在 60℃下加热 30 分钟。通过旋转蒸发 (约 40℃,约 200mbar,约 30 分钟) 将溶剂从合并的反应混合物中除去。将粗产物溶于二氯甲烷,在约 800ml 硅胶中进行色谱分析。使用的溶剂为 2/1 的二氯甲烷 / 乙酸乙酯,1/1 的二氯甲烷 / 乙酸乙酯。将含有产物的级分合并,在减压下浓缩至干燥。这得到 4.91g(3.73mmol,理论值的 70%,69% 纯度) 的所需产物。

[0460] LC-MS(方法 1) :R_t = 1.35min, m/z = 910$(M+H)^+$

[0461] 实施例 15A

[0462] 烯丙基 -N-(叔丁氧羰基)-O-{[(3S)-3-[(叔丁氧羰基) 氨基]-4- 氧代 -4-{[2-(三苯甲基硫烷基) 乙基] 氨基 } 丁基] 氨基甲酰基 }-L- 酪氨酸酯

[0463]



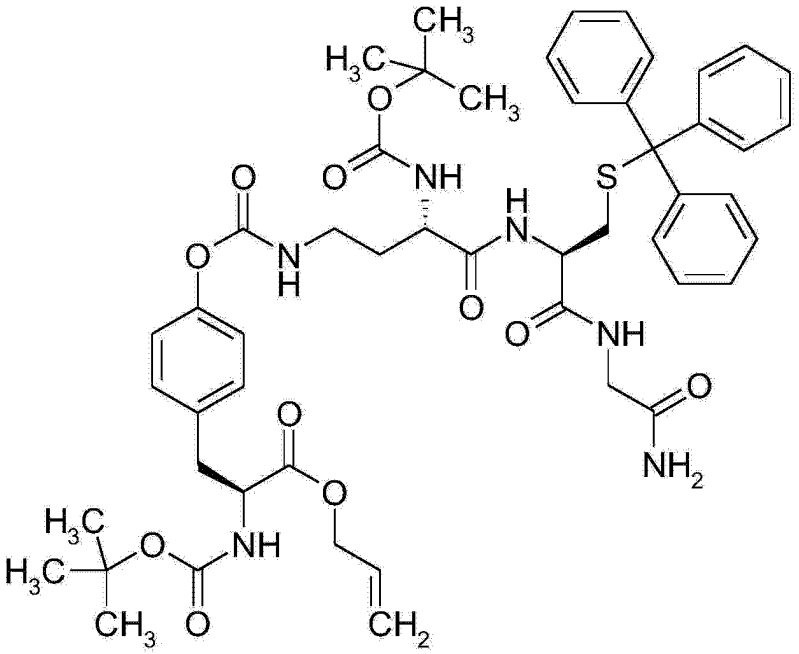
[0464] 将 351mg(0.63mmol) 来自实施例 2A 的化合物溶于 15ml 二氯甲烷。添加 200mg(0.63mmol)2-(三苯甲基硫烷基)乙胺、0.11ml(0.63mmol)N,N-二异丙基乙胺和 238mg(0.63mmol)HATU。在微波合成器中将反应混合物在密封管中在 60℃下加热 30 分钟。在减压下将溶剂从反应混合物中除去。粗产物通过制备型 RP-HPLC 在 C18 柱上使用 9/1 至 1/9 的水甲醇梯度提纯。将含有产物的级分合并,在减压下浓缩至干燥。这得到 98mg(0.110mmol,理论值的 16%)的所需产物。

[0465] LC-MS(方法 1):R_t = 1.45min,m/z = 867$(M+H)^+$

[0466] 实施例 16A

[0467] N-{(2S)-4-{[(4-{(2S)-3-(烯丙基氧基)-2-[(叔丁氧羰基)氨基]-3-氧代丙基}苯氧基)羰基]氨基}-2-[(叔丁氧羰基)氨基]丁酰基}-S-三苯甲基-L-半胱氨酰基甘氨酰胺

[0468]



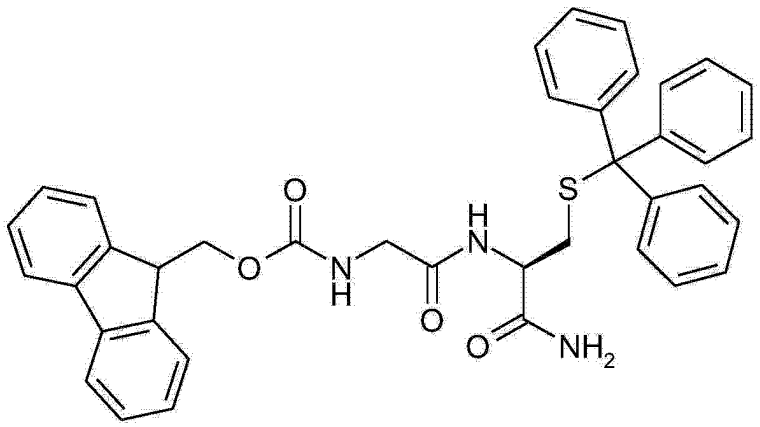
[0469] 将 173mg(0.31mmol) 来自实施例 2A 的化合物溶于 10ml 二氯甲烷。添加 128mg(0.31mmol)S- 三苯甲基 -L- 半胱氨酰基甘氨酰胺、53μl(0.31mmol)N,N- 二异丙基乙胺和 116mg(0.31mmol)HATU。在微波合成器中将反应混合物在密封管中在 60℃下加热 30 分钟。在减压下将溶剂从反应混合物中除去。粗产物通过制备型 RP-HPLC 在 C18 柱上使用 9/1 至 1/9 的水甲醇梯度提纯。将含有产物的级分合并,在减压下浓缩至干燥。这得到 57mg(0.02mmol,理论值的 18%) 的所需产物。

[0470] LC-MS(方法 1) :R_t = 1.31min.,m/z = 968$(M+H)^+$

[0471] 实施例 17A

[0472] N-[(9H- 芴 -9- 基甲氧基) 羰基] 甘氨酰基 -S- 三苯甲基 -L- 半胱氨酰胺

[0473]



[0474] 将 1.00g(3.36mmol)N-[(9H- 芴 -9- 基甲氧基) 羰基] 甘氨酸溶于 30ml 二氯甲烷。添加 1.41g(3.36mmol)S- 三苯甲基 -L- 半胱氨酰基甘氨酰胺、0.59ml(3.36mmol)N,N- 二异丙基乙胺和 1.28g(3.36mmol)HATU。在微波合成器中将反应混合物在密封管中在 60℃下加热 30 分钟。在减压下将溶剂从反应混合物中除去。将粗产物溶于二氯甲烷,在约 300ml 硅胶中进行色谱分析。使用的溶剂为二氯甲烷、20/1 的二氯甲烷 / 甲醇、10/1 的二氯甲烷 / 甲醇。将含有产物的级分合并,在减压下浓缩至干燥。这得到 1.63g(2.06mmol,理论值的

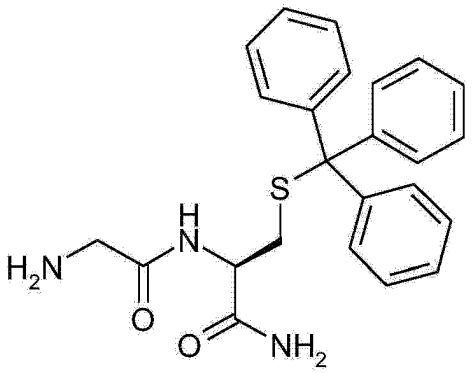
81％）的所需产物。

[0475] LC-MS（方法 1）：R_t = 1.31min.，m/z = 642$(M+H)^+$

[0476] 实施例 18A

[0477] 甘氨酰基 -S- 三苯甲基 -L- 半胱氨酰胺

[0478]



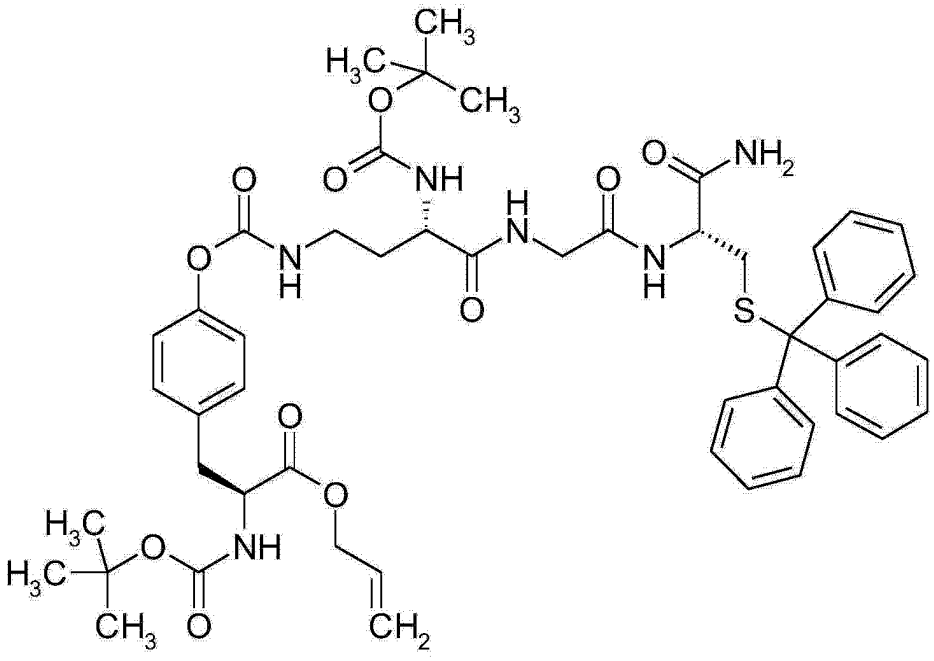
[0479] 将 1.53g(2.38mmol) 来自实施例 17A 的化合物溶于 18ml 二甲基甲酰胺，添加 0.47ml(4.79mmol) 的 DIEA。1 小时的反应时间后，粗产物通过制备型 RP-HPLC 在 C18 柱上使用 9/1 至 1/9 的水甲醇梯度提纯。将含有产物的级分合并，在减压下浓缩至干燥。这得到 416mg(0.97mmol，理论值的 40％）的所需产物。

[0480] LC-MS（方法 1）：R_t = 0.76min，m/z = 418$(M-H)^-$

[0481] 实施例 19A

[0482] N-{(2S)-4-{[(4-{(2S)-3-（烯丙基氧基）-2-[（叔丁氧羰基）氨基]-3- 氧代丙基}苯氧基）羰基]氨基}-2-[（叔丁氧羰基）氨基]丁酰基}甘氨酰基 -S- 三苯甲基 -L- 半胱氨酰胺

[0483]



[0484] 将 559mg(0.99mmol) 来自实施例 2A 的化合物溶于 15ml 二氯甲烷。添加 415mg(0.99mmol) 来自实施例 18A 的化合物、173μl(0.99mmol)N,N- 二异丙基乙胺和

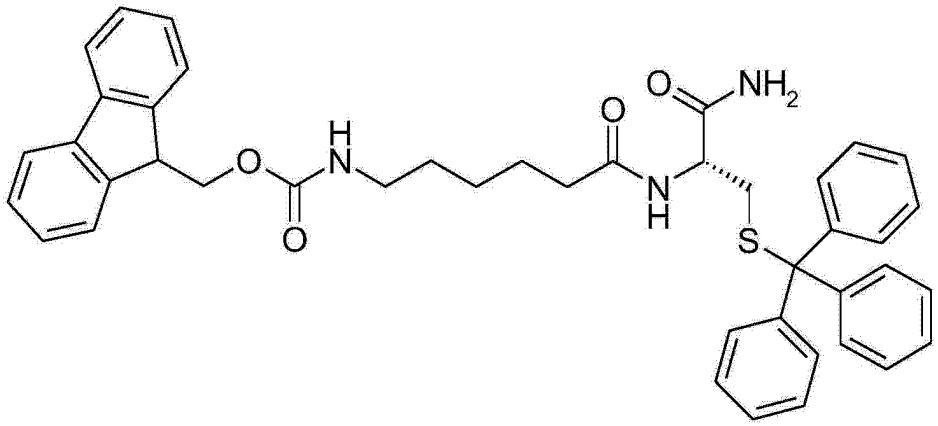
376mg(0.99mmol)HATU。在微波合成器中将反应混合物在密封管中在60℃下加热30分钟。在减压下将溶剂从反应混合物中除去。将粗产物溶于二氯甲烷，在约70ml硅胶中进行色谱分析。使用的溶剂为二氯甲烷、20/1的二氯甲烷/甲醇至5/1的二氯甲烷/甲醇。将含有产物的级分合并，在减压下浓缩至干燥。这得到860mg(0.69mmol，理论值的70％)的所需产物。

[0485] LC-MS(方法1)：R_t = 1.30min，m/z = 968$(M+H)^+$

[0486] 实施例20A

[0487] 9H-芴-9-基甲基-(6-{[(2R)-1-氨基-1-氧代-3-(三苯甲基硫烷基)丙-2-基]氨基}-6-氧代己基)氨基甲酸酯

[0488]



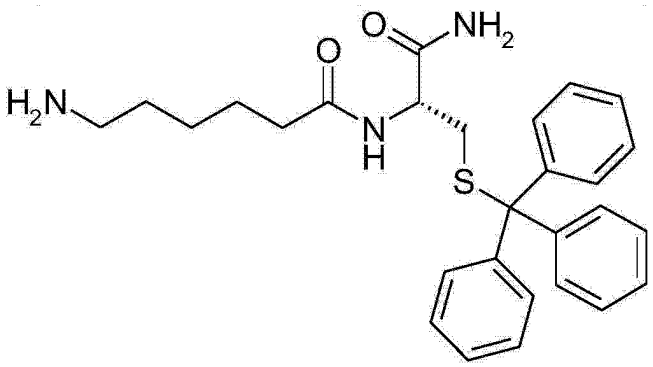
[0489] 将500mg(1.42mmol)6-{[(9H-芴-9-基甲氧基)羰基]氨基}己酸溶于18ml二氯甲烷。添加513mg(1.42mmol)S-三苯甲基-L-半胱氨酰基甘氨酰胺、246μl(1.42mmol)N,N-二异丙基乙胺和537mg(1.42mmol)HATU。在微波合成器中将反应混合物在密封管中在60℃下加热30分钟。在减压下将溶剂从反应混合物中除去。粗产物通过制备型RP-HPLC在C18柱上使用9/1至1/9的水甲醇梯度提纯。将含有产物的级分合并，在减压下浓缩至干燥。这得到678mg(0.70mmol，理论值的49％)的所需产物。

[0490] LC-MS(方法1)：R_t = 1.38min.，m/z = 698$(M+H)^+$

[0491] 实施例21A

[0492] 6-氨基-N-[(2R)-1-氨基-1-氧代-3-(三苯甲基硫烷基)丙-2-基]己酰胺

[0493]



[0494] 将678mg(0.97mmol)来自实施例20A的化合物溶于7ml二甲基甲酰胺，添加0.19ml(1.94mmol)的DIEA。1小时的反应时间后，粗产物通过制备型RP-HPLC在C18柱上

