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(71) Applicants: BAYER AKTIENGESELLSCHAFT

[DE/DE]; Kaiser-Wilhelm-Allee 1, 51373 Leverkusen

(DE). BAYER PHARMA AKTIENGESELLSCHAFT

[DE/DE]; Müllerstr. 178, 13353 Berlin (DE).

(72) Inventors: BIERER, Donald; Horst 9, 42781 Haan (DE).

FLAMME, Ingo; Auf den Schmitten 1, 51580 Reichshof

(DE). ZUBOV, Dmitry; Kippdorf 78, 42857 Remscheid

(DE). NEUBAUER, Thomas; Kleiberweg 5c, 42111 Wup-

pertal (DE). TERSTEEGEN, Adrian; Rübezahlweg 8,

42111 Wuppertal (DE). JUHL, Cathleen; Jürgen-Töpfer

Str. 87, 22763 Hamburg (DE). GLATZ, Marie; Westfalen-

weg 178a, 42111 Wuppertal (DE). DREHER, Jan; Ot-

tenbrucher Str. 24, 42105 Wuppertal (DE). HOLTON, Si-

mon; Niklasstrasse 14a, 14163 Berlin (DE). TERJUNG,

Carsten; Am Knüpp 64, 44799 Bochum (DE). BAU-

MANN, Lars; Haus Düsseldorf 10, 42489 Wülfrath (DE). PO-

ETHKO, Thorsten; Gustav Strasse 5, 58332 Schwelm

(DE). XIONG, Jiancheng; 23-1103 Longchao Xiaoqu

Hankoubei Avenue, Wuhan, Huangpi District 430000 (CN).

QIU, Yibo; 2272 Stone Road, Ann Arbor, Michigan 48105

(US).

(74) Agent: BIP PATENTS; Alfred-Nobel-Str. 10, 40789 Mon-

heim am Rhein (DE).

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(54) Title: MASP INHIBITORY COMPOUNDS AND USES THEREOF

(57) Abstract: The present invention relates to novel Mannose-binding lectin (MBL)-associated serine protease (MASP) inhibitory compounds, as well as analogues and derivatives thereof, to processes for the preparation thereof, to the use thereof alone or in combinations for treatment and/or prevention of diseases and to the use thereof for production of medicaments for treatment and/or prevention of diseases, especially for treatment and/or prevention of renal and cardiovascular disorders and of ischemia reperfusion injuries.

MASP inhibitory compounds and uses thereof

The present invention relates to novel Mannose-binding lectin (MBL)-associated serine protease (MASP) inhibitory compounds, as well as analogues and derivatives thereof, to processes for the preparation thereof, to the use thereof alone or in combinations for treatment and/or prevention of diseases and to the use thereof for production of medicaments for treatment and/or prevention of diseases, especially for treatment and/or prevention of renal and cardiovascular disorders and of ischemia reperfusion injuries.

The complement system consists of a complex cascading network of proteins, receptors and enzymes of which many are circulating in the blood stream. The complement system is an important constituent of innate immunity and essential for the defense against invading pathogens and clearance of dead and virus infected cells. It forms a bridge between innate and adaptive immune responses. Activation of the complement system is also involved in the pathologies of sepsis and ischemia reperfusion injuries, e.g. after myocardial infarction, ischemic kidney injury or organ transplantation. Three branches of the complement system have been identified: the lectin pathway, the classical and the alternative pathway (*Dunkelberger and Song, Complement and its role in innate and adaptive immune responses. Cell Res. 2010; 20(1): 34-50*). The lectin pathway is activated by deposition of lectins which are circulating in the blood stream and under normal conditions have a sentinel function against invading pathogens and dead cells by recognizing foreign and altered carbohydrate surface patterns, respectively, and decorating their surfaces. Mannose-binding lectin (MBL), ficolins and collectins are the major representatives of these lectins which are produced in liver, kidney and other organs (*Garred et al., A journey through the lectin pathway of complement-MBL and beyond. Immunol Rev. 2016;274(1):74-97*). Their deposition is followed by further recruitment of zymogens of essentially two closely related serine proteases from the blood stream, mannose-binding lectin-associated serine protease 1 and 2 (MASP-1 and MASP-2) forming a complex in which the zymogens come into close proximity to each other. The current concept is that under *in vivo* conditions MASP-1 zymogen after recruitment is self-activating and then activates the MASP-2 zymogen by cleavage. Activated MASP-1 furthermore cleaves complement factor C2 into C2a and C2b. Activated MASP-2 also cleaves C2 and complement factor C4 into C4a and C4b which together with C2a forms the C4bC2a complex which serves as complement factor C3 convertase. Constitution of C3 convertase activity and consecutive C3 deposition to target cell surfaces represents the point of convergence of all three complement pathways activating the common downstream cascade that results in generation of inflammatory mediators and target cell lysis. In intact human serum activities of both MASP-enzymes are indispensable for C3 convertase formation (*Héja et al, Revised mechanism of complement lectin-pathway activation revealing the role of serine protease MASP-1 as the exclusive activator of MASP-2. Proc Natl Acad Sci U S A. 2012;109(26):10498-503*).

The microvascular system plays a crucial role during inflammatory and ischemic organ disorders. Barrier function, leukocyte trafficking and coagulation control are closely dependent on the integrity of the

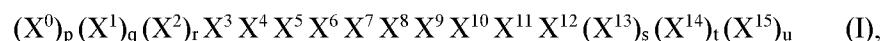
luminal endothelial cell surface in small blood vessels. The luminal endothelial surface is lined by a dense coat of glycosylation extensions from membrane integrated glycoproteins, proteoglycans, and glycolipids which in their entirety are called glycocalyx. Electron microscopic analyses of samples from animal experiments and human pathologies have shown that in particular the endothelial glycocalyx is rapidly and fundamentally being degraded upon ischemic challenge as well as under inflammatory conditions such as in sepsis. These changes lead to exposure of carbohydrate residues to the blood stream that under normal conditions are not detectable (for review see: *Sieve et al., Regulation and function of endothelial glycocalyx layer in vascular diseases. Vascu Pharmacol. 2018; 100: 26-33*). Beside other changes of the cell surface in particular the altered carbohydrate pattern is thought to activate the lectin pathway causing deposition of the pattern recognizing lectins, subsequent C3 deposition and initiation of cell lysis. MBL and C3 deposition was shown to occur after ischemia and acute kidney injury across species including man. The lectin pathway activation was of particular relevance for reperfusion damage as targeted deletion of MBL and MASP-2 protected mice from ischemia reperfusion damages in kidney heart and intestine (*Moller-Kristensen et al., Mannan-binding lectin recognizes structures on ischaemic reperfused mouse kidneys and is implicated in tissue injury. Scand J Immunol. 2005; 61(5): 426-34; Schwaeble et al., Targeting of mannan-binding lectin-associated serine protease-2 confers protection from myocardial and gastrointestinal ischemia/reperfusion injury. Proc Natl Acad Sci U S A. 2011; 108(18): 7523-8*). Moreover, deletion of collectin 11, another MASP activating lectin which is predominantly expressed in the kidney, made mice resistant against ischemic acute kidney injury (*Farrar et al., Collectin-11 detects stress-induced L-fucose pattern to trigger renal epithelial injury. J Clin Invest. 2016; 126(5): 1911-1925*). Selective peptide inhibitors of MASP-1 and MASP-2 have been identified from phage display libraries employing natural trypsin inhibitors from sun flower or grass hoppers as a starting point. These peptides have been shown to inhibit the lectin pathway dependent C3 convertase formation in vitro (*Kocsis et al., Selective inhibition of the lectin pathway of complement with phage display selected peptides against mannose-binding lectin-associated serine protease (MASP)-1 and -2: significant contribution of MASP-1 to lectin pathway activation. J Immunol. 2010; 185(7): 4169-78; Héja et al., Monospecific inhibitors show that both mannan-binding lectin-associated serine protease-1 (MASP-1) and are essential for lectin pathway activation and reveal structural plasticity of MASP-2. J Biol Chem. 2012; 287(24): 20290-300*). However, no evidence for pharmaceutical utility and in vivo efficacy of those peptide inhibitors is available, yet. Similarly, antibodies directed against MASP-2 which interfere with MASP zymogen interaction have been identified and brought to clinical development for atypical hemolytic uremic syndrome and other inflammatory kidney disorders (*ClinicalTrials.gov Identifier: NCT03205995; NCT02682407; NCT03608033*). However, clinical proof for utility in the prevention or treatment of acute, in particular ischemic organ damage is still missing.

WO 2004/075837 discloses anti-MASP antibodies, functionally equivalent fragments thereof and MASP binding peptides for decreasing the morbidity and mortality caused by tissue damage associated with

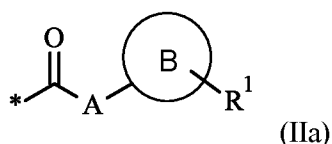
ischemia-reperfusion injury or TAAA repair by inhibition of the complement system. Small peptides such as the sunflower MASP inhibitor-1 (SFMI-1) and sunflower MASP inhibitor-2 (SFMI-2) as well as derivatives thereof for the treatment of diseases related to the complement system, primarily the lectin pathway were first described in WO 2010/136831.

- 5 WO 2015/054298 discloses methods for preserving vision or reducing vision loss in a subject and for inhibiting or reducing photoreceptor cell death in a subject by reducing the activity of MASP-1, MASP-2 or MASP-3. WO 2004/106384, WO 2005/123128, WO 2007/117996 and WO 2014/144542 disclose anti-MASP-2 antibodies for the therapy of diseases associated with MASP-2-dependent complement activation.
- 10 It was the object of the present invention to provide novel peptides, having inhibitory effects on MASP-1 and/or MASP-2 enzymes and other beneficial properties making them suitable as efficient and safe alternatives for the prophylaxis and/or treatment of MASP-1 and/or MASP-2-associated disorders as defined below. It was a further object to provide novel peptides, having an improved inhibitory effect on human MASP-1 and/or MASP-2 enzyme and/or rat MASP-1 and/or MASP-2 enzyme.
- 15 The present invention generally relates to peptides acting as inhibitors of MASP-1 and/or MASP-2 enzymes and methods of making and using the same.

In particular, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, the following structural formula (I):



- 20 or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X^0 represents a group of the formula (IIa)



wherein

- 25 * marks the bond to a terminal amino group of the adjoining amino acid,
- A is a bond or C₁-C₆-alkylene, wherein one CH₂ group in C₁-C₆-alkylene may be exchanged for -O- or -S-, and wherein C₁-C₆-alkylene is up to trisubstituted identically or differently by a radical selected from the group consisting of hydroxyl, methoxy, ethoxy, carboxy, amino and halogen,

B is absent, aryl, heteroaryl, C₃-C₈-cycloalkyl or C₃-C₇-heterocycloalkyl,

wherein aryl, heteroaryl, C₃-C₈-cycloalkyl and C₃-C₇-heterocycloalkyl can be up to trisubstituted identically or differently by a radical selected from the group of C₁-C₄-alkyl, hydroxyl, methoxy, ethoxy, carbonyl, carboxy, amino and halogen, and

5 R¹ is hydrogen, halogen, amino, hydroxyl or C₁-C₂₀-alkyl, wherein C₁-C₂₀-alkyl is up to trisubstituted identically or differently by a radical selected from the group consisting of hydroxyl, carboxy, amino and halogen,

p represents the integer 0 or 1,

10 X¹ represents any natural amino acid or an unnatural amino acid, whereas any natural amino acid and/or unnatural amino acid can be in D- or L-stereoconfiguration,

and in case p is 0 and q is different from 0, the terminal amino group of X¹ is unsubstituted, acetylated or mono- or disubstituted with C₁-C₂₀-alkyl,

q represents an integer of from 0 to 5,

15 X² represents a natural amino acid selected from a list consisting of I, L, M, V and A, or an unnatural amino acid selected from a list consisting of L-N-Methylisoleucine ((N-Me)I), allo-L-Isoleucine (allo-I), L-Cyclobutylalanine (Cba), L-Norvaline (Nva), L-2-Aminobutyric acid (Abu), (2S,3S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-3-methylpentanoic acid, (2S,3S)-2-[2-Oxopiperazin-1-yl]-3-methylpentanoic acid, (2S,3S)-2-[(3S)-2-oxopiperazin-1-yl]-3-methylpentanoic acid, L-Methionine-L-sulfoxide, L-Methionine-sulfone and L-tert-butylglycine,

20 whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration,

and in case p and q are both 0 and r is 1, the terminal amino group of X² is unsubstituted, acetylated or mono- or disubstituted with C₁-C₂₀-alkyl,

r represents the integer 0 or 1,

25 X³ represents the natural amino acid C, or an unnatural amino acid selected from a list consisting of L-Penicillamine (Pen) and L-N-Methylcysteine ((N-Me)C),

and in case p and q and r are all 0, the terminal amino group of X³ is unsubstituted, acetylated or mono- or disubstituted with C₁-C₂₀-alkyl,

- X⁴ represents a natural amino acid selected from a list consisting of S, C, T, R or K, or an unnatural amino acid selected from a list consisting of allo-L-Threonine (allo-T), L- Homoserine (hSer) and L-Ornithine (Orn),
- X⁵ represents the natural amino acid R or N(5)-methyl-L-arginine ((Me)R),
- 5 X⁶ represents a natural amino acid selected from a list consisting of S, C or T, or an unnatural amino acid selected from a list consisting of allo-L-Threonine (allo-T) and L-2,3-diaminopropionic acid (Dap),
- X⁷ represents a natural amino acid selected from a list consisting of L, F or N or an unnatural amino acid selected from a list consisting of 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), L-4-Bromophenylalanine ((4-Bromo)F), 2,5-Difluoro-L-phenylalanine ((2,5-Difluoro)F), L-tert-Butylalanine ((tBu)A), 2-Chloro-L-phenylalanine ((2-Chloro)F), L-2-Bromophenylalanine ((2-Bromo)F), (S)-2-(Amino)-1,6-hexanedioic acid (AAD), (2S)-2-amino-4,4,4-trifluorobutanoic acid, L-2-amino-4-cyanobutyric acid (Cnba), 4-Fluoro-Leucine ((4-Fluoro)L), (S)-(trifluoromethyl)-L-cysteine, (2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid, L-tert-Butylglycine ((tBu)G), 3-(Trimethylsilyl)-L-alanine, 2,5-difluoro-L-phenylalanine, 2-Amino-7-(tert-butoxy)-7-oxoheptanoic acid, 5,5,5-Trifluoro-L-leucine ((Trifluoro)L), 2-Methyl-L-phenylalanine ((2-Me)F), L-Cyclobutylalanine (Cba), L-Cyclopentylalanine (Cpa), L-cyclopropylmethylalanine, L-trifluoromethylalanine, L-difluoromethylalanine, 2-Fluoro-L-phenylalanine ((2-Fluoro)F), (2S)-3-(2,3-difluorophenyl)-2-aminopropanoic acid, (2S)-3-(3-Cyanophenyl)-2-aminopropanoic acid, and (2S)-3-(indol-4-yl)-2-(amino)propanoic acid,
- 10 15 20 X⁸ represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of (1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid, L-Hydroxyproline (Hyp), (3S)-Morpholine-3-carboxylic acid (Morpholine-3-carboxylic), L-Pipecolic acid (Pip), (4aR,6aR,9S,11aS)-11-oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid, trans-4-fluoroproline ((trans-4-Fluoro)P) and (1R,2S,5S)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid,
- 25 30 X⁹ represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), L-Hydroxyproline (Hyp), (2S,4S)-4-Trifluoromethyl-pyrrolidine-2-carboxylic acid ((4-CF₃)P), (2S,4S)-4-fluoroproline ((cis-4-Fluoro)P), trans-4-fluoroproline ((trans-4-Fluoro)P), (2S)-2-amino-4,4,4-trifluorobutanoic acid, L-trans-3-hydroxyproline ((3S-OH)P), (1R,3S,5R)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid, (6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid, rel-(1R,3R,5R,6R)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid, (2S)-2-Amino-4,4,4-trifluorobutanoic acid, (2S,3aS,6aS)-octahydrocyclopenta[b]pyrrole-2-carboxylic acid, trans-4-fluoroproline ((trans-4-Fluoro)P), (2S,4S)-4-fluoroproline ((cis-4-Fluoro)P), L-4,4-difluoroproline ((Difluoro)P), rel-(3R,6R)-1,1-difluoro-5-azaspiro[2.4]heptane-6-

carboxylic acid (enantiomer 1) and rel-(3R,6R)-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2),

X¹⁰ represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of L-Cyclopentylglycine (Cpg), L-Cyclohexylglycine (Chg), (S)-2-amino-3-ethyl-pentanoic acid, 3-Chlorophenylglycine ((3-Chloro-Ph)G), L-tert-butylglycine, allo-L-Isoleucine (allo-I), L-Cyclobutylglycine, L-Norvaline (Nva) and (2S)-2-(Amino)-2-[(1S,3R)-3-hydroxycyclohexyl]acetic acid,

X¹¹ represents the natural amino acid C, or an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen),

X¹² represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of allo-L-Isoleucine (allo-I), (S)-2-Amino-2-cyclobutylacetic acid (Cbg), (2S,3S)-2-((amino)methyl)-3-methylpentanoic acid, L-Phenylglycine (Phg), 2-[(1S,2S)-1-(amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid, 2-methyl-D-alloisoleucine, L-Norvaline (Nva), L-2-Aminobutyric acid (Abu), L-tert-butylglycine and Aminoisobutyric acid (Aib),

and in case u and t and s are all 0, the terminal carboxyl group of X¹² is unsubstituted or amidated,

X¹³ represents a natural amino acid selected from a list consisting of P, A, S, T, G, D, E, Q or N or an unnatural amino acid selected from a list consisting of N-Methyl-Glycine ((N-Me)G), 5-azaspiro[2.4]heptane-6-carboxylic acid, L-2-Aminobutyric acid (Abu), 2-Aminoisobutyric acid (Aib), 2-Methyl-L-Proline (2-Me)P, Hydroxyproline (Hyp), 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), trans-4-fluoroproline ((trans-4-Fluoro)P), (2S,4S)-4-fluoroproline ((cis-4-Fluoro)P), L-4,4-difluoroproline ((Difluoro)P), L-Cyclopentylglycine (Cpg), (S)-2-Amino-2-cyclobutylacetic acid (Cbg) and (2S)-Pyrrolidin-2-ylacetic acid (beta-homo-P),

whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration,

and in case u and t are both 0 and s is different from 0, the terminal carboxyl group of X¹³ is unsubstituted or amidated,

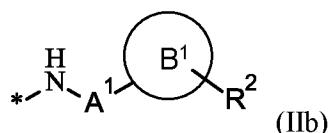
s represents an integer of from 0 to 3,

X¹⁴ represents any natural amino acid or an unnatural amino acid, whereas any natural amino acid and/or unnatural amino acid can be in D- or L-stereoconfiguration,

and in case u is 0 and t is different from 0, the terminal carboxyl group of X¹⁴ is unsubstituted or amidated,

t represents an integer of from 0 to 4,

X¹⁵ represents a group of the formula (IIb)



wherein

5 * marks the bond to a terminal carboxyl group of the adjoining amino acid,

A¹ is a bond or C₁-C₆-alkylene, wherein one CH₂ group in C₁-C₆-alkylene may be exchanged for -O- or -S-, and wherein C₁-C₆-alkylene is up to trisubstituted identically or differently by a radical selected from the group consisting of hydroxyl, methoxy, ethoxy, carboxy, amino and halogen,

10 B¹ is absent, aryl, heteroaryl, C₃-C₈-cycloalkyl or C₃-C₇-heterocycloalkyl,

wherein aryl, heteroaryl, C₃-C₈-cycloalkyl and C₃-C₇-heterocycloalkyl can be up to trisubstituted identically or differently by a radical selected from the group of C₁-C₄-alkyl, hydroxyl, methoxy, ethoxy, carbonyl, carboxy, amino and halogen, and

15 R² is hydrogen, halogen, amino, hydroxyl or C₁-C₂₀-alkyl, wherein C₁-C₂₀-alkyl is up to trisubstituted identically or differently by a radical selected from the group consisting of hydroxyl, carboxy, amino and halogen,

u represents the integer 0 or 1,

with the proviso that at least one of X¹ to X¹⁴ is an unnatural amino acid.

The invention further provides compounds containing a peptide of the following formula (II),

20 (X¹)_q(X²)_rX³X⁴X⁵X⁶X⁷X⁸X⁹X¹⁰X¹¹X¹²(X¹³)_s(X¹⁴)_t (II),

or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein

X¹ represents a natural amino acid or an unnatural amino acid,

q represents the integer 0 or 1,

X² represents the natural amino acid I,

r represents the integer 0 or 1,

X³ represents the natural amino acid C or the unnatural amino acid L-Penicillamine (Pen),

X⁴ represents the natural amino acid S,

5 X⁵ represents the natural amino acid R or the unnatural amino acid N(5)-Methyl-L-arginine ((Me)R),

X⁶ represents the natural amino acid S,

X⁷ represents the natural amino acid L or the unnatural amino acid L-tert-Butylalanine ((tBu)A),

X⁸ represents the natural amino acid P or the unnatural amino acid L-Proline (3,4-₂H),

10 X⁹ represents the natural amino acid P or the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic),

X¹⁰ represents the natural amino acid I,

X¹¹ represents an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen)

15 X¹² represents the natural amino acid I,

X¹³ represents the natural amino acid P,

s represents the integer 0 or 1,

X¹⁴ represents a natural amino acid selected from a list consisting of D, Q and E,

t represents the integer 0 or 1,

20 wherein the N-terminus of the peptide is unsubstituted, acetylated or mono- or disubstituted with C₁-C₂₀-alkyl,

wherein the C-terminus of the peptide is unsubstituted or amidated, and

wherein the peptide is cyclized, preferably via a linkage connecting X³ and X¹¹.

25 Unless otherwise defined herein, scientific and technical terms used in this application shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature

used in connection with, and techniques of, chemistry, molecular biology, cell and cancer biology, immunology, microbiology, pharmacology, and protein and nucleic acid chemistry, described herein, are those well-known and commonly used in the art.

Throughout this specification, the word “comprise” or variations thereof such as “comprises” or “comprising” will be understood to imply the inclusion of a stated integer (or components) or group of integers (or components), but not the exclusion of any other integer (or components) or group of integers (or components). The singular forms “a”, “an” and “the” include the plurals unless the context clearly dictates otherwise. The term “including” and “containing” is used to mean “including but not limited to”, which expressions can be used interchangeably. In particular, the expression “compound containing a peptide” means a compound which contains a defined peptide sequence and which can optionally contain further chemical groups or substituents covalently bound to the peptide, e.g. amino acids, fatty acids, chemical groups to enhance pharmacodynamic or pharmacokinetic properties of the peptide or any other chemical groups. It is also to be understood that the expression “compound containing a peptide” explicitly includes the defined peptide sequence without any further chemical groups or substituents covalently bound to that peptide.

As used herein, the following terms have the meanings ascribed to them unless specified otherwise. “Essentially consisting of” is understood as a peptide being at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the peptide it is compared to.

The terms “protein”, “polypeptide” and “peptide” are used interchangeably to refer broadly to a sequence of two or more amino acids linked together, preferable by peptide (amide) bonds. Peptide (amide) bonds are formed when the carboxyl group of one amino acid reacts with the amino group of another. It should be further understood that the terms “protein”, “polypeptide” and “peptide” do not indicate a specific length of a polymer of amino acids, nor is it intended to imply or distinguish whether the polypeptide is produced using recombinant techniques, chemical or enzymatic synthesis, or is naturally occurring. It should be further understood, that a peptide can contain one or more parts which are no amino acids under the definition of the present application. These parts are preferably present at the N- and C-terminal ends of the peptide.

The term “amino acid” or “any amino acid” as used herein refers to organic compounds containing amine (-NH₂) and carboxyl (-COOH) functional groups, along with a side chain and refers to any and all amino acids, including naturally occurring amino acids (e.g., α -L-amino acids), unnatural amino acids, modified amino acids, and non-natural amino acids. “Natural amino acids” include those found in nature, such as, e.g., the 23 amino acids that combine into peptide chains to form the building-blocks of a vast array of proteins. These are primarily L stereoisomers, although a few D-amino acids occur in bacterial envelopes and some antibiotics. The 20 proteinogenic, natural amino acids in the standard genetic code are listed in

Table 2. The “non-standard” natural amino acids are pyrrolysine (found in methanogenic organisms and other eukaryotes), selenocysteine (present in many non-eukaryotes as well as most eukaryotes), and N-formylmethionine (encoded by the start codon AUG in bacteria, mitochondria and chloroplasts).

“Unnatural” or “non-natural” amino acids are non-proteinogenic amino acids (i.e., those not naturally encoded or found in the genetic code) that either occur naturally or are chemically synthesized. Over 140 natural amino acids are known and thousands of more combinations are possible. Examples of “unnatural” amino acids include β -amino acids (β^3 and β^2), homo-amino acids, proline and pyruvic acid derivatives, 3-substituted alanine derivatives, glycine derivatives, ring-substituted phenylalanine and tyrosine derivatives, linear core amino acids, diamino acids, D-amino acids, and N-methyl amino acids. Unnatural or non-natural amino acids also include modified amino acids. “Modified” amino acids include amino acids (e.g., natural amino acids) that have been chemically modified to include a group, groups, or chemical moiety not naturally present in the amino acid. According to the present invention preferred unnatural amino acids are listed in Table 1. Table 1 displays unnatural amino acids as D- and/or L-stereoisomers, however preferred unnatural amino acids according to the invention are both D- and L-stereoisomers of unnatural amino acids listed in Table 1.

Table 1: Preferred unnatural amino acids

(1R,2R)-2-Amino-1-cyclopentanecarboxylic acid (R,R-ACPC)
(1R,3S)-3-(Amino)cyclopentanecarboxylic acid
(1R,3S,5R)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid
(1S,2S)-2-Amino-1-cyclopentanecarboxylic acid (S,S-ACPC)
(1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid
(1R,2S,5S)-3-Azabicyclo[3.1.0]hexane-2-carboxylic acid
(1S,3R)-3-(Amino)cyclopentanecarboxylic acid
(1S,3R)-3-(Amino)cyclopentanecarboxylic acid
(1S,3R,4R)-2-Azabicyclo[2.2.1]heptane-3-carboxylic acid
(2S)-2-(Amino)-2-[(1S,3R)-3-hydroxycyclohexyl]acetic acid
(2S)-2-(Amino)-2-[(1S,3S)-3-hydroxycyclohexyl]acetic acid
(2R)-Amino-(1-methyl-1H-indazol-5-yl)acetic acid
(2S)-2-Amino-5-methyl-hexanoic acid
(2S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-4-methylpentanoic acid
(2S)-2[(amino)-2-(tetrahydro-2H-pyran-4-yl)]acetic acid
(2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid
(2S)-2-amino-3-(2,3,4,5,6-pentafluorophenyl)propanoic acid
(2S)-2-amino-3-(4-tert-butylphenyl)propanoic acid

(2S)-2-Amino-4-(benzylamino)-4-oxobutanecarboxylic acid
(2S)-2-amino-4,4,4-trifluorobutanoic acid
(2S)-2-Amino-5-methyl-hexanoic acid
(2S)-3-(2,3-difluorophenyl)-2-aminopropanoic acid
(2S)-3-(3-Cyanophenyl)-2-aminopropanoic acid
(2S)-3-(4-carboxyphenyl)-2-aminopropanoic acid
(2S)-3-(indol-4-yl)-2-(amino)propanoic acid
(2S)-3-(Triazol-1-yl)-2-(amino)propanoic acid
(2S)-Amino-(1-methyl-1H-indazol-5-yl)acetic acid
(2S)-Amino-2-[3-(Trifluoromethyl)bicyclo[1.1.1]pent-1-yl]acetic acid
(2S)-Pyrrolidin-2-ylacetic acid (beta-homo-P)
(2S,3aS,6aS)-octahydrocyclopenta[b]pyrrole-2-carboxylic acid
(2S,3S)-2-((Amino)methyl)-3-methylpentanoic acid
(2S,3S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-3-methylpentanoic acid
(2S,3S)-2-[(3S)-2-oxopiperazin-1-yl]-3-methylpentanoic acid
(2S,4S)-4-fluoroproline ((cis-4-Fluoro)P)
(2S,4S)-4-Trifluoromethyl-pyrrolidine-2-carboxylic acid ((4-CF ₃)P)
(3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1)
(3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2)
(4aR,6aR,9S,11aS)-11-oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid
(6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid
(R)-3-Aminoadipic acid
(R)-4-Amino-6-methylheptanoic acid
(R)-Piperidine-3-Carboxylic Acid
(R)-Pyrrolidine-3-Carboxylic Acid
(S)-(1-Piperidin-3-yl)-acetic acid
(S)-(trifluoromethyl)-L-cysteine
(S)-2-(Amino)-1,6-hexanedioic acid (AAD)
(S)-2-Amino-2-cyclobutylacetic acid (Cbg)
(S)-2-Amino-3-ethyl-pentanoic acid
(S)-3-(1-Pyrrolidine-2-yl)-propionic acid
(S)-4-Piperazine-2-carboxylic acid
(S)-Piperidine-3-carboxylic acid
(S)-Pyrrolidine-2-carboxylic acid (beta-P)
[(2R)-4,4-Difluoropyrrolidin-2-yl]acetic acid
1-(Aminomethyl)-cyclopropyl-1-carboxylic acid

1,13-Diamino-4,7,10-trioxatridecan-succinamic acid
12-Amino-4,7,10-trioxadodecanoic acid
14-Amino-3,6,9,12-tetraoxatetradecanoic acid
15-Amino-4,7,10,13-tetraoxa(Pen)tadecanoic acid
17-Amino-3,6,9,12,15-(Pen)taoxaheptadecanoic acid
18-Amino-4,7,10,13,16-(Pen)taoxaoctadecanoic acid
1-Amino-3,6,9,12,15,18,21,24,27-nonaoxatriacontan-30-oic acid
1-Amino-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid
1-Amino-3,6,9,12,15,18,21-heptaoxatetracosan-24-oic acid
1-Amino-3,6,9,12,15,18-hexaoxahenicosan-21-oic acid
1-Aminocyclobutane-1-carboxylic acid (ACBA)
1-Benzyl-L-histidine (H(1-Bn))
1-Methyl-L-histidine (H(1-Me))
2-(Cyclohexylamino)acetic acid
2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic)
2,5-difluoro-L-phenylalanine
2-[(1S,2S)-1-(amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid
2-Amino-1,7-heptanedioic acid
2-Amino-5,5,5-trifluoro-4-methyl-pentanoic acid
2-Amino-7-(tert-butoxy)-7-oxoheptanoic acid
2-Aminoisobutyric acid (Aib)
2-Chloro-L-phenylalanine ((2-Chloro)F)
2-Fluoro-L-phenylalanine ((2-Fluoro)F)
2-Methyl-D-alloisoleucine
2-Methyl-L-phenylalanine ((2-Me)F)
2-Methyl-L-Proline (2-Me)P,
3-(1,3-Benzothiazol-2-yl)-L-alanine ((Bth)A)
3-(Aminomethyl)benzoic acid
3-(Trimethylsilyl)-L-alanine
3-Amino-2,2-dimethylpropionic acid
3-Aminomethylphenylacetic acid
3-Azido-L-Alanine
3-Carboxyphenylalanine
3-Chloro-L-Phenylalanine
3-Chlorophenylglycine ((3-Chloro-Ph)G)
3-Cyano-L-phenylalanine