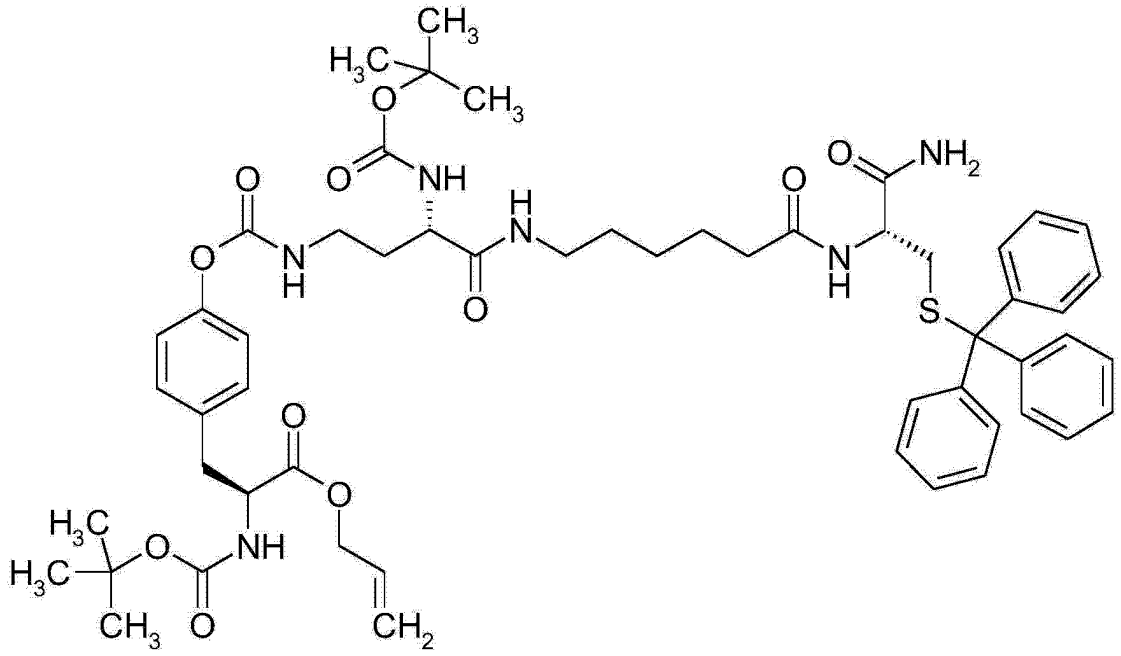
使用 9/1 至 1/9 的水甲醇梯度提纯。将含有产物的级分合并，在减压下浓缩至干燥。这得到 457mg(0.93mmol，理论值的 95％）的所需产物。

[0495] LC-MS（方法 1）：R_t = 0.85min.，m/z = 476 $(M+H)^+$

[0496] 实施例 22A

[0497] 烯丙基 -O-({(3S)-4-[(6-{[(2R)-1- 氨基 -1- 氧代 -3-（三苯甲基硫烷基）丙 -2- 基］氨基 }-6- 氧代己基）氨基]-3-[（叔丁氧羰基）氨基]-4- 氧代丁基 } 氨基甲酰基）-N-（叔丁氧羰基）-L- 酪氨酸酯

[0498]



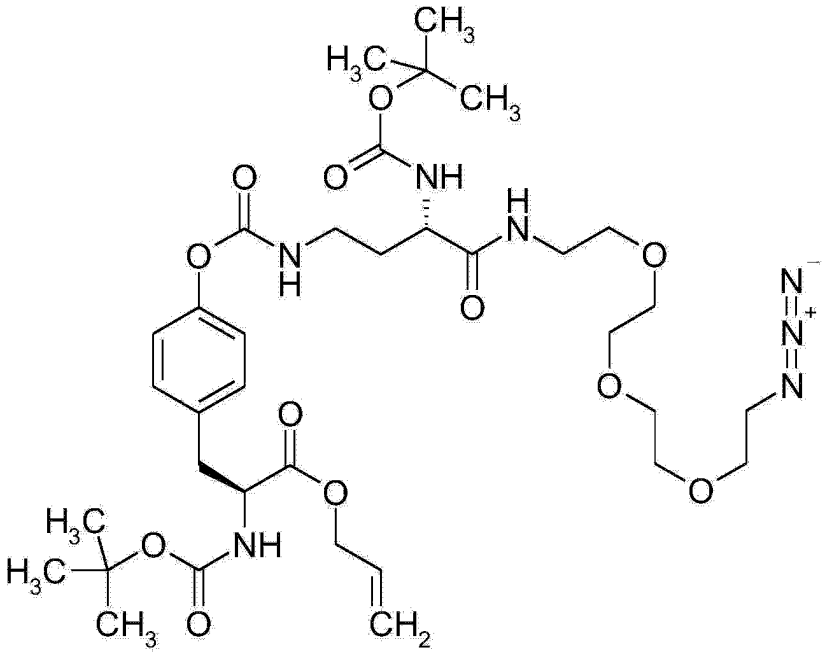
[0499] 将 457mg(0.81mmol) 来自实施例 2A 的化合物溶于 15ml 二氯甲烷。添加 384mg(0.81mmol) 来自实施例 21A 的化合物、141μl(0.81mmol)N,N- 二异丙基乙胺和 307mg(0.81mmol)HATU。在微波合成器中将反应混合物在密封管中在 60℃下加热 30 分钟。在减压下将溶剂从反应混合物中除去。粗产物通过制备型 RP-HPLC 在 C18 柱上使用 9/1 至 1/9 的水甲醇梯度提纯为两部分。将含有产物的级分合并，在减压下浓缩至干燥。这得到 255mg(0.22mmol，理论值的 28％）的所需产物。

[0500] LC-MS（方法 1）：R_t = 1.31min，m/z = 1032 $(M+H)^+$

[0501] 实施例 23A

[0502] 烯丙基 -O-({(14S)-1- 叠氮基 -14-[（叔丁氧羰基）氨基]-13- 氧代 -3,6,9- 三氧杂 -12- 氮杂十六烷 -16- 基 } 氨基甲酰基）-N-（叔丁氧羰基）-L- 酪氨酸酯

[0503]



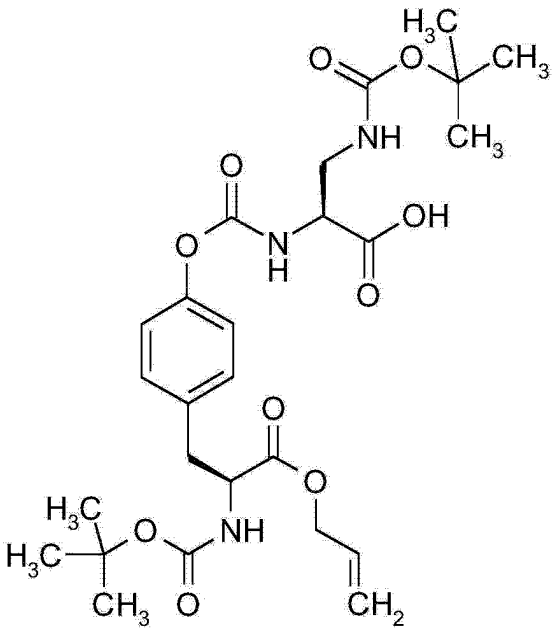
[0504] 将 518mg(0.92mmol) 来自实施例 2A 的化合物溶于 15ml 二氯甲烷。添加 200mg(0.92mmol)2-{2-[2-(2- 叠氮基乙氧基) 乙氧基] 乙氧基 } 乙胺、160μl(0.92mmol) N,N- 二异丙基乙胺和 348mg(0.92mmol)HATU。在微波合成器中将反应混合物在密封管中在 60℃下加热 30 分钟。在减压下将溶剂从反应混合物中除去。粗产物通过制备型 RP-HPLC 在 C18 柱上使用 9/1 至 1/9 的水甲醇梯度提纯。将含有产物的级分合并,在减压下浓缩至干燥。这得到 276mg(0.34mmol,理论值的 37%) 的所需产物。

[0505] LC-MS(方法 1) :R_t = 1.15min, m/z = 766 $(M+H)^+$

[0506] 实施例 24A

[0507] N-[(4-{(2S)-3-(烯丙基氧基)-2-[(叔丁氧羰基) 氨基]-3- 氧代丙基 } 苯氧基) 羰基]-3-[(叔丁氧羰基) 氨基]-L- 丙氨酸

[0508]



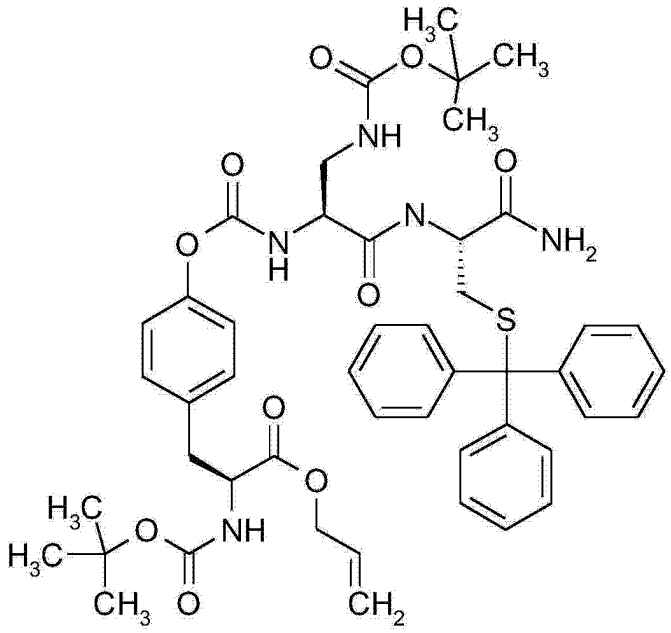
[0509] 将 2.45g(5.0mmol) 来自实施例 1A 的化合物溶于 40ml 二氯乙烷。添加 1.03g(5.0mmol)3-[(叔丁氧羰基)氨基]-L-丙氨酸。将反应混合物加热至 85℃,持续 2 小时。在减压下将溶剂除去。将粗产物溶于二氯甲烷,在约 150ml 硅胶中进行色谱分析。使用的溶剂为 20/1 的二氯甲烷 / 甲醇至 1/1 的二氯甲烷 / 甲醇。将含有产物的级分合并,在减压下浓缩至干燥。这得到 1.23g(2.2mmol,理论值的 44%) 的所需产物。

[0510] LC-MS(方法 1) :R_t = 1.06min., m/z = 550 $(M-H)^-$

[0511] 实施例 25A

[0512] N-[(4-{(2S)-3-(烯丙基氧基)-2-[(叔丁氧羰基)氨基]-3-氧代丙基}苯氧基)羰基]-3-[(叔丁氧羰基)氨基]-L-丙氨酰基-S-三苯甲基-L-半胱氨酰胺

[0513]



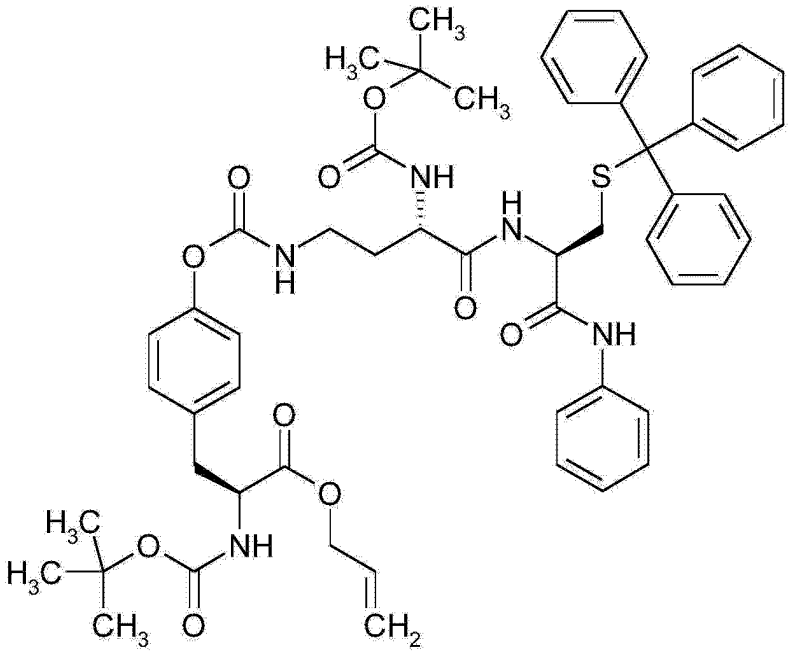
[0514] 将 1.23g(2.23mmol) 来自实施例 24A 的化合物溶于 25ml 二氯甲烷。添加 0.81g(2.23mmol)S-三苯甲基-L-半胱氨酰胺、0.39ml(2.23mmol)N,N-二异丙基乙胺和 0.85g(2.23mmol)HATU。反应混合物在室温下搅拌 3h。溶剂通过旋转蒸发(约 40℃,约 200mbar,约 30 分钟)从反应混合物中被除去。将粗产物溶于二氯甲烷,在约 70ml 硅胶中进行色谱分析。使用的溶剂为 20/1 的二氯甲烷 / 甲醇至 5/1 的二氯甲烷 / 甲醇。将含有产物的级分合并,在减压下浓缩至干燥。这得到 2.38g(2.03mmol,理论值的 91%,76%纯度)的所需产物。

[0515] LC-MS(方法 1) :R_t = 1.37min., m/z = 897 $(M+H)^+$

[0516] 实施例 26A

[0517] 烯丙基-O-({(3S)-4-{[(2R)-1-苯胺基-1-氧代-3-(三苯甲基硫烷基)丙-2-基]氨基}-3-[(叔丁氧羰基)氨基]-4-氧代丁基}氨基甲酰基)-N-(叔丁氧羰基)-L-酪氨酸酯

[0518]



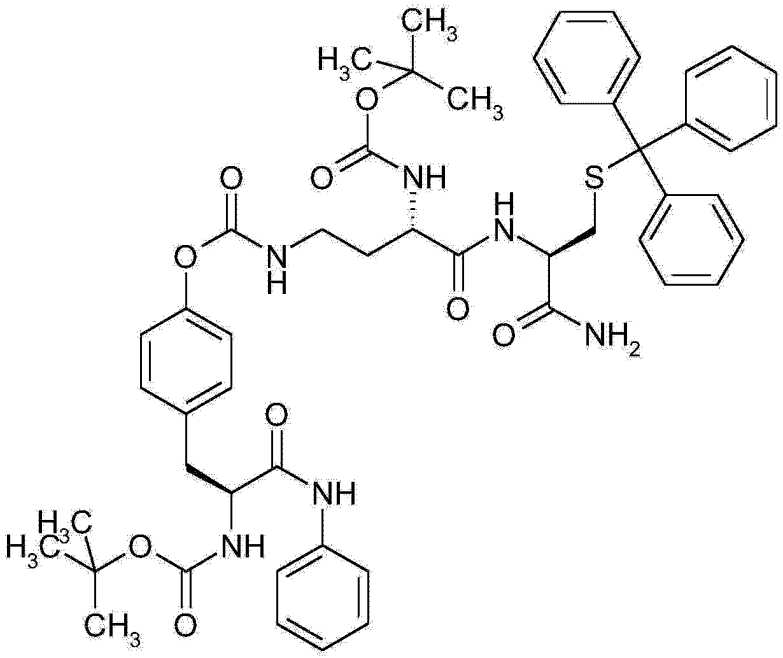
[0519] 将 456mg(0.68mmol) 来自实施例 2A 的化合物溶于 8ml 二氯甲烷。添加 300mg(0.68mmol)N- 苯基 -S- 三苯甲基 -L- 半胱氨酰胺、0.12ml(0.68mmol)N,N- 二异丙基乙胺和 260mg(0.68mmol)HATU。反应混合物在室温下搅拌 4h。在减压下将溶剂从反应混合物中除去。粗产物通过制备型 RP-HPLC 在 C18 柱上使用 9/1 至 1/9 的水甲醇梯度提纯为两部分。将含有产物的级分合并,在减压下浓缩至干燥。这得到 361mg(0.37mmol,理论值的 53%) 的所需产物。

[0520] LC-MS(方法 1) :R_t = 1.48min,m/z = 987$(M+H)^+$

[0521] 实施例 1B

[0522] 叔丁基 -[(2S)-1-{[(2R)-1- 氨基 -1- 氧代 -3-(三苯甲基硫烷基) 丙 -2- 基] 氨基 }-4-{[(4-{(2S)-3- 苯胺基 -2-[(叔丁氧羰基) 氨基]-3- 氧代丙基 } 苯氧基) 羰基] 氨基 }-1- 氧代丁 -2- 基] 氨基甲酸酯

[0523]

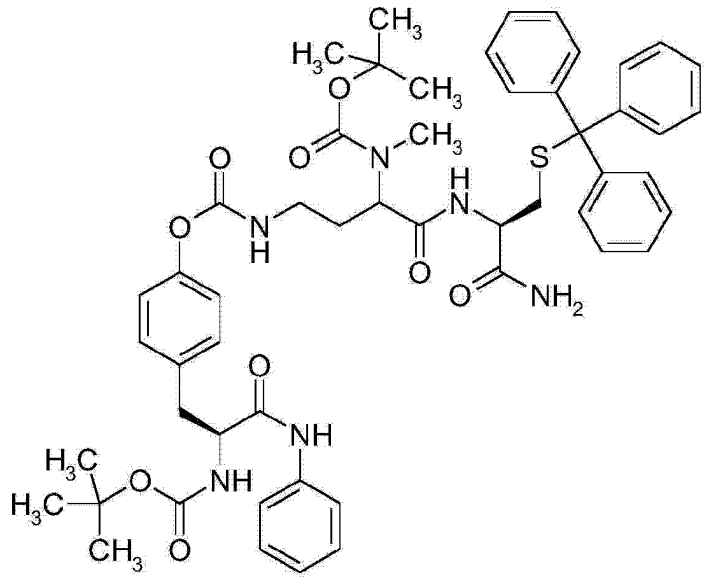
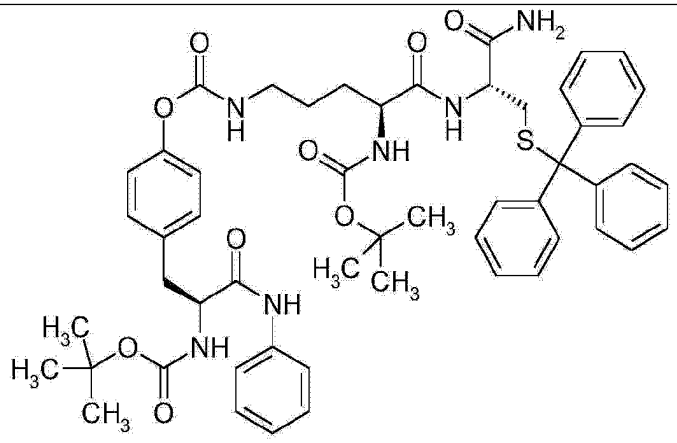
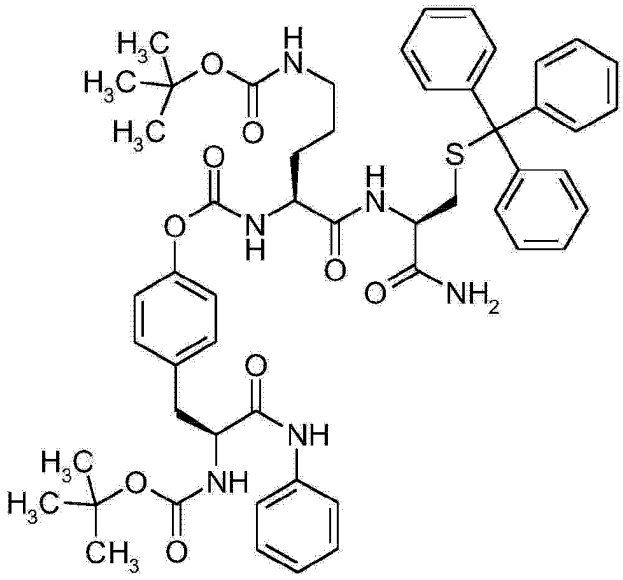


[0524] 将 250mg(0.29mmol) 实施例 1 的化合物溶于 10ml 二氯甲烷。添加 40mg(0.43mmol) 苯胺、164mg(0.43mmol)HATU 和 75μl(0.43mmol)DIEA。在微波合成器中将反应混合物在密封管中在 60℃下加热 30 分钟。将粗产物在减压下浓缩至干燥。将粗产物溶于甲醇,通过制备型 RP-HPLC 在 C18 柱上使用水 / 甲醇梯度提纯,生成 271mg 产物 (理论值的 88%)。

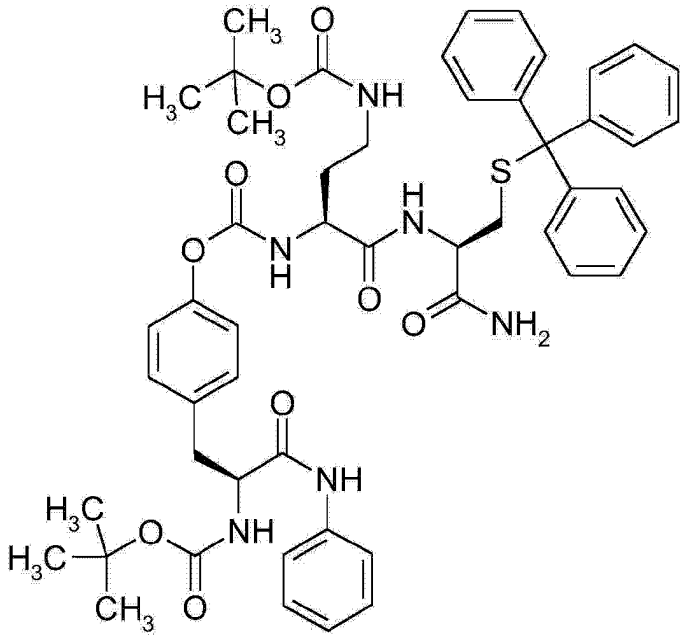
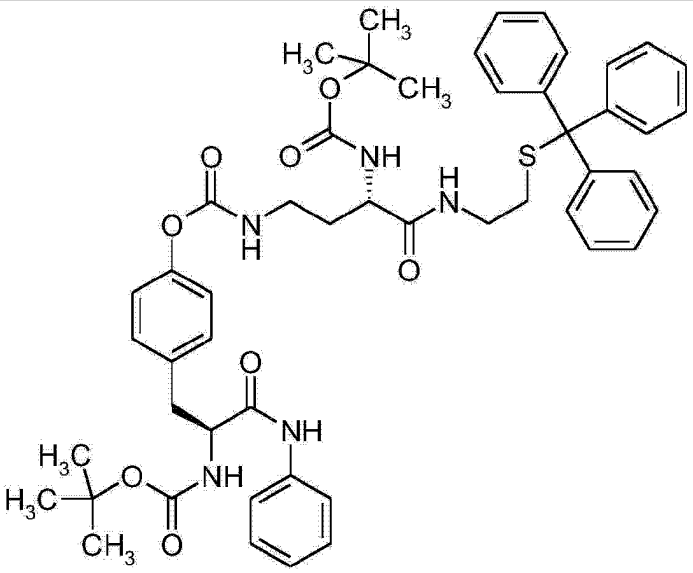
[0525] LC-MS(方法 1) :R_t = 1.31min,m/z = 945$(M+H)^+$

[0526] 使用适当的羧酸 (工作实施例 2-12) 以与实施例 1B 类似的方法来制备下表的实施例。

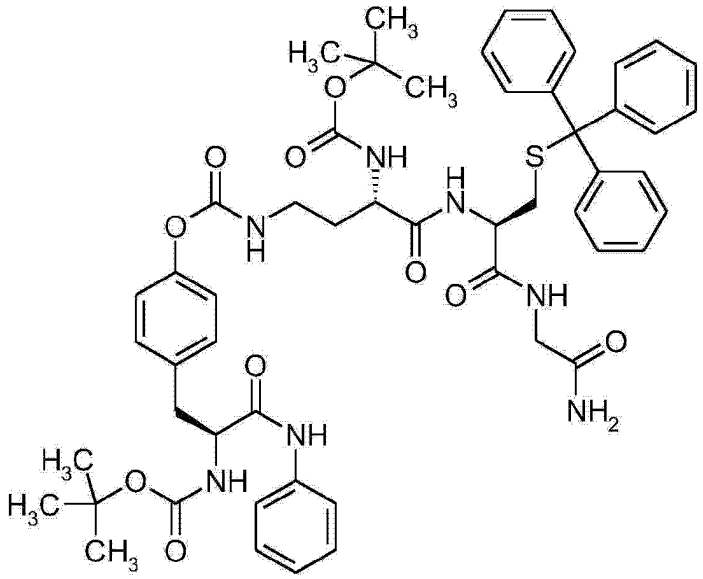
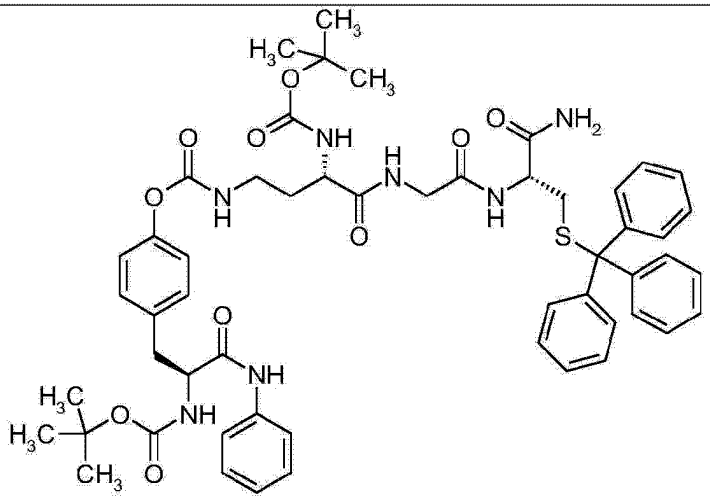
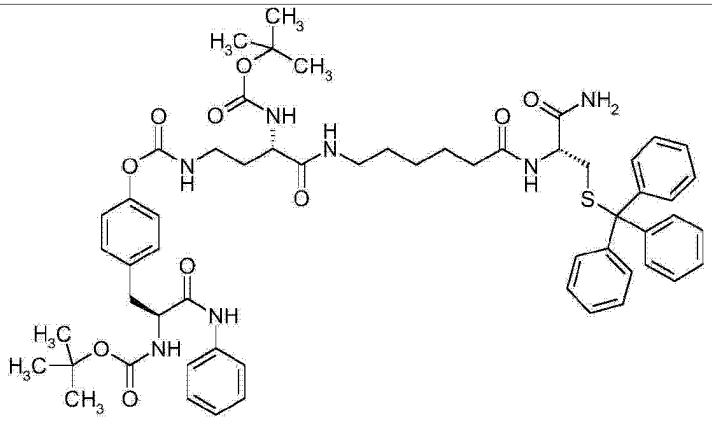
[0527]

实施例	结构	表征
2B		LC-MS（方法 1）：R_t = 1.34 和 1.37 min，m/z = 959 $(M+H)^+$
3B		LC-MS（方法 1）：R_t = 1.32 min.，m/z = 959 $(M+H)^+$
4B		LC-MS（方法 1）：R_t = 1.32 min.，m/z = 959 $(M+H)^+$

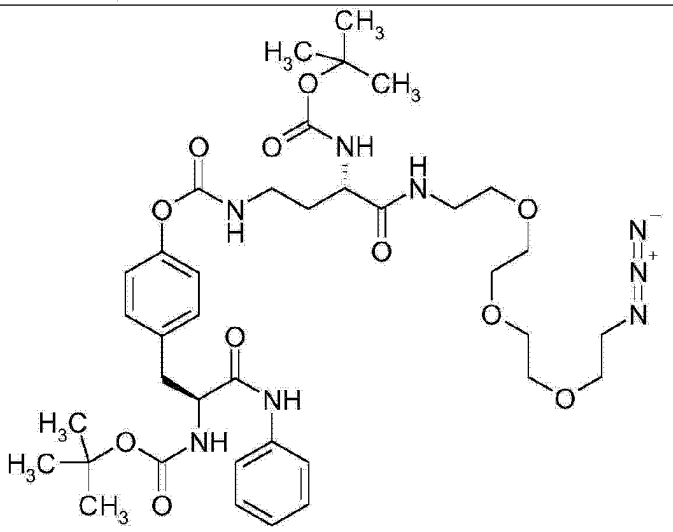
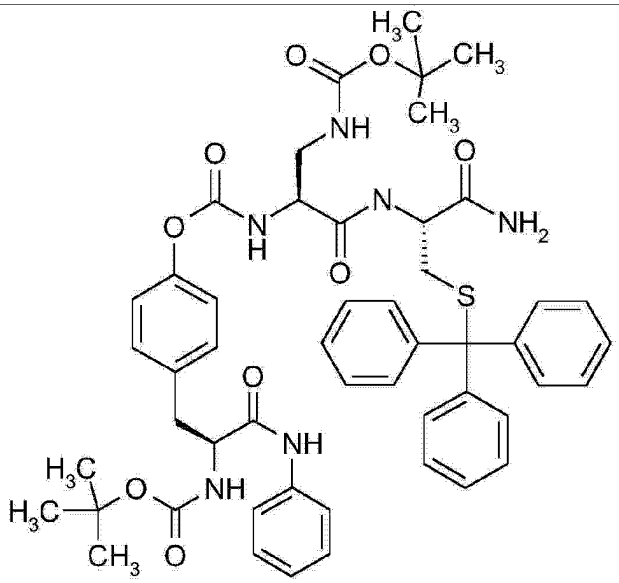
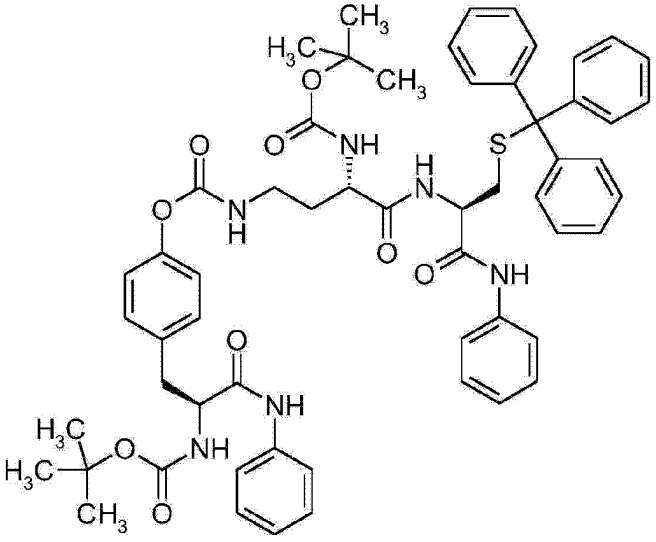
[0528]