3-Ethyl-L-Norvaline
3-Fluoro-L-phenylalanine
3-Methyl-L-phenylalanine
4-(3,5-Dimethyl-1,2-oxazol-4-yl)-L-phenylalanine
4-(Aminomethyl)benzoic acid
4-Aminomethylphenylacetic acid
4-Ethyl-L-norleucine
4-Fluoro-Leucine ((4-Fluoro)L)
4-Fluoro-L-phenylalanine ((4-Fluoro)F)
5,5,5-Trifluoro-L-leucine ((Trifluoro)L)
5-azaspiro[2.4]heptane-6-carboxylic acid
6-Aminohexanoic acid (Ahx)
8-Aminocubane-1-carboxylic acid
9-Amino-4,7-dioxanonanoic acid
allo-L-Isoleucine (allo-I)
allo-L-Threonine (allo-T)
Aminocyclobutanecarboxylic acid (ACBC)
Aminoisobutyric acid (Aib)
beta-Alanine (beta-A)
Cyclohexylalanine (Cha)
D-2-Chlorophenylalanine
D-beta-Proline
D-cyclohexylalanine
D-Hydroxyproline
D-N-Methylalanine
Gamma-Aminobutyric acid (Gamma-Abu)
Hydroxyproline (Hyp)
Iminodiacetic acid
L- Homoserine (hSer)
L-1-Napthylalanine (1-Nal)
L-2,3-Diaminopropionic acid (Dap)
L-2,4-Diaminobutyric acid (Dab)
L-2,6-Difluorophenylalanine
L-2-Amino-4-cyanobutyric acid
L-2-Aminobutyric acid (Abu)
L-2-Bromophenylalanine ((2-Bromo)F)

L-2-Naphthylalanine (2-Nal)
L-2-Pyridylalanine (2-Pal)
L-2-Thienylalanine
L-3-Bromophenylalanine ((3-Bromo)F)
L-3-Methylhistidine (H(3-Me))
L-3-Pyridylalanine (3-Pal)
L-4,4-difluoroproline ((Difluoro)P)
L-4-Aminophenylalanine ((4-Amino)F)
L-4-Bromophenylalanine
L-4-Pyridylalanine
L-Citrulline (Cit)
L-Cyclobutylalanine (Cba)
L-Cyclobutylglycine
L-Cyclohexylalanine
L-Cyclohexylglycine
L-Cyclohexylglycine (Chg)
L-Cyclopentylalanine
L-cyclopentylalanine (Cpa)
L-Cyclopentylglycine (Cpg)
L-Cyclopropylmethylalanine
L-Difluoromethylalanine
L-Dihydroorotic acid (Hoo)
L-Homocysteine
L-Hydroxyproline (Hyp)
L-Methionine-L-sulfoxide
L-Methionine-sulfone
L-N,N-Dimethylalanine ((N,N-diMe)A)
L-N-Methylalanine
L-N-Methylcysteine ((N-Me)C)
L-N-Methylisoleucine ((N-Me)I)
L-N-Methylphenylalanine ((N-Me)F)
L-Norleucine (Nle)
L-Norvaline (Nva)
L-Ornithine (Orn)
L-Penicillamine (Pen)
L-Phenylglycine (Phg)

L-Pipecolic acid (Pip)
L-Propargylglycine
L-Pyroglutamic acid (Pyr)
L-tert-Butylalanine ((tBu)A)
L-tert-Butylglycine ((tBu)G)
L-trans-3-Hydroxyproline ((3S-OH)P)
L-Trifluoromethylalanine
Morpholine-3-carboxylic
N(5)-methyl-L-arginine ((Me)R)
N-e-Isopropyl-L-lysine
N-Methyl-Alanine (N-Me)A
N-Methyl-Glycine ((N-Me)G)
N-Phenylglycine ((N-Ph)G)
Palmitic acid (Palm)
rel-(1R,2S)-2-Amino-1-cyclopentanecarboxylic acid (ACPC)
rel-(1R,3R,5R,6R)-6-(Trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid
rel-(1R,3S)-3-[(Amino)methyl]cyclohexanecarboxylic acid
rel-(3R,6R)-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1)
rel-(3R,6R)-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2)
S-2-amino-3-ethyl-pentanoic acid
S-3-1-Pyrrolidin-2-yl-propionic acid
Tranexamic acid (Tranexamic)
trans-2-(3-(Amino)cyclohexyl)acetic acid
trans-4-Fluoroproline ((trans-4-Fluoro)P)
3-Amino-3-methylbutyric acid

More preferred unnatural amino acid are selected from a list consisting of N-Methyl-Alanine (N-Me)A, N-Methyl-Glycine ((N-Me)G), (1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid, L-3-Bromophenylalanine ((3-Bromo)F), L-N,N-Dimethylalanine ((N,N-diMe)A), N,N-Dimethylglycine ((N,N-diMe)G), N-Phenylglycine ((N-Ph)G), (R)-Piperidine-3-carboxylic acid, (S)-Piperidine-3-carboxylic acid, L-tert-Butylalanine ((tBu)A), L-2-Pyridylalanine (2-Pal), L-3-Pyridylalanine (3-Pal), L-4-Pyridylalanine (4-Pal), 3-(Aminomethyl)benzoic acid, 3-Amino-2,2-dimethylpropionic acid, 3-Amino-3-methylbutyric acid, 4-(Aminomethyl)benzoic acid, L-2-Aminobutyric acid (Abu), 1-Aminocyclobutane-1-carboxylic acid (ACBA), 6-Aminohexanoic acid (Ahx), 2-Aminoisobutyric acid (Aib), L-2-Thienylalanine (beta-2-thienylalanine), beta-Alanine (beta-A), beta-Proline (beta-P), L-Citrulline (Cit), L-2,4-Diaminobutyric acid (Dab), L-2,3-Diaminopropionic acid (Dap), Gamma-Aminobutyric acid (Gamma-Abu), L-3-Methylhistidine (3-Me)H,

L-Dihydroorotic acid (Hoo), L-Norleucine (Nle), N-Methyl-L-proline ((N-Me)P), L-Norvaline (Nva), L-Ornithine (Orn), L-Pipecolic acid (Pip), (2S)-2[(Amino)-2-(tetrahydro-2H-pyran-4-yl)]acetic acid; 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), L-N-Methylcysteine ((N-Me)C), N(5)-methyl-L-arginine ((Me)R), L-Penicillamine (Pen) and Tranexamic acid (Tranexamic).

- 5 Most preferred unnatural amino acid are selected from a list consisting of N-Methyl-L-Alanine (N-Me)A, N-Methyl-Glycine ((N-Me)G), L-Norleucine (Nle), L-Norvaline (Nva), L-Ornithine (Orn), N(5)-methyl-L-arginine ((Me)R), L-tert-Butylalanine ((tBu)A), 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen).

10 It should be further understood, that a peptide according to the invention can contain one or more chemical groups which are no amino acid under the definition of the present invention. These chemical groups can be present at the N- and/or C-terminal ends of a peptid and are represented by formula X^0 and X^{15} . It should be understood that all amino acids and chemical groups of the peptids of the present invention are connected via peptide (amide) bonds. Generally peptides are formed by linking α -amino and carboxy groups of α -amino acids, which are then linked by α -peptide bonds. According to the present invention a peptide bond
15 can be formed by any carboxyl- and amino group being present in a respective natural or unnatural amino acid. For example, α -amino acids which contain a second amino group in addition to the α -amino group (e.g. L-lysine) or α -amino acids which, in addition to the α -carboxy group, contain a second carboxy group, (eg. L-aspartic acid and L-glutamic acid) can be connected via the additional amino- or carboxy group.

In accordance with the understanding of a person skilled in the art, the peptide sequences disclosed herein
20 represent sequences of amino acids, which are connected via α -peptide bonds. An amino acid linked via a peptide bond, which is not an α -peptide bond, are marked by a “*”. The “*” is either on the left or the right side of the amino acid, to illustrate wether the additional amino group or the additional carboxy group of that amino acid is used for the peptide bond to the adjoining amino acid (e.g. (*L), (E*), (*Dap) etc.).

In accordance with the understanding of a person skilled in the art, the peptide sequences disclosed herein
25 are shown proceeding from left to right, with the left end of the sequence being the “N-terminus” (“amino terminus”, “N-terminal end”) of the peptide and the right end of the sequence being the “C-terminus” (“carboxy terminus”, “C-terminal end”) of the peptide. This terminology N-terminus (amino terminus, N-terminal end)” applies irrespective of whether the peptide actually contains an amino group at the N-terminus. This terminology C-terminus (carboxy terminus, C-terminal end) applies irrespective of whether
30 the peptide actually contains a carboxy group at the C-terminus. The term “terminal amino group” refers to any amino group present at the N-terminus. The term “terminal carboxyl group” refers to any carboxyl group present at the C-terminus.

According to the present invention the N-terminus can be formed by X^0 , in case p represents 1. Alternatively the N-terminus can be formed by X^1 , in case q represents at least 1 and p represents 0. Alternatively

the N-terminus can be formed by X^2 , in case r represents 1 and p and q both represent 0. In case p, q and r all represent 0, the N-terminus is formed by X^3 in case of a linear peptide. In case the peptide of the present invention is cyclized via a linkage connecting X^3 and X^{11} and p, q and r all represent 0, the peptid does not comprise an N-terminus.

- 5 According to the present invention the C-terminus can be formed by X^{15} , in case u represents 1. Alternatively the the C-terminus can be formed by X^{14} , in case t represents an integer of at least 1 and u represents 0. Alternatively the C-terminus can be formed by X^{13} in case s represents 1 and t and u both represent 0. In case s, t and u all represent 0, the C-terminus is formed by X^{12} .

10 In the present invention the names of naturally occurring and non-naturally occurring aminoacyl residues used herein are preferably following the naming conventions suggested by the IUPAC Commission on the Nomenclature of Organic Chemistry and the IUPAC-IUB Commission on Biochemical Nomenclature as set out in *Nomenclature of α -Amino Acids (Recommendations, 1974)*, *Biochemistry*, 14(2), (1975).

15 In the present specification naturally occurring proteinogenic amino acids are usually designated by their conventional single-letter abbreviations. Alternatively, they can also be referred to by their three-letter abbreviations (e.g. in particular in the sequence listings) or by their full name as shown in Table 2 below:

Table 2: Standard Abbreviations for Natural Amino Acids

3-Letter	1-Letter	Amino Acid
Ala	A	Alanine
Arg	R	Arginine
Asn	N	Asparagine
Asp	D	Aspartic acid
Cys	C	Cysteine
Glu	E	Glutamic acid
Gln	Q	Glutamine
Gly	G	Glycine
His	H	Histidine
Ile	I	Isoleucine

3-Letter	1-Letter	Amino Acid
Leu	L	Leucine
Lys	K	Lysine
Met	M	Methionine
Phe	F	Phenylalanine
Pro	P	Proline
Ser	S	Serine
Thr	T	Threonine
Trp	W	Tryptophan
Tyr	Y	Tyrosine
Val	V	Valine

20 In the case of non-proteinogenic or non-naturally occurring amino acids, unless they are referred to by their full name (e.g. ornithine, etc.), frequently employed three- to six-character codes are employed for residues thereof, including those abbreviations as indicated in the abbreviation list below (Table 3).

The term "L-amino acid" as used herein refers to the "L" isomeric form of an amino acid, and conversely the term "D-amino acid" refers to the "D" isomeric form of an amino acid. It is further a conventional

manner to indicate the L-amino acid with capital letters such as Ala / A, Arg / R, etc. and the D-amino acid with small letters such as ala / a, arg / r, etc.

The three-letter code in the form as indicated in Table 2 above, i.e. Ala, Arg, Asn etc. and as generally used in the present specification, shall generally comprise the D- and L- form as well as homo- and nor-forms, unless explicitly indicated otherwise. The prefix “nor” refers to a structural analog that can be derived from a parent compound by the removal of one carbon atom along with the accompanying hydrogen atoms. The prefix “homo” indicates the next higher member in a homologous series. A reference to a specific isomeric form will be indicated by the capital prefix L- or D- as described above (e.g. D-Arg, L-Arg etc.). A specific reference to homo- or nor-forms will accordingly be explicitly indicated by a respective prefix (e.g. homo-Arg, homo-R, nor-Arg, nor-R, homo-Cys, homo-C etc.).

The term “C₁-C₆-alkyl” means a linear or branched, saturated, monovalent hydrocarbon group having 1, 2, 3, 4, 5 or 6 carbon atoms, e.g. a methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl, tert-butyl, pentyl, isopentyl, 2-methylbutyl, 1-methylbutyl, 1-ethylpropyl, 1,2-dimethylpropyl, neo-pentyl, 1,1-dimethylpropyl, hexyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 1-ethylbutyl, 2-ethylbutyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 2,3-dimethylbutyl, 1,2-dimethylbutyl or 1,3-dimethylbutyl group, or an isomer thereof. Particularly, said group has 1, 2, 3 or 4 carbon atoms (“C₁-C₄-alkyl”), e.g. a methyl, ethyl, propyl, isopropyl, butyl, sec-butyl isobutyl, or tert-butyl group, more particularly 1, 2 or 3 carbon atoms (“C₁-C₃-alkyl”), e.g. a methyl, ethyl, n-propyl or isopropyl group. Particularly preferred is methyl, ethyl, n-propyl. Most preferred is methyl.

The term “C₁-C₂₀-alkyl” means a linear or branched, saturated, monovalent hydrocarbon group having 1, to 20 carbon atoms, e.g. a methyl, ethyl, propyl, isopropyl, butyl, sec-butyl isobutyl, tert-butyl or pentyl, isopentyl, hexyl, isohexyl, heptyl, isoheptyl, octyl and isooctyl, nonyl, decyl, dodecyl or eicosyl.

The term “C₁-C₄-alkylene” means a straight-chain or branched hydrocarbon bridge having 1 to 4 carbon atoms, e.g. methylene, ethylene, propylene, (α -methylethylene, β -methylethylene, α -ethylethylene, β -ethylethylene, butylene, α -methylpropylene, β -methylpropylene and γ -methylpropylene.

The term “C₁-C₆-alkylene” means a straight-chain or branched hydrocarbon bridge having 1 to 6 carbon atoms, e.g. methylene, ethylene, propylene, (α -methylethylene, β -methylethylene, α -ethylethylene, β -ethylethylene, butylene, α -methylpropylene, β -methylpropylene, γ -methylpropylene, α -ethylpropylene, β -ethylpropylene, γ -ethylpropylene, pentylene and hexylene.

The term “C₃-C₈-cycloalkyl” means a saturated hydrocarbon ring which contains 3, 4, 5, 6, 7 or 8 carbon atoms. Said C₃-C₈-cycloalkyl group is for example, a monocyclic hydrocarbon ring, e.g. a cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or cyclooctyl group, a bicyclic hydrocarbon ring, e.g. a

bicyclo[4.2.0]octyl or octahydropentalenyl, or a bridged or caged saturated ring groups such as norborane or adamantane, and cubane.

The term “C₃-C₇-heterocycloalkyl” means a saturated heterocycle with 4, 5, 6 or 7 which contains one or two identical or different ring heteroatoms from the series N, O and S, it being possible for said heterocycloalkyl group to be attached to the rest of the molecule via any one of the carbon atoms or, if present, a nitrogen atom. Said C₃-C₇-heterocycloalkyl group, without being limited thereto, can be a 4-membered ring, such as azetidiny, oxetanyl or thietanyl, for example; or a 5-membered ring, such as tetrahydrofuranyl, 1,3-dioxolanyl, thiolanyl, pyrrolidinyl, imidazolidinyl, pyrazolidinyl, 1,1-dioxidothiolanyl, 1,2-oxazolidinyl, 1,3-oxazolidinyl or 1,3-thiazolidinyl, for example; or a 6 membered ring, such as tetrahydropyranyl, tetrahydrothiopyranyl, piperidinyl, morpholinyl, dithianyl, thiomorpholinyl, piperazinyl, hexahydropyrimidinyl, 1,3-dioxanyl, 1,4-dioxanyl or 1,2-oxazinanyl, for example, or a 7 membered ring, such as azepanyl, 1,4-diazepanyl or 1,4-oxazepanyl, for example.

The term “aryl” means an unsaturated or partially unsaturated cycle having 6 to 10 carbon atoms. Preferred aryl radicals are phenyl and naphthyl.

The term “heteroaryl” means a monovalent, monocyclic, bicyclic or tricyclic aromatic ring having 5, 6, 8, 9, 10, 11, 12, 13 or 14 ring atoms (a “5 to 14 membered heteroaryl” group), particularly 5, 6, 9 or 10 ring atoms, which contains at least one ring heteroatom and optionally one, two or three further ring heteroatoms from the series: N, O and/or S, and which is bound via a ring carbon atom or optionally via a ring nitrogen atom (if allowed by valency). Said heteroaryl group can be a 5-membered heteroaryl group, such as, for example, thienyl, furanyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl or tetrazolyl; or a 6-membered heteroaryl group, such as, for example, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl or triazinyl; or a tricyclic heteroaryl group, such as, for example, carbazolyl, acridinyl or phenazinyl; or a 9-membered heteroaryl group, such as, for example, benzofuranyl, benzothienyl, benzoxazolyl, benzisoxazolyl, benzimidazolyl, benzothiazolyl, benzotriazolyl, indazolyl, indolyl, isoindolyl, indoliziny or purinyl; or a 10-membered heteroaryl group, such as, for example, quinolinyl, quinazolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinoxalinyl or pteridinyl.

In general, and unless otherwise mentioned, the heteroaryl or heteroarylene groups include all possible isomeric forms thereof, e.g.: tautomers and positional isomers with respect to the point of linkage to the rest of the molecule. Thus, for some illustrative non-restricting examples, the term pyridinyl includes pyridine-2-yl, pyridine-3-yl and pyridine-4-yl; or the term thienyl includes thien-2 yl-and thien-3-yl.

Among sequences disclosed herein are sequences incorporating either an “-OH” moiety or an “-NH₂” moiety at the carboxy terminus (C-terminus) of the sequence. An “-OH” or an “-NH₂” moiety at the C-terminus of the sequence indicates a hydroxy group or an amino group, corresponding to the presence of a carboxy group or an amido (-(C=O)-NH₂) group at the C-terminus, respectively. In each sequence of the

invention, a C-terminal “-OH” moiety may be substituted for a C-terminal “-NH₂” moiety, which is also referred to as “amidated C-terminus” in the present invention, and vice-versa. However, among said alternatives a C-terminal “-OH” moiety is preferred.

The term “acetylated” (also abbreviated “Ac”) refers to an acetyl protection of the N-terminal moiety through acetylation of the N-terminus of a peptide (N-terminus of the peptide is acetylated).

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein

X⁰ is a chemical group selected from the list consisting of, (1S,2S,4S)-Bicyclo[2.2.1]hept-5-en-2-ylacetic acid, (2,4-Dioxoimidazolidin-1-yl)acetic acid, 2-(Thiomorpholine)acetic acid ((2-Thiomorpholine)acetyl), 2-(N-Isopropyl-N-methylamino)acetic acid, (S)-3-Methylvaleric acid ((S)-3-Methylpentanoic acid), 2-(3-Pyridyl)acetic acid, 2-(Cyclohexylamino)acetic acid (2-(Cyclohexylamino)acetyl), 2-(Diethylamino)acetic acid (2-(Diethylamino)acetyl), 2-(Morpholine)acetic acid (2-(Morpholine)acetyl), 2-(N-Methyl-N-cyclopropylamino)acetic acid (2-(N-Methyl-N-cyclopropylamino)acetyl), 2-(Piperidin)acetic acid (2-(Piperidin)acetyl), 2-(Pyrrolidine)acetic acid (2-(Pyrrolidine)acetyl), 2-Hydroxyacetic acid (2-Hydroxyacetyl), 2-Hydroxyisobutyric acid (2-Hydroxyisobutyric), 3-(Aminomethyl)benzoic acid, 3-Methoxypropionic acid, 4-(Aminomethyl)benzoic acid, 4-Methylpentanoic acid (4-Methylvaleric), 5-Chorothiophene-carboxylic acid, 1-(Aminomethyl)-cyclopropyl-1-carboxylic acid (ACMP), Adipic acid, (S)-Azetidine-2-carboxylic acid, Benzoic acid (Benzoic), 4-(3,5-Dimethyl-1,2-oxazol-4-yl)-L-phenylalanine, Cyclobutanecarboxylic acid (Cyclobutanecarboxylic), 2-(Cyclobutyl)acetic acid (Cyclobutylacetic), Cyclobutylacetic acid (Cyclobutylacetic), Cyclohexylacetic acid (Cyclohexylacetic), Cyclohexanecarboxylic acid (Cyclohexylcarboxylic), Cyclopentanecarboxylic acid, Cyclopentylacetic acid (Cyclopentylacetic), Cyclopropanecarboxylic acid (Cyclopropanecarboxylic), Cyclopropylacetic acid, D-(+)-Biotin, Fumaric acid, 3-Phenylpropanoic acid (Hydrocinnamic), Isobutyric acid, Isovaleric acid (Isovaleric), L-(+)-Lactic acid (Lactic), Phenylacetic acid, Piperidin-4-ylacetic acid, Pivalic acid (Pivalic), Suberic acid, tert-Butylacetic acid, Tetrahydropyranyl-4-acetic acid, Tetrahydro-2H-pyran-3-ylacetic acid and trans-2-(3-((t-butoxy)carbonylamino)cyclohexyl)acetic acid),

p represents the integer 0 or 1,

X¹ represents a natural amino acid selected from the list consisting of A, F, G, H, I, K, L, P, R, S, T, V, W and Y or an unnatural amino acid selected from a list consisting of N-Methyl-Alanine (N-Me)A, N-Methyl-Glycine ((N-Me)G), (1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid, L-3-Bromophenylalanine ((3-Bromo)F), L-N,N-Dimethylalanine ((N,N-diMe)A), N,N-Dimethylglycine ((N,N-diMe)G), N-Phenylglycine ((N-Ph)G), (R)-Piperidine-3-carboxylic acid, (S)-Piperidine-3-carboxylic acid, L-tert-Butylalanine ((tBu)A), L-2-Pyridylalanine (2-Pal), L-3-Pyridylalanine (3-Pal), L-4-

Pyridylalanine (4-Pal), 3-(Aminomethyl)benzoic acid, 3-Amino-2,2-dimethylpropionic acid, 3-Amino-3-methylbutyric acid, 4-(Aminomethyl)benzoic acid, L-2-Aminobutyric acid (Abu), 1-Aminocyclobutane-1-carboxylic acid (ACBA), 6-Aminohexanoic acid (Ahx), 2-Aminoisobutyric acid (Aib), L-2-Thienylalanine (beta-2-thienylalanine), beta-Alanine (beta-A), beta-Proline (beta-P), L-Citrulline (Cit),
 5 L-2,4-Diaminobutyric acid (Dab), L-2,3-Diaminopropionic acid (Dap), Gamma-Aminobutyric acid (Gamma-Abu), L-3-Methylhistidine (3-Me)H, L-Dihydroorotic acid (Hoo), L-Norleucine (Nle), N-Methyl-L-proline ((N-Me)P), L-Norvaline (Nva), L-Ornithine (Orn), L-Pipecolic acid (Pip), (2S)-2[(Amino)-2-(tetrahydro-2H-pyran-4-yl)]acetic acid and Tranexamic acid (Tranexamic),

whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration,

q represents an integer of from 0 to 5,

X² represents the natural amino acid I or an unnatural amino acid selected from a list consisting of L-N-Methylisoleucine ((N-Me)I), allo-L-Isoleucine (allo-I), L-Cyclobutylalanine (Cba), L-Norvaline (Nva), L-2-Aminobutyric acid (Abu), (2S,3S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-3-methylpentanoic acid, (2S,3S)-2-[2-Oxopiperazin-1-yl]-3-methylpentanoic acid and (2S,3S)-2-[(3S)-2-oxopiperazin-1-yl]-3-methylpentanoic acid,

whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration,

r represents the integer 0 or 1,

X³ represents the natural amino acid C or the unnatural amino acid L-Penicillamine (Pen),

X⁴ represents a natural amino acid selected from a list consisting of S, C, T, R and K,

X⁵ represents the natural amino acid R or the unnatural amino acid N(5)-methyl-L-arginine ((Me)R),

X⁶ represents the natural amino acid S, or an unnatural amino acid selected from a list consisting of allo-L-Threonine (allo-T) and L-2,3-diaminopropionic acid (Dap),

X⁷ represents a natural amino acid selected from a list consisting of L and N, or an unnatural amino acid selected from a list consisting of 2,5-Difluoro-L-phenylalanine ((2,5-Difluoro)F), L-2-Bromophenylalanine ((2-Bromo)F), 2-Chloro-L-phenylalanine ((2-Chloro)F), L-4-Bromophenylalanine ((4-Bromo)F), 4-Fluoro-L-Leucine ((4-Fluoro)L), L-tert-Butylalanine ((tBu)A), L-tert-Butylglycine ((tBu)G), 5,5,5-Trifluoro-L-leucine ((Trifluoro)L), (S)-2-(Amino)-1,6-hexanedioic acid (AAD), (2S)-2-amino-4,4,4-trifluorobutanoic acid, L-2-amino-4-cyanobutyric acid (Cnba), (S)-(trifluoromethyl)-L-cysteine, (2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid, (2S)-3-(2,3-difluorophenyl)-2-aminopropanoic acid,

2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), 3-(Trimethylsilyl)-L-alanine and 2-Amino-7-(tert-butoxy)-7-oxoheptanoic acid,

X⁸ represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of (1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid, (1R,2S,5S)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid, trans-4-fluoroproline ((trans-4-Fluoro)P), L-Hydroxyproline (Hyp), (3S)-Morpholine-3-carboxylic acid (Morpholine-3-carboxylic), L-Pipecolic acid (Pip) and (4aR,6aR,9S,11aS)-11-Oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid,

X⁹ represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), (6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid, rel-(1R,3R,5R,6R)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid, (2S,4S)-4-Trifluoromethyl-pyrrolidine-2-carboxylic acid ((4-CF₃)P), (2S,3aS,6aS)-octahydrocyclopenta[b]pyrrole-2-carboxylic acid, (1R,3S,5R)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid, (2S)-2-Amino-4,4,4-trifluorobutanoic acid, L-trans-3-hydroxyproline ((3S-OH)P), trans-4-fluoroproline ((trans-4-Fluoro)P), L-Hydroxyproline (Hyp), (2S,4S)-4-Fluoroproline ((cis-4-Fluoro)P), (3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1) and (3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2),

X¹⁰ represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of (2S)-2-(Amino)-2-[(1S,3R)-3-hydroxycyclohexyl]acetic acid, 3-Chlorophenylglycine ((3-Chloro-Ph)G), (S)-2-amino-3-ethyl-pentanoic acid, allo-L-Isoleucine (allo-I), L-Cyclohexylglycine (Chg), L-Cyclopentylglycine (Cpg), L-Cyclobutylglycine and L-Norvaline (Nva),

X¹¹ represents the natural amino acid C, or an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen),

X¹² represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of 2-[(1S,2S)-1-(amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid, (S)-2-Amino-2-cyclobutylacetic acid (Cbg), allo-L-Isoleucine (allo-I), L-Phenylglycine (Phg), 2-methyl-D-alloisoleucine and (2S,3S)-2-((amino)methyl)-3-methylpentanoic acid,

and in case u and t and s are all 0, the terminal carboxyl group of X¹² is unsubstituted or amidated,

X¹³ represents a natural amino acid selected from a list consisting of P, A and D or an unnatural amino acid selected from a list consisting of 2-Methyl-L-Proline (2-Me)P, N-Methyl-Glycine ((N-Me)G), trans-4-fluoroproline ((trans-4-Fluoro)P), L-2-Aminobutyric acid (Abu), 2-Aminoisobutyric acid (Aib), Hydroxyproline (Hyp) and 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic),

whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration,

and in case u and t are both 0 and s is different from 0, the terminal carboxyl group of X¹³ is unsubstituted or amidated,

5 s represents an integer of from 0 to 3,

X¹⁴ represents a natural amino acid selected from a list consisting of D, E, G, K, N, P and Q or an unnatural amino acid selected from a list consisting of 3-Carboxyphenylalanine ((3-Carboxy)F), (2S)-2-Amino-4-(benzylamino)-4-oxobutanecarboxylic acid ((N-Benzyl)D), N-Methyl-Glycine ((N-Me)G), 6-Aminohexanoic acid (Ahx), (2S)-Pyrrolidin-2-ylacetic acid (beta-homo-P), L-2,3-Diaminopropionic acid (Dap), L-Ornithine (Orn) and Tranexamic acid (Tranexamic),
10

and in case u is 0 and t is different from 0, the terminal carboxyl group of X¹⁴ is unsubstituted or amidated,

whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration,

15 t represents an integer of from 0 to 4,

X¹⁵ is a chemical group selected from the list consisting of (1R,3S)-3-(Amino)cyclopentanecarboxylic acid, (1S,3R)-3-(Amino)cyclopentanecarboxylic acid, (R)-4-Amino-6-methylheptanoic acid, (S)-(1-Piperidin-3-yl)-acetic acid, (S)-3-(1-Pyrrolidine-2-yl)-propionic acid, (S)-3-(2H-tetrazol-5-yl)propanoic acid, (S)-Pyrrolidine-3-carboxylic acid, 5-Azaspiro[2.4]heptane-1-carboxylic acid
20 and (2S)-3-(Triazol-1-yl)-2-(amino)propanoic acid,

u represents the integer 0 or 1,

with the proviso that the peptide contains at least one unnatural amino acid.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein
25

X⁰ is a chemical group selected from the list consisting of (1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid, (2-Thiomorpholine)acetic acid, (N-Isopropyl-N-methylamino)acetic acid, 2-(3-Pyridyl)acetic acid, 2-(Cyclohexylamino)acetic acid, 2-(Diethylamino)acetic acid, 2-(Morpholine)acetic acid, 2-(Piperidin)acetic acid, 2-(Pyrrolidine)acetic acid, 3-(Aminomethyl)benzoic acid, 4-(Aminomethyl)benzoic acid, 1-(Aminomethyl)-cyclopropyl-1-carboxylic acid (ACMP),
30

Azetidine-2-carboxylic acid, Benzoic acid, Cyclobutylacetic acid, Cyclopropylacetic acid, Phenylacetic acid, Piperidin-4-ylacetic acid, Tetrahydro-2H-pyran-3-ylacetic acid, Tranexamic acid and trans-2-(3-((t-butoxy)carbonylamino)cyclohexyl)acetic acid,

p represents the integer 0 or 1,

5 X¹ represents a natural amino acid selected from a list consisting of A, F, G, H, I, K, L, P, R, S, T, V, W and Y or an unnatural amino acid selected from a list consisting of L-N,N-Dimethylalanine ((N,N-diMe)A), N-Methyl-Alanine (N-Me)A, N-Methyl-Glycine ((N-Me)G), L-2-Pyridylalanine (2-Pal), L-3-Pyridylalanine (3-Pal), L-4-Pyridylalanine (4-Pal), L-2-Aminobutyric acid (Abu), 6-Aminohexanoic acid (Ahx), 2-Aminoisobutyric acid (Aib), 3-(Aminomethyl)benzoic acid, 3-
10 Amino-2,2-dimethylpropionic acid, 3-Amino-3-methylbutyric acid, 4-(Aminomethyl)benzoic acid, beta-2-thienylalanine, (S)-Pyrrolidine-2-carboxylic acid (beta-P), L-Citrulline (Cit), L-2,4-diaminobutyric acid (Dab), L-2,3-diaminopropionic acid (Dap), Gamma-Aminobutyric acid (Gamma-Abu), L-3-Methylhistidine (H(3-Me)), L-Dihydroorotic acid (Hoo), L-Norleucine (Nle), L-Norvaline (Nva), L-Ornithine (Orn), L-Pipecolic acid (Pip), N-Methyl-L-proline ((N-Me)P), Tranexamic acid (Tranexamic), (1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid and (2S)-
15 2[(amino)-2-(tetrahydro-2H-pyran-4-yl)]acetic acid,

whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration,

q represents an integer of from 0 to 5,

20 X² represents the natural amino acid I or an unnatural amino acid selected from a list consisting of L-N-Methylisoleucine ((N-Me)I), L-Cyclobutylalanine (Cba), L-Norvaline (Nva) and L-2-Aminobutyric acid (Abu),

whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration,

25 r represents the integer 0 or 1,

X³ represents the natural amino acid C or the unnatural amino acid L-Penicillamine (Pen),

X⁴ represents a natural amino acid selected from a list consisting of S and T,

X⁵ represents the natural amino acid R,

X⁶ represents the natural amino acid S or the unnatural amino acid allo-L-Threonine (allo-T),

- X^7 represents a natural amino acid selected from a list consisting of N and L, or an unnatural amino acid selected from a list consisting of 2,5-Difluoro-L-phenylalanine ((2,5-Difluoro)F), L-2-Bromophenylalanine ((2-Bromo)F), 2-Chloro-L-phenylalanine ((2-Chloro)F), 4-Fluoro-L-Leucine ((4-Fluoro)L), L-tert-Butylalanine ((tBu)A), (S)-(trifluoromethyl)-L-cysteine, (2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid and 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), 3-(Trimethylsilyl)-L-alanine,
- X^8 represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of (1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid and (4aR,6aR,9S,11aS)-11-oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid,
- X^9 represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), (2S,4S)-4-Trifluoromethyl-pyrrolidine-2-carboxylic acid ((4-CF₃)P), (6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid, rel-(1R,3R,5R,6R)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid, (2S,3aS,6aS)-octahydrocyclopenta[b]pyrrole-2-carboxylic acid, rel-(3R,6R)-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1) and rel-(3R,6R)-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2),
- X^{10} represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of (2S)-2-(Amino)-2-[(1S,3R)-3-hydroxycyclohexyl]acetic acid, (S)-2-amino-3-ethyl-pentanoic acid, L-Cyclohexylglycine (Chg) and L-Cyclopentylglycine (Cpg),
- X^{11} represents the natural amino acid C, or an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen),
- X^{12} represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of allo-L-Isoleucine (allo-I), (S)-2-Amino-2-cyclobutylacetic acid (Cbg) and 2-[(1S,2S)-1-(amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid,
- and in case u and t and s are all 0, the terminal carboxyl group of X^{12} is unsubstituted or amidated,
- X^{13} represents a natural amino acid selected from a list consisting of P and D or an unnatural amino acid selected from a list consisting of 2-Aminoisobutyric acid (Aib), 2-Methyl-L-Proline (2-Me)P and trans-4-fluoroproline ((trans-4-Fluoro)P),
- whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration,
- and in case u and t are both 0 and s is different from 0, the terminal carboxyl group of X^{13} unsubstituted or amidated,

s represents an integer of from 0 to 3,

X¹⁴ represents a natural amino acid selected from a list consisting of D, Q, N, E and P or an unnatural amino acid selected from a list consisting of 3-Carboxyphenylalanine ((3-Carboxy)F), N-Methyl-Glycine ((N-Me)G), (2S)-Pyrrolidin-2-ylacetic acid (beta-homo-P), L-2,3-Diaminopropionic acid (Dap), L-Ornithine (Orn) and Tranexamic acid (Tranexamic),

whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration,

and in case u is 0 and t is different from 0, the terminal carboxyl group of X¹⁴ is unsubstituted or amidated,

t represents an integer of from 0 to 4,

X¹⁵ is a chemical group selected from the list consisting of (1R,3S)-3-(Amino)cyclopentanecarboxylic acid, (1S,3R)-3-(Amino)cyclopentanecarboxylic acid, (R)-4-Amino-6-methylheptanoic acid, (S)-(1-Piperidin-3-yl)-acetic acid, (S)-3-(1-Pyrrolidine-2-yl)-propionic acid, (S)-3-(2H-tetrazol-5-yl)propanoic acid, (S)-Pyrrolidine-3-carboxylic acid, 5-Azaspiro[2.4]heptane-1-carboxylic acid and (2S)-3-(Triazol-1-yl)-2-(amino)propanoic acid,

u represents the integer 0 or 1,

with the proviso that the peptide contains at least one unnatural amino acid.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein

p represents an integer of 0,

X¹ represents a natural amino acid selected from the list consisting of A and G or an unnatural amino acid selected from a list consisting of N-Methyl-L-Alanine (N-Me)A, N-Methyl-Glycine ((N-Me)G), L-Norleucine (Nle), L-Norvaline (Nva) and L-Ornithine (Om),

q represents the integer 0 or 1,

X² represents the natural amino acid I,

r represents the integer 0 or 1,

X³ represents the natural amino acid C or the unnatural amino acid L-Penicillamine (Pen),

X^4 represents the natural amino acid S,

X^5 represents the natural amino acid R or the unnatural amino acid N(5)-methyl-L-arginine ((Me)R),

X^6 represents the natural amino acid S,

X^7 represents the natural amino acid L or the unnatural amino acid L-tert-Butylalanine ((tBu)A),

5 X^8 represents the natural amino acid P,

X^9 represents the natural amino acid P or the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic),

X^{10} represents the natural amino acid I,

10 X^{11} represents the natural amino acid C, or an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen),

X^{12} represents the natural amino acid I,

and in case u and t and s are all 0, the terminal carboxyl group of X^{12} is unsubstituted or amidated,

X^{13} represents the natural amino acid P,

15 and in case u and t are both 0 and s is different from 0, the terminal carboxyl group of X^{13} is unsubstituted or amidated,

s represents the integer 0 or 1,

X^{14} represents a natural amino acid selected from a list consisting of D, Q and E,

and in case u is 0 and t is different from 0, the terminal carboxyl group of X^{14} is unsubstituted or amidated,

20 t represents the integer 0 or 1,

u represents an integer of 0,

with the proviso that the peptide contains at least one unnatural amino acid.

According to a further embodiment, the invention provides a compound containing a peptide of the following formula (II),

25 $(X^1)_q(X^2)_rX^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}(X^{13})_s(X^{14})_t$ (II),

or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein

X¹ represents a natural amino acid selected from a list consisting of A and G, or an unnatural amino acid selected from a list consisting of N-Methyl-L-Alanine (N-Me)A, N-Methyl-Glycine ((N-Me)G), L-Norleucine (Nle), L-Norvaline (Nva) and L-Ornithine (Om),

5 q represents the integer 0 or 1,

X² represents the natural amino acid I,

r represents the integer 0 or 1,

X³ represents the natural amino acid C or the unnatural amino acid L-Penicillamine (Pen),

X⁴ represents the natural amino acid S,

10 X⁵ represents the natural amino acid R or the unnatural amino acid N(5)-Methyl-L-arginine ((Me)R),

X⁶ represents the natural amino acid S,

X⁷ represents the natural amino acid L or the unnatural amino acid L-tert-Butylalanine ((tBu)A),

15 X⁸ represents the natural amino acid P or the unnatural amino acid L-Proline (3,4-₂H),

X⁹ represents the natural amino acid P or the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic),

X¹⁰ represents the natural amino acid I,

20 X¹¹ represents an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen),

X¹² represents the natural amino acid I,

X¹³ represents the natural amino acid P,

s represents the integer 0 or 1,

X¹⁴ represents a natural amino acid selected from a list consisting of D, Q and E,

25 t represents the integer 0 or 1,

wherein the N-terminus of the peptide is unsubstituted, acetylated or mono- or disubstituted with C₁-C₂₀-alkyl,

wherein the C-terminus of the peptide is unsubstituted or amidated, and

wherein the peptide is cyclized via a linkage connecting X³ and X¹¹.

- 5 According to a further embodiment, the invention provides a compound containing a peptide of formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein

X¹ represents a natural amino acid or an unnatural amino acid,

q represents the integer 0 or 1,

- 10 X² represents the natural amino acid I,

r represents the integer 0 or 1,

X³ represents the natural amino acid C or the unnatural amino acid L-Penicillamine (Pen),

X⁴ represents the natural amino acid S,

- 15 X⁵ represents the natural amino acid R or the unnatural amino acid N(5)-Methyl-L-arginine ((Me)R),

X⁶ represents the natural amino acid S,

X⁷ represents the natural amino acid L or the unnatural amino acid L-tert-Butylalanine ((tBu)A),

X⁸ represents the natural amino acid P or the unnatural amino acid L-Proline (3,4-₂H),

- 20 X⁹ represents the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic),

X¹⁰ represents the natural amino acid I,

X¹¹ represents the natural amino acid C, or an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen),

X¹² represents the natural amino acid I,

- 25 X¹³ represents the natural amino acid P,

s represents the integer 0 or 1,

X¹⁴ represents a natural amino acid selected from a list consisting of D, Q and E,

t represents the integer 0 or 1,

wherein the N-terminus of the peptide is unsubstituted, acetylated or mono- or disubstituted with C₁-C₂₀-alkyl,

wherein the C-terminus of the peptide is unsubstituted or amidated, and

wherein the peptide is cyclized via a linkage connecting X³ and X¹¹.

According to a further embodiment, the invention provides a compound containing a peptide of formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein

X¹ represents a natural amino acid selected from a list consisting of A and G, or an unnatural amino acid selected from a list consisting of N-Methyl-L-Alanine (N-Me)A, N-Methyl-Glycine ((N-Me)G), L-Norleucine (Nle), L-Norvaline (Nva) and L-Ornithine (Orn),

q represents the integer 0 or 1,

X² represents the natural amino acid I,

r represents the integer 0 or 1,

X³ represents the natural amino acid C or the unnatural amino acid L-Penicillamine (Pen),

X⁴ represents the natural amino acid S,

X⁵ represents the natural amino acid R or the unnatural amino acid N(5)-Methyl-L-arginine ((Me)R),

X⁶ represents the natural amino acid S,

X⁷ represents the natural amino acid L or the unnatural amino acid L-tert-Butylalanine ((tBu)A),

X⁸ represents the natural amino acid P or the unnatural amino acid L-Proline (3,4-₂H),

X⁹ represents the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic),

X^{10} represents the natural amino acid I,

X¹¹ represents the natural amino acid C, or an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen),

X^{12} represents the natural amino acid I,

5 X^{13} represents the natural amino acid P,

s represents the integer 0 or 1,

X^{14} represents a natural amino acid selected from a list consisting of D, Q and E,

t represents the integer 0 or 1,

wherein the N-terminus of the peptide is unsubstituted or acetylated,

10 wherein the C-terminus of the peptide is unsubstituted or amidated, and

wherein the peptide is cyclized via a linkage connecting X³ and X¹¹.

In particular, the invention provides compounds containing a peptide of the formula (I) or compounds containing a peptide of the formula (II).

According to the present invention, the following definitions of X¹ to X¹⁴ apply to peptides of formula (I)

15 and peptides of formula (II), definitions of positions X⁰ and X¹⁵ apply to peptides of formula (I).

According to the present invention X^0 , if present, can be a chemical group which is not an amino acid, according to the definition herein.

According to a further embodiment of the invention, X⁰ represents a chemical group selected from the list consisting of 2-cyanobenzoic acid, (1S,2S,4S)-Bicyclo[2.2.1]hept-5-en-2-ylacetic acid, (2,4-Dioxoimidazolidin-1-yl)acetic acid, 2-(Thiomorpholine)acetic acid ((2-Thiomorpholine)acetyl), 2-(N-Isopropyl-N-methylamino)acetic acid, (S)-3-Methylvaleric Acid ((S)-3-Methylpentanoic acid), 2-(3-Pyridyl)acetic acid, 2-(Cyclohexylamino)acetic acid (2-(Cyclohexylamino)acetyl), 2-(Diethylamino)acetic acid (2-(Diethylamino)acetyl), 2-(Morpholine)acetic acid (2-(Morpholine)acetyl), 2-(N-Methyl-N-cyclopropylamino)acetic acid (2-(N-Methyl-N-cyclopropylamino)acetyl), 2-(Piperidin)acetic acid (2-(Piperidin)acetyl), 2-(Pyrrolidine)acetic acid (2-(Pyrrolidine)acetyl), 2-Hydroxyacetic acid (2-Hydroxyacetyl), 2-Hydroxyisobutyric acid (2-Hydroxyisobutyric), 3-(Aminomethyl)benzoic acid, 3-Methoxypropionic acid, 4-(Aminomethyl)benzoic acid, 4-Methylpentanoic acid (4-Methylvaleric), 5-Chorothiophene-carboxylic acid, 1-(Aminomethyl)-cyclopropyl-1-carboxylic acid (ACMP), Adipic acid, (S)-Azetidine-2-carboxylic acid, Benzoic acid (Benzoic), 4-(3,5-Dimethyl-1,2-oxazol-4-yl)-L-phenylalanine, Cyclobutanecarboxylic

acid (Cyclobutanecarboxylic), 2-(Cyclobutyl)acetic acid (Cyclobutylacetic), Cyclobutylacetic acid (Cyclobutylacetic), Cyclohexylacetic acid (Cyclohexylacetic), Cyclohexanecarboxylic acid (Cyclohexylcarboxylic), Cyclopentanecarboxylic acid, Cyclopentylacetic acid (Cyclopentylacetic), Cyclopropanecarboxylic acid (Cyclopropanecarboxylic), Cyclopropylacetic acid, D-(+)-Biotin, Fumaric acid, 3-Phenylpropanoic acid (Hydrocinnamic), Isobutyric acid, Isovaleric acid (Isovaleric), L-(+)-Lactic acid (Lactic), Phenylacetic acid, Piperidin-4-ylacetic acid, Pivalic acid (Pivalic), Suberic acid, tert-Butylacetic acid, Tetrahydropyranyl-4-acetic acid, Tetrahydro-2H-pyran-3-ylacetic acid and trans-2-(3-((t-butoxy)carbonylamino)cyclohexyl)acetic acid).

According to a further embodiment of the invention, X^0 represents a chemical group selected from the list consisting of 2-cyanobenzoic acid, (1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid, (2-Thiomorpholine)acetic acid, (N-Isopropyl-N-methylamino)acetic acid, 2-(3-Pyridyl)acetic acid, 2-(Cyclohexylamino)acetic acid, 2-(Diethylamino)acetic acid, 2-(Morpholine)acetic acid, 2-(Piperidin)acetic acid, 2-(Pyrrolidine)acetic acid, 3-(Aminomethyl)benzoic acid, 4-(Aminomethyl)benzoic acid, 1-(Aminomethyl)-cyclopropyl-1-carboxylic acid (ACMP), Azetidine-2-carboxylic acid, Benzoic acid, Cyclobutylacetic acid, Cyclopropylacetic acid, Phenylacetic acid, Piperidin-4-ylacetic acid, Tetrahydro-2H-pyran-3-ylacetic acid, Tranexamic acid and trans-2-(3-((t-butoxy)carbonylamino)cyclohexyl)acetic acid.

According to a further embodiment of the invention, X^0 is a chemical group selected from an unnatural amino acid as defined above, 2-cyanobenzoic acid, a substituted benzoic acid or phenyl acetic acids.

According to a further embodiment of the invention, X^0 is Hoo, ODD or Ahx.

According to a further embodiment of the invention, p represents an integer of 1.

According to a further embodiment of the invention, p represents an integer of 0.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X^1 represents any natural amino acid, or an unnatural amino acid, preferably selected from a list consisting of unnatural amino acids listed in table 1, whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration.

According to a further embodiment of the invention, X^1 represents a natural amino acid selected from a list consisting of A, F, G, H, I, K, L, P, R, S, T, V, W and Y, or an unnatural amino acid selected from a list consisting of N-Methyl-Alanine (N-Me)A, N-Methyl-Glycine ((N-Me)G), (1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid, L-3-Bromophenylalanine ((3-Bromo)F), L-N,N-Dimethylalanine ((N,N-diMe)A), N,N-Dimethylglycine ((N,N-diMe)G), N-Phenylglycine ((N-Ph)G), (R)-Piperidine-3-

carboxylic acid, (S)-Piperidine-3-carboxylic acid, L-tert-Butylalanine ((tBu)A), L-2-Pyridylalanine (2-Pal), L-3-Pyridylalanine (3-Pal), L-4-Pyridylalanine (4-Pal), 3-(Aminomethyl)benzoic acid, 3-Amino-2,2-dimethylpropionic acid, 3-Amino-3-methylbutyric acid, 4-(Aminomethyl)benzoic acid, L-2-Amino-butyric acid (Abu), 1-Aminocyclobutane-1-carboxylic acid (ACBA), 6-Aminohexanoic acid (Ahx), 2-Aminoisobutyric acid (Aib), L-2-Thienylalanine (beta-2-thienylalanine), beta-Alanine (beta-A), beta-Pro-
line (beta-P), L-Citrulline (Cit), L-2,4-Diaminobutyric acid (Dab), L-2,3-Diaminopropionic acid (Dap),
Gamma-Aminobutyric acid (Gamma-Abu), L-3-Methylhistidine (3-Me)H, L-Dihydroorotic acid (Hoo),
L-Norleucine (Nle), N-Methyl-L-proline ((N-Me)P), L-Norvaline (Nva), L-Ornithine (Orn), L-Pipecolic
acid (Pip), (2S)-2[(Amino)-2-(tetrahydro-2H-pyran-4-yl)]acetic acid and Tranexamic acid (Tranexamic),
whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by
the stereoisomer in D-stereoconfiguration.

According to a further embodiment of the invention, X¹ represents a natural amino acid selected from a
list consisting of A and G, or an unnatural amino acid selected from a list consisting of N-Methyl-L-
Alanine (N-Me)A, N-Methyl-Glycine ((N-Me)G), L-Norleucine (Nle), L-Norvaline (Nva) and L-Orni-
thine (Orn), whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be
replaced by the stereoisomer in D-stereoconfiguration.

According to a further embodiment of the invention, X¹ represents a natural amino acid selected from a
list consisting of A and G, or an unnatural amino acid selected from a list consisting of N-Methyl-L-
Alanine (N-Me)A, N-Methyl-Glycine ((N-Me)G), L-Norleucine (Nle), L-Norvaline (Nva) and L-Orni-
thine (Orn).

According to a further embodiment of the invention, X¹ represents the natural amino acid A or the unnat-
ural amino acid N-Methyl-Glycine ((N-Me)G).

According to a further embodiment of the invention, in case p is 0 and q is different from 0, the terminal
amino group of X¹ is unsubstituted, acetylated or mono- or disubstituted with C₁-C₄-alkyl, preferably
monosubstituted, with C₁-C₂₀-alkyl.

According to a further embodiment of the invention, X¹ is methylated.

According to a further embodiment of the invention, X¹ is acetylated.

According to a further embodiment of the invention, q represents an integer of 0.

According to a further embodiment of the invention, q represents an integer of 1.

According to a further embodiment, the invention provides compounds containing a peptide, which may
be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula

(II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X² represents a natural amino acid selected from a list consisting of I, L, M, V and A, or an unnatural amino acid selected from a list consisting of L-N-Methylisoleucine ((N-Me)I), allo-L-Isoleucine (allo-I), L-Cyclobutylalanine (Cba), L-Norvaline (Nva), L-2-Aminobutyric acid (Abu), (2S,3S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-3-methylpentanoic acid, (2S,3S)-2-[2-Oxopiperazin-1-yl]-3-methylpentanoic acid, (2S,3S)-2-[(3S)-2-oxopiperazin-1-yl]-3-methylpentanoic acid, L-Methionine-L-sulfoxide, L-Methionine-sulfone and L-tert-butylglycine, whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration.

According to a further embodiment of the invention, X² represents the natural amino acid I or an unnatural amino acid selected from a list consisting of L-N-Methylisoleucine ((N-Me)I), allo-L-Isoleucine (allo-I), L-Cyclobutylalanine (Cba), L-Norvaline (Nva), L-2-Aminobutyric acid (Abu), (2S,3S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-3-methylpentanoic acid, (2S,3S)-2-[2-Oxopiperazin-1-yl]-3-methylpentanoic acid and (2S,3S)-2-[(3S)-2-oxopiperazin-1-yl]-3-methylpentanoic acid, whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration.

According to a further embodiment of the invention, X² represents the natural amino acid I or an unnatural amino acid selected from a list consisting of L-N-Methylisoleucine ((N-Me)I), L-Cyclobutylalanine (Cba), L-Norvaline (Nva) and L-2-Aminobutyric acid (Abu), whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration.

According to a further embodiment of the invention, X² represents the natural amino acid I.

According to a further embodiment of the invention, in case p and q are both 0 and r is 1, the terminal amino group of X² is unsubstituted, acetylated or mono- or disubstituted with C₁-C₂₀-alkyl, preferably monosubstituted, with C₁-C₄-alkyl.

According to a further embodiment of the invention, r represents an integer of 0.

According to a further embodiment of the invention, r represents an integer of 1.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X³ represents the natural amino acid C, or an unnatural amino acid selected from a list consisting of L-Penicillamine (Pen) and L-N-Methylcysteine ((N-Me)C).

According to a further embodiment of the invention, X³ represents the natural amino acid C or the unnatural amino acid L-Penicillamine (Pen).

According to a further embodiment of the invention, X³ represents the natural amino acid C.

According to a further embodiment of the invention, in case p and q and r are all 0, the terminal amino group of X³ is unsubstituted, acetylated or mono- or disubstituted with C₁-C₄-alkyl, preferably monosubstituted, with C₁-C₂₀-alkyl.

- 5 According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X⁴ represents a natural amino acid selected from a list consisting of S, C, T, R or K, or an unnatural amino acid selected from a list consisting of allo-L-Threonine (allo-T), L- Homoserine (hSer) and L-
10 Ornithine (Orn).

According to a further embodiment of the invention, X⁴ represents a natural amino acid selected from a list consisting of S, C, T, R and K.

According to a further embodiment of the invention, X⁴ represents a natural amino acid selected from a list consisting of S or T.

- 15 According to a further embodiment of the invention, X⁴ represents the natural amino acid S.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X⁵ represents the natural amino acid R or the unnatural amino acid N(5)-methyl-L-arginine
20 ((Me)R).

According to a further embodiment of the invention, X⁵ represents the natural amino acid R or the unnatural amino acid N(5)-methyl-L-arginine ((Me)R).

According to a further embodiment of the invention, X⁵ represents the natural amino acid R.

According to a further embodiment, the invention provides compounds containing a peptide, which may
25 be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X⁶ represents a natural amino acid selected from a list consisting of S, C or T, or an unnatural amino acid selected from a list consisting of allo-L-Threonine (allo-T) and L-2,3-diaminopropionic acid (Dap).

- 30 According to a further embodiment of the invention, X⁶ represents the natural amino acid S or an unnatural amino acid selected from a list consisting of allo-L-Threonine (allo-T) and L-2,3-diaminopropionic acid (Dap).

According to a further embodiment of the invention, X⁶ represents the natural amino acid S or the unnatural amino acid allo-L-Threonine (allo-T).

According to a further embodiment of the invention, X⁶ represents the natural amino acid S.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X⁷ represents a natural amino acid selected from a list consisting of L, F or N or an unnatural amino acid selected from a list consisting of 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), L-4-Bromophenylalanine ((4-Bromo)F), 2,5-Difluoro-L-phenylalanine ((2,5-Difluoro)F), L-tert-Butylalanine ((tBu)A), 2-Chloro-L-phenylalanine ((2-Chloro)F), L-2-Bromophenylalanine ((2-Bromo)F), (S)-2-(Amino)-1,6-hexanedioic acid (AAD), (2S)-2-amino-4,4,4-trifluorobutanoic acid, L-2-amino-4-cyanobutyric acid (Cnba), 4-Fluoro-Leucine ((4-Fluoro)L), (S)-(trifluoromethyl)-L-cysteine, (2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid, L-tert-Butylglycine ((tBu)G), 3-(Trimethylsilyl)-L-alanine, 2,5-difluoro-L-phenylalanine, 2-Amino-7-(tert-butoxy)-7-oxoheptanoic acid, 5,5,5-Trifluoro-L-leucine ((Trifluoro)L), 2-Methyl-L-phenylalanine ((2-Me)F), L-Cyclobutylalanine (Cba), L-Cyclopentylalanine (Cpa), L-cyclopropylmethylalanine, L-trifluoromethylalanine, L-difluoromethylalanine, 2-Fluoro-L-phenylalanine ((2-Fluoro)F), (2S)-3-(2,3-difluorophenyl)-2-aminopropanoic acid, (2S)-3-(3-Cyanophenyl)-2-aminopropanoic acid, 2-Amino-5,5,5-trifluoro-4-methyl-pentanoic acid, (2S)-2-Amino-5-methyl-hexanoic acid and (2S)-3-(indol-4-yl)-2-(amino)propanoic acid.

According to a further embodiment of the invention, X⁷ represents a natural amino acid selected from a list consisting of L and N, or an unnatural amino acid selected from a list consisting of 2,5-Difluoro-L-phenylalanine ((2,5-Difluoro)F), L-2-Bromophenylalanine ((2-Bromo)F), 2-Chloro-L-phenylalanine ((2-Chloro)F), L-4-Bromophenylalanine ((4-Bromo)F), 4-Fluoro-L-Leucine ((4-Fluoro)L), L-tert-Butylalanine ((tBu)A), L-tert-Butylglycine ((tBu)G), 5,5,5-Trifluoro-L-leucine ((Trifluoro)L), (S)-2-(Amino)-1,6-hexanedioic acid (AAD), (2S)-2-amino-4,4,4-trifluorobutanoic acid, L-2-amino-4-cyanobutyric acid (Cnba), (S)-(trifluoromethyl)-L-cysteine, (2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid, (2S)-3-(2,3-difluorophenyl)-2-aminopropanoic acid, 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), 3-(Trimethylsilyl)-L-alanine and 2-Amino-7-(tert-butoxy)-7-oxoheptanoic acid.

According to a further embodiment of the invention, X⁷ represents a natural amino acid selected from a list consisting of N or L, or an unnatural amino acid selected from a list consisting of 2,5-Difluoro-L-phenylalanine ((2,5-Difluoro)F), L-2-Bromophenylalanine ((2-Bromo)F), 2-Chloro-L-phenylalanine ((2-Chloro)F), 4-Fluoro-L-Leucine ((4-Fluoro)L), L-tert-Butylalanine ((tBu)A), (S)-(trifluoromethyl)-L-cysteine, (2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid and 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), 3-(Trimethylsilyl)-L-alanine.

According to a further embodiment of the invention, X⁷ represents the natural amino acid L or the unnatural amino acid L-tert-Butylalanine ((tBu)A).

According to a further embodiment of the invention, X⁷ represents the unnatural amino acid L-tert-Butylalanine ((tBu)A).

- 5 According to a further embodiment of the invention, X⁷ represents the natural amino acid L.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X⁸ represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of L-Proline (3,4-₂H), (1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid, (1R,2S,5S)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid, L-Hydroxyproline (Hyp), (3S)-Morpholine-3-carboxylic acid (Morpholine-3-carboxylic), L-Pipecolic acid (Pip), (4aR,6aR,9S,11aS)-11-oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid, trans-4-fluoroproline ((trans-4-Fluoro)P) and (1R,2S,5S)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid.

- 15 According to a further embodiment of the invention, X⁸ represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of L-Proline (3,4-₂H), (1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid, trans-4-fluoroproline ((trans-4-Fluoro)P), L-Hydroxyproline (Hyp), (3S)-Morpholine-3-carboxylic acid (Morpholine-3-carboxylic), L-Pipecolic acid (Pip) and (4aR,6aR,9S,11aS)-11-Oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid.

- 20 According to a further embodiment of the invention, X⁸ represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of L-Proline (3,4-₂H), (1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid and (4aR,6aR,9S,11aS)-11-oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid.

According to a further embodiment of the invention, X⁸ represents the natural amino acid P or the unnatural amino acid L-Proline (3,4-₂H).

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According to a further embodiment of the invention, X⁸ represents the natural amino acid P.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X⁹ represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), L-Hydroxyproline (Hyp), (2S,4S)-4-Trifluoromethyl-pyrrolidine-2-carboxylic acid ((4-CF₃)P), (2S,4S)-4-fluoroproline ((cis-4-Fluoro)P), trans-4-fluoroproline ((trans-4-

- 30

Fluoro)P), (2S)-2-amino-4,4,4-trifluorobutanoic acid, L-trans-3-hydroxyproline ((3S-OH)P), (1R,3S,5R)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid, (6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid, rel-(1R,3R,5R,6R)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid, (2S)-2-Amino-4,4,4-trifluorobutanoic acid, (2S,3aS,6aS)-octahydrocyclopenta[b]pyrrole-2-carboxylic acid, trans-4-fluoroproline ((trans-4-Fluoro)P), (2S,4S)-4-fluoroproline ((cis-4-Fluoro)P), L-4,4-difluoroproline ((Difluoro)P), rel-(3R,6R)-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1) and rel-(3R,6R)-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2).

According to a further embodiment of the invention, X⁹ represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), (6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid, rel-(1R,3R,5R,6R)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid, (2S,4S)-4-Trifluoromethyl-pyrrolidine-2-carboxylic acid ((4-CF₃)P), (2S,3aS,6aS)-octahydrocyclopenta[b]pyrrole-2-carboxylic acid, (1R,3S,5R)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid, (2S)-2-Amino-4,4,4-trifluorobutanoic acid, L-trans-3-hydroxyproline ((3S-OH)P), trans-4-fluoroproline ((trans-4-Fluoro)P), L-Hydroxyproline (Hyp), (2S,4S)-4-Fluoroproline ((cis-4-Fluoro)P), (3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1) and (3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2).

According to a further embodiment of the invention, X⁹ represents the natural amino acid P, or an unnatural amino acid selected from a list consisting of 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), (2S,4S)-4-Trifluoromethyl-pyrrolidine-2-carboxylic acid ((4-CF₃)P), (6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid, rel-(1R,3R,5R,6R)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid, (2S,3aS,6aS)-octahydrocyclopenta[b]pyrrole-2-carboxylic acid, rel-(3R,6R)-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1) and rel-(3R,6R)-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2).

According to a further embodiment of the invention, X⁹ represents the natural amino acid P or the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic).

According to a further embodiment of the invention, X⁹ represents the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic).

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X¹⁰ represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of L-Cyclopentylglycine (Cpg), L-Cyclohexylglycine (Chg), (S)-2-amino-3-ethyl-pentanoic acid, 3-Chlorophenylglycine ((3-Chloro-Ph)G), L-tert-butylglycine, allo-L-Isoleucine (allo-I), L-Cyclobutylglycine, L-Norvaline (Nva) and (2S)-2-(Amino)-2-[(1S,3R)-3-hydroxycyclohexyl]acetic acid.