实施例	结构	表征
5B		LC-MS（方法 1）: R_t = 1.30 min, m/z = 945 $(M+H)^+$
6B		LC-MS（方法 1）: R_t = 1.43 min, m/z = 903 $(M+H)^+$

[0529]

实施例	结构	表征
7B		LC-MS（方法 1）：R_t = 1.29 min, m/z=1002 $(M+H)^+$
8B		LC-MS（方法 1）：R_t = 1.27 min, m/z=1002 $(M+H)^+$
9B		LC-MS（方法 1）：R_t = 1.29 min, m/z=1058 $(M+H)^+$

[0530]

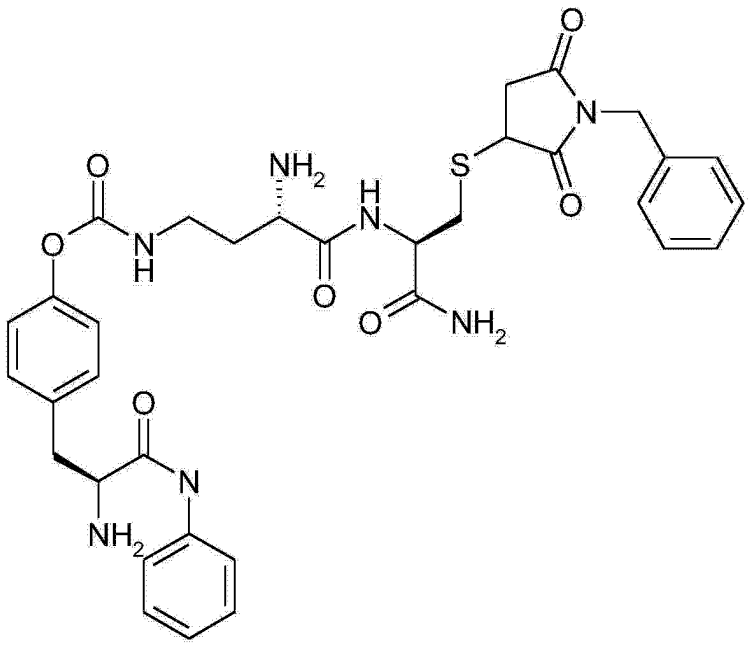
实施例	结构	表征
10B		LC-MS（方法 2）：R_t = 2.42 min, m/z = 801 $(M+H)^+$
11B		LC-MS（方法 1）：R_t = 1.36 min, m/z = 931 $(M+H)^+$
12B		LC-MS（方法 1）：R_t = 1.48 min, m/ =1022 $(M+H)^+$

[0531] 实施例 1C

[0532] O-{[(3S)-3- 氨 基 -4-({(2R)-1- 氨 基 -3-[(1- 苯 甲 基 -2,5- 二 氧 代 吡 咯

烷-3-基)硫烷基]-1-氧代丙-2-基}氨基)-4-氧代丁基]氨基甲酰基}-N-苯基-L-酪氨酰胺

[0533]

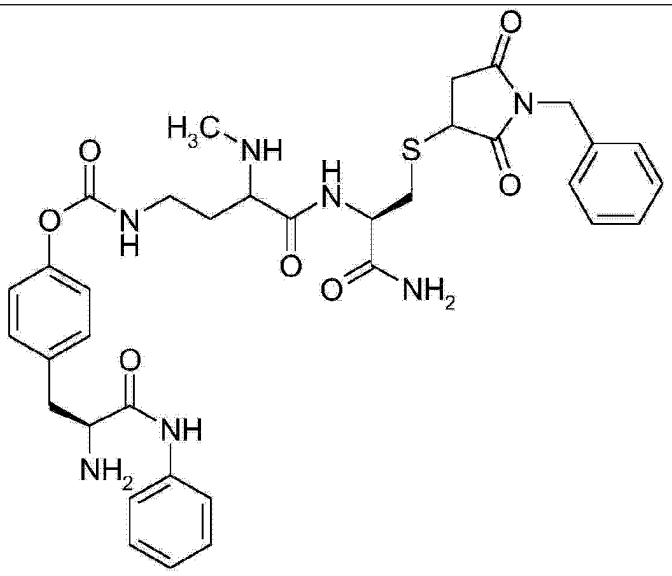
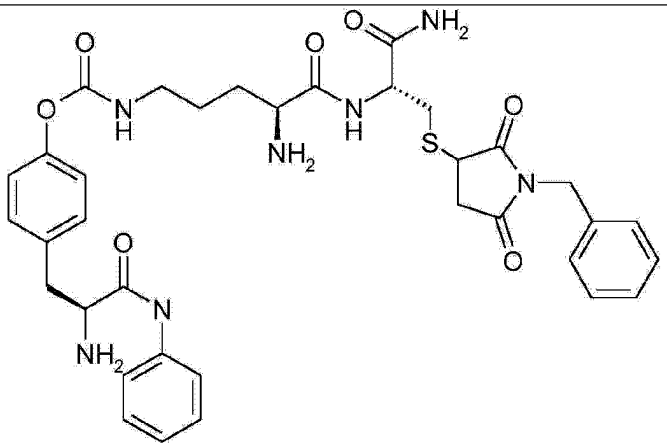
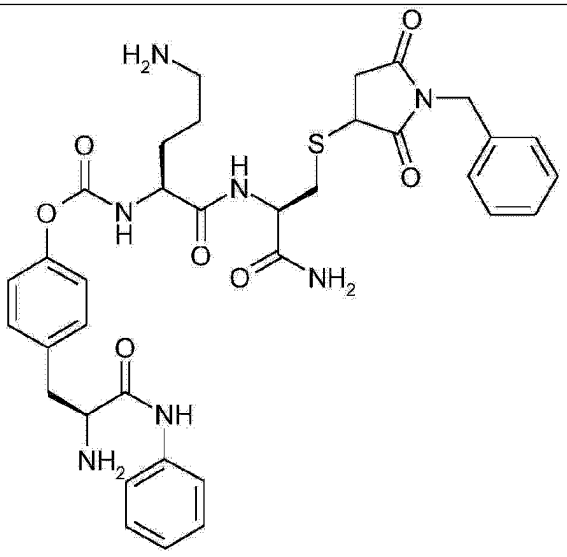


[0534] 将238mg(0.25mmol)的实施例1B的化合物溶于10ml二氯乙烷。添加0.12ml三乙基硅烷、约10ml三氟乙酸和约0.5ml水。添加0.5ml水。反应混合物在室温下搅拌约30min。添加100ml二氯乙烷,在减压下将反应混合物蒸发至约1ml的溶剂体积。添加约100ml水,反应混合物用约50ml二氯甲烷萃取三次。将15ml的乙酸加入到水相中。冷冻水相并冻干。将冻干物(lyophylistae)溶于约50ml甲醇,添加0.183mg(0.98mmol)N-苯甲基马来酰亚胺。反应混合物在室温下搅拌过夜。将反应混合物蒸干,重新溶于约5ml甲醇,通过制备型RP-HPLC在C18上用水/甲醇梯度提纯。在自动级分收集器上将这些级分收集在20ml的试管中。为了确保足够的酸性,在收集之前每个小瓶用0.5ml乙酸充满。合并所有的含有实施例1C的化合物的级分。在旋转蒸发器上在30℃的水浴温度和约50mbar下部分地除去乙腈,持续约30min。添加0.5ml乙酸后,冻干剩余溶液。总产率为168mg(0.24mmol,理论值的98%)的所需产物。

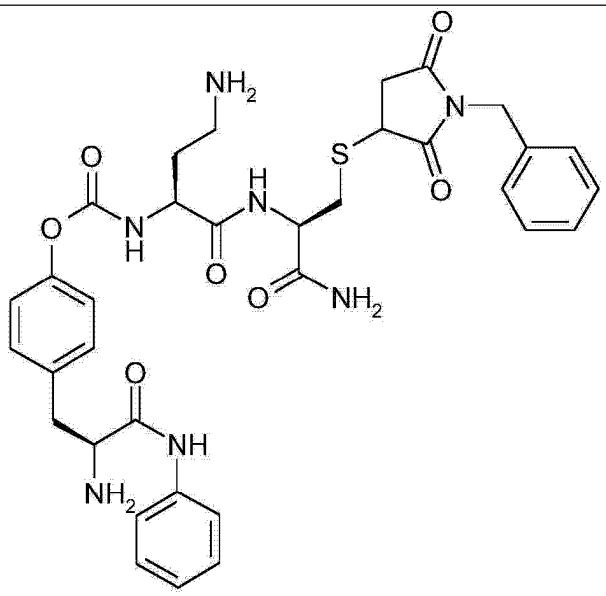
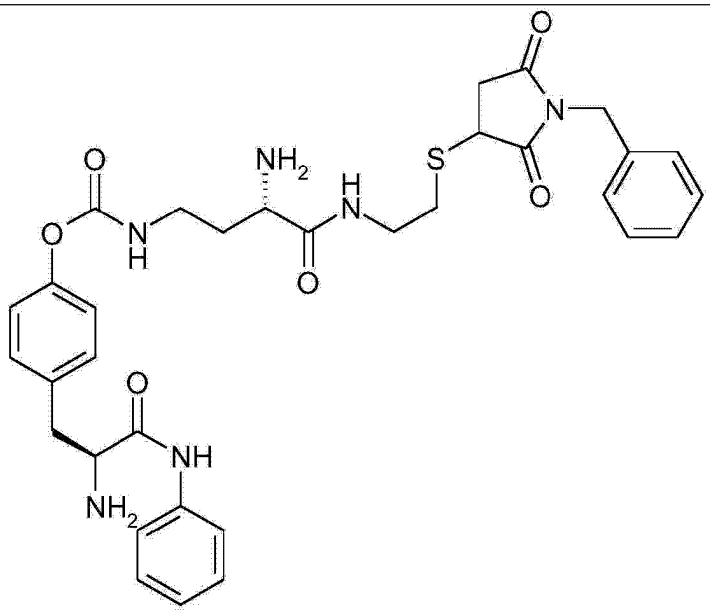
[0535] LC-MS(方法1):R_t = 0.55min,m/z = 690$(M+H)^+$

[0536] 使用适当的前体(实施例2B-9B)以与实施例1C类似的方法来制备下表的实施例。

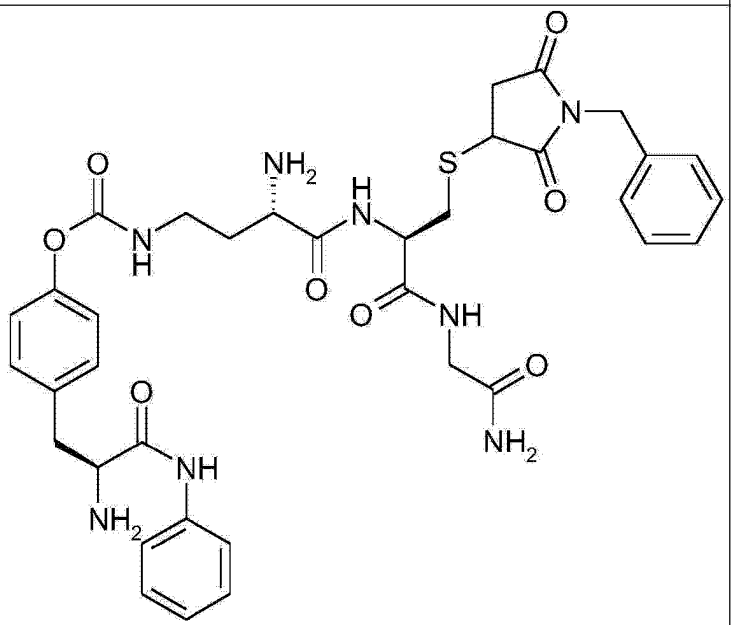
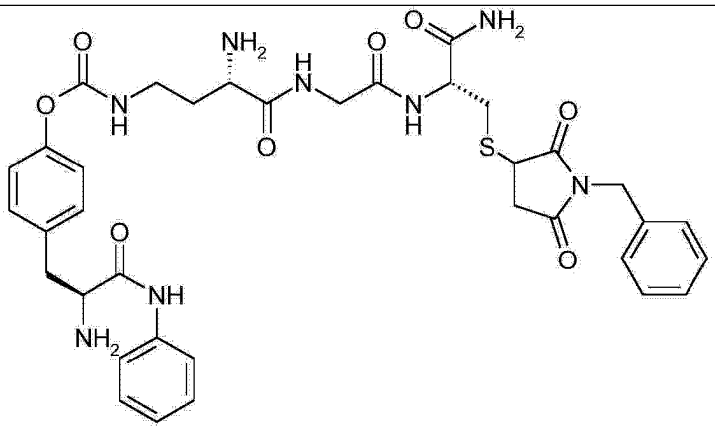
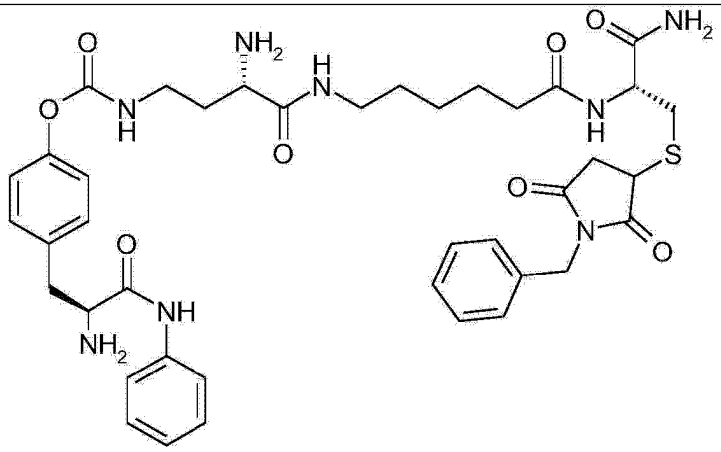
[0537]

实施例	结构	表征
2C		LC-MS(方法1): R_t=0.64 min., m/z=704 $(M+H)^+$
3C		LC-MS(方法1): R_t = 0.63 min., m/z=704 $(M+H)^+$
4C		LC-MS(方法1): R_t = 0.61 min., m/z=704 $(M+H)^+$

[0538]

实施例	结构	表征
5C		LC-MS(方法1): R_t = 0.55 min., m/z=690 $(M+H)^+$
6C		LC-MS(方法1): R_t = 0.67 min., m/z=647 $(M+H)^+$

[0539]