According to a further embodiment of the invention, X¹⁰ represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of (2S)-2-(Amino)-2-[(1S,3R)-3-hydroxycyclohexyl]acetic acid, 3-Chlorophenylglycine ((3-Chloro-Ph)G), (S)-2-amino-3-ethyl-pentanoic acid, allo-L-Isoleucine (allo-I), L-Cyclohexylglycine (Chg), L-Cyclopentylglycine (Cpg), L-Cyclobutylglycine and L-Norvaline (Nva).

According to a further embodiment of the invention, X¹⁰ represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of (2S)-2-(Amino)-2-[(1S,3R)-3-hydroxycyclohexyl]acetic acid, (S)-2-amino-3-ethyl-pentanoic acid, L-Cyclohexylglycine (Chg) and L-Cyclopentylglycine (Cpg).

According to a further embodiment of the invention, X¹⁰ represents the natural amino acid I.

10 According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X¹¹ represents the natural amino acid C, or an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen).

15 According to a further embodiment of the invention, X¹¹ represents the natural amino acid C, or an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen).

According to a further embodiment of the invention, X¹¹ represents an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen).

20 According to a further embodiment of the invention, X¹¹ represents the unnatural amino acid L-Penicillamine (Pen).

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X⁹ represents the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic) and X¹¹ represents the unnatural amino acid L-N-Methylcysteine ((N-Me)C) or the unnatural amino acid L-Penicillamine (Pen).

According to a further embodiment of the invention, X⁹ represents the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic) and X¹¹ represents the unnatural amino acid L-Penicillamine (Pen).

According to a further embodiment of the invention, X⁹ represents the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic) and X¹¹ represents an unnatural amino acid selected from a list consisting of L-N-Methyleysteine ((N-Me)C) and L-Penicillamine (Pen).

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X¹² represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of allo-L-Isoleucine (allo-I), (S)-2-Amino-2-cyclobutylacetic acid (Cbg), (2S,3S)-2-((amino)methyl)-3-methylpentanoic acid, L-Phenylglycine (Phg), 2-[(1S,2S)-1-(amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid, 2-methyl-D-alloisoleucine, L-Norvaline (Nva), L-2-Aminobutyric acid (Abu), L-tert-butylglycine and Aminoisobutyric acid (Aib).

According to a further embodiment of the invention, X¹² represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of 2-[(1S,2S)-1-(amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid, (S)-2-Amino-2-cyclobutylacetic acid (Cbg), allo-L-Isoleucine (allo-I), L-Phenylglycine (Phg), 2-methyl-D-alloisoleucine and (2S,3S)-2-((amino)methyl)-3-methylpentanoic acid.

According to a further embodiment of the invention, X¹² represents the natural amino acid I, or an unnatural amino acid selected from a list consisting of allo-L-Isoleucine (allo-I), (S)-2-Amino-2-cyclobutylacetic acid (Cbg) and 2-[(1S,2S)-1-(amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid.

According to a further embodiment of the invention, X¹² represents the natural amino acid I.

According to a further embodiment of the invention, in case u and t and s are all 0, the terminal carboxyl group of X¹² is unsubstituted or amidated.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X¹³ represents a natural amino acid selected from a list consisting of P, A, S, T, G, D, E, Q or N or an unnatural amino acid selected from a list consisting of N-Methyl-Glycine ((N-Me)G), 5-azaspiro[2.4]heptane-6-carboxylic acid, L-2-Aminobutyric acid (Abu), 2-Aminoisobutyric acid (Aib), 2-Methyl-L-Proline (2-Me)P, Hydroxyproline (Hyp), 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), trans-4-fluoroproline ((trans-4-Fluoro)P), (2S,4S)-4-fluoroproline ((cis-4-Fluoro)P), L-4,4-difluoroproline ((Difluoro)P), L-Cyclopentylglycine (Cpg), (S)-2-Amino-2-cyclobutylacetic acid (Cbg) and (2S)-Pyrrolidin-2-ylacetic acid (beta-homo-P), whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration.

According to a further embodiment of the invention, X^{13} represents a natural amino acid selected from a list consisting of P, A and D or an unnatural amino acid selected from a list consisting of 2-Methyl-L-Proline (2-Me)P, N-Methyl-Glycine ((N-Me)G), trans-4-fluoroproline ((trans-4-Fluoro)P), L-2-Amino-butyrinic acid (Abu), 2-Aminoisobutyric acid (Aib), Hydroxyproline (Hyp) and 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic), whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration.

According to a further embodiment of the invention, X^{13} represents a natural amino acid selected from a list consisting of P or D or an unnatural amino acid selected from a list consisting of 2-Aminoisobutyric acid (Aib), 2-Methyl-L-Proline (2-Me)P and trans-4-fluoroproline ((trans-4-Fluoro)P), whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration.

According to a further embodiment of the invention, X^{13} represents the natural amino acid P.

According to a further embodiment of the invention, s represents an integer of 0.

According to a further embodiment of the invention, s represents an integer of 1.

According to a further embodiment of the invention, in case u and t are both 0 and s is different from 0, the terminal carboxyl group of X^{13} is unsubstituted or amidated.

According to a further embodiment of the invention, in case t is 0 and s is 1, the terminal carboxyl group of X^{13} is unsubstituted or amidated.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein X^{14} represents any natural amino acid or an unnatural amino acid, whereas any natural amino acid and/or unnatural amino acid can be in D- or L-stereoconfiguration.

According to a further embodiment of the invention, X^{14} represents a natural amino acid selected from a list consisting of D, E, G, K, N, P and Q or an unnatural amino acid selected from a list consisting of 3-Carboxyphenylalanine ((3-Carboxy)F), (2S)-2-Amino-4-(benzylamino)-4-oxobutanecarboxylic acid ((N-Benzyl)D), N-Methyl-Glycine ((N-Me)G), 6-Aminohexanoic acid (Ahx), (2S)-Pyrrolidin-2-ylacetic acid (beta-homo-P), L-2,3-Diaminopropionic acid (Dap), L-Ornithine (Orn) and Tranexamic acid (Tranexamic), whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration.

According to a further embodiment of the invention, X¹⁴ represents a natural amino acid selected from a list consisting of D, Q, N, E and P or an unnatural amino acid selected from a list consisting of 3-Carboxyphenylalanine ((3-Carboxy)F), N-Methyl-Glycine ((N-Me)G), (2S)-Pyrrolidin-2-ylacetic acid (beta-homo-P), L-2,3-Diaminopropionic acid (Dap), L-Ornithine (Orn) and Tranexamic acid (Tranexamic),
5 whereas each natural amino acid and/or unnatural amino acid in L-stereoconfiguration can be replaced by the stereoisomer in D-stereoconfiguration.

According to a further embodiment of the invention, X¹⁴ represents a natural amino acid selected from a list consisting of D, Q or I.

According to a further embodiment of the invention, X¹⁴ represents the natural amino acid D.

10 According to a further embodiment of the invention, the C-terminal "-OH" moiety at X¹⁴ is substituted for a C-terminal "-NH₂" moiety.

According to a further embodiment of the invention, q represents an integer of 0.

According to a further embodiment of the invention, q represents an integer of 1.

According to a further embodiment of the invention, in case s and t represent the integer 1, the terminal
15 carboxyl group of X¹⁴ is unsubstituted or amidated.

According to a further embodiment of the invention, in case s and t represent the integer 1, the terminal carboxyl group of X¹⁴ is unsubstituted.

According to the present invention X¹⁵, if present, can be a chemical group which is not an amino acid.

According to a further embodiment of the invention, X¹⁵ is a chemical group selected from the list consisting
20 of -NH₂, -NH-C₁-C₆-alkyl, (1R,3S)-3-(Amino)cyclopentanecarboxylic acid, (1S,3R)-3-(Amino)cyclopentanecarboxylic acid, (R)-4-Amino-6-methylheptanoic acid, (S)-(1-Piperidin-3-yl)-acetic acid, (S)-3-(1-Pyrrolidine-2-yl)-propionic acid, (S)-3-(2H-tetrazol-5-yl)propanoic acid, (S)-Pyrrolidine-3-carboxylic acid, 5-Aza-spiro[2.4]heptane-1-carboxylic acid and (2S)-3-(Triazol-1-yl)-2-(amino)propanoic acid.

According to a further embodiment of the invention, X¹⁵ is a chemical group selected from -NH₂ or -NH-
25 C₁-C₆-alkyl, preferably -NH₂.

According to a further embodiment of the invention, q represents an integer of 0.

According to a further embodiment of the invention, q represents an integer of 1.

According to a further embodiment of the invention, the N-terminus of the peptide is unsubstituted, acetylated or mono- or disubstituted with C₁-C₂₀-alkyl.

According to a further embodiment of the invention, the N-terminus of the peptide is unsubstituted, acetylated or mono- or disubstituted with C₁-C₄-alkyl.

According to a further embodiment of the invention, the N-terminus of the peptide is unsubstituted or acetylated.

- 5 According to a further embodiment of the invention, the C-terminus is unsubstituted or amidated.

According to a further embodiment of the invention, the N-terminus and C-terminus is unsubstituted.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt,
10 which are selected from the group consisting of example 29, 44, 45, 67, 75, 109, 166, 185, 443 and 466 or which are selected from the group consisting of example 29, 44, 45, 67, 75, 109, 166, 185, 443, 466 and 499.

The peptide of formula (I) or (II) can be linear or cyclized. The term "cyclized," as used herein, refers to a reaction in which one part of a polypeptide molecule (e.g. Cys, (N-Me)Cys or Pen) becomes linked to
15 another part of the polypeptide molecule (e.g. another Cys, (N-Me)Cys or Pen) to form a closed ring, such as by forming a disulfide bridge (-S-S-) or other similar bonds such as a carbon-sulphur bond (e.g. (-CH₂-S-) or (-CH₂)₂-S-), a sulphur-carbon bond (e.g. (-S-CH₂-) or (-S-(CH₂)₂-)), a carbon-sulphur-carbon bond (-CH₂-S-CH₂-) or a carbon-carbon bond (e.g. (-CH₂-CH₂-). According to the present invention a linkage between positions X³ and X¹¹ of the peptide is preferred. An amino acid followed by a "+" in a sequence
20 of a peptide according to the invention refers to a cyclic peptide, wherein the amino acid is linked to another amino acid followed by a "+" by a disulfide bridge forming a closed ring. Alternatively two linked chemical groups of a polypeptide molecule can be depicted by a connecting line.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a
25 derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein the peptide is cyclized.

According to a further embodiment of the invention, the peptide of formula (I) or formula (II) is cyclized via a linkage connecting X³ and X¹¹.

According to a further embodiment of the invention, the peptide of formula (I) or formula (II) is cyclized
30 via a linkage connecting X³ and X¹¹ and wherein X³ and X¹¹ are connected via a linker of the formula -S-S-, -CH₂-S-, -S-CH₂-, -CH₂-CH₂-, -S-(CH₂)₂-, -(CH₂)₂-S- or -CH₂-S-CH₂-.

According to a further embodiment of the invention, the peptide of formula (I) or formula (II) is cyclized via a linkage connecting X^3 and X^{11} and wherein X^3 and X^{11} are connected via a disulfide bond of the formula -S-S-.

The peptide of the present invention can be substituted with a suitable watersoluble polymer characterized by repeating units. Suitable polymers may be selected from the group consisting of polyalkyloxy polymers, hyaluronic acid and derivatives thereof, polyvinyl alcohols, polyoxazolines, polyanhydrides, poly(ortho esters), polycarbonates, polyurethanes, polyacrylic acids, polyacrylamides, polyacrylates, polymethacrylates, polyorganophosphazenes, polysiloxanes, polyvinylpyrrolidone, polycyanoacrylates, and polyesters.

The peptides of the present invention can be substituted with at least one polyethylene group (PEG group).

The at least one PEG group is preferably bound to the N-terminal and/or C-terminal end. The PEG group can be bound to any suitable functional group of a chemical group and/or amino acid of the peptide, e.g. hydroxyl group, carboxyl group, amino group, thiol group, preferably an amino or carboxy group. Preferably the peptide according to the invention contains one PEG group bound to the N-terminal end or one PEG group bound to the C-terminal end. More preferably the one PEG group is bound to the N-terminal or C-terminal end via an amide bond.

PEGylation of peptides may enhance their solubility, reduce immunogenicity, improve stability and/or increase half life by reducing renal clearance, which 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 PEG group according to the invention is any group containing at least two ethylene oxide units to form an oligomer or polymer ethylene oxide. The PEG group is covalently coupled to a peptide of the present invention (PEGylation), which is then referred to as a PEGylated peptide. Generally, this type of modification to a molecule is well known in the art.

PEG group may consist of an interconnecting moiety, a polymer moiety, and an end group. Interconnecting moieties may consist of a C_1 - C_{20} alkyl, which is optionally interrupted or terminated by hetero atoms or functional groups selected from the group consisting of -O-, -S-, $N(R^X)$, C(O), $C(O)R^X$, $C(O)N(R^X)$, $N(R^X)C(O)$, one or more C_3 - C_8 -cycloalkyl, C_3 - C_7 -heterocycloalkyl, aryl or heteraryl, wherein R^X is hydrogen or C_1 - C_6 -alkyl, which is optionally interrupted or terminated by one or more of the abovementioned atoms or groups, which further have a hydrogen as terminal atom. Polymer moieties consist of at least two ethylene oxide units, optionally interrupted or terminated by one or more hetero atoms or functional groups selected from the group consisting of -O-, -S-, $N(R^X)$, C(O), $C(O)R^X$, $C(O)N(R^X)$, $N(R^X)C(O)$, C_1 - C_6 -alkyl, C_3 - C_8 -cycloalkyl, C_3 - C_7 -heterocycloalkyl, aryl or heteraryl, wherein R^X is hydrogen or C_1 - C_6 -alkyl. The end group may consist of a hetero atom or functional groups selected from OH, SH, $N(R^X)_2$,

$C(O)R^X$; COR^X , $COOR^X$, $C(O)N(R^X)$, $N(R^X)C(O)NH_2$ wherein R^X is hydrogen or C_1 - C_6 -alkyl. Preferred end groups are $COOH$ and $C(O)NH_2$.

Examples of PEG spacer according to the present invention comprise PEG1(10 atoms) having 1 ethylene glycol unit ($n=1$; 10 atoms), PEG2(13 atoms) having 2 ethylene glycol units ($n=2$; 13 atoms), PEG3(16 atoms) having 3 ethylene glycol units ($n=3$; 16 atoms), PEG4(19 atoms) having 4 ethylene glycol units ($n=4$; 19 atoms), PEG5(22 atoms) having 5 ethylene glycol units ($n=5$; 22 atoms), PEG6(25 atoms) having 6 ethylene glycol units ($n=6$; 25 atoms) etc..

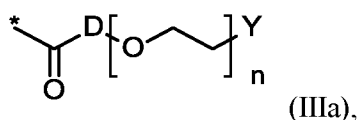
It is noted that the PEG groups as defined herein may be indicated with different names by commercial suppliers, which shall not exclude such identical compounds with different names from the present invention.

According to a further embodiment, the invention provides compounds containing a peptide, which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein the peptide is substituted by at least one PEG group.

According to a further embodiment of the invention, the peptide is substituted by one PEG group.

According to a further embodiment of the invention, the peptide is substituted by one PEG group, wherein the PEG group is bound to the C-terminal end, preferably via an amide bond.

According to a further embodiment of the invention, the peptide is substituted by at least one PEG group, whereas the at least one or more PEG group is a group of the formula (IIIa)



wherein

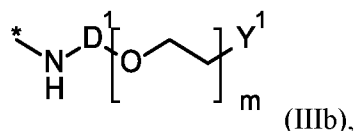
* marks the attachment to a nitrogen or oxygen atom,

D is C_1 - C_4 -alkylene,

Y is selected from the group consisting of hydroxyl, methoxy, ethoxy, carboxy, carboxamide or amino and

n represents an integer of from 2 to 15,

or a group of the formula (IIIb)



wherein

* marks the bond to a carbonyl group,

5 D^1 is C_1 - C_4 -alkylene,

Y^1 is selected from the group consisting of hydroxyl, methoxy, ethoxy, carboxy, carboxamide or amino and

m represents an integer of from 2 to 15,

or TTDS.

10 According to a further embodiment of the invention, the peptide is substituted by at least one PEG group, whereas the at least one or more PEG group selected from a list consisting of PEG1(10 atoms), PEG2(13 atoms), PEG3(16atoms), PEG4(19 atoms), PEG4- $\text{CH}_2\text{CO}_2\text{H}$ (15 atoms), PEG5(22 atoms), PEG5- $\text{CH}_2\text{CO}_2\text{H}$ (18 atoms), PEG7(25 atoms), PEG8(28 atoms), PEG9(31 atoms) and TTDS.

The peptide of the present invention can comprise at least one C_8 - C_{20} fatty acid. Generally, such fatty acid
 15 may be branched or cyclic. The at least one C_8 - C_{20} fatty acid is preferably bound to the N-terminal and/or C-terminal end. The C_8 - C_{20} fatty acid can be bound to any suitable functional group of a chemical group and/or amino acid of the peptide, e.g. hydroxyl group, carboxyl group, amino group, thiol group, preferably an amino or carboxy group. Preferably the peptide according to the invention contains one C_8 - C_{20} fatty acid bound to the N-terminal end or one C_8 - C_{20} fatty acid bound to the C-terminal end. More preferably
 20 the one C_8 - C_{20} fatty acid is bound to the N-terminal or C-terminal end via an amide bond. Preferably the fatty acid side chain formed by X^0 or X^{15} is a fatty acid $> \text{C}_8$, more preferably a fatty acid $\geq \text{C}_{12}$, more preferably a fatty acid $\geq \text{C}_{14}$. It is further preferred that the fatty acid side chain formed by X^0 or X^{15} is a C_{12} - C_{18} fatty acid, preferably a C_{12} - C_{16} fatty acid, or a C_{14} - C_{18} fatty acid, or a C_{14} - C_{16} fatty acid. Most preferred is a C_{16} fatty acid such as palmitic acid (palmitoyl, Palm) and a C_{18} fatty acid such as 1,18-
 25 Octadecanedioic acid (ODD).

It is further understood that the moiety at the N-terminus or C-terminus may be a bond, e.g., a covalent bond, particularly in situations where the amino terminus or carboxy terminus is bound to a linker or to another chemical moiety.

Chemical groups, unnatural amino acids or moieties may be abbreviated herein as shown in Table 3.

Table 3: Abbreviations/expressions and nomenclature used for chemical groups, unnatural amino acids or further moieties in the sequences

Abbreviation/Expression	Abbreviation/Expression Definition
Cyclohexylcarboxylic	Cyclohexanecarboxylic acid
(2S)-Amino-2-[3-(Trifluoromethyl)bicyclo[1.1.1]pent-1-yl]acetic acid	(2S)-Amino-2-[3-(Trifluoromethyl)bicyclo[1.1.1]pent-1-yl]acetic acid
(S)-3-(2H-tetrazol-5-yl)propanoic acid	(S)-3-(2H-tetrazol-5-yl)propanoic acid
(1R,3S)-3-(Amino)cyclopentanecarboxylic acid	(1R,3S)-3-(Amino)cyclopentanecarboxylic acid
(1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid	(1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid
(1R,3S,5R)-2-Azabicyclo[3.1.0]hexane-3-carboxylic acid	(1R,3S,5R)-2-Azabicyclo[3.1.0]hexane-3-carboxylic acid
(1S,2S,4S)-Bicyclo[2.2.1]hept-5-en-2-ylacetic acid	(1S,2S,4S)-Bicyclo[2.2.1]hept-5-en-2-ylacetic acid
(1R,2S,5S)-3-Azabicyclo[3.1.0]hexane-2-carboxylic acid	(1R,2S,5S)-3-Azabicyclo[3.1.0]hexane-2-carboxylic acid
(1S,3R)-3-(Amino)cyclopentanecarboxylic acid	(1S,3R)-3-(Amino)cyclopentanecarboxylic acid
(1S,3R,4R)-2-Azabicyclo[2.2.1]heptane-3-carboxylic acid	(1S,3R,4R)-2-Azabicyclo[2.2.1]heptane-3-carboxylic acid
(2,4-Dioxoimidazolidin-1-yl)acetic acid	(2,4-Dioxoimidazolidin-1-yl)acetic acid
(2-CN)F	2-Cyano-L-phenylalanine
(2-Fluoro)F	2-Fluoro-L-phenylalanine
(2-Me)F	2-Methyl-L-phenylalanine
(4-Pyranoyl)G	(2S)-(tetrahydro-2H-pyran-4-yl)ethanoic acid
(4-CF ₃)P	(2S,4S)-4-Trifluoromethyl-pyrrolidine-2-carboxylic acid
(3,3-Difluorocyclobutyl)acetic acid	(3,3-difluorocyclobutyl)acetic acid
(3-CN)F	3-Cyano-L-phenylalanine
(3-Fluoro)F	3-Fluoro-L-phenylalanine
(4-Fluoro)F	4-Fluoro-L-phenylalanine
5-Azaspiro[2.4]heptane-1-carboxylic acid	5-Azaspiro[2.4]heptane-1-carboxylic acid
D-(+)Biotin	D-(+)Biotin
(N,N-diMe)A	L-N,N-Dimethylalanine
(N,N-diMe)G	N,N-Dimethylglycine
(N-Isopropyl-N-methylamino)acetyl	2-(N-Isopropyl-N-methylamino)acetic acid
((N-Me)A)	L-N-Methylalanine

Abbreviation/Expression	Abbreviation/Expression Definition
((N-Me)a)	D-N-Methylalanine
((N-Me)C)	L-N-Methylcysteine
(N-Me)F	L-N-Methylphenylalanine
(N-Me)G	N-Methyl-Glycine
(N-Me)I	L-N-Methylisoleucine
(N-Ph)G	N-Phenylglycine
(R)-3-Aminoadipic acid	(R)-3-Aminoadipic acid
(R)-4-Amino-6-methylheptanoic acid	(R)-4-Amino-6-methylheptanoic acid
(R)-Piperidine-3-Carboxylic Acid	(R)-Piperidine-3-Carboxylic Acid
(R)-Pyrrolidine-3-Carboxylic Acid	(R)-Pyrrolidine-3-Carboxylic Acid
(S)-(1-Piperidin-3-yl)-acetic acid	(S)-(1-Piperidin-3-yl)-acetic acid
(S)-2-Amino-3-ethyl-pentanoic acid	(S)-2-Amino-3-ethyl-pentanoic acid
(S)-3-(1-Pyrrolidine-2-yl)-propionic acid	(S)-3-(1-Pyrrolidine-2-yl)-propionic acid
(S)-3-(2H-Tetrazol-5-yl)propionic acid	(S)-3-(2H-Tetrazol-5-yl)propionic acid
(S)-3-Methylpentanoic acid	(S)-3-Methylvaleric Acid
(S)-4-Piperazine-2-carboxylic acid	(S)-4-Piperazine-2-carboxylic acid
(S)-Piperidine-3-carboxylic acid	(S)-Piperidine-3-carboxylic acid
(S)-Pyrrolidine-3-carboxylic acid	(S)-Pyrrolidine-3-carboxylic acid
[(2R)-4,4-Difluoropyrrolidin-2-yl]acetic acid	[(2R)-4,4-Difluoropyrrolidin-2-yl]acetic acid
[(6S)-5-Azaspiro[2.4]hept-6-yl]acetic acid	[(6S)-5-Azaspiro[2.4]hept-6-yl]acetic acid
1,1-Dioxidotetrahydro-2H-thiopyran-4-yl)acetic acid	1,1-Dioxidotetrahydro-2H-thiopyran-4-yl)acetic acid
1-Nal	L-1-Napthylalanine
2-(3-Pyridyl)acetic acid	2-(3-Pyridyl)acetic acid
2-(Aminomethyl)benzoic acid	2-(Aminomethyl)benzoic acid
2-(Cyclohexylamino)acetyl	2-(Cyclohexylamino)acetic acid
2-(Diethylamino)acetyl	2-(Diethylamino)acetic acid
2-(Morpholine)acetyl	2-(Morpholine)acetic acid
2-(N-Methyl-N-cyclopropylamino)acetyl	2-(N-Methyl-N-cyclopropylamino)acetic acid
2-(Piperidin)acetyl	2-(Piperidin)acetic acid
2-(Pyrrolidine)acetyl	2-(Pyrrolidine)acetic acid
2-(Thian-4-yl)acetic acid	2-(Thian-4-yl)acetic acid
2-(Thiomorpholine)acetyl	2-(Thiomorpholine)acetic acid
(2,3-Difluoro)F	2,3-Difluoro-L-phenylalanine

Abbreviation/Expression	Abbreviation/Expression Definition
2-aminobenzoic acid	2-Aminobenzoic acid
2-Azaspiro[3.3]heptane-6-carboxylic acid	2-Azaspiro[3.3]heptane-6-carboxylic acid
(2-Bromo)F	L-2-Bromophenylalanine
(2-Chloro)F	2-Chloro-L-phenylalanine
2-Cyanobenzoic acid	2-Cyanobenzoic acid
2-Fluorobenzoic acid	2-Fluorobenzoic acid
2-Hydroxyacetyl	2-Hydroxyacetic acid
2-Hydroxyisobutyric	2-Hydroxyisobutyric acid
2-Methylbenzoic acid	2-Methylbenzoic acid
(2-Me)F	2-Methyl-L-phenylalanine
2-Nal	L-2-Napthylalanine
2-Pal	L-2-Pyridylalanine
beta-homo-P	(2S)-Pyrrolidin-2-ylacetic acid
3-(2-Oxoimidazolidin-1-yl)benzoic acid	3-(2-Oxoimidazolidin-1-yl)benzoic acid
3-(3-Oxo-1,3,4,5,6,7-hexahydro-2H-indazol-2-yl)benzoic acid	3-(3-Oxo-1,3,4,5,6,7-hexahydro-2H-indazol-2-yl)benzoic acid
3-(Aminomethyl)benzoic acid	3-(Aminomethyl)benzoic acid
3-(Isobutyrylamino)benzoic acid	3-(Isobutyrylamino)benzoic acid
3-(Trifluoromethyl)bicyclo[1.1.1]pent-1-yl]acetic acid	3-(Trifluoromethyl)bicyclo[1.1.1]pent-1-yl]acetic acid
3-[(E)-(2-carboxycyclohexen-1-yl)azo]benzoic acid	3-[(E)-(2-carboxycyclohexen-1-yl)azo]benzoic acid
3-Acetamidobenzoic acid	3-Acetamidobenzoic acid
3-Amino-2,2-dimethylpropionic acid	3-Amino-2,2-dimethylpropionic acid
3-Amino-3-methylbutyric acid	3-Amino-3-methylbutyric acid
3-Aminobenzoic acid	3-Aminobenzoic acid
3-Aminomethylphenylacetic acid	3-Aminomethylphenylacetic acid
(3-Bromo)F	L-3-Bromophenylalanine
3-Carboxybenzoic acid	3-Carboxybenzoic acid
(3-Chloro)F	3-Chloro-L-Phenylalanine
(3-Chloro-Ph)G	3-Chlorophenylglycine
3-Cyanobenzoic acid	3-Cyanobenzoic acid
3-Fluorobenzoic acid	3-Fluorobenzoic acid
(3-Me)F	3-Methyl-L-phenylalanine

Abbreviation/Expression	Abbreviation/Expression Definition
3-Methoxypropionic acid	3-Methoxypropionic acid
3-methylbenzoic acid	3-Methylbenzoic acid
3-Pal	L-3-Pyridylalanine
4-(Aminomethyl)benzoic acid	4-(Aminomethyl)benzoic acid
4-Aminobenzoic acid	4-Aminobenzoic acid
4-Aminomethylphenylacetic acid	4-Aminomethylphenylacetic acid
(4-Amino)F	L-4-Aminophenylalanine
(4-Bromo)F	L-4-Bromophenylalanine
4-Carboxybenzoic acid	4-Carboxybenzoic acid
4-Cyanobenzoic acid	4-Cyanobenzoic acid
4-Fluorobenzoic acid	4-Fluorobenzoic acid
(4-Fluoro)L	4-Fluoro-L-Leucine
4-Methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxylic acid	4-Methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxylic acid
4-methylbenzoic acid	4-Methylbenzoic acid
4-Methylvaleric	4-Methylpentanoic acid
4-Pal	L-4-Pyridylalanine
4-Piperidinepropanoic acid	4-Piperidinepropanoic acid
4-Tetrahydropyranoyl	4-Tetrahydropyranoyl
5-Chlorothiophene-2-carboxylic acid	5-Chlorothiophene-2-carboxylic acid
8-aminocubane-1-carboxylic acid	8-Aminocubane-1-carboxylic acid
AAD	(S)-2-(Amino)-1,6-hexanedioic acid
Abu	L-2-Aminobutyric acid
ACBA	1-Aminocyclobutane-1-carboxylic acid
ACBC	Aminocyclobutanecarboxylic acid
ACMP	1-(Aminomethyl)-cyclopropyl-1-carboxylic acid
S,S-ACPC	(1S,2S)-2-Amino-1-cyclopentanecarboxylic acid
R,R-ACPC	(1R,2R)-2-Amino-1-cyclopentanecarboxylic acid
ACPC	rel-(1R,2S)-2-Amino-1-cyclopentanecarboxylic acid
Adipic acid	Adipic acid
Ahx	6-Aminohexanoic acid
Aib	2-Aminoisobutyric acid
(tBu)A	L-tert-Butylalanine
allo-I	allo-L-Isoleucine

Abbreviation/Expression	Abbreviation/Expression Definition
allo-T	allo-L-Threonine
(2-Me)P	2-Methyl-L-Proline
(Me)R	N(5)-methyl-L-arginine
Azetidine-2-carboxylic acid	(S)-Azetidine-2-carboxylic acid
Benzoic	Benzoic acid
beta-2-thienylalanine	L-2-Thienylalanine
beta-A	beta-Alanine
4-(3,5-Dimethyl-1,2-oxazol-4-yl)-L-phenylalanine	4-(3,5-Dimethyl-1,2-oxazol-4-yl)-L-phenylalanine
(2S)-2-amino-3-(4-tert-butylphenyl)propanoic acid	(2S)-2-amino-3-(4-tert-butylphenyl)propanoic acid
(2S)-2-Amino-5-methyl-hexanoic acid	(2S)-2-Amino-5-methyl-hexanoic acid
(2S)-2-Amino-4,4,4-trifluorobutanoic acid	(2S)-2-Amino-4,4,4-trifluorobutanoic acid
(2S)-3-(4-carboxyphenyl)-2-aminopropanoic acid	(2S)-3-(4-carboxyphenyl)-2-aminopropanoic acid
(2S)-2-amino-3-(4-carbamoylphenyl)propanoic acid	(2S)-2-amino-3-(4-carbamoylphenyl)propanoic acid
(2S)-2-Amino-3-(2,3,4,5,6-(pentafluorophenyl)propanoic acid	(2S)-2-Amino-3-(2,3,4,5,6-(pentafluorophenyl)propanoic acid
(2S)-3-(3-Cyanophenyl)-2-aminopropanoic acid	(2S)-3-(3-Cyanophenyl)-2-aminopropanoic acid
3-Azido-L-Alanine	3-Azido-L-Alanine
2-Amino-5,5,5-trifluoro-4-methyl-pentanoic acid	2-Amino-5,5,5-trifluoro-4-methyl-pentanoic acid
(1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid	(1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid
Cba	L-Cyclobutylalanine
Cbg	(S)-2-Amino-2-cyclobutylacetic acid
Cha	L-Cyclohexylalanine
Cha	D-cyclohexylalanine
Chg	L-Cyclohexylglycine
(Difluoro)P	L-4,4-Difluoroproline
(cis-Fluoro)P	(2S,4S)-4-Fluoroproline
Cit	L-Citrulline
Cnba	L-2-Amino-4-cyanobutyric acid

Abbreviation/Expression	Abbreviation/Expression Definition
3,3-dimethyl-1,3-azasilolidine-5-carboxylic acid	3,3-dimethyl-1,3-azasilolidine-5-carboxylic acid
(S)-(trifluoromethyl)-L-cysteine	(S)-(trifluoromethyl)-L-cysteine
(2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid	(2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid
(2S)-3-(indol-4-yl)-2-(amino)propanoic acid	(2S)-3-(indol-4-yl)-2-(amino)propanoic acid
(2S)-3-(2,3-difluorophenyl)-2-aminopropanoic acid	(2S)-3-(2,3-difluorophenyl)-2-aminopropanoic acid
CpA	L-Cyclopentylalanine
Cpg	L-Cyclopentylglycine
Cyclobutanecarboxylic	Cyclobutanecarboxylic acid
Cyclobutylacetic	2-(Cyclobutyl)acetic acid
Cyclobutylglycine	L-Cyclobutylglycine
Cyclohexylacetic	Cyclohexylacetic acid
Cyclopentanecarboxylic acid	Cyclopentanecarboxylic acid
Cyclopentylacetic	Cyclopentylacetic acid
Cyclopropanecarboxylic	Cyclopropanecarboxylic acid
Cyclopropylacetic acid	Cyclopropylacetic acid
(2-Chloro)f	D-2-Chlorophenylalanine
Dab	L-2,4-Diaminobutyric acid
Dap	L-2,3-Diaminopropionic acid
beta-p	D-beta-Proline
hyp	D-Hydroxyproline
(N-Benzyl)D	(2S)-2-Amino-4-(benzylamino)-4-oxobutanecarboxylic acid
Freidinger's Lactam	(2S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-4-methylpentanoic acid
Fumaric acid	Fumaric acid
Gamma-Abu	Gamma-Aminobutyric acid
(tBu)G	L-tert-Butylglycine
(1-Bn)H	1-Benzyl-L-histidine
(1-Me)H	1-Methyl-L-histidine
(3-Me)H	L-3-Methylhistidine
Homo-C	L-Homocysteine
Hoo	L-Dihydroorotic acid
Hydrocinnamic	3-Phenylpropanoic acid

Abbreviation/Expression	Abbreviation/Expression Definition
Hyp	L-Hydroxyproline
Ida	Iminodiacetic acid
Isobutyric acid	Isobutyric acid
Isonipecotic acid	Isonipecotic acid
Isovaleric	Isovaleric acid
K(ISP)	N-ε-Isopropyl-L-lysine
(3-Et)Nva	3-Ethyl-L-Norvaline
Lactic	L-(+)-Lactic acid
(Bth)A	3-(1,3-Benzothiazol-2-yl)-L-alanine
beta-P	(S)-Pyrrolidine-2-carboxylic acid
L-Propargylglycine	L-Propargylglycine
Morpholine-3-carboxylic	(3S)-Morpholine-3-carboxylic acid
Nicotinic acid	Nicotinic acid
Nle	L-Norleucine
(N-Me)P	N-Methyl-L-proline
Nva	L-Norvaline
ODD	1,18-Octadecanedioic acid
Oic	2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid
Orn	L-Ornithine
Palm	Palmitic acid
PEG1(10 atoms)	9-Amino-4,7-dioxanonoic acid
PEG2(13 atoms)	12-Amino-4,7,10-trioxadodecanoic acid
PEG3(16 atoms)	15-Amino-4,7,10,13-tetraoxa(Pen)tridecanoic acid
PEG4(19 atoms)	18-Amino-4,7,10,13,16-(Pen)tetraoxatetradecanoic acid
PEG4-CH ₂ CO ₂ H (15 atoms)	14-Amino-3,6,9,12-tetraoxatetradecanoic acid
PEG5(22 atoms)	1-Amino-3,6,9,12,15,18-hexaoxaheneicosan-21-oic acid
PEG5-CH ₂ CO ₂ H (18 atoms)	17-Amino-3,6,9,12,15-(Pen)heptaheptadecanoic acid
PEG7(25 atoms)	1-Amino-3,6,9,12,15,18,21-heptaotetacosan-24-oic acid
PEG8(28 atoms)	1-Amino-3,6,9,12,15,18,21,24-octaooctacosan-27-oic acid
PEG9(31 atoms)	1-Amino-3,6,9,12,15,18,21,24,27-nonaotriacontan-30-oic acid
Pen	L-Penicillamine
Phenylacetic acid	Phenylacetic acid
Phg	L-Phenylglycine

Abbreviation/Expression	Abbreviation/Expression Definition
Picolinic acid	Picolinic acid
Pip	L-Pipecolic acid
Piperidin-4-ylacetic acid	Piperidin-4-ylacetic acid
Pivalic	Pivalic acid
(3S-OH)P	L-trans-3-Hydroxyproline
Pyr	L-Pyroglutamic acid
S-2-amino-3-ethyl-pentanoic acid	S-2-amino-3-ethyl-pentanoic acid
Pyrrolidineacetyl	2-(Pyrrolidin-1-yl)acetic acid
S-3-1-Pyrrolidin-2-yl-propionic acid	S-3-1-Pyrrolidin-2-yl-propionic acid
Suberic acid	Suberic acid
tert-Butylacetic acid	tert-Butylacetic acid
Tetrahydropyranyl-4-acetic acid	Tetrahydropyranyl-4-acetic acid
TFA	Trifluoroacetic acid
Thi	L-2-Thienylalanine
(2S,3S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-3-methylpentanoic acid	(2S,3S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-3-methylpentanoic acid
(2S,3S)-2-[2-Oxopiperazin-1-yl]-3-methylpentanoic acid	(2S,3S)-2-[2-Oxopiperazin-1-yl]-3-methylpentanoic acid
(2S,3S)-2-[(3S)-2-oxopiperazin-1-yl]-3-methylpentanoic acid	(2S,3S)-2-[(3S)-2-oxopiperazin-1-yl]-3-methylpentanoic acid
(1R,3S,5R)-2-Azabicyclo[3.1.0]hexane-3-carboxylic acid	(1R,3S,5R)-2-Azabicyclo[3.1.0]hexane-3-carboxylic acid
3-(Trimethylsilyl)-L-alanine	3-(Trimethylsilyl)-L-alanine
(6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid	(6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid
rel-(1R,3R,5R,6R)-6-(Trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid	rel-(1R,3R,5R,6R)-6-(Trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid
(4aR,6aR,9S,11aS)-11-Oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid	(4aR,6aR,9S,11aS)-11-Oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid
(2S)-2[(Amino)-2-(tetrahydro-2H-pyran-4-yl)]acetic acid	(2S)-2[(Amino)-2-(tetrahydro-2H-pyran-4-yl)]acetic acid
(2S,3S)-2-((Amino)methyl)-3-methylpentanoic acid	(2S,3S)-2-((Amino)methyl)-3-methylpentanoic acid
2-[(1S,2S)-1-(Amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid	2-[(1S,2S)-1-(Amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid

Abbreviation/Expression	Abbreviation/Expression Definition
(2S)-Amino-(1-methyl-1H-indazol-5-yl)acetic acid	(2S)-Amino-(1-methyl-1H-indazol-5-yl)acetic acid
(2R)-Amino-(1-methyl-1H-indazol-5-yl)acetic acid	(2R)-Amino-(1-methyl-1H-indazol-5-yl)acetic acid
(3-Carboxy)F	3-Carboxyphenylalanine
2-Methyl-D-alloisoleucine	2-Methyl-D-alloisoleucine
4-Ethyl-L-norleucine	4-Ethyl-L-norleucine
L-2,6-Difluorophenylalanine	L-2,6-Difluorophenylalanine
(2,5-Difluoro)F	2,5-Difluoro-L-phenylalanine
(2S,3aS,6aS)-Octahydrocyclopenta[b]pyrrole-2-carboxylic acid	(2S,3aS,6aS)-Octahydrocyclopenta[b]pyrrole-2-carboxylic acid
(2S)-2-(Amino)-2-[(1S,3R)-3-hydroxycyclohexyl]acetic acid	(2S)-2-(Amino)-2-[(1S,3R)-3-hydroxycyclohexyl]acetic acid
(2S)-2-(Amino)-2-[(1S,3S)-3-hydroxycyclohexyl]acetic acid	(2S)-2-(Amino)-2-[(1S,3S)-3-hydroxycyclohexyl]acetic acid
2-Amino-7-(tert-butoxy)-7-oxoheptanoic acid	2-Amino-7-(tert-butoxy)-7-oxoheptanoic acid
(2S)-3-(Triazol-1-yl)-2-(amino)propanoic acid	(2S)-3-(Triazol-1-yl)-2-(amino)propanoic acid
Tetrahydro-2H-pyran-3-ylacetic acid	Tetrahydro-2H-pyran-3-ylacetic acid
rel-(1R,3S)-3-[(Amino)methyl]cyclohexanecarboxylic acid	rel-(1R,3S)-3-[(Amino)methyl]cyclohexanecarboxylic acid
(3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1)	(3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1)
(3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2)	(3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2)
Tranexamic	Tranexamic acid
trans-2-(3-(Amino)cyclohexyl)acetic acid	trans-2-(3-(Amino)cyclohexyl)acetic acid
(trans-4-Fluoro)P	trans-4-Fluoroproline
(Trifluoro)l/L	5,5,5-Trifluoro-DL-leucine
TTDS	1,13-Diamino-4,7,10-trioxatridecan-succinamic acid
(R)-Pyrrolidine-3-acetic acid	(R)-Pyrrolidine-3-acetic acid
Pro-D2	L-Proline (3,4- ² H) (deuterated)
(2S)-2-(morpholin-4-yl)propanoic acid	(2S)-2-(morpholin-4-yl)propanoic acid
L-dehydroproline	3,4-dehydro-L-proline

Abbreviation/Expression	Abbreviation/Expression Definition
(3S)-2-azaspiro[4.4]nonane-3-carboxylic acid	(3S)-2-azaspiro[4.4]nonane-3-carboxylic acid
(2R)-2-amino-3-(trifluoromethylsulfonyl)propanoic acid	(2R)-2-amino-3-(trifluoromethylsulfonyl)propanoic acid
Arg(13C6,15N4)	L-Arginine-N-Fmoc, Pbf-OH (13C6, 15N4)

The term “mimetic”, used in context with some amino acids in the definition of several moieties of the peptide according to formula (I) or formula (II) of the present invention, represents a respective amino acid mimetic, such as e.g. an arginine mimetic, an isoleucine mimetic or a proline mimetic. Generally, a “protein mimetic” indicates a molecule such as a peptide, a modified peptide or any other molecule that biologically mimics the action or activity of some other protein. In context with the use of the term “mimetic” in connection with a certain amino acid said term “mimetic” analogously indicates any other amino acid, amino acid analogue, amino acid derivative, amino acid conjugate or the like, which biologically mimics the action or activity of the respective amino acid.

Proline mimetics according to the present invention comprise in particular (1S,2S,5R)-3-Azabicyclo[3.1.0]hexane-2-carboxylic acid, Hyp, Morpholine-3-carboxylic, Pip, (4aR,6aR,9S,11aS)-11-Oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid or (trans-4-Fluoro)P, (1R,2S,5S)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid, Oic, Hyp, (4-CF₃)P, (cis-4-Fluoro)P, 3,3-dimethyl-1,3-azasilolidine-5-carboxylic acid, (3S-OH)P, (1R,3S,5R)-2-Azabicyclo[3.1.0]hexane-3-carboxylic acid, (6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid, rel-(1R,3R,5R,6R)-6-(Trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid, (2S,3aS,6aS)-Octahydrocyclopenta[b]pyrrole-2-carboxylic acid or difluoroproline, (3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1), (3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2) and substituted prolines.

Isoleucine mimetics according to the present invention comprise in particular (N-Methyl)-I, allo-Ile, Cba, Nva, Abu, Leu, Cpg, cyclohexyl-Gly, (S)-2-Amino-3-ethyl-pentanoic acid, 3-Chloro-Phg, allo-Ile, Chg, Cyclobutylglycine, allo-Ile, Cbg, (2S,3S)-2-((Amino)methyl)-3-methylpentanoic acid, Phg, 2-[(1S,2S)-1-(Amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid, 2-Methyl-D-alloisoleucine, Nva, Abu or Ala.

Leucine mimetics according to the present invention comprise in particular (tBu)A, (2-Chloro)F, (2-Bromo)F, AAD, (2S)-2-Amino-4,4,4-trifluorobutanoic acid, Cnba, (4-Fluoro)L, (S)-(trifluoromethyl)-L-cysteine, (2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid, Gly(tBu), 3-(Trimethylsilyl)-L-alanine, 2,5-difluoro-L-phenylalanine, 2-Amino-7-(tert-butoxy)-7-oxoheptanoic acid, 5,5,5-Trifluoro-L-leucine ((Trifluoro)L), (2-Me)F, Cba, Cpa, cyclopropylmethylalanine, trifluoromethylalanine or difluoromethylalanine, (2-Fluoro)F, (2S)-3-(2,3-difluorophenyl)-2-aminopropanoic acid, (2S)-3-(3-Cyanophenyl)-2-

aminopropanoic acid, 2-Amino-5,5,5-trifluoro-4-methyl-pentanoic acid, (2S)-2-Amino-5-methyl-hexanoic acid or (2S)-3-(indol-4-yl)-2-(amino)propanoic acid.

The invention further comprises analogues and derivatives of the described peptides. The term “analogue” or “derivative” of a peptide or an amino acid sequence according to the present invention comprises in particular any amino acid sequence having a sequence identity of at least 80% or at least 85%, preferably at least 90%, more preferably at least 95%, and even more preferably of at least 99% identity to said sequence, and same or comparable properties or activity. Sequence identity can be determined by common techniques, such as visual comparison or by means of any computer tool generally used in the field. Examples comprise BLAST programs used with default parameters.

- 10 An analogue or derivative of a peptide or an amino acid sequence of the invention may result from changes derived from mutation or variation in the sequences of peptides of the invention, including the deletion or insertion of one or more amino acids or the substitution of one or more amino acids, or even to alternative splicing. Several of these modifications may be combined. Preferably, an analogue of an amino acid sequence of the invention comprises conservative substitutions relative to the sequence of amino acids.
- 15 The term “conservative substitution” as used herein denotes that one or more amino acids are replaced by another, biologically similar residue. Examples include substitution of amino acid residues with similar characteristics, e.g., small amino acids, acidic amino acids, polar amino acids, basic amino acids, hydrophobic amino acids and aromatic amino acids. See, for example, the scheme in Table 4 below, wherein conservative substitutions of amino acids are grouped by physicochemical properties. I: neutral, hydrophilic; II: acids and amides; III: basic; IV: hydrophobic; V: aromatic, bulky amino acids, VI: neutral or hydrophobic; VII: acidic; VIII: polar.
- 20

Table 4: Amino Acids grouped according to their physicochemical properties

I	II	III	IV	V	VI	VII	VIII
Ala	Asn	His	Met	Phe	Ala	Glu	Met
Ser	Asp	Arg	Leu	Tyr	Leu	Asp	Ser
Thr	Glu	Lys	Ile	Trp	Ile		Thr
Pro	Gln		Val		Pro		Cys
Gly			Cys		Gly		Asn
					Val		Gln

- A peptide analogue or derivative may also comprise one or more additional modifications such as, e.g., conjugation to another compound to form an amino acid conjugate.
- 25

Such a modification may, alternatively or additionally, result from conjugation to the side-chains of one or more amino acid residues in a peptide of the present invention for example a chemical group as defined above in context with the chemical group X^0 or a polymeric moiety such as a PEG group. Such modification may, for example, increase solubility and/or half-life *in vivo* (e.g. in plasma) and/or bioavailability of the peptide and are also known to reduce clearance (e.g. renal clearance) of therapeutic proteins and peptides. Suitable modifications are well known to a skilled person and comprise in particular, without being limited thereto, PEGylation of one or more side chains of the peptide of the present invention. Therein, "PEGylation" represents the act of coupling (e.g., covalently) a Polyethylene glycol (PEG) structure to the peptide of the invention. The skilled person knows well possible PEGs for coupling to the amino acid side chains of small peptides for forming a respective conjugate, e.g. from WO 2015/200916 A1, which are herein incorporated by reference.

All peptides of this invention unless otherwise noted are TFA salts. The invention comprises further pharmaceutically acceptable salts of the peptides as defined herein and salt free forms. Therein, pharmaceutically acceptable salts represent salts or zwitterionic forms of the peptides or compounds of the present invention which are water or oil-soluble or dispersible, which are suitable for treatment of diseases without undue toxicity, irritation, and allergic response; which are commensurate with a reasonable benefit/risk ratio, and which are effective for their intended use. The salts can be prepared during the final isolation and purification of the compounds or separately by reacting an amino group with a suitable acid. Representative acid addition salts include acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, carbonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, formate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate (isethionate), lactate, maleate, mesitylenesulfonate, methanesulfonate, naphthyl-20 enesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, sulfate, tartrate, trichloroacetate, trifluoroacetate, phosphate, glutamate, bicarbonate, para-toluenesulfonate, and undecanoate. Preferred acid addition salts include trifluoroacetate, formate, hydrochloride, and acetate.

Also, amino groups in the compounds of the present invention can be quaternized with methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides; dimethyl, diethyl, dibutyl, and diamyl sulfates; decyl, lauryl, myristyl, and steryl chlorides, bromides, and iodides; and benzyl and phenethyl bromides. Examples of acids which can be employed to form therapeutically acceptable addition salts include inorganic acids such as hydrochloric, hydrobromic, sulfuric, and phosphoric, and organic acids such as oxalic, maleic, succinic, and citric. A pharmaceutically acceptable salt may suitably be a salt chosen, e.g., among acid addition salts and basic salts. Examples of acid addition salts include chloride salts, citrate salts and acetate salts.

Examples of basic salts include salts where the cation is selected from alkali metal cations, such as sodium or potassium ions, alkaline earth metal cations, such as calcium or magnesium ions, as well as substituted ammonium ions, such as ions of the type $N(R^1)(R^2)(R^3)(R^4)^+$, where R^1 , R^2 , R^3 and R^4 independently from

each other will typically designate hydrogen, optionally substituted C₁₋₆-alkyl or optionally substituted C₂₋₆-alkenyl. Examples of relevant C₁₋₆-alkyl groups include methyl, ethyl, 1-propyl and 2-propyl groups. Examples of C₂₋₆-alkenyl groups of possible relevance include ethenyl, 1-propenyl and 2-propenyl. Therein, salts where the cation is selected among sodium, potassium and calcium are preferred.

- 5 Other examples of pharmaceutically acceptable salts are described in *"Remington's Pharmaceutical Sciences"*, 17th edition, Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, USA, 1985 (and more recent editions thereof), in the *"Encyclopaedia of Pharmaceutical Technology"*, 3rd edition, James Swarbrick (Ed.), Informa Healthcare USA (Inc.), NY, USA, 2007. Also, for a review on suitable salts, see *Handbook of Pharmaceutical Salts: Properties, Selection, and Use* by Stahl and Wermuth (Wiley- VCH, 2002). Other suitable
- 10 base salts are formed from bases which form non-toxic salts. Representative examples include the aluminum, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine, and zinc salts, preferably choline. Hemisalts of acids and bases may also be formed, e.g., hemisulphate and hemicalcium salts.

- The invention further comprises solvates of the peptides as defined herein. Therein the term "solvate"
- 15 refers to a complex of defined stoichiometry formed between a solute (e.g., a peptide according to the invention or pharmaceutically acceptable salt thereof) and a solvent. The solvent in this connection may, for example, be water, ethanol or another pharmaceutically acceptable, typically small-molecular organic species, such as, but not limited to, acetic acid or lactic acid. When the solvent in question is water, such a solvate is normally referred to as a hydrate.

- 20 The compounds according to the invention have useful pharmacological properties and can be used for prevention and treatment of disorders in humans and animals.

- In the context of the present invention, the term "treatment" or "treat" includes the inhibition, delay, arrest, amelioration, attenuation, limitation, reduction, suppression, reversal or cure of a disease, a condition, a disorder, an injury or health impairment, of the development, course or the progression of such states
- 25 and/or the symptoms of such states. Here, the term "therapy" is understood to be synonymous with the term "treatment".

- In the context of the present invention, the terms "prevention", "prophylaxis" or "precaution" are used synonymously and refer to the avoidance or reduction of the risk to get, to contract, to suffer from or to have a disease, a condition, a disorder, an injury or a health impairment, a development or a progression
- 30 of such states and/or the symptoms of such states.

The treatment or the prevention of a disease, a condition, a disorder, an injury or a health impairment may take place partially or completely.

The compounds according to the invention are particularly suitable for the treatment and/or prevention of cardiovascular, cardiopulmonary, renal, pulmonary, fibrotic, thromboembolic, and inflammatory disorders.

Accordingly, the compounds according to the invention can be used in medicaments for the treatment and/or prevention of cardiovascular and cardiopulmonary disorders and their sequels such as, for example inflammatory heart diseases, myocarditis, endocarditis, pericarditis, rheumatic fever without and with heart involvement, acute rheumatic pericarditis, acute rheumatic endocarditis, acute rheumatic myocarditis, chronic rheumatic heart diseases with and without endocarditis, valvulitis, pericarditis, ischemic heart diseases such as unstable angina pectoris and acute myocardial infarction, atrial and ventricular arrhythmias and impaired conduction such as, for example, grade I-III atrioventricular blocks, supraventricular tachyarrhythmia, atrial fibrillation, atrial flutter, ventricular fibrillation, ventricular flutter, ventricular tachyarrhythmia, Torsade de pointes tachycardia, atrial and ventricular extrasystoles, AV-junctional extrasystoles, sick sinus syndrome, stroke due to occlusion and stenosis of cerebral arteries (Cerebral infarction following e.g. thromboembolic, atherosclerotic, infectious and inflammatory vascular lesions), for the treatment and/or prevention of stroke due to intracerebral or intracranial haemorrhage, peripheral ischemic tissue damage (e.g. atherosclerotic gangrene) due to diseases of arteries, arterioles and capillaries (e.g. thromboembolic, atherosclerotic, infectious and inflammatory vascular lesions, endarteritis deformans or obliterans, and aneurysm dissection), phlebitis and thrombophlebitis, for preventing postprocedural disorders of the circulatory system, e.g. systemic inflammatory response syndrome, vasoplegia after surgery, postcardiotomy syndrome, postprocedural hypotension and heart failure, for preventing and treating ischemia reperfusion injury and organ dysfunction for example after thrombolysis therapies, percutaneous transluminal angioplasties (PTA), percutaneous transluminal coronary angioplasties (PTCA), bypass operations and heart, lung, liver and kidney transplants, and for the prevention and treatment of delayed graft function after kidney transplantation.

The compounds according to the invention are furthermore suited for the treatment of shock such as cardiogenic shock, septic shock and anaphylactic shock by preventing MASP mediated end organ damages.

Moreover, the compounds according to the invention have antiinflammatory action and can therefore be used as antiinflammatories for treatment and/or prevention of sepsis (SIRS), multiple organ failure (MODS, MOF), inflammatory disorders of the kidney, chronic bowel inflammations (IBD, Crohn's Disease, UC), pancreatitis, peritonitis, rheumatoid disorders, inflammatory skin disorders and inflammatory eye disorders.

By virtue of their activity profile, the compounds according to the invention are particularly suitable for the treatment and/or prevention of cardiovascular, pulmonary, cerebral and renal sequels of sepsis and systemic inflammatory response syndrome.

The compounds according to the invention are particularly suitable for the treatment and/or prevention of ischemia and/or reperfusion-related damage to the heart and the kidney and other organs in the context of

resuscitation and surgical interventions such as but not restricted to bypass operations, heart valve surgery, and aortic aneurysm surgery,

The compounds according to the invention can additionally also be used for preventing ischaemic and/or reperfusion-related damage to organs or tissues and also as additives for perfusion and preservation solutions of organs, organ parts, tissues or tissue parts of human or animal origin, in particular for surgical interventions or in the field of transplantation medicine.

Furthermore, the compounds according to the invention are suitable for the treatment and/or prevention of diseases of the blood and blood-forming organs and the immune system including but not limited to acquired haemolytic anaemia, haemolytic-uraemic syndrome, paroxysmal nocturnal haemoglobinuria [Marchiafava-Micheli], coagulation defects, purpura and other haemorrhagic conditions, disseminated intravascular coagulation [defibrination syndrome], essential (haemorrhagic) thrombocythaemia, purpura fulminans, thrombotic thrombocytopenic purpura, allergic purpura, allergic vasculitis, lymphopenia and Igranulocytosis, and sarcoidosis.

Furthermore, the compounds according to the invention are suitable for the treatment and/or prevention of sequels of diabetes mellitus such as renal complications of diabetes mellitus, diabetic nephropathy, intracapillary glomerulonephrosis, ophthalmic complications of diabetes mellitus, diabetic retinopathy, neurological complications, diabetic polyneuropathy, and circulatory complications such as microangiopathy and gangrene.

Moreover, the compounds according to the invention are suitable for the treatment and/or prevention of inflammatory diseases of the nervous system such as multiple sclerosis, meningitis and encephalitis, bacterial and viral meningitis and encephalitis, postimmunization encephalitis, inflammatory polyneuropathy, and polyneuropathy in infectious and parasitic diseases.

The compounds according to the invention are furthermore suitable for the treatment and/or prevention of diseases of the eye and its adnexa, such as acute and subacute iridocyclitis, choroidal degeneration, chorioretinal inflammation, chorioretinal inflammation in infectious and parasitic diseases, background retinopathy and retinal vascular changes, proliferative retinopathy, degeneration of macula and posterior pole, peripheral retinal degeneration, age-related macular degeneration (AMD) including dry (non-exudative) and wet (exudative, neovascular) AMD, choroidal neovascularization (CNV), choroidal neovascular membranes (CNVM), cystoid macular oedema (CME), epiretinal membranes (ERM) and macular perforations, myopia-associated choroidal neovascularization, angioid and vascular streaks, retinal detachment, diabetic retinopathy, diabetic macular oedema (DME), atrophic and hypertrophic lesions in the retinal pigment epithelium, retinal vein occlusion, choroidal retinal vein occlusion, macular oedema, macular oedema associated with retinal vein occlusion, postprocedural disorders of eye and adnexa, e.g. keratopathy following cataract surgery.

Furthermore, the compounds according to the invention are suitable for the treatment and/or prevention of diseases of the respiratory system including but not restricted to viral, bacterial, and mycotic pneumonia, radiation pneumonitis, pneumoconiosis, allergic alveolitis, airway disease due to specific organic dust, e.g. farmer lung, bronchitis, pneumonitis and pulmonary oedema due to chemicals, gases, fumes and vapours, drug-induced interstitial lung disorders, adult respiratory distress syndrome (ARDS) and acute lung injury (ALI), acute oedema of the lung, interstitial pulmonary diseases with fibrosis, rheumatoid lung disease, respiratory disorders in other diffuse connective tissue disorders, such as associated to systemic lupus erythematosus, scleroderma and Wegener granulomatosis.

Furthermore, the compounds according to the invention are suitable for treatment and/or prevention of microvascular injury, thrombosis and consecutive thromboembolic events caused by viral infections such as, but not restricted to, Influenza viruses (e.g. caused by strains of serotypes H1N1, H5N1, H7N9), and Corona viruses (e.g. SARS-CoV, the pathogen of *severe acute respiratory syndrome (SARS)*, MERS-CoV, the pathogen of *Middle East respiratory syndrome (MERS)*, and SARS-CoV-2 the pathogen of *COVID-19 pandemic*).

Furthermore, the compounds according to the invention are suitable for the treatment and/or prevention of diseases of the digestive system including but not restricted to noninfective enteritis and colitis such as Crohn disease and ulcerative colitis, pancreatitis (including acute alcohol- and drug induced pancreatitis), cholecystitis, inflammatory liver diseases, hepatorenal syndrome, postprocedural disorders of the liver, e.g. after liver surgery.

By virtue of their activity profile, the compounds according to the invention are particularly suitable for the treatment and/or prevention of diseases of the genitourinary system including but not restricted to acute renal failure, acute kidney injury (AKI), surgery associated AKI, sepsis associated AKI, contrast media and chemotherapy induced AKI, ischaemia and infarction of the kidney, complications such as hypersensitivity in the context of hemodialysis and hemodiafiltration, cystitis, irradiation cystitis, inflammatory diseases of the prostate, and endometriosis.

The compounds according to the invention are furthermore suitable for the treatment and/or prevention of sequels of burns and injuries including but not restricted to early complications of trauma, traumatic anuria, crush syndrome, renal failure following crushing, traumatic ischaemia of muscle, traumatic brain injury, organ damage after exposure to electric current, radiation and extreme ambient air temperature and pressure, after exposure to smoke, fire and flames, after contact with venomous animals and plants.

By virtue of their activity profile, the compounds according to the invention are furthermore suitable for the treatment of inflammatory skin diseases for example dermal lupus erythematosus, bullous disorders

and acantholytic skin diseases such as pemphigus subtypes, papulosquamous disorders such as psoriasis, dermatitis and eczema, urticaria and erythema.

According to a further embodiment, the invention provides a compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue or pharmaceutically acceptable salts or solvates thereof for the use in the prophylaxis and/or treatment of diseases.

According to a further embodiment, the invention provides a compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue or pharmaceutically acceptable salts or solvates thereof for the use in the prophylaxis and/or treatment of MASP-associated disorders.

According to a further embodiment, the invention provides a compound containing a peptidewhich may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, which acts as a MASP-1 and/or MASP-2 inhibitor and/or which inhibits C3 deposition, for the use in the prophylaxis and/or treatment of MASP-associated disorders.

According to a further embodiment, the invention provides a compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue or pharmaceutically acceptable salts or solvates thereof for the use in the prophylaxis and/or treatment of cardiovascular and cardiopulmonary disorders, shock, inflammatory disorders, cardiovascular, pulmonary, cerebral and renal sequels of sepsis, ischemia and/or reperfusion-related damage, acute kidney injury, transplant protection and delayed graft function, diseases of the blood and blood-forming organs and the immune system, sequels of diabetes mellitus, inflammatory diseases of the nervous system, diseases of the eye, diseases of the skin, diseases of the respiratory, digestive or genitourinary system and sequels of burns and injuries.

According to a further embodiment, the invention provides a compound containing a peptidewhich may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue or pharmaceutically acceptable salts or solvates thereof for the use in the prophylaxis and/or treatment of diseases of the genitourinary system including but not restricted to acute renal failure, acute kidney injury (AKI), surgery associated AKI, sepsis associated AKI, contrast media and chemotherapy induced AKI, ischaemia and infarction of the kidney, complications such as hypersensitivity in the context of hemodialysis and hemodiafiltration, cystitis, irradiation cystitis, inflammatory diseases of the prostate, and endometriosis.

The invention further relates to a method of treating or ameliorating MASP-associated disorders, as defined above, in a subject or patient by administering at least one peptide, derivative or analogue as defined herein or a pharmaceutically acceptable salt or solvate thereof, a complex or a pharmaceutical composition as defined above, to said subject or patient in need thereof.