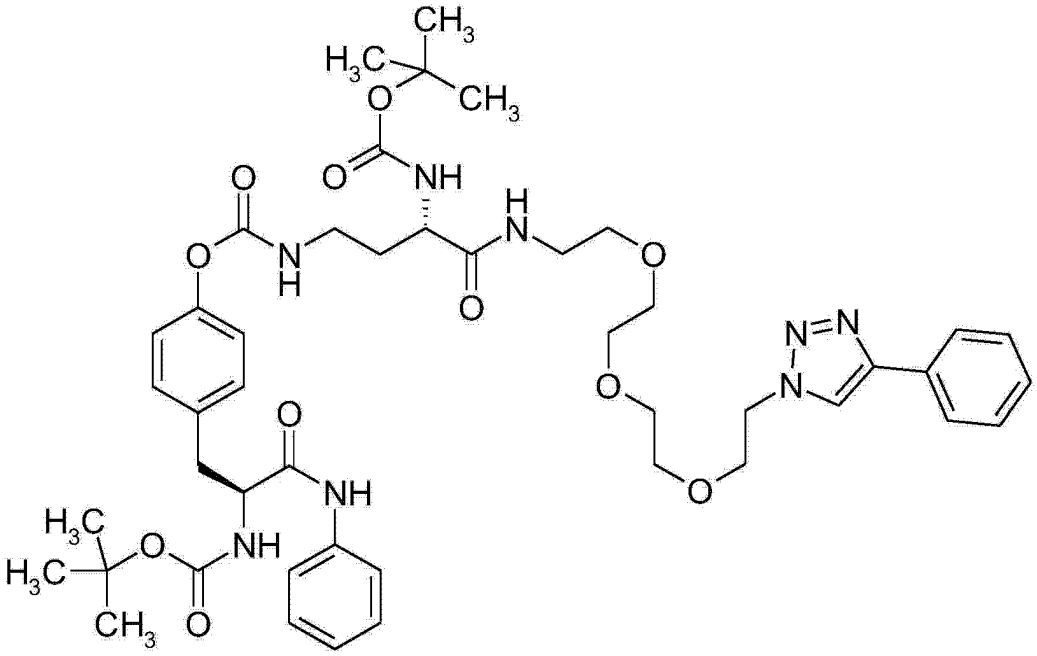
实施例	结构	表征
7C		LC-MS(方法1): R_t = 0.61 min., m/z=747 $(M+H)^+$
8C		LC-MS(方法1): R_t = 0.61 min., m/z=747 $(M+H)^+$
9C		LC-MS(方法2): R_t = 1.53 min., m/z=803 $(M+H)^+$

[0540] 实施例 10Ca

[0541] Nα-(叔丁氧羰基)-O-{[(14S)-14-[(叔丁氧羰基)氨基]-13-氧代-1-(4-苯基-1H-1,2,3-三唑-1-基)-3,6,9-三氧杂-12-氮杂十六烷-16-基]氨基甲酰基}-N-苯

基 -L- 酪氨酰胺

[0542]



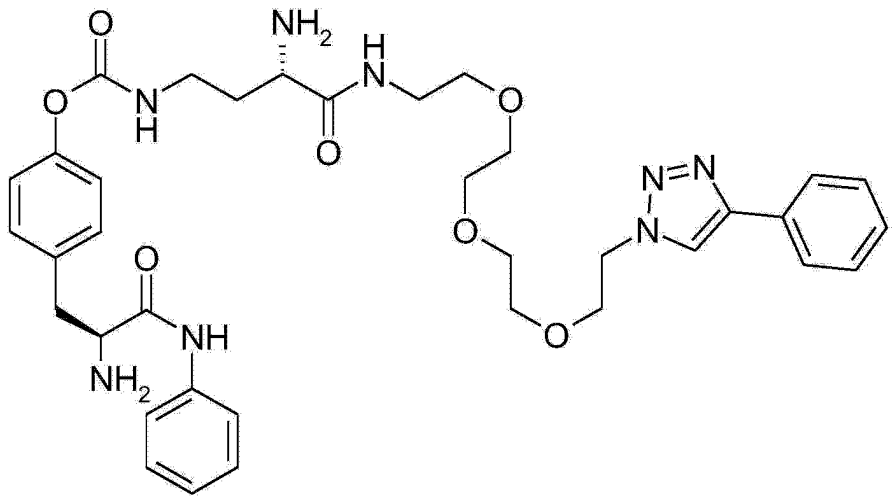
[0543] 将 40mg(0.05mmol) 的实施例 10B 的化合物溶于 4ml DMSO 与 1ml 水的混合物中。添加 10mg(0.10mmol) 苯乙炔、0.8mg 硫酸铜 (copper(II)sulfate)(0.005mmol)、445mg(2.25mmol) 抗坏血酸钠和 1.8mg(0.01mmol)1,10- 菲咯啉 (phenantroline)。通过添加 3-4 滴 10％的硫酸将反应混合物的 pH 调至 4，反应混合物搅拌过夜。反应混合物用约 10ml 水稀释，用约 10ml 的乙酸乙酯萃取 2 次。将合并的有机相蒸干，重新溶于约 5ml 甲醇中，通过制备型 RP-HPLC 在 C18 上使用水 / 甲醇梯度提纯。将含有产物的级分合并，在减压下浓缩至干燥。这得到 36mg(0.04mmol，理论值的 79％) 的所需产物。

[0544] LC-MS(方法 1) :R_t = 1.12min，m/z = 903$(M+H)^+$

[0545] 实施例 10Cb

[0546] O-{[(14S)-14- 氨基 -13- 氧代 -1-(4- 苯基 -1H-1,2,3- 三唑 -1- 基)-3,6,9- 三氧杂 -12- 氮杂十六烷 -16- 基] 氨基甲酰基 }-N- 苯基 -L- 酪氨酰胺

[0547]

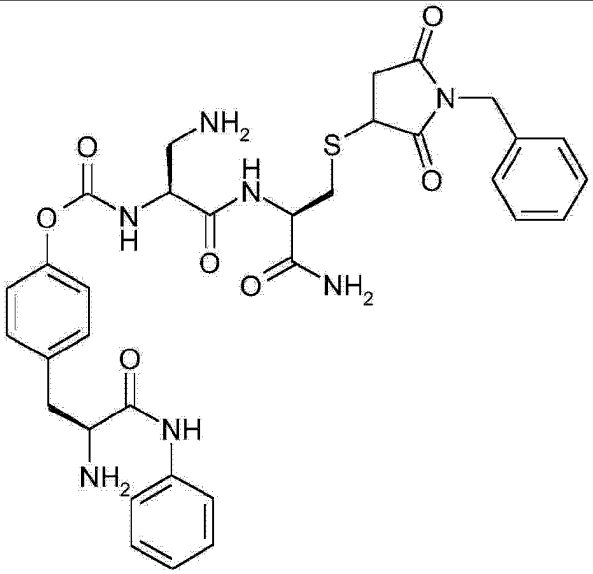


[0548] 将 36mg(0.04mmol) 的实施例 10Ca 的化合物溶于 2.5ml 二氯乙烷。添加 0.02ml 三乙基硅烷、约 2.5ml 三氟乙酸和约 0.1ml 水。反应混合物在室温下搅拌约 30min。将反应混合物蒸干，重新溶于约 15ml 水中。反应混合物用约 10ml 的二氯甲烷萃取 3 次。添加约 0.5ml 乙酸后，将水相冻干。将冻干物重新溶于约 5ml 甲醇中，通过制备型 RP-HPLC 在 C18 上使用水 / 甲醇梯度提纯。将含有产物的级分合并，在减压下浓缩至干燥。这得到 13mg(0.02mmol，理论值的 45％) 的所需产物。

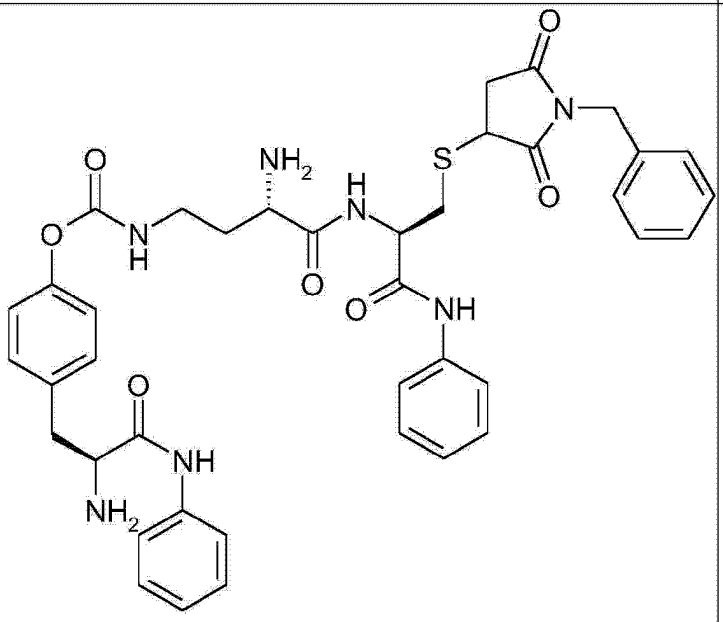
[0549] LC-MS(方法 1) :R_t = 0.57min，m/z = 703$(M+H)^+$

[0550] 使用适当的前体 (实施例 11B 和 12B) 以与实施例 1C 类似的方法来制备下表的实施例。

[0551]

实施例	结构	表征
11C		**LC-MS (方法 1): R_t = 0.60 min., m/z = 676 $(M+H)^+$**

[0552]

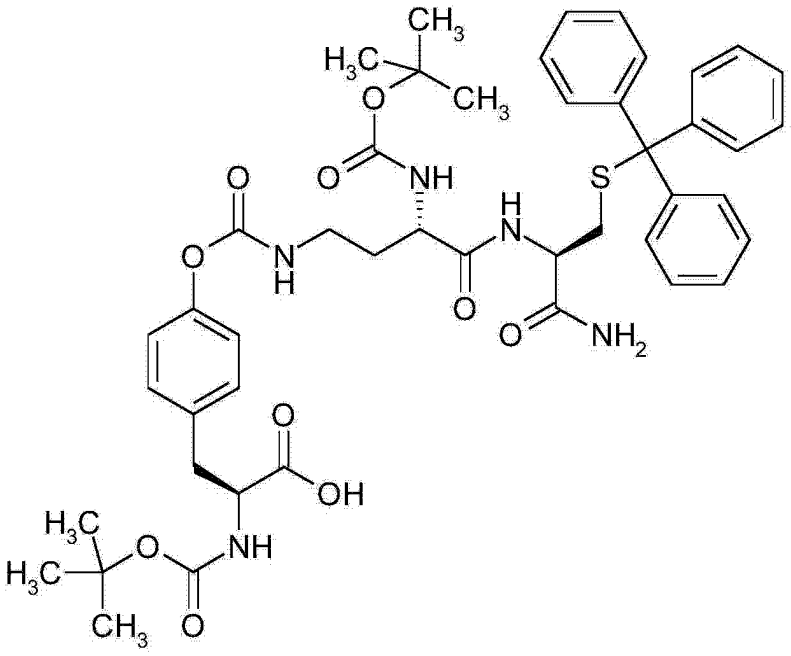
实施例	结构	表征
12C		LC-MS（方法 2）：R_t = 1.69 min.，m/z = 767 $(M+H)^+$

[0553] 工作实施例

[0554] 实施例 1

[0555] O-({(3S)-4-{[(2R)-1- 氨基 -1- 氧代 -3-（三苯甲基硫烷基）丙 -2- 基] 氨基 }-3-[（叔丁氧羰基）氨基]-4- 氧代丁基 } 氨基甲酰基)-N-（叔丁氧羰基)-L- 酪氨酸

[0556]



[0557] 将 4.14g(4.55mmol) 的来自实施例 3A 的化合物溶于 90ml 四氢呋喃。添加 3.17ml(22.8mmol) 三乙胺、0.86ml(22.8mmol) 甲酸和 0.526g(0.455mmol) 四（三苯基膦）钯 (0)。反应混合物在室温下搅拌过夜。反应物用约 100ml 水稀释，用约 100ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取，经硫酸钠干燥，在减压下浓缩至干燥。将粗产物溶于二

氯甲烷，在约 500ml 硅胶中进行色谱分析。使用的溶剂为二氯甲烷、20/1 的二氯甲烷 / 甲醇和 1/1 的二氯甲烷 / 甲醇。将含有产物的级分合并，在减压下浓缩至干燥。这得到 2.62g 的 94.5％纯度的粗产物。产物通过制备型 RP-HPLC 在 C18 上使用水 / 甲醇梯度进一步提纯，生成 2.35g(2.70mmol，理论值的 59％) 的纯产物。

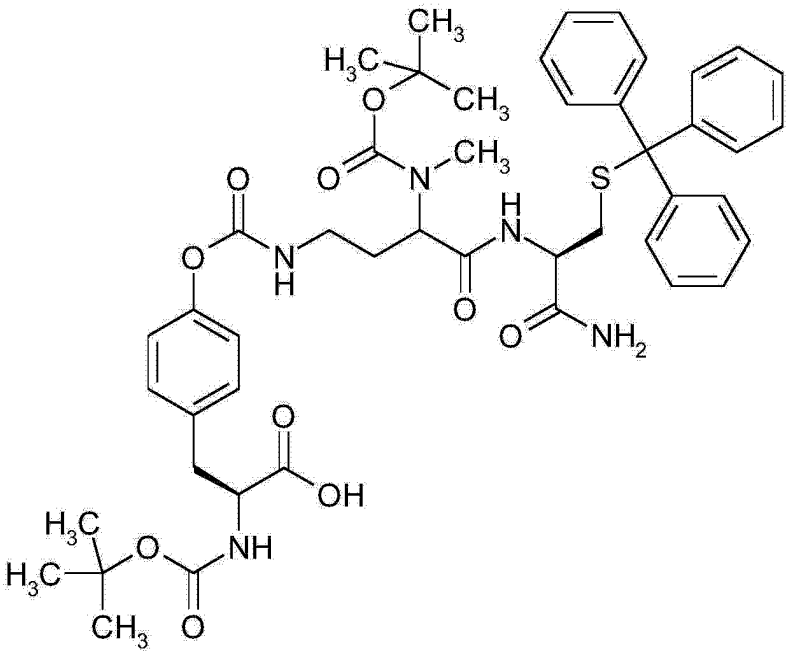
[0558] LC-MS(方法 1) :R_t = 1.22min，m/z = 871 $(M+H)^+$

[0559] ^{1}H-NMR(400MHz, DMSO-d_6, δ /ppm) : δ = 7.92(d, 1H), 7.65(t, 1H), 7.28-7.35(m, 12H), 7.25-7.28(t, 3H), 7.15-7.20(m, 4H), 6.95(d, 2H), 4.29(q, 1H), 4.00(m, 1H), 3.92(m, 1H), 3.11(m, 3H), 2.90(m, 1H), 2.36(m, 2H), 1.84(m, 1H), 1.68(m, 1H), 1.34(d, 18H)。

[0560] 实施例 2

[0561] O-[(4-{[(2R)-1- 氨基 -1- 氧代 -3-(三苯甲基硫烷基) 丙 -2- 基] 氨基 }-3-[(叔丁氧羰基)-(甲基) 氨基]-4- 氧代丁基) 氨基甲酰基]-N-(叔丁氧羰基)-L- 酪氨酸