- 5 As used herein, the terms “patient”, “subject” or “individual” may be used interchangeably and refer to either a human or a non-human animal. These terms include mammals such as humans, primates, livestock animals (e.g., bovines, porcines), companion animals (e.g., canines, felines) and rodents (e.g., mice and rats). The term “mammal” refers to any mammalian species such as a human, mouse, rat, dog, cat, hamster, guinea pig, rabbit, livestock, and the like.
- 10 According to the invention the at least one peptide, derivative or analogue as defined herein or the pharmaceutically acceptable salt or solvate thereof, or the complex as defined above is administered to a patient or subject in a therapeutically effective amount, wherein a “therapeutically effective amount” of a compound of the present invention is meant to describe a sufficient amount of a compound of the present invention to treat an MASP-associated disorder as defined herein. In particular embodiments, the therapeutically effective amount will achieve a desired benefit/risk ratio applicable to any medical treatment.
- 15

The present invention particularly comprises the following embodiments, wherein the substituents or moieties of the peptide according to formula (I) or formula (II) as defined below, may independently have the meanings as described below.

- A peptide or a compound containing a peptide, or derivative or analogue thereof as defined herein or the pharmaceutically acceptable salt or solvate thereof or the complex or the pharmaceutical composition (as defined below), are hereinafter commonly also referred to as “MASP inhibitory peptide of the present invention”.
- 20

- In some embodiments, a MASP inhibitory peptide of the present invention binds to MASP-1 and/or MASP-2, e.g. human MASP-1 and/or MASP-2. In certain embodiments, a MASP inhibitory peptide of the present invention specifically binds to human MASP-1 and/or MASP-2. As used herein, “specifically binds” refers to a specific binding agent's preferential interaction with a given ligand over other agents in a sample. For example, a specific binding agent that specifically binds a given ligand binds the given ligand, under suitable conditions, in an amount or a degree that is observable over that of any nonspecific interaction with other components in the sample. Suitable conditions are those that allow interaction between a given specific binding agent and a given ligand. These conditions include pH, temperature, concentration, solvent, time of incubation, and the like, and may differ among given specific binding agent and ligand pairs, but may be readily determined by those skilled in the art. In some embodiments, a MASP inhibitory peptide of the present invention binds MASP-1 and/or MASP-2 with greater specificity than a MASP inhibitory peptide reference compound (e.g. any one of the MASP inhibitory peptide reference compounds provided herein).
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- 30

The invention thus further relates to a complex comprising at least one peptide or compound containing a peptide, derivative or analogue as defined herein bound to MASP-1 or MASP-2.

In some embodiments, a MASP inhibitory peptide of the present invention exhibits specific binding to MASP-1 and/or MASP-2, especially human MASP-1 and/or MASP-2, that is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 700%, 1000%, or 10000% higher than a selected MASP inhibitory peptide reference compound.

In some embodiments, a MASP inhibitory peptide of the present invention exhibits specific binding to MASP-1 and/or MASP-2, especially human MASP-1 and/or MASP-2, that is at least about 1, 2, 3, 4, 5-fold, or at least about 10, 20, 50, or 100-fold higher than a selected MASP inhibitory peptide reference compound.

10 In some embodiments, a MASP inhibitory peptide of the present invention exhibits a binding affinity to MASP-1 and/or MASP-2 that is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 700%, 1000%, or 10000% higher than a selected MASP inhibitory peptide reference compound.

15 In some embodiments, a MASP inhibitory peptide of the present invention exhibits a binding affinity to MASP-1 and/or MASP-2 that is at least about 1, 2, 3, 4, 5-fold, or at least about 10, 20, 50, 100 or 1000-fold higher than a selected MASP inhibitory peptide reference compound.

In some embodiments, a MASP inhibitory peptide of the present invention exhibits an inhibition of MASP-1 and/or MASP-2 (e.g., rat or human MASP-1 and/or MASP-2) activity. In some embodiments, the activity is an *in vitro* or an *in vivo* activity, e.g. an *in vitro* or *in vivo* activity described herein. In some
20 embodiments, a MASP inhibitory peptide of the present invention inhibits at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 700%, 1000%, or 10000% of the MASP-1 and/or MASP-2 activity inhibited by a selected MASP inhibitory peptide reference compound.

In certain embodiments, the MASP inhibitory peptide of the present invention exhibits 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, or 200-fold
25 greater MASP-1 and/or MASP-2 inhibition than a selected MASP inhibitory peptide reference compound.

In further particular embodiments, the MASP-1 and/or MASP-2 inhibitory activity of the MASP inhibitory peptides according to the present invention is determined by measurement of their IC_{50} for MASP-1 and/or MASP-2 (e.g., rat human MASP-1 and/or MASP-2). Determination of the IC_{50} for MASP-1 and/or MASP-2 can be done with the biochemical assays shown herein. It is particularly preferred that a MASP inhibitory
30 peptide of the present invention exhibits an IC_{50} for MASP-1 and/or MASP-2 of $< 1,000$ nM, preferably ≤ 500 nM, more preferably ≤ 300 nM, more preferably ≤ 250 nM, more preferably ≤ 200 nM, more preferably

≤ 150 nM, more preferably ≤ 100 nM, more preferably ≤ 75 nM, more preferably ≤ 50 nM, more preferably ≤ 45 nM, more preferably ≤ 40 nM, more preferably ≤ 35 nM, more preferably ≤ 30 nM.

In some embodiments, a MASP inhibitory peptide of the present invention has a lower IC_{50} (i.e. higher binding affinity) for MASP-1 and/or MASP-2, (e.g., rat or human MASP-1 and/or MASP-2) compared to a selected MASP inhibitory peptide reference compound. In some embodiments, a MASP inhibitory peptide according to the present invention has an IC_{50} in a MASP-1 and/or MASP-2 competitive binding assay which is at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 700%, 1000% or 10000% lower than that of a selected MASP inhibitory peptide reference compound.

In some embodiments, a MASP inhibitory peptide of the present invention exhibits at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99%, or greater than 99%, 100%, 200%, 300%, 400%, 500%, 700%, 1000% or 10000% greater *in vitro* inhibition of human MASP-1 and/or MASP-2 activity as that of a selected MASP inhibitory peptide reference compound.

In some embodiments, a MASP inhibitory peptide of the present invention exhibits at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99%, or greater than 99%, 100%, 200%, 300%, 400%, 500%, 700%, 1000% or 10000% greater *in vivo* inhibition of human MASP-1 and/or MASP-2 activity as that of a selected MASP inhibitory peptide reference compound.

As used herein, in certain embodiments, a MASP inhibitory peptide having a “MASP-1 and/or MASP-2 inhibitory activity” means that the compound has the ability to inhibit C3 deposition *in vitro* or in subjects (e.g. mice or humans), when administered thereto (e.g. by the parenteral route, e.g. by injection, or by the pulmonary, nasal, sublingual, lingual, buccal, dermal, transdermal, conjunctival, optic route or as implant or stent orally administered), in a dose-dependent and time-dependent manner.

In some embodiments, a MASP inhibitory peptide of the present invention exhibits an inhibition of C3 deposition (e.g., human C3 deposition. In some embodiments, the inhibition of C3 deposition is determined by an *in vitro* or an *in vivo* inhibition. In some embodiments, a MASP inhibitory peptide of the present invention inhibits at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 700%, 1000%, or 10000% of the C3 deposition inhibited by a selected MASP inhibitory peptide reference compound.

In certain embodiments, the MASP inhibitory peptide of the present invention exhibits 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, or 200-fold greater inhibition of C3 deposition than a selected MASP inhibitory peptide reference compound.

In further particular embodiments, the MASP-1 and/or MASP-2 inhibitory activity of the MASP inhibitory peptides according to the present invention is determined by measurement of their IC_{50} for inhibition

of C3 deposition *in vitro* or in subjects (e.g. mice or humans). Determination of the IC_{50} for C3-deposition can be done with the C3 Human Deposition assay shown herein. It is particularly preferred that a MASP inhibitory peptide of the present invention exhibits an IC_{50} for C3 deposition of $< 1,000$ nM, preferably ≤ 500 nM, more preferably ≤ 300 nM, more preferably ≤ 250 nM, more preferably ≤ 200 nM, more preferably ≤ 150 nM, more preferably ≤ 100 nM, more preferably ≤ 75 nM, more preferably ≤ 50 nM, more preferably ≤ 45 nM, more preferably ≤ 40 nM, more preferably ≤ 35 nM, more preferably ≤ 30 nM.

In some embodiments, a MASP inhibitory peptide of the present invention exhibits at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99%, or greater than 99%, 100%, 200% 300%, 400%, 500%, 700%, 1000% or 10000% greater *in vitro* inhibition of C3-deposition as that of a selected MASP inhibitory peptide reference compound.

In some embodiments, a MASP inhibitory peptide of the present invention exhibits at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99%, or greater than 99%, 100%, 200% 300%, 400%, 500%, 700%, 1000% or 10000% greater *in vivo* inhibition of C3-deposition as that of a selected MASP inhibitory peptide reference compound.

It is particularly preferred that a peptide according to the present invention acts as a MASP inhibitory peptide with its activity being determined in accordance with at least one of the specific assays and/or the *in vivo* studies according to the examples of the present invention.

Due to their aforesaid MASP-1 and/or MASP-2 inhibitory activity and inhibitory activity of C3-deposition, a compound containing the peptide or the peptide of the present invention (including analogues, derivatives, and pharmaceutically acceptable salts or solvates thereof as well as the above mentioned complex) are suitable for the use in the prophylaxis and/or treatment of MASP-1 and/or MASP-2-associated disorders.

According to a further embodiment, the invention provides a compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, which acts as a MASP-1 and/or MASP-2 inhibitor and/or which inhibits C3 deposition.

The compounds according to the invention can be used alone or in combination with other active compounds if necessary. The present invention further relates to medicaments containing at least one of the compounds according to the invention and one or more further active compounds, in particular for the treatment and/or prophylaxis of the aforementioned diseases. As suitable combination active compounds, we may mention for example and preferably:

- compounds that inhibit the degradation of cyclic guanosine monophosphate (cGMP) and/or cyclic adenosine monophosphate (cAMP), for example inhibitors of phosphodiesterases (PDE) 1, 2, 3, 4

and/or 5, in particular PDE 4 inhibitors such as roflumilast or revamilast and PDE 5 inhibitors such as sildenafil, vardenafil, tadalafil, udenafil, dasantafil, avanafil, mirodenafil or lodenafil;

- NO-independent but haem-dependent stimulators of guanylate cyclase, in particular riociguat, nelociguat, vericiguat and the compounds described in WO 00/06568, WO 00/06569, WO 02/42301, WO 03/095451, WO 2011/147809, WO 2012/004258, WO 2012/028647 and WO 2012/059549;
- NO-independent and haem-independent activators of guanylate cyclase, in particular Runcaciguat, BI 703704 and the compounds described in WO2012/139888, WO 2001/019780 and WO2014/012934;
- prostacyclin analogs and IP receptor agonists, for example and preferably iloprost, beraprost, treprostinil, epoprostenol, NS-304, selexipag, or ralinepag;
- endothelin receptor antagonists, for example and preferably bosentan, darusentan, ambrisentan, macicentan or sitaxsentan;
- vasopressin receptor antagonists, for example tolvaptan, conivaptan, relcovaptan;
- human neutrophil elastase (HNE) inhibitors, for example and preferably sivelestat or DX-890 (Reltran);
- compounds which inhibit the signal transduction cascade, in particular from the group of the tyrosine kinase inhibitors, for example and preferably dasatinib, nilotinib, bosutinib, regorafenib, sorafenib, sunitinib, cediranib, axitinib, telatinib, imatinib, brivanib, pazopanib, vatalanib, gefitinib, erlotinib, lapatinib, canertinib, lestaurtinib, pelitinib, semaxanib, masitinib, or tandutinib;
- signal transduction modulators from the group of ASK1 kinase inhibitors, for example selonsertib;
- Rho kinase inhibitors, for example and preferably fasudil, Y-27632, SLX-2119, BF-66851, BF-66852, BF-66853, KI-23095 or BA-1049;
- active ingredients which reduce vascular wall permeability (oedema formation), by way of example and with preference inhibitors of the ALK1-Smad1/5 signalling pathway, inhibitors of the VEGF and/or PDGF signalling pathways, cyclooxygenase inhibitors, inhibitors of the kallikrein-kinin system or inhibitors of the sphingosine-1-phosphate signalling pathways;
- corticosteroids, for example cortisone, cortisol, prednisolone, methylprednisolone, triamcinolone or dexamethasone;
- active ingredients which reduce damage to organs under oxidative stress, by way of example and with preference inhibitors of the complement system, especially antagonists of the complement C5a receptor, anti C5 antibodies or agonists of the 5-HT1A receptor;
- modulators, stimulators and enhancers of the transcription factor Nrf2, for example CXA-10, Oltipraz, dimethyl fumarate or Bardoxolone;
- adrenomedullin and adrenomedullin derivatives, for example pegylated adrenomedullin, and adrenomedullin stabilizing agents, for example adrecizumab;
- compounds which inhibit hypoxia inducible factor prolyl hydroxylase (HIF-PH inhibitors), for example molidustat, vadadustat, roxadustat, daprodustat or desidustat;

- compounds which inhibit induction of cell death and apoptosis pathway, for example QPI-1002;
- C-Met agonists and hepatocyte growth factor mimetics, for example refanalin;
- alkaline phosphatase and recombinant alkaline phosphatase;
- compounds which inhibit inflammatory response and T cell proliferation, for example CD28 antago-
5 nistic compounds such as Reltecimod;
- compounds which modulate the activation of Th17 T cells, for example modulators of the RORc/ROR-gamma transcription factor;
- compounds antagonizing the Th17 T cell response for example anti IL-17 and anti IL-23 antibodies, for example Ixekizumab, Secukinumab, Brodalumab, Ustekinumab, Guselkumab or PTG-200;
- 10 • antithrombotic agents, for example and preferably from the group of platelet aggregation inhibitors, anticoagulants or profibrinolytic substances;
- In a preferred embodiment of the invention, the compounds according to the invention are administered in combination with a platelet aggregation inhibitor, for example and preferably aspirin, clopidogrel, ticlopidine or dipyridamole.
- 15 • In a preferred embodiment of the invention, the compounds according to the invention are administered in combination with a thrombin inhibitor, for example and preferably ximelagatran, melagatran, dabigatran, bivalirudin or Clexane.
- In a preferred embodiment of the invention, the compounds according to the invention are administered in combination with a GPIIb/IIIa antagonist, for example and preferably tirofiban or abciximab.
- 20 • In a preferred embodiment of the invention, the compounds according to the invention are administered in combination with a factor Xa inhibitor, for example and preferably rivaroxaban, apixaban, fidexaban, razaxaban, fondaparinux, idraparinux, DU-176b, PMD-3112, YM-150, KFA-1982, EMD-503982, MCM-17, MLN-1021, DX 9065a, DPC 906, JTV 803, SSR-126512 or SSR-128428.
- In a preferred embodiment of the invention, the compounds according to the invention are adminis-
25 tered in combination with heparin or a low molecular weight (LMW) heparin derivative.
- In a preferred embodiment of the invention, the compounds according to the invention are administered in combination with direct inhibitors of coagulation factor XI, inhibitors of coagulation factor XI expression, and anti-coagulation factor XI antibodies such as Xisomab 3G3;
- In a preferred embodiment of the invention, the compounds according to the invention are adminis-
30 tered in combination with a mineralocorticoid-receptor antagonist, for example and preferably spiro-nolactone, eplerenone or finerenone.
- In a preferred embodiment of the invention, the compounds according to the invention are adminis-
35 tered in combination with a diuretic, for example and preferably furosemide, bumetanide, Torsemide, bendroflumethiazide, chlorthiazide, hydrochlorthiazide, hydroflumethiazide, methyclothiazide, polythiazide, trichlormethiazide, chlorthalidone, indapamide, metolazone, quinethazone, acetazolamide, dichlorphenamide, methazolamide, glycerol, isosorbide, mannitol, amiloride or triamterene.

- In a preferred embodiment of the invention, the compounds according to the invention are administered in combination with a PPAR-gamma agonist, for example and preferably pioglitazone or rosiglitazone.
- In a preferred embodiment of the invention, the compounds according to the invention are administered
5 in combination with a PPAR-delta agonist, for example and preferably GW 501516 or BAY 68-5042.

According to a further embodiment, the invention provides a pharmaceutical composition comprising at least one compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, in combination with one or more further active ingredients selected from the group consisting of inhibitors of phosphodiesterases, stimulators or activators of
10 guanylate cyclase, IP receptor agonists, mineralocorticoid-receptor antagonist, diuretic, PPAR-gamma agonist, PPAR-delta agonist, corticosteroids, active ingredients which reduce damage to organs under oxidative stress, compounds which inhibit induction of cell death and apoptosis pathway, compounds which inhibit inflammatory response and T cell proliferation, antithrombotic agents, platelet aggregation inhibitor, thrombin inhibitor, GPIIb/IIIa antagonist, factor Xa inhibitor, heparin or a low molecular weight
15 (LMW) heparin derivative and inhibitors of coagulation factor XI.

The invention further relates to a kit-of-parts combination comprising at least one peptide, derivative or analogue as defined herein or a pharmaceutically acceptable salt or solvate thereof, a complex or a pharmaceutical composition as defined above, and at least one selected from a reagent, medical device, instruction letter or any combination thereof.
20

The invention further relates to a medical device comprising at least one peptide, derivative or analogue as defined herein or a pharmaceutically acceptable salt or solvate thereof, a complex or a pharmaceutical composition as defined above, for delivery of the peptide, derivative, analogue or complex thereof or of the pharmaceutical composition to a subject.

25 The pharmaceutical composition, kit-of-parts combination or medical device as defined above is in particular for the use in the prophylaxis and/or treatment of the disorders or diseases as defined as defined herein.

It is possible for the MASP inhibitory peptide of the present invention to have systemic and/or local activity. For this purpose, they can be administered in a suitable manner, such as, for example, via the oral, parenteral, pulmonary, nasal, sublingual, lingual, buccal, rectal, vaginal, dermal, transdermal, conjunctival, otic route or as an implant or stent.
30

For these administration routes, it is possible for the compounds according to the invention to be administered in suitable administration forms.

For oral administration, it is possible to formulate the compounds according to the invention to dosage forms known in the art that deliver the compounds of the invention rapidly and/or in a modified manner, such as, for example, tablets (uncoated or coated tablets, for example with enteric or controlled release coatings that dissolve with a delay or are insoluble), orally-disintegrating tablets, films/wafers, films/ly-
5 ophyllisates, capsules (for example hard or soft gelatine capsules), sugar-coated tablets, granules, pellets, powders, emulsions, suspensions, aerosols or solutions. It is possible to incorporate the compounds according to the invention in crystalline and/or amorphised and/or dissolved form into said dosage forms.

Parenteral administration can be effected with avoidance of an absorption step (for example intravenous, intraarterial, intracardial, intraspinal or intralumbal) or with inclusion of absorption (for example intra-
10 muscular, subcutaneous, intracutaneous, percutaneous or intraperitoneal, intraocular). Administration forms which are suitable for parenteral administration are, inter alia, preparations for injection and infusion in the form of solutions, suspensions, emulsions, lyophilisates or sterile powders.

Examples which are suitable for other administration routes are pharmaceutical forms for inhalation [inter alia powder inhalers, nebulizers], nasal drops, nasal solutions, nasal sprays; tablets/films/wafers/capsules
15 for lingual, sublingual or buccal administration; suppositories; eye drops, eye ointments, eye baths, ocular inserts, ear drops, ear sprays, ear powders, ear-rinses, ear tampons; vaginal capsules, topical application, aqueous suspensions (lotions, mixturae agitandae), lipophilic suspensions, emulsions, ointments, creams, transdermal therapeutic systems (such as, for example, patches), milk, pastes, foams, dusting powders, implants or stents.

20 According to a further embodiment, the invention provides a pharmaceutical composition comprising at least one compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, in combination with one or more inert, nontoxic, pharmaceutically suitable excipients.

25 The compounds according to the invention can be incorporated into the stated administration forms. This can be effected in a manner known per se by mixing with pharmaceutically suitable excipients. Pharmaceutically suitable excipients include, inter alia,

- fillers and carriers (for example cellulose, microcrystalline cellulose (such as, for example, Avicel®), lactose, mannitol, starch, calcium phosphate (such as, for example, Di-Cafos®)),
- 30 • ointment bases (for example petroleum jelly, paraffins, triglycerides, waxes, wool wax, wool wax alcohols, lanolin, hydrophilic ointment, polyethylene glycols),
- bases for suppositories (for example polyethylene glycols, cacao butter, hard fat),
- solvents (for example water, ethanol, isopropanol, glycerol, propylene glycol, medium chain-length triglycerides fatty oils, liquid polyethylene glycols, paraffins),

- surfactants, emulsifiers, dispersants or wetters (for example sodium dodecyl sulfate), lecithin, phospholipids, fatty alcohols (such as, for example, Lanette[®]), sorbitan fatty acid esters (such as, for example, Span[®]), polyoxyethylene sorbitan fatty acid esters (such as, for example, Tween[®]), polyoxyethylene fatty acid glycerides (such as, for example, Cremophor[®]), polyoxethylene fatty acid esters, polyoxyethylene fatty alcohol ethers, glycerol fatty acid esters, poloxamers (such as, for example, Pluronic[®]),
- buffers, acids and bases (for example phosphates, carbonates, citric acid, acetic acid, hydrochloric acid, sodium hydroxide solution, ammonium carbonate, trometamol, triethanolamine),
- isotonicity agents (for example glucose, sodium chloride),
- adsorbents (for example highly-disperse silicas),
- viscosity-increasing agents, gel formers, thickeners and/or binders (for example polyvinylpyrrolidone, methylcellulose, hydroxypropylmethylcellulose, hydroxypropylcellulose, carboxymethylcellulose-sodium, starch, carbomers, polyacrylic acids (such as, for example, Carbopol[®]); alginates, gelatine),
- disintegrants (for example modified starch, carboxymethylcellulose-sodium, sodium starch glycolate (such as, for example, Explotab[®]), cross-linked polyvinylpyrrolidone, croscarmellose-sodium (such as, for example, AcDiSol[®])),
- flow regulators, lubricants, glidants and mould release agents (for example magnesium stearate, stearic acid, talc, highly-disperse silicas (such as, for example, Aerosil[®])),
- coating materials (for example sugar, shellac) and film formers for films or diffusion membranes which dissolve rapidly or in a modified manner (for example polyvinylpyrrolidones (such as, for example, Kollidon[®]), polyvinyl alcohol, hydroxypropylmethylcellulose, hydroxypropylcellulose, ethylcellulose, hydroxypropylmethylcellulose phthalate, cellulose acetate, cellulose acetate phthalate, polyacrylates, polymethacrylates such as, for example, Eudragit[®])),
- capsule materials (for example gelatine, hydroxypropylmethylcellulose),
- synthetic polymers (for example polylactides, polyglycolides, polyacrylates, polymethacrylates (such as, for example, Eudragit[®]), polyvinylpyrrolidones (such as, for example, Kollidon[®]), polyvinyl alcohols, polyvinyl acetates, polyethylene oxides, polyethylene glycols and their copolymers and blockcopolymers),
- plasticizers (for example polyethylene glycols, propylene glycol, glycerol, triacetine, triacetyl citrate, dibutyl phthalate),
- penetration enhancers,
- stabilisers (for example antioxidants such as, for example, ascorbic acid, ascorbyl palmitate, sodium ascorbate, butylhydroxyanisole, butylhydroxytoluene, propyl gallate),
- preservatives (for example parabens, sorbic acid, thiomersal, benzalkonium chloride, chlorhexidine acetate, sodium benzoate),
- colourants (for example inorganic pigments such as, for example, iron oxides, titanium dioxide),
- flavourings, sweeteners, flavour- and/or odour-masking agents.

The present invention furthermore relates to a pharmaceutical composition comprising at least one peptide, derivative or analogue as defined herein or a pharmaceutically acceptable salt or solvate thereof or a complex as defined above.

5 In particular, the present invention relates to a pharmaceutical composition comprising at least one peptide, derivative or analogue as defined herein or a pharmaceutically acceptable salt or solvate thereof or a complex as defined above, conventionally together with one or more pharmaceutically suitable excipient(s), and to their use according to the present invention.

10 A pharmaceutical composition according to the present invention may comprise at least one additional active ingredient, such as preferably an additional active ingredient which is active in the prophylaxis and/or treatment of the disorders or diseases as defined herein.

15 The at least one peptide, derivative or analogue as defined herein or the pharmaceutically acceptable salt or solvate thereof or the complex or the pharmaceutical compositions as defined above may be administered enterally or parenterally, including intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous, intradermal and intraarticular injection and infusion, orally, intravaginally, intraperitoneally, intrarectally, topically or buccally. Suitable formulations for the respective administration routes are well known to a skilled person and include, without being limited thereto: pills, tablets, enteric-coated tablets, film tablets, layer tablets, sustained-release or extended-release formulations for oral administration, plasters, topical extended-release formulations, dragees, pessaries, gels, ointments, syrup, granules, suppositories, emulsions, dispersions, microcapsules, microformulations, nanoformulations, liposomal formulations, capsules, enteric-coated capsules, powders, inhalation powders, microcrystalline formulations, inhalation sprays, powders, drops, nose drops, nasal sprays, aerosols, ampoules, solutions, juices, suspensions, infusion solutions or injection solutions, etc.

25 According to a further embodiment, the invention provides a pharmaceutical composition comprising at least one compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, in combination with one or more inert, nontoxic, pharmaceutically suitable excipients for the use in the prophylaxis and/or treatment of cardiovascular and cardiopulmonary disorders, shock, inflammatory disorders, cardiovascular, pulmonary, cerebral and renal sequels of sepsis, ischemia and/or reperfusion-related damage, acute kidney injury, transplant protection and delayed graft function, diseases of the blood and blood-forming organs and the immune system, sequels of diabetes mellitus, inflammatory diseases of the nervous system, diseases of the eye, diseases of the skin, diseases of the respiratory, digestive or genitourinary system and sequels of burns and injuries.

30 According to a further embodiment, the invention provides a pharmaceutical composition comprising at least one compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting

of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, in combination with one or more further active ingredients selected from the group consisting of inhibitors of phosphodiesterases, stimulators or activators of guanylate cyclase, IP receptor agonists, mineralocorticoid-receptor antagonist, diuretic, PPAR-gamma agonist, PPAR-delta agonist, corticosteroids, active ingredients which reduce damage to organs under oxidative stress, compounds which inhibit induction of cell death and apoptosis pathway, compounds which inhibit inflammatory response and T cell proliferation, antithrombotic agents, platelet aggregation inhibitor, thrombin inhibitor, GPIIb/IIIa antagonist, factor Xa inhibitor, heparin or a low molecular weight (LMW) heparin derivative and inhibitors of coagulation factor XI for the use in the prophylaxis and/or treatment of cardiovascular and cardiopulmonary disorders, shock, inflammatory disorders, cardiovascular, pulmonary, cerebral and renal sequels of sepsis, ischemia and/or reperfusion-related damage, acute kidney injury, transplant protection and delayed graft function, diseases of the blood and blood-forming organs and the immune system, sequels of diabetes mellitus, inflammatory diseases of the nervous system, diseases of the eye, diseases of the skin, diseases of the respiratory, digestive or genitourinary system and sequels of burns and injuries.

According to a further embodiment, the invention provides a method for treatment and/or prevention of cardiovascular and cardiopulmonary disorders, shock, inflammatory disorders, cardiovascular, pulmonary, cerebral and renal sequels of sepsis, ischemia and/or reperfusion-related damage, acute kidney injury, transplant protection and delayed graft function, diseases of the blood and blood-forming organs and the immune system, sequels of diabetes mellitus, inflammatory diseases of the nervous system, diseases of the eye, diseases of the skin, diseases of the respiratory, digestive or genitourinary system and sequels of burns and injuries in humans and animals by administration of an effective amount of a pharmaceutical composition comprising at least one compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, or of a pharmaceutical composition comprising at least one compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, in combination with one or more inert, nontoxic, pharmaceutically suitable excipients and/or one or more further active ingredients.

The suitable dosage of the MASP inhibitory peptide of the present invention can be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including: a) the disorder being treated and the severity of the disorder; b) activity of the specific compound employed; c) the specific composition employed, the age, body weight, general health, sex and diet of the patient; d) the time of administration, route of administration, and rate of excretion of the specific hepcidin analogue employed; e) the duration

of the treatment; f) drugs used in combination or coincidental with the MASP inhibitory peptide employed, and like factors well known in the medical arts.

In particular embodiments, the total daily dose of the MASP inhibitory peptide of the invention to be administered to a subject or patient in single or divided doses may be in amounts, for example, from 0.0001 to 300 mg/kg body weight daily or 1 to 300 mg/kg body weight daily, or from about 0.0001 to about 100 mg/kg body weight per day, such as from about 0.0005 to about 50 mg/kg body weight per day, such as from about 0.001 to about 10 mg/kg body weight per day, e.g. from about 0.01 to about 1 mg/kg body weight per day, administered in one or more doses, such as from one to three doses. Generally, the MASP inhibitory peptide of the invention may be administered continuously (e.g. by intravenous administration or another continuous drug administration method), or may be administered to a subject at intervals, typically at regular time intervals, depending on the desired dosage and the pharmaceutical composition selected by the skilled practitioner for the particular subject. Regular administration dosing intervals include, e.g., once daily, twice daily, once every two, three, four, five or six days, once or twice weekly, once or twice monthly, and the like.

The invention further comprises the use of the MASP inhibitory peptide as described herein for the manufacture of a medicament, in particular for the manufacture of a medicament for the prophylaxis and/or treatment of a disorder or disease as defined herein.

The invention further comprises a process for manufacturing the peptides of the present invention, derivative or analogue or the pharmaceutically acceptable salt or solvate thereof or a complex, each as described herein.

The process for manufacturing comprises the steps as shown in the examples of the present invention.

Generally, the MASP inhibitory peptide of the present invention may be manufactured synthetically, or semi-recombinantly.

According to a further embodiment, the invention provides a process for preparing a compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue or pharmaceutically acceptable salts or solvates thereof by using solid phase peptide synthesis.

According to a further embodiment, the invention provides a process for preparing a compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, containing the steps

1. Use of a 2-chlorotrityl-type resin with a loading of 0.2 – 1.0 mmol/g, or a Wang-type resin with a loading of 0.2 – 1.0 mmol/ gram,

2. Loading the c-terminal amino acid of the sequence onto the resin,
 3. Removal of fmoc protection with a 15-25% piperidine solution in DMF or NMP,
 4. Coupling of the next amino acid in the sequence with coupling reagents such as HBTU, HATU or DIC/Oxyma using stoichiometries between 3-8 equivalents,
 5. Repeating steps 3 and 4 until the sequence is completed,
 6. Cleavage of the peptide from the solid support using a cleavage cocktail that involves TFA and a thiol scavenger,
 7. Cyclization of two cysteines in the sequence under oxidative conditions (air or I₂),
 8. Purification of the cleaved peptide using reversed-phase HPLC.
- 10 According to a further embodiment, the invention provides a process for preparing a compound containing a peptide which may be isolated and/or purified, comprising, essentially consisting of, or consisting of, formula (I) or formula (II) or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, containing the steps
1. Use of a 2-chlorotrityl-type resin with a loading of 0.2 – 1.0 mmol/g, or a Wang-type resin with a loading of 0.2 – 1.0 mmol/ gram,
 2. Loading the c-terminal amino acid of the sequence onto the resin,
 3. Removal of fmoc protection with a 15-25% piperidine solution in DMF or NMP,
 4. Coupling of the next amino acid in the sequence with coupling reagents such as HBTU, HATU or DIC/Oxyma using stoichiometries between 3-8 equivalents,
 5. Repeating steps 3 and 4 until the sequence is completed,
 6. Cleavage of the peptide from the solid support using a cleavage cocktail that involves TFA and a thiol scavenger,
 7. Cyclization of two cysteines in the sequence under oxidative conditions (air or I₂),
 8. Purification of the cleaved peptide using reversed-phase HPLC,
 9. Conversion to the HCl salt.

The at least one peptide, derivative or analogue as defined herein or the pharmaceutically acceptable salt or solvate thereof or the complex as defined herein may also be used as a biochemical agent in a biochemical assay, such as e.g. in a diagnostic assay to measure responsiveness to MASP inhibitors or in any biochemical assay being based on MASP inhibitor binding.

- 30 The present invention also includes polynucleotides comprising a sequence encoding a MASP inhibitory peptide according to the present invention, as well as a vector comprising a polynucleotide comprising a sequence encoding a MASP inhibitory peptide according to the present invention.

The invention is further illustrated by the following examples, which relate to certain specific embodiments of the present invention. The examples were carried out using well known standard techniques

within the routine to those of skill in the art, unless indicated otherwise. The following examples are for illustrative purposes only and do not purport to be wholly definitive as to conditions or scope of the invention. As such, they should not be construed in any way as limiting the scope of the present invention.

Examples

5 List of Abbreviations used in Experimental Section:

	Å	Angstroms
	aq.	Aqueous, aqueous solution
	bar	Unit of pressure
	BPR	Back-pressure regulator
10	conc	Concentrated
	d	Doublet (NMR)
	dd	Doublet of doublet (NMR)
	DCM	Dichloromethane
	DEA	Diethylamine
15	DIPEA	N,N-diisopropylethylamine (Hünig's base)
	DMAP	4-Dimethylaminopyridine
	DMF	N,N-dimethylformamide
	DMSO	Dimethylsulfoxide
	dt	Doublet of triplet (NMR)
20	EA	Ethyl acetate
	ee	Enantiomeric excess
	ent	Enantiomeric
	eq	Equivalent(s)
	equiv	Equivalent(s) (ion chromatography)
25	ESI	Electrospray-ionisation (mass spectroscopy)
	Fmoc-OSu	1-[(9H-Fluoren-9-ylmethoxy)carbonyl]oxy}pyrrolidine-2,5-dione
	GC-MS	Gas chromatography coupled with mass spectrometry
	h	Hour(s)
	HATU	O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium-hexafluorophosphate
30	HPLC	High pressure liquid chromatography
	IC	Ion chromatography
	ID	Internal diameter
	L	Liter
	LC-MS	Liquid chromatography coupled to mass spectroscopy
35	LiHMDS	Lithium bis(trimethylsilyl)amide

	lit.	Literature
	m	Multiplet (NMR)
	MALDI	Matrix Assisted Laser Desorption/Ionization (mass spectrometry)
	Me	Methyl
5	min	Minute(s)
	mm	millimeter
	μm	micrometer (micron)
	M	Molar
	MPLC	Medium pressure liquid chromatography
10	MS	Mass spectroscopy
	MTBE	tert-Butyl methyl ether
	MTP	Microtiter plate
	m/z	mass-to-charge ratio (mass spectrometry)
	nm	nanometer
15	NMP	N-Methyl-2-pyrrolidone
	NMR	Nuclear magnetic resonance spectroscopy
	PBS	Phosphate buffered saline
	PE	Petroleum ether
	PEG	Polyethylene glycol
20	pos	Positive
	ppm	parts per million
	Pr	Propyl
	Psi	Pounds per square inch (pressure)
	q / quart	Quartet (NMR)
25	qd	Quartet of doublet (NMR)
	quint	Quintet (NMR)
	rac	racemic
	Rf	Retention factor (TLC)
	RP	reversed-phase (for liquid chromatography)
30	Rt	Retention time (chromatography)
	s	Singlet (NMR)
	s	Seconds (time)
	SPPS	solid phase peptide synthesis
	t	Triplet (NMR)
35	TBTU	O (Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate
	tBu	tert-Butyl
	TEA	triethylamine

THF	Tetrahydrofuran
TLC	Thin layer chromatography
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet

- 5 Further abbreviations can be found in Table 2 and 3.

Analytical LC-MS Methods

Method 1

Equipment type MS: ThermoFisherScientific LTQ-Orbitrap-XL; Equipment type HPLC: Agilent 1200SL;
Column: Agilent, POROSHELL 120, 3 x 150 mm, SB – C18 2.7 µm; eluent A: 1 L water + 0.1% trifluoro-
10 acetic acid; eluent B: 1 L acetonitrile + 0.1% trifluoroacetic acid; gradient: 0.0 min 2% B → 1.5 min 2% B
→ 15.5 min 98% B → 18.0 min 98% B; oven: 40°C; flow rate: 0.75 mL/min; UV-detection: 210 nm

Method 2

Equipment type MS: ThermoFisherScientific LTQ-Orbitrap-XL; Equipment type HPLC: Agilent 1200SL;
Column: Agilent, POROSHELL 120; 3 x 150 mm, SB – C18 2.7 µm; eluent A: 1 L water + 0.1% trifluoro-
15 acetic acid; eluent B: 1 L acetonitrile + 0.1% trifluoroacetic acid; gradient: 0.0 min 5% B → 0.3 min 5% B
→ 7.0 min 98% B → 10 min 98% B; oven: 40°C; flow rate: 0.75 mL/min; UV-detection: 210 nm

Method 3

Equipment type MS: Waters TOF instrument; Equipment type UPLC: Waters Acquity I-CLASS; Column:
YMC, TRIART C18, 75 x 1 mm, 3.0 µm x 12nm ; eluent A: 1 L water + 0.01% formic acid; eluent B: 1
20 L acetonitrile + 0.01% formic acid; gradient: 0.0 min 1% B → 2.0 min 1% B → 8.0 min 95% B → 10.0
min 95% B; oven: 50°C; flow rate: 0.63 mL/min; UV-detection: 210 nm

Method 4

Equipment type MS: Waters TOF instrument; Equipment type UPLC: Waters Acquity I-CLASS; Column:
YMC, TRIART C18, 75 x 1 mm, 3.0 µm 12nm ; eluent A: 1 L water + 0.01% formic acid; eluent B: 1 L
25 acetonitrile + 0.01% formic acid; gradient: 0.0 min 1% B → 1.0 min 1% B → 15.0 min 50% B → 18.0
min 95% B; oven: 50°C; flow rate: 0.63 mL/min; UV-detection: 210 nm

Method 5

Equipment type MS: Waters TOF instrument; Equipment type UPLC: Waters Acquity I-CLASS; Column:
Waters, HSST3, 2.1 x 50 mm, C18 1.8 µm; eluent A: 1 L water + 0.01% formic acid; eluent B: 1 L
30 acetonitrile + 0.01% formic acid; gradient: 0.0 min 2% B → 0.5 min 2% B → 7.5 min 95% B → 10.0 min
95% B; oven: 50°C; flow rate: 1.00 mL/min; UV-detection: 210 nm

Method 6

Equipment type MS: Waters Synapt G2S; Equipment type UPLC: Waters Acquity I-CLASS; Column: Waters, BEH300, 2.1 x 150 mm, C18 1.7 µm; eluent A: 1 L water + 0.01% formic acid; eluent B: 1 L acetonitrile + 0.01% formic acid; gradient: 0.0 min 2% B → 1.5 min 2% B → 8.5 min 95% B → 10.0 min 95% B; oven: 50°C; flow rate: 0.50 mL/min; UV-detection: 220 nm

Method 7

Instrument type MS: Agilent 6410 Triple Quad; Instrument type HPLC: Agilent 1200; Column: Gemini-NX C18 5µm 110Å 150 x 4.6 mm; eluent A: 0.1%TFA in H₂O; eluent B: 0.1%TFA in ACN; gradient: 0.0 min 20% B → 20 min 50% B → 20.1 min 90% B → 23 min 90% B; oven temperature: 50°C; flow rate: 1.0 mL/min; UV-detection: 220 nm

Method 8

Instrument type MS: Agilent 6410 Triple Quad; Instrument type HPLC: Agilent 1200; Column: Discovery BIO Wide Pore C18 5µm 300Å x 4.6 mm; eluent A: 0.1%TFA in H₂O; eluent B: 0.1%TFA in ACN; gradient: 0.0 min 10% B → 20 min 80% B → 20.1 min 90% B → 23 min 90% B; oven temperature: 50°C; flow rate: 1.0 mL/min; UV-detection: 220 nm

Method 9

Instrument: Waters ACQUITY SQD UPLC System; column: Waters Acquity UPLC HSS T3 1.8 µm 50 x 1 mm; eluent A: 1 L water + 0.25 mL 99% formic acid, eluent B: 1 L acetonitrile + 0.25 mL 99% 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 nm

Method 10

Instrument MS: Thermo Scientific FT-MS; Instrument UHPLC: Thermo Scientific UltiMate 3000; Column: Waters, HSST3, 2.1 x 75 mm, C18 1.8 µm; eluent A: 1 L water + 0.01% formic acid; eluent B: 1 L acetonitrile + 0.01% formic acid; gradient: 0.0 min 10% B → 2.5 min 95% B → 3.5 min 95% B; oven: 50°C; flow rate: 0.90 mL/min; UV-detection: 210 nm/ Optimum Integration Path 210-300 nm

Method 11

MS Instrument: Agilent MS Quad 6150; HPLC: Agilent 1290; Column: Waters Acquity UPLC HSS T3 1.8 µm 50 x 2.1 mm; eluent A: 1 L water + 0.25 mL formic acid, eluent B: 1 L acetonitrile + 0.25 mL formic acid; gradient: 0.0 min 90% A → 0.3 min 90% A → 1.7 min 5% A → 3.0 min 5% A oven: 50°C; flow rate: 1.20 mL/min; UV-detection: 205 – 305 nm.

Method 12

Instrument: Waters ACQUITY SQD UPLC System; column: Waters Acquity UPLC HSST3 1.8 µm 50 x 1 mm; eluent A: 1 L water + 0.25 mL 99% formic acid, eluent B: 1 L acetonitrile + 0.25 mL 99% formic

acid; gradient: 0.0 min 95% A → 6.0 min 5% A → 7.5 min 5% A oven: 50°C; flow rate: 0.35 mL/min; UV-detection: 210 nm.

Method 13

Instrument: Waters Single Quad MS System; Instrument Waters UPLC Acquity; column: Waters BEH C18 1.7 µm 50 x 2.1 mm; eluent A: 1 L water + 1.0 mL (25% ammonia)/L, eluent B: 1 L acetonitrile; gradient: 0.0 min 92% A → 0.1 min 92% A → 1.8 min 5% A → 3.5 min 5% A; oven: 50°C; flow rate: 0.45 mL/min; UV-detection: 210 nm.

Method 14

System MS: Waters TOF instrument; System UPLC: Waters Acquity I-CLASS; Column: Waters Acquity UPLC Peptide BEH C18 300Å, 1.7 µm 150 x 2.1 mm; Eluent A: 1 l Water + 0.100 ml 99% Formic acid, Eluent B: 1 l Acetonitrile + 0.100 ml 99% Formic acid; Gradient: 0.0 min 90% A → 0.25 min 90% A → 8.0 min 45% A → 10.0 min 2% → 12.0 min 2% A Oven: 50°C; Flow: 0.475 ml/min; UV-Detection: 210 nm.

Method 15

MS instrument type: Agilent G6110A; HPLC instrument type: Agilent 1200 Series LC; UV DAD; column: Chromolith Flash RP-18e 25 x 2.0mm; mobile phase A: 0.0375% TFA in water (v/v), mobile phase B: 0.01875% TFA in acetonitrile (V/V); gradient: 0.01 min 5% B → 0.80 min 95% B → 1.20 min 95% B → 1.21 min 5% B → 1.5 min 5% B; flow rate: 1.50 mL/min; oven temperature: 50 °C; UV detection: 220 nm & 254 nm.

MALDI Method

Exact mass measurements were performed on selected peptides using a Matrix Assisted Laser Desorption/Ionization (MALDI) mass spectrometry method on a Bruker autoflex maX LRF MALDI MS Time-of-Flight (ToF-MS) system. Samples were prepared on a Bruker MALDI target plate using α-cyano-4-hydroxycinnamic acid (CAS 28166-41-8) as the matrix. A solution of the sample peptide 0.1 to 1.0 mg in 1.0 mL acetonitrile-water (50/50 or 30/70) and a stock solution of the matrix (10 mg/mL) in 50% acetonitrile in water containing 0.05% trifluoroacetic acid are prepared. 1.0 µL of each solution is placed onto the MALDI target plate and allowed to dry. The sample is then ready for analysis. Recommended sample preparations for MALDI target plates can be found in the documentation provided by Bruker.

Analytical Ion Chromatography Method

Method: IC – Quantitative

Quantitative Measurement of Cations and Anions using external standards; Instrument: Thermo Scientific ICS 5000+; Capillary IC Columns: IonPac AS11-HC und IonPac CS16; eluent: gradient eluent [H]⁺ [OH]⁻; Detector: Conductivity detection; routine anions possible: acetate, bromide, citrate, chloride,

fluoride, formate, lactate, mesylate, phosphate, sulfate, tartrate, trifluoroacetate; routine cations possible: ammonium, barium, calcium, potassium, lithium, sodium, magnesium, choline.

Analytical Gas Chromatography Mass Spec Method

GC-MS Method 1

- 5 Instrument: Thermo Scientific DSQII, Thermo Scientific Trace GC Ultra; column: Restek RTX-35MS, 15 m x 200 μ m x 0.33 μ m; constant flow with Helium: 1.20 mL/min; oven: 60°C; Inlet: 220°C; gradient: 60°C, 30°C/min $\rightarrow$ 300°C (3.33 min hold).

NMR

- 10 The ^1H -NMR data of selected compounds are listed in the form of ^1H -NMR peaklists. Therein, for each signal peak the δ value in ppm is given, followed by the signal intensity, reported in round brackets. The δ value-signal intensity pairs from different peaks are separated by commas. Therefore, a peaklist is described by the general form: $\delta 1$ (intensity1), $\delta 2$ (intensity2), ... , δi (intensityi), ... , δn (intensityn).

The intensity of a sharp signal correlates with the height (in cm) of the signal in a printed NMR spectrum. When compared with other signals, this data can be correlated to the real ratios of the signal intensities.

- 15 In the case of broad signals, more than one peak, or the center of the signal along with their relative intensity, compared to the most intense signal displayed in the spectrum, are shown. A ^1H -NMR peaklist is similar to a classical ^1H -NMR readout, and thus usually contains all the peaks listed in a classical NMR interpretation. Moreover, similar to classical ^1H -NMR printouts, peaklists can show solvent signals, signals derived from stereoisomers of the particular target compound, peaks of impurities, ^{13}C satellite peaks, and/or spinning sidebands. The peaks of stereoisomers, and/or peaks of impurities are typically
- 20 displayed with a lower intensity compared to the peaks of the target compound (e.g., with a purity of >90%). Such stereoisomers and/or impurities may be typical for the particular manufacturing process, and therefore their peaks may help to identify a reproduction of the manufacturing process on the basis of "by-product fingerprints". An expert who calculates the peaks of the target compound by known methods
- 25 (MestReC, ACD simulation, or by use of empirically evaluated expectation values), can isolate the peaks of the target compound as required, optionally using additional intensity filters. Such an operation would be similar to peak-picking in classical ^1H -NMR interpretation. A detailed description of the reporting of NMR data in the form of peaklists can be found in the publication "Citation of NMR Peaklist Data within Patent Applications" (cf. <http://www.researchdisclosure.com/searching-disclosures>, Research Disclosure
- 30 Database Number 605005, 2014, 01 Aug 2014). In the peak picking routine, as described in the Research Disclosure Database Number 605005, the parameter "MinimumHeight" can be adjusted between 1% and 4%. However, depending on the chemical structure and/or depending on the concentration of the measured compound it may be reasonable to set the parameter "MinimumHeight" <1%.

Preparation Examples

General Methods for the Synthesis

Solid Phase Peptide Synthesis (SPPS) was carried out either using an automatic peptide synthesizer or performed manually by hand. Peptide synthesis was typically carried out in scale ranges from 0.1 to 1.0 mmol. When peptide synthesis was carried out by hand, the general procedures Methods C, D and E described below were used. Automated peptide synthesis was performed on a Symphony X peptide synthesizer (Gyros Protein Technologies).

Fmoc-protected amino acids (or intermediate amino acids used to prepare Fmoc amino acids) were purchased from Novabiochem, Bachem, Iris Biotech, Sigma-Aldrich, Alfa, Enamine, Amatek, Anichem, ACBR, ABC Laboratory, AP Bioscience, Combiblocks, ArZa Bioscience, Ark Pharm, Acroteinchem, Apollo Scientific, Biofine, Broadpharm, VWR, or Gyros Protein Technologies (Fluorenylmethoxycarbonyl = Fmoc), GL Biochem (Shanghai) Ltd, Chengdu Aminotop Pharmaceutical Technology Ltd, Suzhou Highfine Biotech Co., Ltd, WuXi AppTec, or sourced through other Chinese vendors. Some special fmoc-protected amino acids were synthesized internally, and these synthetic methods are described herein. Some of the fmoc amino acids synthesized internally are also commercially available. In some cases where the Fmoc-protected amino acid was not commercially available but the Boc-protected (tert-Butyloxycarbonyl = Boc) unnatural amino acid was commercially available, the fmoc-protected amino acid was prepared from the Boc-protected amino acid by deprotection and reprotection using methods commonly employed in the art. CAS Numbers for commercially available, unnatural amino acids used in the synthesis of peptides of this invention have in most cases been included in Table 5. In cases where a racemic amino acid (Fmoc or Boc) was purchased, one skilled in the art should recognize that the enantiomers can be separated using chiral chromatography, and this was in fact sometimes done to obtain the enantiomerically pure amino acid prior to peptide synthesis.

The following unnatural amino acids have been used in preparing peptides of the invention. The Fmoc- or Boc- protected amino acids were either obtained through commercial sources (CAS Number is available) or, synthesized internally by methods described herein. Table 5 shows the CAS number of the chemical groups / amino acids which were used for the peptide synthesis (right column) and the corresponding chemical group / amino acid present in the peptides (left column).

Table 5: Availability of unnatural amino acids and chemical groups of this Invention

Abbreviation/Expression Definition	CAS Number for Fmoc-/Boc-protected amino acid, chemical group or building block used for peptide synthesis
Cyclohexanecarboxylic acid	98-89-5

Abbreviation/Expression Definition	CAS Number for Fmoc-/Boc-protected amino acid, chemical group or building block used for peptide synthesis
(2S)-Amino-2-[3-(Trifluoromethyl)bicyclo[1.1.1]pent-1-yl]acetic acid	914082-67-0
(S)-3-(2H-tetrazol-5-yl)propanoic acid	954147-35-4
(1R,3S)-3-(Amino)cyclopentanecarboxylic acid	220497-67-6
(1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid	291775-59-2
(1R,3S,5R)-2-Azabicyclo[3.1.0]hexane-3-carboxylic acid	1932624-93-5
(1S,2S,4S)-Bicyclo[2.2.1]hept-5-en-2-ylacetic acid	14734-13-5
(1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid	400720-05-0 (Boc); 854113-43-2
(1S,3R)-3-(Amino)cyclopentanecarboxylic acid	220497-66-5
(1S,3R,4R)-2-Azabicyclo[2.2.1]heptane-3-carboxylic acid	291775-53-6; 291775-53-6 (Boc)
(2,4-Dioxoimidazolidin-1-yl)acetic acid	94738-31-5; 94738-31-5 (NH)
2-Cyano-L-phenylalanine	401933-16-2
2-Fluoro-L-phenylalanine	205526-26-7
2-Methyl-L-phenylalanine	211637-75-1
(2S)-(tetrahydro-2H-pyran-4-yl)ethanoic acid	368866-31-3
(2S,4S)-4-Trifluoromethyl-pyrrolidine-2-carboxylic acid	1242934-32-2
(3,3-difluorocyclobutyl)acetic acid	1373503-48-0
3-Cyano-L-phenylalanine	205526-36-9
3-Fluoro-L-phenylalanine	8560-68-8
4-Fluoro-L-phenylalanine	169243-86-1
5-Azaspiro[2.4]heptane-1-carboxylic acid	150543-61-6
D-(+)Biotin	58-85-5
L-N,N-Dimethylalanine	2812-31-9
N,N-Dimethylglycine	1118-68-9
2-(N-Isopropyl-N-methylamino)acetic acid	4747-21-1
L-N-Methylalanine	84000-07-7
D-N-Methylalanine	138774-92-2
L-N-Methylcysteine	944797-51-7
L-N-Methylphenylalanine	77128-73-5
N-Methyl-Glycine	77128-70-2
L-N-Methylisoleucine	138775-22-1
N-Phenylglycine	103-01-5
(R)-3-Aminoadipic acid	197006-18-1

Abbreviation/Expression Definition	CAS Number for Fmoc-/Boc-protected amino acid, chemical group or building block used for peptide synthesis
(R)-4-Amino-6-methylheptanoic acid	269078-75-3
(R)-Piperidine-3-Carboxylic Acid	193693-67-3; 163438-09-3 (Boc)
(R)-Pyrrolidine-3-Carboxylic Acid	68464-02-8
(S)-(1-Piperidin-3-yl)-acetic acid	1217646-18-8
(S)-2-Amino-3-ethyl-pentanoic acid	1310680-47-7
(S)-3-(1-Pyrrolidine-2-yl)-propionic acid	1013997-51-7
(S)-3-(2H-Tetrazol-5-yl)propionic acid	954147-35-4
(S)-3-Methylvaleric Acid	1730-92-3
(S)-4-Piperazine-2-carboxylic acid	1217628-46-0
(S)-Piperidine-3-carboxylic acid	193693-68-4
(S)-Pyrrolidine-3-carboxylic acid	193693-66-2; 140148-70-5 (Boc)
[(2R)-4,4-Difluoropyrrolidin-2-yl]acetic acid	1402687-79-9
[(6S)-5-Azaspiro[2.4]hept-6-yl]acetic acid	Omegachem (OC-0701)
1,1-Dioxidotetrahydro-2H-thiopyran-4-yl)acetic acid	1224869-02-6
L-1-Naphthylalanine	96402-49-2
2-(3-Pyridyl)acetic acid	501-81-5
2-(Aminomethyl)benzoic acid	219640-94-5
2-(Cyclohexylamino)acetic acid	108-91-8
2-(Diethylamino)acetic acid	109-89-7
2-(Morpholine)acetic acid	110-91-8
2-(N-Methyl-N-cyclopropylamino)acetic acid	5163-20-2
2-(Piperidin)acetic acid	110-89-4
2-(Pyrrolidine)acetic acid	123-75-1
2-(Thian-4-yl)acetic acid	137103-09-4
2-(Thiomorpholine)acetic acid	123-90-0
2,3-Difluoro-L-phenylalanine	1260605-30-8
2-Aminobenzoic acid	150256-42-1
2-Azaspiro[3.3]heptane-6-carboxylic acid	2138525-84-3
L-2-Bromophenylalanine	220497-47-2
2-Chloro-L-phenylalanine	198560-41-7
2-Cyanobenzoic acid	3839-22-3
2-Fluorobenzoic acid	445-29-4
2-Hydroxyacetic acid	7732-18-5

Abbreviation/Expression Definition	CAS Number for Fmoc-/Boc-protected amino acid, chemical group or building block used for peptide synthesis
2-Hydroxyisobutyric acid	594-61-6
2-Methylbenzoic acid	118-90-1
2-Methyl-L-phenylalanine	211637-75-1
L-2-Naphthylalanine	112883-43-9
L-2-Pyridylalanine	185379-40-2
(2S)-Pyrrolidin-2-ylacetic acid	19693-60-6
3-(2-Oxoimidazolidin-1-yl)benzoic acid	884504-86-3
3-(3-Oxo-1,3,4,5,6,7-hexahydro-2H-indazol-2-yl)benzoic acid	885949-86-0
3-(Aminomethyl)benzoic acid	117445-22-4
3-(Isobutyrylamino)benzoic acid	28533-44-0
3-(Trifluoromethyl)bicyclo[1.1.1]pent-1-yl]acetic acid	914082-67-0
3-[(E)-(2-carboxycyclohexen-1-yl)azo]benzoic acid	885949-86-0
3-Acetamidobenzoic acid	587-48-4
3-Amino-2,2-dimethylpropionic acid	1076197-00-6
3-Amino-3-methylbutyric acid	244031-65-0; 129765-95-3 (Boc)
3-Aminobenzoic acid	185116-42-1
3-Aminomethylphenylacetic acid	631915-50-9
L-3-Bromophenylalanine	220497-48-3
3-Carboxybenzoic acid	121-91-5
3-Chloro-L-Phenylalanine	198560-44-0
3-Chlorophenylglycine	10242-05-4
3-Cyanobenzoic acid	1877-72-1
3-Fluorobenzoic acid	455-38-9
3-Methyl-L-phenylalanine	211637-74-0
3-Methoxypropionic acid	2544-06-1
3-Methylbenzoic acid	99-04-7
L-3-Pyridylalanine	175453-07-3
4-(Aminomethyl)benzoic acid	33233-67-9
4-Aminobenzoic acid	185116-43-2
4-Aminomethylphenylacetic acid	176504-01-1; 71420-92-3 (Boc)
L-4-Aminophenylalanine	95753-56-3
L-4-Bromophenylalanine	198561-04-5
4-Carboxybenzoic acid	100-21-0

Abbreviation/Expression Definition	CAS Number for Fmoc-/Boc-protected amino acid, chemical group or building block used for peptide synthesis
4-Cyanobenzoic acid	619-65-8
4-Fluorobenzoic acid	456-22-4
4-Fluoro-L-Leucine	1643928-01-1
4-Methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxylic acid	141762-02-9
4-Methylbenzoic acid	99-94-5
4-Methylpentanoic acid	646-07-1
L-4-Pyridylalanine	169555-95-7
4-Piperidinepropanoic acid	154938-68-8
4-Tetrahydropyranoyl	368866-31-3
5-Chlorothiophene-2-carboxylic acid	24065-33-6
8-Aminocubane-1-carboxylic acid	Enamine EN300-88072 (MFCD09971721)
(S)-2-(Amino)-1,6-hexanedioic acid	159751-47-0
L-2-Aminobutyric acid	135112-27-5
1-Aminocyclobutane-1-carboxylic acid	885951-77-9
Aminocyclobutanecarboxylic acid	885951-77-9
1-(Aminomethyl)-cyclopropyl-1-carboxylic acid	1263045-62-0
(1S,2S)-2-Amino-1-cyclopentanecarboxylic acid	359586-64-4
(1R,2R)-2-Amino-1-cyclopentanecarboxylic acid	359586-69-9
rel-(1R,2S)-2-Amino-1-cyclopentanecarboxylic acid	352707-76-7
Adipic acid	124-04-9
6-Aminohexanoic acid	88574-06-5
2-Aminoisobutyric acid	94744-50-0
L-tert-Butylalanine	139551-74-9
allo-L-Isoleucine	251316-98-0
allo-L-Threonine	201481-37-0
2-Methyl-L-Proline	167275-47-0
N(5)-methyl-L-arginine	1135616-49-7
(S)-Azetidine-2-carboxylic acid	136552-06-2; 51077-14-6 (Boc)
Benzoic acid	65-85-0
L-2-Thienylalanine	130309-35-2
beta-Alanine	35737-10-1

Abbreviation/Expression Definition	CAS Number for Fmoc-/Boc-protected amino acid, chemical group or building block used for peptide synthesis
Bromoacetyl	79-08-3
4-(3,5-Dimethyl-1,2-oxazol-4-yl)-L-phenylalanine	1381790-25-5
(2S)-2-amino-3-(4-tert-butylphenyl)propanoic acid	143415-62-7
(2S)-2-Amino-5-methyl-hexanoic acid	180414-94-2
(2S)-2-Amino-4,4,4-trifluorobutanoic acid	181128-48-3
(2S)-3-(4-carboxyphenyl)-2-aminopropanoic acid	183070-44-2
(2S)-2-amino-3-(4-carbamoylphenyl)propanoic acid	205126-71-2
(2S)-2-Amino-3-(2,3,4,5,6-(pentafluorophenyl)propanoic acid	205526-32-5
(2S)-3-(3-Cyanophenyl)-2-aminopropanoic acid	205526-36-9
3-Azido-L-Alanine	684270-46-0
2-Amino-5,5,5-trifluoro-4-methyl-pentanoic acid	777946-04-0
(1R,2S,5S)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid	167611-99-6 (Boc); 1219181-14-2 (rac)
L-Cyclobutylalanine	478183-62-9
(S)-2-Amino-2-cyclobutylacetic acid	1391630-31-1
L-Cyclohexylalanine	135673-97-1
D-cyclohexylalanine	144701-25-7
L-Cyclohexylglycine	161321-36-4
L-4,4-Difluoroproline	
(2S,4S)-4-Fluoroproline	203866-19-7
L-Citrulline	133174-15-9
L-2-Amino-4-cyanobutyric acid	913253-24-4
3,3-dimethyl-1,3-azasilolidine-5-carboxylic acid	268224-29-9 (Boc)
(S)-(trifluoromethyl)-L-cysteine	1994331-25-7
(2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid	2350203-57-3
(2S)-3-(indol-4-yl)-2-(amino)propanoic acid	220499-20-7
(2S)-3-(2,3-difluorophenyl)-2-aminopropanoic acid	266360-64-9
L-Cyclopentylalanine	371770-32-0
L-Cyclopentylglycine	220497-61-0
Cyclobutanecarboxylic acid	3721-95-7
2-(Cyclobutyl)acetic acid	6540-33-6
L-Cyclobutylglycine	49607-08-1
Cyclohexylacetic acid	5292-21-7
Cyclopentanecarboxylic acid	3400-45-1

Abbreviation/Expression Definition	CAS Number for Fmoc-/Boc-protected amino acid, chemical group or building block used for peptide synthesis
Cyclopentylacetic acid	1123-00-8
Cyclopropanecarboxylic acid	1759-53-1
Cyclopropylacetic acid	5239-82-7
D-2-Chlorophenylalanine	205526-22-3
L-2,4-Diaminobutyric acid	125238-99-5; 607366-21-2 (ivDde); 851392-68-2 (MTT)
L-2,3-Diaminopropionic acid	162558-25-0; 607366-20-1 (ivDde); 654670-89-0 (MTT)
D-beta-Proline	193693-61-7
D-Hydroxyproline	464193-92-8
(2S)-2-Amino-4-(benzylamino)-4-oxobutanecarboxylic acid	100-46-9
(2S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-4-methylpentanoic acid	957507-85-6
Fumaric acid	110-17-8
Gamma-Aminobutyric acid	116821-47-7; 57294-38-9 (Boc)
L-tert-Butylglycine	132684-60-7
1-Benzyl-L-histidine	84030-19-3
1-Methyl-L-histidine	202920-22-7
L-3-Methylhistidine	84030-19-3
L-Homocysteine	167015-23-8
L-Dihydroorotic acid	5988-19-2
3-Phenylpropanoic acid	501-52-0
L-Hydroxyproline	122996-47-8
Iminodiacetic acid	142-73-4
Isobutyric acid	79-31-2
Isonipecotic acid	148928-15-8
Isovaleric acid	503-74-2
N-ε-Isopropyl-L-lysine	201003-48-7
3-Ethyl-L-Norvaline	1310680-47-7
L-(+)-Lactic acid	79-33-4
3-(1,3-Benzothiazol-2-yl)-L-alanine	959583-56-3
(S)-Pyrrolidine-2-carboxylic acid	193693-60-6
L-Propargylglycine	1435854-95-7
(3S)-Morpholine-3-carboxylic acid	281655-37-6