[0562]



[0563] 将 2.2g(2.38mmol) 的来自实施例 8A 的化合物溶于 48ml 四氢呋喃。添加 1.66ml(11.9mmol) 三乙胺、0.45ml(11.9mmol) 甲酸和 0.275g(0.238mmol) 四 (三苯基膦) 钯 (0)。反应混合物在室温下搅拌过夜。反应物用约 50ml 水稀释，用约 50ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取，经硫酸钠干燥，在减压下浓缩至干燥。将粗产物溶于二氯甲烷，在约 100g 硅胶中进行色谱分析。使用的溶剂为二氯甲烷、50/1 的二氯甲烷 / 甲醇和 4/1 的二氯甲烷 / 甲醇。将含有产物的级分合并，在减压下浓缩至干燥。这得到 1.44g(1.61mmol，理论值的 68％) 的产物，产物为非对映异构体的混合物。

[0564] LC-MS(方法 1) :R_t = 1.20 和 1.24min，m/z = 884 $(M+H)^+$

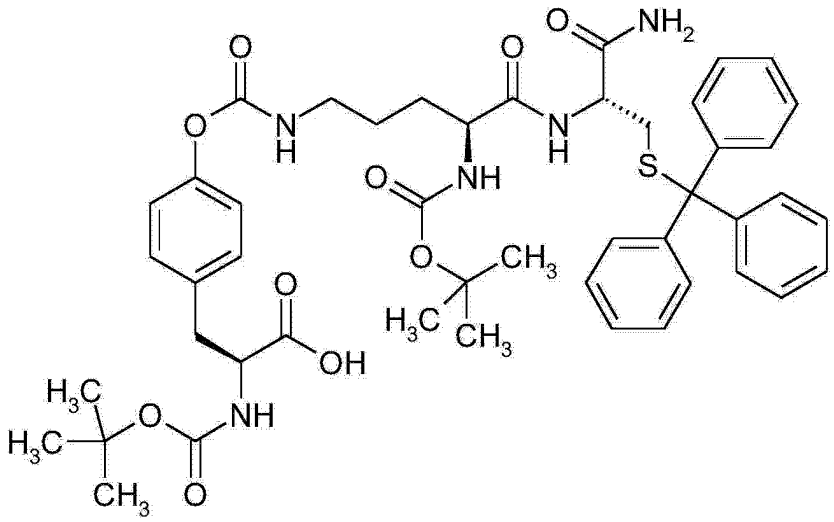
[0565] ^{1}H-NMR(400MHz, DMSO-d_6, δ /ppm) : δ = 8.00(m, 1H), 7.65-7.90(m, 4H), 7.18-7.35(m, 18H), 7.10(m, 2H), 6.96(m, 4H), 4.60(m, 1H), 4.46(m, 1H), 4.30(m, 2H), 4.05(m, 2H), 3.00(m, 4H), 2.75(m, 6H), 2.36(m, 3H), 2.00(m, 2H), 1.82(m, 2H), 1.40(m, 3H), 1.35(s, 18H)。

[0566] 实施例 3

[0567] N^2-(叔丁氧羰基)-N^5-[(4-{(2S)-2-[(叔丁氧羰基) 氨基]-2- 羧基乙基 } 苯氧

基）羰基]-L- 鸟氨酰基 -S- 三苯甲基 -L- 半胱氨酰胺

[0568]



[0569] 将 3.06g(2.33mmol) 的来自实施例 10A 的化合物溶于 46ml 四氢呋喃。添加 1.63ml(11.6mmol) 三乙胺、0.44ml(11.6mmol) 甲酸和 0.265g(0.233mmol) 四（三苯基膦）钯 (0)。反应混合物在室温下搅拌过夜。反应物用约 50ml 水稀释，用约 50ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取，经硫酸钠干燥，在减压下浓缩至干燥。将粗产物溶于二氯甲烷，在约 500ml 硅胶中进行色谱分析。使用的溶剂为二氯甲烷、40/1 的二氯甲烷 / 甲醇和 1/1 的二氯甲烷 / 甲醇。将含有产物的级分合并，在减压下浓缩至干燥。这得到 1.40g 的 86％纯度的粗产物。产物通过制备型 RP-HPLC 在 C18 柱上使用水 / 甲醇梯度进一步提纯，生成 2 个级分 :0.93g 产物（理论值的 45％）。

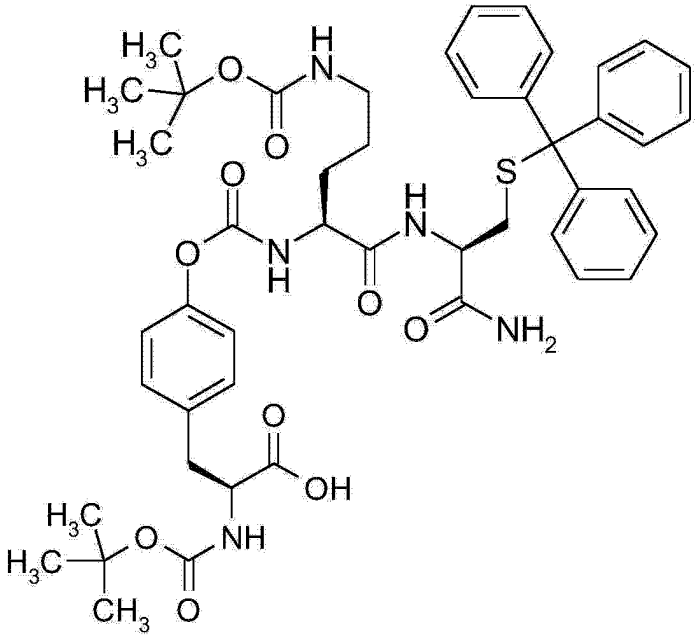
[0570] LC-MS（方法 1）:R_t = 1.18min，m/z = 885$(M+H)^+$

[0571] ^{1}H-NMR(400MHz,DMSO-d_6, δ /ppm) : δ = 7.89(d,1H),7.65(t,1H),7.25-7.35(m,12H),7.20-7.25(m,6H),7.10-7.20(m,3H),6.95(d,2H),4.29(m,1H),4.05(m,1H),3.88(m,1H),3.11(d,1H),3.00(m,4H),2.75(m,2H),2.36(m,3H),1.64(m,1H),1.51(m,3H),1.36(s,9H),1.32(s,9H).

[0572] 实施例 4

[0573] N^5-（叔丁氧羰基）-N^2-[(4-{(2S)-2-[（叔丁氧羰基）氨基]-2- 羧基乙基} 苯氧基）羰基]-L- 鸟氨酰基 -S- 三苯甲基 -L- 半胱氨酰胺

[0574]



[0575] 将 5.27g(5.65mmol) 的来自实施例 12A 的化合物溶于约 60ml 四氢呋喃。添加 2.1ml(15.2mmol) 三乙胺、0.57ml(15.2mmol) 甲酸和 0.35g(0.30mmol) 四 (三苯基膦) 钯 (0)。反应混合物在室温下搅拌过夜。反应物用约 60ml 水稀释，用约 50ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取，经硫酸钠干燥，在减压下浓缩至干燥。将粗产物溶于二氯甲烷，在约 500ml 硅胶中进行色谱分析。使用的溶剂为二氯甲烷、20/1 的二氯甲烷 / 甲醇和 1/1 的二氯甲烷 / 甲醇。将含有产物的级分合并，在减压下浓缩至干燥。粗产物通过制备型 RP-HPLC 在 C18 柱上使用水 / 甲醇梯度进一步提纯，生成 1.37g(理论值的 24%) 的产物。

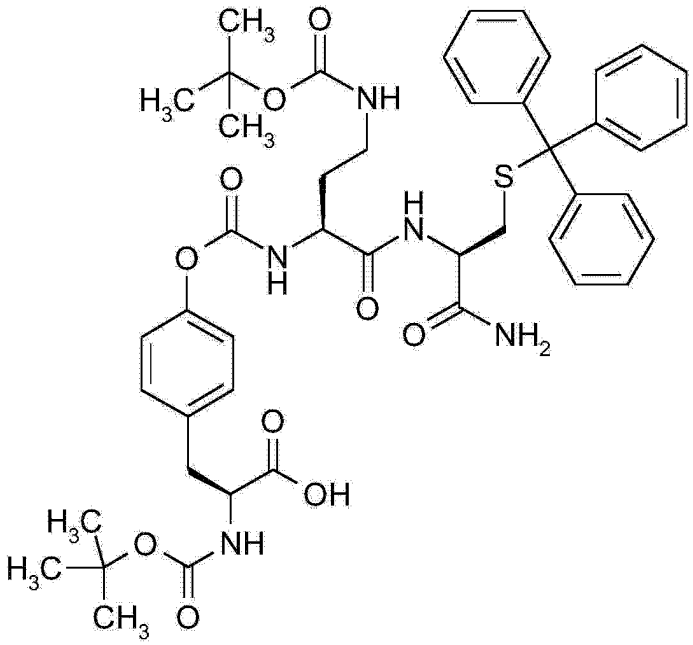
[0576] LC-MS(方法 1) :R_t = 1.17min，m/z = 885(M+H)$^+$

[0577] ^{1}H-NMR(400MHz，DMSO-d_6，δ /ppm) ：δ = 12.6(bs，1H)，8.05(d，1H)，7.97(d，1H)，7.06-7.39(m，20H)，6.97(d，2H)，6.79(t，1H)，4.30(dd，1H)，4.07(m，1H)，4.00(m，1H)，2.85 - 3.04(m，3H)，2.30 - 2.40(m，2H)，1.65(m，1H)，1.41 - 1.60(m，4H)，1.37(s，9H)，1.32(s，9H)。

[0578] 实施例 5

[0579] N-(叔丁氧羰基)-O-{[(4R，7S)-4- 氨基甲酰基 -13，13- 二甲基 -6，11- 二氧代 -1，1，1- 三苯基 -12- 氧杂 -2- 硫杂 -5，10- 二氮杂十四烷 -7- 基] 氨基甲酰基 }-L- 酪氨酸

[0580]



[0581] 将 4.91g(5.40mmol) 的来自实施例 14A 的化合物溶于约 110ml 四氢呋喃。添加 3.8ml(27mmol) 三乙胺、1.02ml(27mmol) 甲酸和 0.62g(0.54mmol) 四 (三苯基膦) 钯 (0)。反应混合物在室温下搅拌过夜。反应物用约 60ml 水稀释，用约 50ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取，经硫酸钠干燥，在减压下浓缩至干燥。将粗产物溶于二氯甲烷，在约 500ml 硅胶中进行色谱分析。使用的溶剂为二氯甲烷、40/1 的二氯甲烷 / 甲醇和 1/1 的二氯甲烷 / 甲醇。将含有产物的级分合并，在减压下浓缩至干燥。粗产物通过制备型 RP-HPLC 在 C18 柱上使用水 / 甲醇梯度进一步提纯，生成 1.96g(理论值的 42%) 的产物。

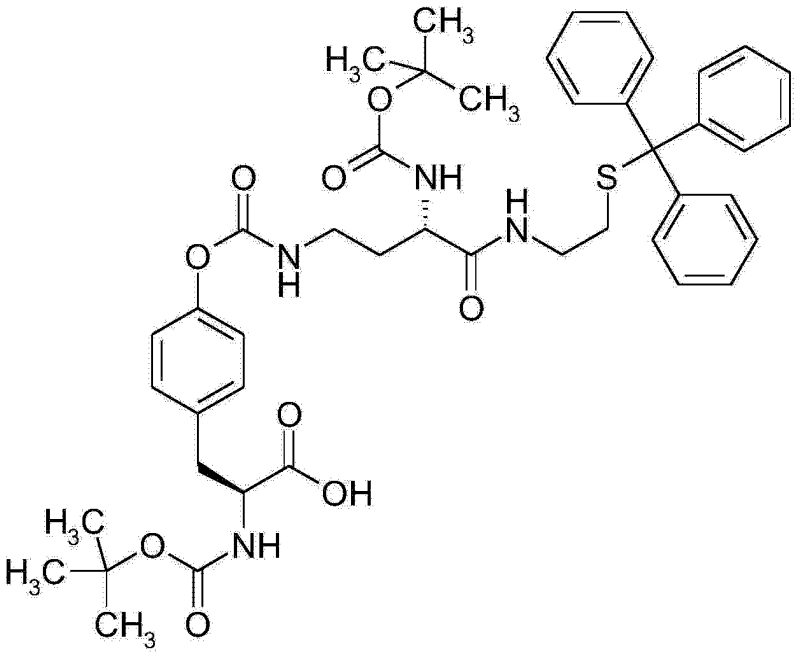
[0582] LC-MS(方法 1) :R_t = 1.20min，m/z = 871 $(M+H)^+$

[0583] ^{1}H-NMR(400MHz, DMSO-d_6, δ /ppm) : δ = 12.6(bs, 1H), 8.05(t, 2H), 7.16 - 7.39(m, 19H), 7.12(d, 1H), 6.98(d, 2H), 6.83(t, 1H), 4.32(dd, 1H), 4.00 - 4.11(m, 2H), 2.92 - 3.12(m, 3H), 2.81(m, 1H), 2.30 - 2.40(m, 2H), 1.82(m, 1H), 1.67(m, 1H), 1.38(s, 9H), 1.32(s, 9H)。

[0584] 实施例 6

[0585] N-(叔丁氧羰基)-O-{[(3S)-3-[(叔丁氧羰基) 氨基]-4- 氧代 -4-{[2-(三苯甲基硫烷基) 乙基] 氨基 } 丁基] 氨基甲酰基 }-L- 酪氨酸

[0586]



[0587] 将 98mg(0.1mmol) 的来自实施例 15A 的化合物溶于约 4ml 四氢呋喃。添加 70μl(0.5mmol) 三乙胺、19μl(0.5mmol) 甲酸和 11mg(0.01mmol) 四(三苯基膦)钯(0)。反应混合物在室温下搅拌过夜。反应物用约 5ml 水稀释,用约 5ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取,经硫酸钠干燥,在减压下浓缩至干燥。粗产物通过制备型 RP-HPLC 在 C18 柱上使用水 / 甲醇梯度提纯,生成 67mg(理论值的 79%)的产物。

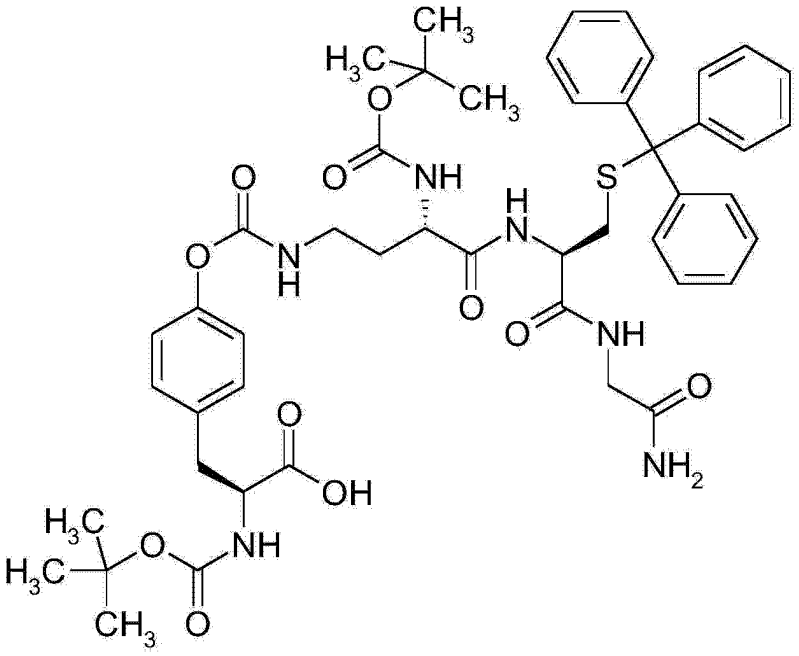
[0588] LC-MS(方法 1):R_t = 1.32min,m/z = 827$(M+H)^+$

[0589] ^{1}H-NMR(400MHz,DMSO-d_6,δ/ppm):δ = 12.6(bs,1H),7.85(t,1H),7.59(m,1H),7.29-7.37(m,12H),7.18-7.27(m,5H),7.07(bs,1H),6.98(d,2H),6.88(d,1H),4.07(m,1H),3.90(m,1H),2.93-3.09(m,5H),2.81(m,1H),2.20(t,2H),1.78(m,1H),1.64(m,1H),1.36(s,9H),1.32(s,9H)。

[0590] 实施例 7

[0591] N-[(2S)-2-[(叔丁氧羰基)氨基]-4-{[(4-{(2S)-2-[(叔丁氧羰基)氨基]-2-羧基乙基}苯氧基)羰基]氨基}丁酰基]-S-三苯甲基-L-半胱氨酰基甘氨酰胺

[0592]



[0593] 将 60mg(0.031mmol) 的来自实施例 16A 的化合物溶于约 3ml 四氢呋喃。添加 22μl(0.16mmol) 三乙胺、6μl(0.16mmol) 甲酸和 4mg(0.003mmol) 四 (三苯基膦) 钯 (0)。反应混合物在室温下搅拌过夜。反应物用约 5ml 水稀释，用约 5ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取，经硫酸钠干燥，在减压下浓缩至干燥。粗产物通过制备型 RP-HPLC 在 C18 柱上使用水 / 甲醇梯度提纯，生成 26mg(理论值的 86%) 的产物。

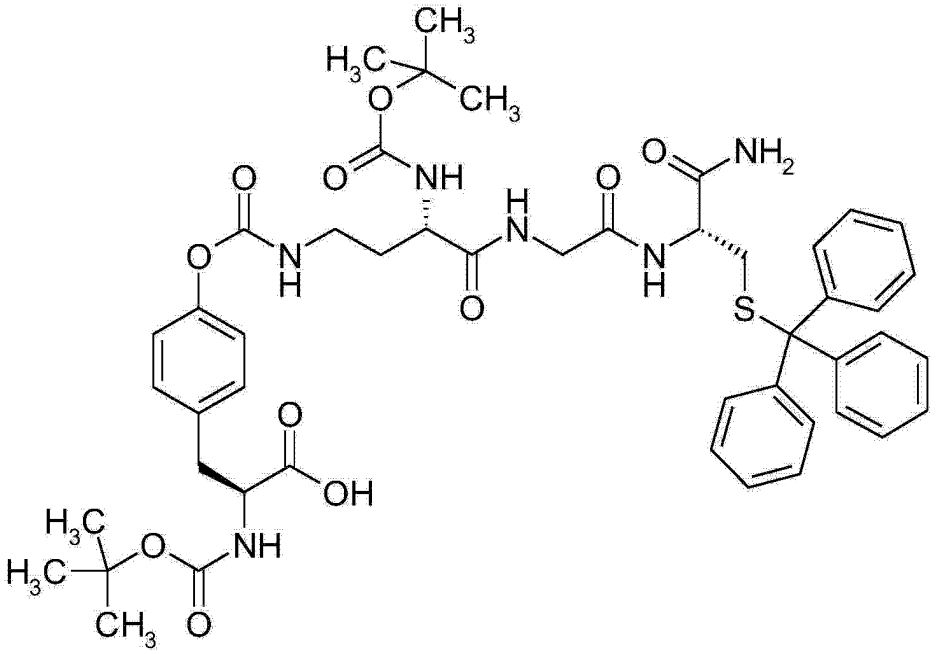
[0594] LC-MS(方法 2) :R_t = 2.55min., m/z = 927 $(M+H)^+$

[0595] ^{1}H-NMR(400MHz, DMSO-d_6, δ/ppm) : d = 12.6(bs, 1H), 8.08(m, 2H), 7.63(t, 1H), 7.18-7.38(m, 18H), 7.03-7.15(m, 3H), 6.99(d, 2H), 4.28(dd, 1H), 3.95-4.10(m, 2H), 3.64(dd, 1H), 3.51(m, 1H), 3.04-3.13(m, 2H), 3.00(dd, 1H), 2.81(m, 1H), 2.42(d, 2H), 1.84(m, 1H), 1.67(m, 1H), 1.36(s, 9H), 1.32(s, 9H)。

[0596] 实施例 8

[0597] N-[(2S)-2-[(叔丁氧羰基) 氨基]-4-{[(4-{(2S)-2-[(叔丁氧羰基) 氨基]-2- 羧基乙基 } 苯氧基) 羰基] 氨基 } 丁酰基] 甘氨酰基 -S- 三苯甲基 -L- 半胱氨酰胺

[0598]



[0599] 将 860mg(0.89mmol) 的来自实施例 19A 的化合物溶于约 20ml 四氢呋喃。添加 620μl(4.45mmol) 三乙胺、168μl(4.45mmol) 甲酸和 103mg(0.089mmol) 四 (三苯基膦) 钯 (0)。反应混合物在室温下搅拌过夜。反应物用约 50ml 水稀释，用约 50ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取，经硫酸钠干燥，在减压下浓缩至干燥。粗产物通过制备型 RP-HPLC 在 C18 柱上使用水 / 甲醇梯度提纯，生成 329mg(理论值的 38%) 的产物。

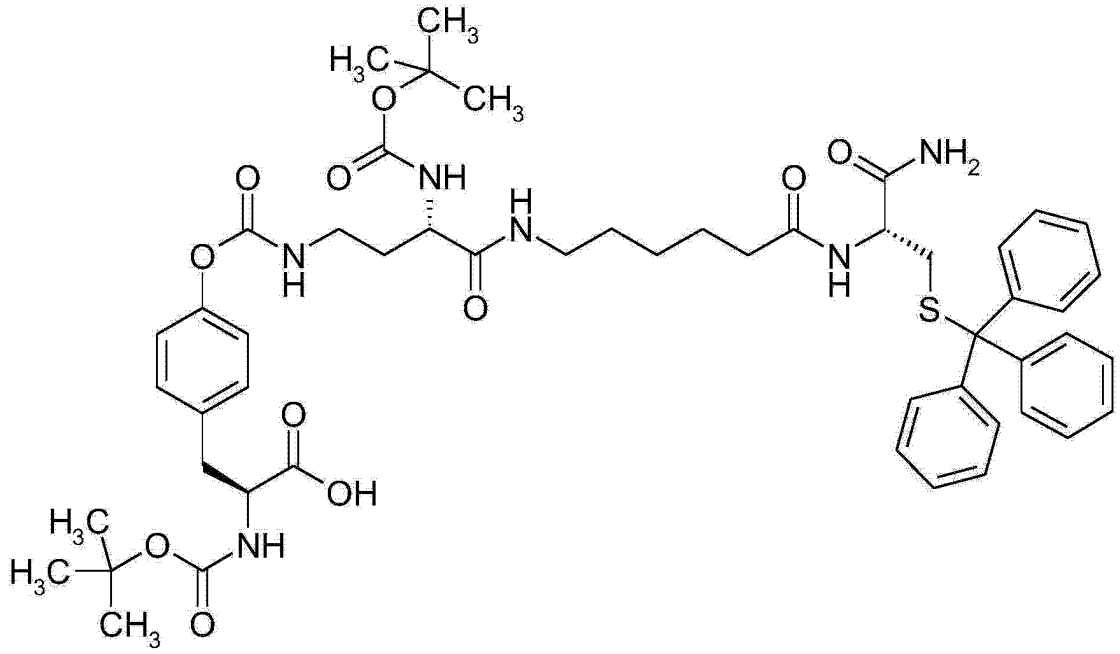
[0600] LC-MS(方法 1) :R_t = 1.16min，m/z = 927$(M+H)^+$

[0601] ^{1}H-NMR(400MHz，DMSO-d_6，δ/ppm) :d = 8.16(d,1H)，8.04(t,1H)，7.64(t,1H)，7.20-7.39(m,15H)，7.15(d,3H)，7.07(d,1H)，6.95(d,2H)，4.28(dd,1H)，4.02(dd,1H)，3.91(m,1H)，3.76(m,2H)，2.99-3.15(m,3H)，-2.88(m,1H)，2.29-2.42(m,2H)，1.86(m,1H)，1.68(m,1H)，1.37(s,9H)，1.33(s,9H)。

[0602] 实施例 9

[0603] O-({(3S)-4-[(6-{[(2R)-1- 氨基 -1- 氧代 -3-(三苯甲基硫烷基) 丙 -2- 基] 氨基 }-6- 氧代己基) 氨基]-3-[(叔丁氧羰基) 氨基]-4- 氧代丁基 } 氨基甲酰基)-N-(叔丁氧羰基)-L- 酪氨酸

[0604]



[0605] 将 250mg(0.24mmol) 的来自实施例 22A 的化合物溶于约 5ml 四氢呋喃。添加 170μl(1.22mmol) 三乙胺、48μl(1.22mmol) 甲酸和 28mg(0.024mmol) 四(三苯基膦)钯(0)。反应混合物在室温下搅拌过夜。反应物用约 20ml 水稀释,用约 20ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取,经硫酸钠干燥,在减压下浓缩至干燥。粗产物通过制备型 RP-HPLC 在 C18 柱上使用水 / 甲醇梯度提纯,生成 167mg(理论值的 65%) 的产物。

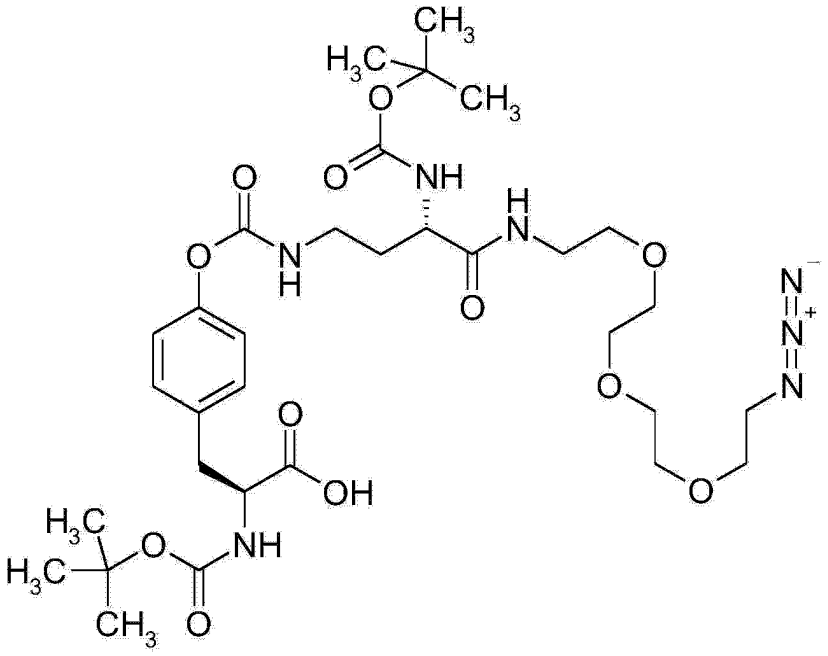
[0606] LC-MS(方法 1):R_t = 1.19min,m/z = 983$(M+H)^+$

[0607] ^{1}H-NMR(400MHz,DMSO-d_6, δ/ppm):d = 12.6(bs,1H),8.00(d,1H),7.75(t,1H),7.63(t,1H),7.18 - 7.37(m,19H),7.12(d,1H),7.08(s,1H),7.00(d,2H),4.31(m,1H),4.06(m,1H),3.93(m,1H),2.92 - 3.11(m,6H),2.81(dd,1H),2.30(m,2H),2.10(t,2H),1.79(m,1H),1.66(m,1H),1.40 - 1.54(m,3H),1.37(s,9H),1.32(s,9H),1.23(m,2H)。

[0608] 实施例 10

[0609] O-({(14S)-1- 叠氮基 -14-[(叔丁氧羰基)氨基]-13- 氧代 -3,6,9- 三氧杂 -12- 氮杂十六烷 -16- 基}氨基甲酰基)-N-(叔丁氧羰基)-L- 酪氨酸

[0610]



[0611] 将 276mg(0.344mmol) 的来自实施例 23A 的化合物溶于约 15ml 四氢呋喃。添加 235μl(1.68mmol) 三乙胺、63μl(1.68mmol) 甲酸和 39mg(0.034mmol) 四（三苯基膦）钯(0)。反应混合物在室温下搅拌过夜。反应物用约 20ml 水稀释，用约 20ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取，经硫酸钠干燥，在减压下浓缩至干燥。粗产物通过制备型 RP-HPLC 在 C18 柱上使用水 / 甲醇梯度提纯，生成 167mg（理论值的 65%）的产物。

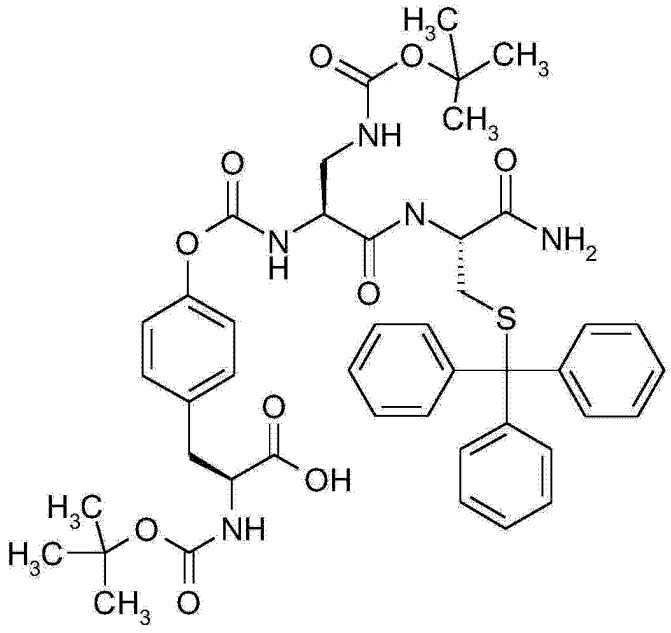
[0612] LC-MS（方法 1）：R_t = 0.98min，m/z = 726$(M+H)^+$

[0613] ^{1}H-NMR（400MHz，DMSO-d_6，δ/ppm）：d = 7.85(t,1H),7.61(t,1H),7.15(d,2H),6.91-6.99(m,3H),3.96(m,1H),3.86(bs,1H),3.59(dd,2H),3.46-3.57(m,9H),3.40(m,4H),3.13-3.29(m,2H),2.98-3.11(m,3H),2.82-2.92(m,1H),1.79(m,1H),1.66(m,1H),1.38(s,9H),1.34(s,9H)。

[0614] 实施例 11

[0615] 3-[（叔丁氧羰基）氨基]-N-[(4-{(2S)-2-[（叔丁氧羰基）氨基]-2-羧基乙基}苯氧基）羰基]-L-丙氨酰基-S-三苯甲基-L-半胱氨酰胺

[0616]



[0617] 将 2.38g(2.03mmol) 的来自实施例 25A 的化合物溶于约 35ml 四氢呋喃。添加 1.42ml(10mmol) 三乙胺、0.38ml(10mmol) 甲酸和 0.24g(0.20mmol) 四 (三苯基膦) 钯 (0)。反应混合物在室温下搅拌过夜。反应物用约 20ml 水稀释，用约 30ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取，经硫酸钠干燥，在减压下浓缩至干燥。将粗产物溶于二氯甲烷，在约 70ml 硅胶中进行色谱分析。使用的溶剂为 10/1 的二氯甲烷 / 甲醇至 1/1 的二氯甲烷 / 甲醇。将含有产物的级分合并，在减压下浓缩至干燥，生成 0.72g(理论值的 41％) 的产物。

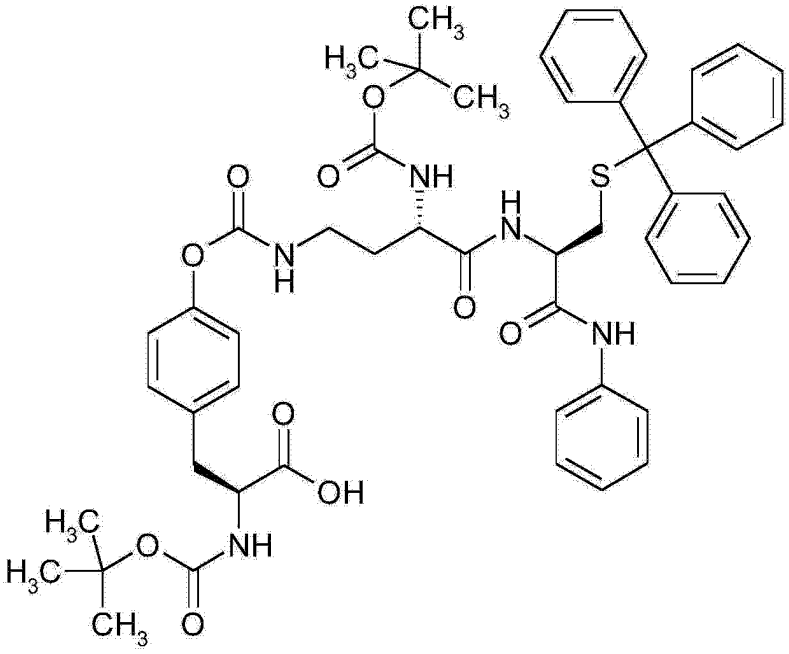
[0618] LC-MS(方法 1) :R_t = 1.18min，m/z = 855(M-H)$^-$

[0619] ^{1}H-NMR(400MHz,DMSO-d_6, δ /ppm) : δ = 8.15(m,1H),7.75(m,1H),7.16 - 7.39(m,19H),6.99(d,2H),6.80(m,1H),4.25(m,2H),),4.13(m,2H),),4.00(m,2H),2.92 - 3.12(m,3H),2.81(m,1H),2.40(m,2H),1.38(s,9H),1.32(s,9H),1.10(m,4H)。

[0620] 实施例 12

[0621] O-({(3S)-4-{[(2R)-1- 苯胺基 -1- 氧代 -3-(三苯甲基硫烷基) 丙 -2- 基] 氨基 }-3-[(叔丁氧羰基) 氨基]-4- 氧代丁基 } 氨基甲酰基)-N-(叔丁氧羰基)-L- 酪氨酸

[0622]



[0623] 将 405mg(0.41mmol) 的来自实施例 26A 的化合物溶于约 10ml 四氢呋喃。添加 0.29ml(2.05mmol) 三乙胺、78μl(2.05mmol) 甲酸和 47mg(0.04mmol) 四(三苯基膦)钯(0)。反应混合物在室温下搅拌过夜。反应物用约 10ml 水稀释,用约 10ml 二氯甲烷萃取 2 次。合并的有机相用盐水萃取,经硫酸钠干燥,在减压下浓缩至干燥。粗产物通过制备型 RP-HPLC 在 C18 柱上使用水 / 甲醇梯度提纯,生成 306mg(理论值的 79%) 的产物。

[0624] LC-MS(方法 1):R_t = 1.39min, m/z = 947 $(M+H)^+$

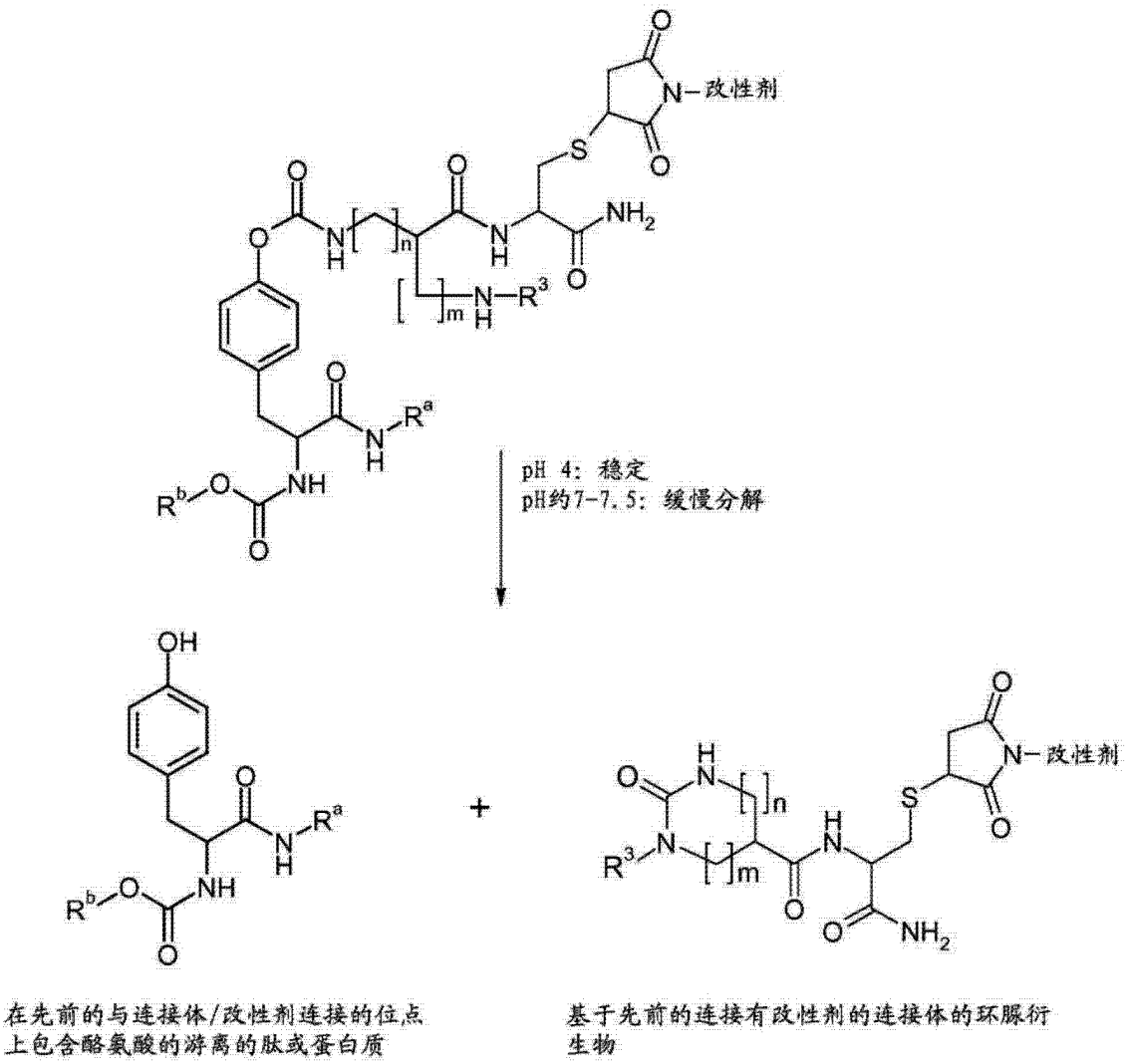
[0625] ^{1}H-NMR(400MHz, DMSO-d_6, δ/ppm):δ = 8.08(d,1H), 7.60(m,1H), 7.55(m,1H), 7.28-7.35(m,16H), 7.22-7.6(m,4H), 7.07(m,2H), 6.92(m,2H), 4.60(m,1H), 4.05(m,4H), 2.85-3.20(m,4H), 2.80(m,1H), 2.45(m,2H), 1.85(m,1H), 1.66(m,1H), 1.35(d,18H), 1.28(m,2H)。

[0626] B. 载体连接体活性的评估

[0627] 可使用以下测定系统,证明本发明的化合物用作载体连接体的合适性。为了阐明不同连接体的不同动力学,合成基于酪氨酸的分子的简单衍生物,并在不同时间点监测在 pH4 和 pH7.4 的缓冲液中的断裂。以基于酪氨酸的连接体结构的精确组成为基础,伴随有游离酪氨酸 OH 基团释放的环脲的形成具有不同的断裂动力学。这些可容易地在体外测量,并被用作体内动力学的预报器 (predictor)。方案 3 示出了示例性的前药的分解,所述前药释放含有酪氨酸的肽和基于先前的连接有改性剂的连接体的环脲衍生物。

[0628] 方案 3

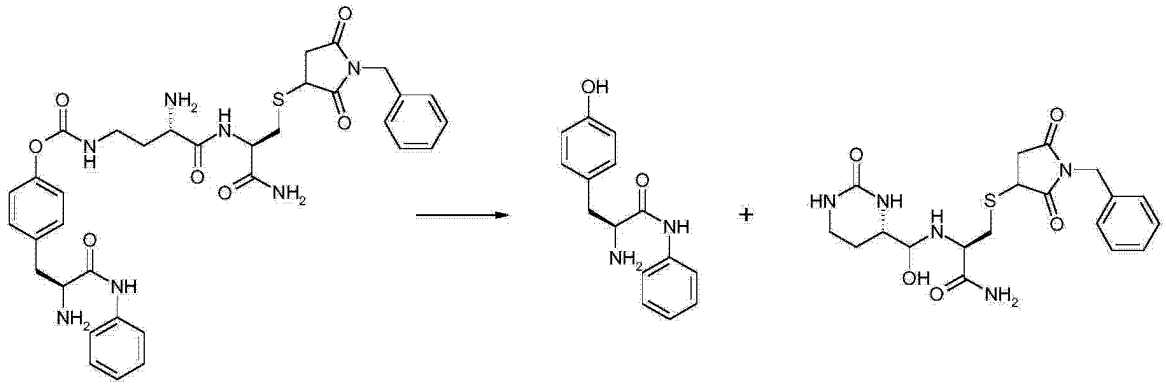
[0629]



[0630] 对于实施例 1C，O-{[(3S)-3- 氨基 -4-({(2R)-1- 氨基 -3-[(1- 苯基 -2,5- 二氧代吡咯烷 -3- 基）硫烷基]-1- 氧代丙 -2- 基 } 氨基)-4- 氧代丁基] 氨基甲酰基 }-N- 苯基 -L- 酪氨酰胺，断裂反应如下：

[0631] 方案 4

[0632]



[0633] 1）试验描述（体外）

[0634] 对于关于不同连接体的稳定性的动力学研究，将 0.3mg 的干燥的试验化合物溶于 0.5ml 乙腈中。为了更好地稀释，对样品进行超声约 10 秒。然后添加 1.0ml 的缓冲溶液，再

次对样品进行超声。

[0635] 使用的所述溶液 / 缓冲液的化学组成 :

[0636] pH4 :用 1N 盐酸将 1 升的去离子水调整至 pH4,

[0637] pH7.4 :将 90g 氯化钠、13.61g 磷酸二氢钾和 83.35g 的 1M 氢氧化钠溶液溶于 1 升的去离子水。该溶液用水稀释,比率为 1:10。

[0638] 所述试验化合物的浓度是通过在室温下在 24 小时时间内每小时进行 HPLC 来分析。所述试验化合物的量通过峰面积来确定。

[0639] HPLC 方法 :Agilent1100, 具有 DAD(G1315B), 二元液相泵 (binary pump) (g1312A), 自动进样器 (G1329A), 柱恒温器 (G1330B), 柱 :Kromasil100C18/250mm x4mm/5μm,柱温 :30℃,洗脱液 A :水 +5ml 高氯酸 /1,洗脱液 B :乙腈,梯度 :0-1.0min90% A、10% B ;1.0-20.0min10% A、90% B ;20.0-21.0min10% A、90% B ;21.0-23.0min90% A、10% B ;23.0-25.0min90% A、10% B ;流速 :1.5ml/min,检测 :210nm,注射体积 :10μl。

[0640] 所述试验化合物的断裂的结果示于表 1 中。

[0641] 表 1 :

[0642]

实施例编号	断裂的% pH 4, 0 h	断裂的% pH 4, 24 h	断裂的% pH 7.4, 0 h	断裂的% pH 7.4, 6 h	断裂的% pH 7.4, 24 h
1C	0	1	0	7	21
2C	0	0	0	6	21
3C	0	0	0	2	11
4C	0	0	0	23	65
5C	0	0	0	75	100
6C	0	0	0	6	20
7C	0	0	0	6	23
8C	0	0	0	6	23
9C	0	0	0	7	25
10Cb	0	0	0	4	14
11C	0	19	0	100	100
12C	0	0	0	29	76

[0643] 数据示出了实施例 11C 被非常快速地断裂,甚至在 pH4 下。实施例 4C、实施例 5C 和实施例 12C 被快速断裂,而实施例 3C 和实施例 10Cb 被缓慢地断裂。所有其他的实施例具有中等的断裂动力学。

[0644] C. 药物组合物的示例性实施方案

[0645] 本发明的化合物可以以下的方式转变为药物制剂：

[0646] 静脉注射用（i.v.）溶液：

[0647] 将本发明的化合物以低于饱和溶解度的浓度溶于生理上可接受的溶剂（例如pH4-pH7的缓冲液、等渗氯化钠溶液、葡萄糖溶液5％和/或PEG400溶液30％）中。所述溶液通过过滤进行灭菌，并充入无菌且无热原的注射容器中。

[0648] 皮下注射用（s.c.）溶液

[0649] 将本发明的化合物以低于饱和溶解度的浓度溶于生理上可接受的溶剂（例如pH4-pH7的缓冲液、等渗氯化钠溶液、葡萄糖溶液5％和/或PEG400溶液30％）中。所述溶液通过过滤进行灭菌，并充入无菌且无热原的注射容器中。

Abstract

The invention relates to novel tyrosine based linkers that allow the releasable connection of peptides or proteins with other molecular entities, e.g. polyethylene glycol, to processes for their preparation and their use for preparing medicaments for the treatment and/or prophylaxis of diseases.