Abbreviation/Expression Definition	CAS Number for Fmoc-/Boc-protected amino acid, chemical group or building block used for peptide synthesis
Nicotinic acid	59-67-6
L-Norleucine	77284-32-3
N-Methyl-L-proline	475-11-6
L-Norvaline	135112-28-6
1,18-Octadecanedioic acid	871-70-5
2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid	130309-37-4
L-Ornithine	109425-55-0; 269062-80-8 (DDE); 1198321-33-3 (ivDDE); 147290-11-7 (Alloc)
Palmitic acid	57-10-3
9-Amino-4,7-dioxanonanoic acid	872679-70-4
12-Amino-4,7,10-trioxadodecanoic acid	867062-95-1
15-Amino-4,7,10,13-tetraoxa(Pen)tadecanoic acid	557756-85-1
18-Amino-4,7,10,13,16-(Pen)taoxaoctadecanoic acid	882847-32-7
14-Amino-3,6,9,12-tetraoxatetradecanoic acid	437655-95-3
1-Amino-3,6,9,12,15,18-hexaoxahenicosan-21-oic acid	882847-34-9
17-Amino-3,6,9,12,15-(Pen)taoxaheptadecanoic acid	635287-26-2
1-Amino-3,6,9,12,15,18,21-heptaotatetracosan-24-oic acid	1863885-74-8
1-Amino-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid	756526-02-0
1-Amino-3,6,9,12,15,18,21,24,27-nonaoxatriacontan-30-oic acid	1191064-81-9
L-Penicillamine	201531-88-6
Phenylacetic acid	103-82-2
L-Phenylglycine	102410-65-1
Picolinic acid	98-98-6
L-Pipecolic acid	86069-86-5
Piperidin-4-ylacetic acid	157688-46-5 (Boc); 180181-05-9
Pivalic acid	75-98-9
L-trans-3-Hydroxyproline	4298-08-2
L-Pyroglutamic acid	53100-44-0
S-2-amino-3-ethyl-pentanoic acid	1310680-47-7
S-3-1-Pyrrolidin-2-yl-propionic acid	MFCD09952617
Suberic acid	505-48-6
tert-Butylacetic acid	1070-83-3

Abbreviation/Expression Definition	CAS Number for Fmoc-/Boc-protected amino acid, chemical group or building block used for peptide synthesis
Tetrahydropyranyl-4-acetic acid	85064-61-5
Trifluoroacetic acid	76-05-1
L-2-Thienylalanine	130309-35-2
(2S,3S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-3-methylpentanoic acid	301840-23-3
(2S,3S)-2-[2-Oxopiperazin-1-yl]-3-methylpentanoic acid	-
(2S,3S)-2-[(3S)-2-oxopiperazin-1-yl]-3-methylpentanoic acid	-
(1R,3S,5R)-2-Azabicyclo[3.1.0]hexane-3-carboxylic acid	1932624-93-5
3-(Trimethylsilyl)-L-alanine	359766-59-9
(6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid	2170726-27-7
rel-(1R,3R,5R,6R)-6-(Trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid	1986905-54-7
(4aR,6aR,9S,11aS)-11-Oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid	1637768-88-7
(2S)-2[(Amino)-2-(tetrahydro-2H-pyran-4-yl)]acetic acid	368866-31-3
(2S,3S)-2-((Amino)methyl)-3-methylpentanoic acid	671233-52-6
2-[(1S,2S)-1-(Amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid	1238864-23-7
(2S)-Amino-(1-methyl-1H-indazol-5-yl)acetic acid	-
(2R)-Amino-(1-methyl-1H-indazol-5-yl)acetic acid	-
3-Carboxyphenylalanine	-
2-Methyl-D-alloisoleucine	118904-37-3
4-Ethyl-L-norleucine	1998613-43-6
L-2,6-Difluorophenylalanine	1235005-44-3
2,5-Difluoro-L-phenylalanine	1004959-90-3
(2S,3aS,6aS)-Octahydrocyclopenta[b]pyrrole-2-carboxylic acid	87269-87-2
(2S)-2-(Amino)-2-[(1S,3R)-3-hydroxycyclohexyl]acetic acid	-
(2S)-2-(Amino)-2-[(1S,3S)-3-hydroxycyclohexyl]acetic acid	-
2-Amino-7-(tert-butoxy)-7-oxoheptanoic acid	159751-46-9
(2S)-3-(Triazol-1-yl)-2-(amino)propanoic acid	-
Tetrahydro-2H-pyran-3-ylacetic acid	1395922-27-6
rel-(1R,3S)-3-[(Amino)methyl]cyclohexanecarboxylic acid	2138238-33-0
(3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1)	-

Abbreviation/Expression Definition	CAS Number for Fmoc-/Boc-protected amino acid, chemical group or building block used for peptide synthesis
(3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 2)	-
Tranexamic acid	167690-53-1; 27687-14-5 (Boc)
trans-2-(3-(Amino)cyclohexyl)acetic acid	347185-01-7
trans-4-Fluoroproline	203866-20-0
1,13-Diamino-4,7,10-trioxatridecan-succinamic acid	172089-14-4
4-Piperazine-2-carboxylic acid	1034574-30-5
(2-Pyrrolidine-1-yl) acetic acid	37386-15-5
(S)-2-(Amino)-1,6-hexanedioic acid (AAD)	159751-47-0
Freidinger's Lactam	145484-45-3
(R)-Pyrrolidine-3-acetic acid	2137100-59-3
(2S)-2-(morpholin-4-yl)propanoic acid	110582-65-5
L-dehydroproline	135837-63-7
(3S)-2-azaspiro[4.4]nonane-3-carboxylic acid	394734-78-2
(2R)-2-amino-3-(trifluoromethylsulfanyl)propanoic acid	1994331-25-7
Arg(13C6,15N4)	1217461-89-6

Solid-phase resins were purchased from Novabiochem, Bachem, Iris Biotech, Pcas Biomatrix, GL Biochem (Shanghai) Ltd, CEM, or Protein Technologies. The resin loading was 0.3 – 1.0 mmol/g. Peptides were synthesized on 2-Chlorotrityl resin, on Wang resin, or on Rink amide-type resins depending on the desired C-terminus. Cleavage of the fluorenylmethoxycarbonyl (Fmoc) protecting group was achieved using 20% piperidine in dimethylformamide at room temperature. Each Fmoc cleavage step was carried out twice. Amino acids were coupled on an automated synthesizer (Symphony X) using 8 equivalents of the Fmoc-amino acid, with 8 equivalents of DIC (Diisopropylcarbodiimide) (0.5 M in DMF) and 8 equivalents of Oxyma (Ethyl cyanohydroxyiminoacetate) (0.5M in DMF). Amino acid couplings were conducted at room temperature and under a nitrogen atmosphere, when the Symphony X was used. When expensive or self-prepared fmoc-amino acids were used, the coupling was performed manually using 3 equivalents of the Fmoc-amino acid, with 3 equivalents of DIC (0.5 M in DMF) and 3 equivalents of Oxyma (0.5M in DMF). Each amino acid coupling step was carried out twice (double coupling). Alternatively, peptides were prepared by SPPS manually using 3 equivalents of the Fmoc-amino acid, 2.85 equivalents of HBTU ((2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, Hexafluorophosphate Benzotriazole Tetramethyl Uronium) (0.5 M in DMF) and 6 equivalents of DIPEA (0.5M in DMF). The coupling reaction was monitored using the ninhydrin test.

Peptides were completely deprotected using trifluoroacetic acid (TFA)/thioanisole (TA)/1,2-ethanedithiol (EDT) (90:7:3) or with 92.5%TFA/2.5%EDT/2.5%TIS (triisopropylsilane)/2.5%H₂O. For peptides containing methionine, the peptides were treated with a solution of 1.6% EDT and 1.2% trimethylsilylbromide in TFA for 2 h at room temperature to reduce oxidized methionine.

5 Disulfide Cyclization:

Disulfide bridges were formed by shaking peptides in 0.1 M ammonium bicarbonate buffer (pH 7.83) at a concentration of 0.5 mg/mL overnight. The solution was then lyophilized. Alternatively, disulfide bridges were formed by shaking peptides in mixture of acetonitrile/water (often 3:7) adjusted to pH 9.0 with solid ammonium bicarbonate buffer at a concentration of 1-3 mg/mL overnight. Alternatively, 10 disulfide bridges were prepared by oxidation with iodine (I₂) (0.1 M in MeOH) at a concentration of 1 – 1.3 mg/mL in acetonitrile/water (1:1) at 20°C for 2min, followed by treatment with sodium thiosulfate (0.1 M in water) followed by lyophilization.

Optional Acetylation:

N-terminal acetylation was performed using 10 equivalents acetic anhydride in DMF (2 mL) and 2.5 equivalents DIPEA by shaking the suspension at RT for 1 h on an orbital shaker. The solvent was removed and the resin was washed with DMF (5x) and DCM (5x). The procedure was then repeated again. Alternatively, N-terminal acetylation was performed using 10 mL of a capping solution consisting of acetic anhydride/N-methyl morpholine (NMM)/DMF (10:5:85) by shaking the suspension at RT for 30 min on an orbital shaker.

Peptide cleavage:

20 A cleavage cocktail containing TFA/EDT/Thioanisole (90:3:7) was prepared. The cleavage cocktail (2 mL) was added to the peptide containing resin and the suspension was shaken on an orbital shaker for 2.5 hours. Cold ether (-20°C) was added to precipitate the peptide. The resulting solution was centrifuged under nitrogen (Sigma 2-16KL), and the resulting solid obtained after decantation was washed with cold ether 3 more times, by centrifugation and decantation. The resulting solid was purified by preparative HPLC.

25 Alternatively, a cleavage cocktail containing TFA/EDT/TIS/H₂O (92.5:2.5:2.5:2.5) was prepared. The cleavage cocktail (6 mL (0.3 mmol scale)) was added to the peptide containing resin and the suspension was shaken on an orbital shaker for 2.5 hours. Cold tert-butyl methyl ether (-20°C) was added to precipitate the peptide. The resulting solution was centrifuged at 3000 rpm for 3 min, and the resulting solid obtained after decantation was washed with cold tert-butyl methyl ether 3 more times (20 mL x 3), by 30 centrifugation and decantation. The resulting crude peptide was dried over vacuum for 2 hours and then purified by preparative HPLC.

Preparative HPLC:

An Agilent 1260 Prep reversed-phase HPLC or a Knauer AZURA Prep reversed-phase HPLC was used for purification. The column is chosen based on the results of a column screen. The peptide is dissolved in 10 – 30% ACN/water (typically the starting point of the gradient). Water and acetonitrile both contain 0.1% TFA. Flow rate 20 mL/min, 10-30% ACN/water to 85-90% ACN/water was typically used. Fractions were analysed by HPLC (Agilent 1260 Infinity) using a Chromolith Speedrod column, 5-95% ACN/water gradient over 8 min) and by one or more of the following LC-MS methods: Method 1, Method 2, Method 3, Method 4, Method 5, Method 6.

Alternatively, a Gilson GX-281 Prep reversed-phase HPLC was used for purification. The column was chosen based on the results of a column screen. The peptide is dissolved in 10 – 30% ACN/water (typically the starting point of the gradient). The water contained 0.075% TFA. Normally a Luna column (25 x 200 mm, C18 10 μ m, 110 Å) or a Gemini column (30 x 150 mm, C18 5 μ m, 110 Å) was used. Conditions for prep HPLC: flow rate 20 mL/min, 10-30% ACN/water to 85-90% ACN/water, wavelength 214/254 nm, oven temperature 30°C. Fractions were analysed by HPLC (Agilent 1260 Infinity) using Method W1 or Method 7. The peptides were thereafter analyzed by one or more of the following methods: Method 1, Method 2, Method 3, Method 4, Method 5, Method 6.

Disulfide mimetics, wherein the -S-S- disulfide bond is replaced by a -CH₂-S-, -S-CH₂-, -CH₂-CH₂-, -S-(CH₂)₂-, -(CH₂)₂-S- or a -CH₂-S-CH₂- can be prepared according to procedure described in the following references in combination with methods described herein: (1) Hong-Kui Cui, Ye Guo, Yao He, Feng-Liang Wang, Hao-Nan Chang, Yu-Jia Wang, Fang-Ming Wu, Chang-Lin Tian, Lei Liu *Angew. Chem. Int. Ed.* **2013**, 52, 9558–9562; (2) Ye Guo, De-Meng Sun, Feng-Liang Wang, Yao He, Lei Liu, Chang-Lin Tian *Angew. Chem. Int. Ed.* **2015**, 54, 14276–14281; (3) Yang Xu, Tao Wang, Chao-Jian Guan, Yi-Ming Li, Lei Liu, Jing Shi, Donald Bierer *Tetrahedron Letters* **2017**, 58, 1677–1680; (4) Tao Wang, Yi-Fu Kong, Yang Xu, Jian Fan, Hua-Jian Xu, Donald Bierer, Jun Wang, Jing Shi, Yi-Ming Li *Tetrahedron Letters* **2017**, 58, 3970–3973; (5) Tao Wang, Jian Fan, Xiao-Xu Chen, Rui Zhao, Yang Xu, Donald Bierer, Lei Liu, Yi-Ming Li, Jing Shi, Ge-Min Fang *Org. Lett.* **2018**, 20, 6074–6078; (6) Jan-Patrick Fischer, Ria Schönaauer, Sylvia Els-Heindl, Donald Bierer, Johannes Koebberling, Bernd Riedl, Annette G. Beck-Sickinger *J Pep. Sci.* **2019**; e3147; (7) Dong-Liang Huang, Jing-Si Bai, Meng Wu, Xia Wang, Bernd Riedl, Elisabeth Pook, Carsten Alt, Marion Erny, Yi-Ming Li, Donald Bierer, Jing Shi, Ge-Min Fang *Chem. Commun.*, **2019**, 55, 2821–2824; (8) Shuai-Shuai Sun, Junyou Chen, Rui Zhao, Donald Bierer, Jun Wang, Ge-Min Fang, Yi-Ming Li *Tetrahedron Letters* **2019**, 60, 1197–1201; (9) C. M. B. K. Kourra and N. Cramer *Chem. Sci.*, **2016**, 7, 7007–7012.

All peptides of this invention unless otherwise noted are TFA Salts.

General Method for the Automated SPPS of Masp Peptides (Method A)

The synthesis of ((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-NH₂ (Example 165) is representative.

The peptide was synthesized using standard Fmoc chemistry.

Automated SPPS was performed on a Symphony X peptide synthesizer (Protein Technologies). Chem-Matrix Rink Amide resin was typically used (loading 0.5 mmol/gram) on a 0.1 mmol scale. The resin was placed into the reaction vessel and placed onto the instrument. The following solutions were prepared and used during the synthesis:

- 1) Fmoc Amino Acids: 0.2 M (8 eq)
- 2) Activator 1: 0.5 M DIC in DMF (7.5 eq)
- 3) Activator 2: 0.5 M Oxyma in DMF (7.5 eq)
- 4) Fmoc Deprotection : 20% Piperidine in DMF
- 5) Acetic anhydride (10 equivalents) in 2 mL DMF

Double couplings were typically performed for each amino acid. For expensive unnatural Fmoc or Boc amino acids, in-house synthesized Fmoc amino acids, or N-methylated amino acid, the sequence was interrupted and this amino acid was coupled manually (double coupling, but typically with less reagent (3-5 equiv). After this coupling was completed, the synthesis was typically continued on the synthesizer. If an N-methyl amino acid was added to the sequence, typically the next amino acid was coupled manually as well. All steps were performed at room temperature under nitrogen. Fmoc-Pen and Fmoc-Oic were typically coupled using automated SPPS. Fmoc(N-Me)G was typically coupled manually. Ahx was always coupled manually.

The resin was swelled and washed with DMF (3 x 3 mL, 10 min). If the resin contained Fmoc, then the Fmoc was removed with 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Asp(t-Bu) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Asp(Ot-Bu) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 5 Fmoc-Pro (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Pro (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained
10 and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 15 Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained
20 and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 25 Fmoc-Pen(Trt) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Pen(Trt) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution
30 was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 5 Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained
10 and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 15 Fmoc-Oic (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Oic (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained
20 and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 25 Fmoc-Pro (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Pro (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained
30 and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 5 Fmoc-Leu (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Leu (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained
10 and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 15 Fmoc-Ser(t-Bu) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Ser(t-Bu) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution
20 was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 25 Fmoc-Arg(Pbf) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Arg(Pbf) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution
30 was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 5 Fmoc-Ser(t-Bu) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Ser(t-Bu) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution
10 was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 15 Fmoc-Cys(Trt) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Cys(Trt) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution
20 was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

- 25 Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained
30 and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Manual Coupling during automated SPPS:

- 5 Fmoc-(N-Me)G (0.2 M in DMF, 5 equiv) was added to the resin. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with shaking (Thermomixer, rt) for 2 hours. The solution was filtered and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-(N-Me)G (0.2 M in DMF, 5 equiv) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to
- 10 proceed with shaking (Thermomixer, rt) for 2 hours. The solution was filtered and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

- 15 If additional amino acids were present in the sequence, they were coupled using the steps above.

Test Cleavage:

When manual couplings were performed, a test cleavage was typically performed to monitor the reactions. The test cleavage cocktail was TFA/EDT/Thioanisol (90:3:7); 1,5 h shaking on a Thermomixer at room temperature and 750 rpm. Analysis was performed by LC-MS using one of the methods above.

- 20 Full Cleavage:

The resin containing the peptide was placed into a syringe and 3.0 mL of cleavage buffer TFA/EDT/Thioanisol (90:3:7) was added to the resin. The mixture was shaken at room temperature for 2.5 hours. The solution was collected by filtration and the peptide was precipitate by adding cold diethyl ether (-20°C). The solution was centrifuged (3000 rpm) in a Falcon tube (60 mL) under a nitrogen atmosphere. The ether

25 was decanted and the solid residue was washed repeatedly with cold diethyl ether (5 x 10 mL). The solid residue was then dried.

Disulfide Cyclization:

The crude peptide was dissolved in 0.1M ammonium bicarbonate (pH 7.8-8.2) at a concentration of 1 mg/2 mL. The solution was allowed to shake on an orbital shaker overnight in a round-bottomed flask open to the air. The solution was then lyophilized to obtain a white powder.

5 Column Screening for HPLC Purification:

The peptide was dissolved in 5% CH₃CN and 95% water. Column screening was performed on each peptide to determine which preparative HPLC method to use for purification. The following analytical columns were screened.

Two methods are available for column screening:

- 10 1) 5-60% ACN_8 Min_1 mL/min_25°C
 2) 30-85% ACN_8 Min_1 mL/min_25°C

Available columns (50 mm x ID 4,6 mm) (available also as as Prep columns):

- 1) Aeris C18 (Phenomenex)
 2) X-Bridge C18 (Waters)
15 3) Kinetex C18 (Coreshell Material) (Phenomenex)
 4) YMC Triart C18 (elution with 100% water possible)
 5) Kinetix Biphenyl (Phenomenex)
 6) X-Select C18 (positive charge)
 7) Jupiter Proteo C18 (Phenomenex)
20 8) Luna C18 (Phenomenex)

For peptides of this invention, one of the following 5 preparative columns was used:

- 1) Column: Phenomenex, Aeris Peptide 5μ XB-C18, AXIA Packed, 21,2x250mm + Cartridge 5μ
 2) Column: Phenomenex, Kinetex C18 5μ 21,5x250mm + Cartridge 5μ
 3) Column: Phenomenex, Kinetex 5μ Biphenyl 100A, AXIA Packed, 21,2x250mm + Cartridge 5μ
25 4) Column: YMC Actus Triart Prep. C18 12nm, S-10μm 250x20mm + Cartridge 3μm (10x4mm)
 5) Column: Waters, Xbridge Prep.C18 5μ OBD 19x250mm + Cartridge 10μ

Once the column was chosen, one of the following methods was used:

- 1) method: Gradient 5-60% ACN in water (0,10% TFA)
 2) method: Gradient 30-85% ACN in water (0,10% TFA)
30 3) focused gradient based on results of the column screening.

Flow rate 20 mL/min

The combined fractions were analyzed by HPLC (5-95 in 8in, Chromolith SpeedROD & YMC C18 5-95 in 18 min) and using one of the LC-MS methods described above.

For Example 165, the crude peptide was dissolved in CH₃CN/water (1:1) and purified on a Phenomenex Kinetex column C18 5μ, 21.5 x 250 mm + 5μ Cartridge, Flow rate: 20 mL/min, Method: 5-60% ACN in water (each containing 0.10% TFA) over 48 min. The combined fractions were lyophilized to provide 39 mg of example 165 (95% pure).

Table 6: Note of materials used and conditions

#	Materials	Coupling reagents	Coupling time
1	Rink Amide ChemMatrix resin, loading 0.5mmol/g (0.1 mmol)	---	
2	Fmoc-Asp(t-Bu)-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
3	Fmoc-Pro-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
4	Fmoc-Ile-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
5	Fmoc-Pen(Trt)-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
6	Fmoc-Ile-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
7	Fmoc-Oic-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
8	Fmoc-Pro-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
9	Fmoc-Leu-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
10	Fmoc-Ser(tBu)-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
11	Fmoc-Arg(Pbf)-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
12	Fmoc-Ser(t-Bu)-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
13	Fmoc-Cys(Trt)-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
14	Fmoc-Ile-OH (3.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
15	Fmoc-(N-Me)G-OH (8 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min

10 General Method for the Automated SPPS of Masp Peptides (Method B)

The synthesis of ((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+I-((5-Azaspiro[2.4]heptane-1-carboxylic acid)-OH (example 164) is representative.

The peptide was synthesized using standard Fmoc chemistry.

When possible, preloaded Wang resin with the first amino acid (e.g. preloaded with Fmoc-Asp(t-Bu)-OH, Fmoc-Pro-OH, etc were used, with a typical loading of 0.55 - 0,7 mmol/g. When the first amino acid was not available, it was added manually.

When the first amino acid was not available as a preloaded resin, it was added manually and chlorotrityl resin was used for the synthesis.

Loading of first amino acid on 2-chlorotrityl resin:

2-Chlorotritylresin (500 mg, 0.775 mmol) was allowed to swell with 10 mL of DCM in a 50 mL Falcon tube for 15 min 5-[(9H-fluoren-9-ylmethoxy)carbonyl]-5-azaspiro[2.4]heptane-1-carboxylic acid (281 mg, 0.775 mmol) was dissolved in DCM with DIEA (6 equiv, 0.81 mL) added, and the solution was added to the resin. The solution was purged with argon and shaken overnight at room temperature. The mixture was filtered and washed with DMF (3 x 5 mL) and DCM (3 x 5 mL). Methanol was added (5 mL), the mixture was shaken for 30 minutes, and then filtered. The resin was washed with DMF (3 x 5 mL) and DCM (3 x 5 mL). Methanol was again added (5 mL), the mixture was shaken for 30 minutes, and then filtered. The resin was washed with DMF (3 x 5 mL) and DCM (3 x 5 mL). The loading was determined to be 0.42 mmol/g based on the determination of loading procedure described below.

The resin was then used for automated SPPS on the Symphony X synthesizer from Protein Technologies on 0.1 mmol scale.

Determination of Resin Loading:

To determine the loading of a resin the FMOC protecting group is cleaved from a defined amount of resin and afterwards the concentration of the resulting fluorenyl compound in the supernatant cleavage solution is measured via photometry at 301nm. This correlates directly with the amount of amino acid loaded on the resin.

- 1) 1-3 mg of resin are weighed into a 2 mL Eppendorf tube or similar (note the exact amount)
- 2) 1000 μ L of a solution of 20% piperidine in DMF are added.
- 3) The mixture is agitated for 30 minutes to cleave the FMOC group.
- 4) 100 μ L of the supernatant solution are then transferred into a quartz glass cuvette and diluted with 900 μ L of 20% piperidine / DMF
- 5) A blank sample of 1000 μ L piperidine / DMF is prepared in a second cuvette.
- 6) After determining the blank value from the reference sample, the extinction of the test sample at $\lambda = 301\text{nm}$ is then measured in a UV-Vis photometer (Thermo Scientific Evolution 201).
- 7) For greater accuracy, multiple test samples (typically two) can be fashioned; the arithmetic mean of the measured extinctions is then used for calculation.

Calculation of resin loading:

The resin loading L_{301} in mmol/g is calculated by the following formula:

$$L_{301} = \frac{E(301nm) \cdot V}{\varepsilon(301nm) \cdot D \cdot m} \cdot VF \cdot 1000$$

E = extinction

5 ε = extinction coefficient at 301nm wavelength (7800 L/mol*cm)

m = amount of resin used (g)

V = volume of sample (L)

D = layer thickness of the cuvette (cm)

VF = dilution factor (= 10)

10 Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

The coupling of the subsequent Fmoc amino acids proceeded as described in Method A.

The following solutions were prepared and used during the synthesis:

- 15 1) Fmoc Amino Acids: 0.2 M (8 eq)
 2) Activator 1: 0.5 M DIC in DMF (7,5 eq)
 3) Activator 2: 0.5 M Oxyma in DMF (7,5 eq)
 4) Fmoc Deprotection : 20% Piperidine in DMF
 5) Acetic anhydride (10 equivalents) in 2 mL DMF
- 20 Double couplings were typically performed for each amino acid. For expensive unnatural Fmoc or Boc amino acids, in-house synthesized Fmoc amino acids, or N-methylated amino acid, the sequence was interrupted and this amino acid was coupled manually (double coupling, but typically with less reagent (3-5 equiv). After this coupling was completed, the synthesis was typically continued on the synthesizer. If an N-methyl amino acid was added to the sequence, typically the next amino acid was coupled manually as well. All steps were performed at room temperature under nitrogen. Fmoc-Pen and Fmoc-Oic were typically coupled using automated
- 25 SPPS. Fmoc(N-Me)G was typically coupled manually. Ahx was always coupled manually.

Fmoc cleavage:

- The resin was swelled and washed with DMF (3 x 3 mL, 10 min). If the resin contained Fmoc, then the Fmoc was removed with 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF
- 30 (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Pen(Trt) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Pen(Trt) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Oic (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Oic (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Pro (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Pro (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Leu (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Leu (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Ser(t-Bu) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Ser(t-Bu) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Arg(Pbf) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Arg(Pbf) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Ser(t-Bu) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Ser(t-Bu) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Cys(Trt) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Cys(Trt) (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Coupling:

Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-Ile (4.0 mL) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with nitrogen bubbling for 2 hours. The solution was drained and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

Manual Coupling during automated SPPS:

Fmoc-(N-Me)G (0.2 M in DMF, 5 equiv) was added to the resin. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with shaking (Thermomixer, rt) for 2 hours. The solution was filtered and washed with DMF (1 x 3 mL, 30 sec). The coupling step was repeated. Fmoc-(N-Me)G (0.2 M in DMF, 5 equiv) was added. Activator 1 solution (DIC, 1.5 mL) and Activator 2 solution (Oxyma, 1.5 mL) were added and the coupling was allowed to proceed with shaking (Thermomixer, rt) for 2 hours. The solution was filtered and washed with DMF (6 x 3 mL, 30 sec).

Fmoc cleavage:

The Fmoc protecting group was removed by adding 20% piperidine solution (2 x 3 mL, 10 min). The resin was washed with DMF (6 x 3 mL, 30 sec).

If additional amino acids were present in the sequence, they were coupled using the steps above.

Test Cleavage:

When manual couplings were performed, a test cleavage was typically performed to monitor the reactions. The test cleavage cocktail was TFA/EDT/Thioanisol (90:3:7); 1,5 h shaking on a Thermomixer at room temperature and 750 rpm. Analysis was performed by LC-MS using one of the methods above.

Full Cleavage:

The resin containing the peptide was placed into a syringe and 3.0 mL of cleavage buffer TFA/EDT/Thioanisol (90:3:7) was added to the resin. The mixture was shaken at room temperature for 2.5 hours. The solution was collected by filtration and the peptide was precipitated by adding cold diethyl ether (-20°C).

The solution was centrifuged (3000 rpm) in a Falcon tube (60 mL) under a nitrogen atmosphere. The ether was decanted and the solid residue was washed repeatedly with cold diethyl ether (5 x 10 mL). The solid residue was then dried.

Disulfide Cyclization:

The crude peptide was dissolved in 0.1M ammonium bicarbonate (pH 7.8-8.2) at a concentration of 1 mg/2 mL. The solution was allowed to shake on an orbital shaker overnight in a round-bottomed flask open to the air. The solution was then lyophilized to obtain a white powder.

Column Screening for HPLC Purification:

The peptide was dissolved in 5% CH₃CN and 95% water. Column screening was performed on each peptide to determine which preparative HPLC method to use for purification. The following analytical columns were screened.

Two methods are available for column screening:

- 1) 5-60% ACN_8 Min_1 mL/min_25°C
- 2) 30-85% ACN_8 Min_1 mL/min_25°C

Available columns (50mm x ID 4,6 mm) (available also as as Prep columns):

- 1) Aeris C18 (Phenomenex)
- 2) X-Bridge C18 (Waters)
- 3) Kinetex C18 (Coreshell Material) (Phenomenex)
- 4) YMC Triart C18 (elution with 100% water possible)
- 5) Kinetix Biphenyl (Phenomenex)

- 6) X-Select C18 (positive charge)
- 7) Jupiter Proteo C18 (Phenomenex)
- 8) Luna C18 (Phenomenex)

For peptides of this invention, one of the following 5 preparative columns was used:

- 5
 - 1) Column: Phenomenex, Aeris Peptide 5 μ XB-C18, AXIA Packed, 21,2 x 250mm + Cartridge 5 μ
 - 2) Column: Phenomenex, Kinetex C18 5 μ 21,5x250mm + Cartridge 5 μ
 - 3) Column: Phenomenex, Kinetex 5 μ Biphenyl 100A, AXIA Packed, 21,2 x 250mm + Cartridge 5 μ
 - 4) Column: YMC Actus Triart Prep. C18 12nm, S-10 μ m 250 x 20mm + Cartridge 3 μ m (10x4mm)
 - 5) Column: Waters, Xbridge Prep.C18 5 μ OBD 19 x 250mm + Cartridge 10 μ

- 10 Once the column was chosen, one of the following methods was used:

- 1) method: Gradient 5-60% ACN in water (0,10% TFA)
- 2) method: Gradient 30-85% ACN in water (0,10% TFA)
- 3) focused gradient based on results of the column screening.

Flow rate 20 mL/min

- 15 The combined fractions were analyzed by HPLC (5-95 in 8in, Chromolith SpeedROD & YMC C18 5-95 in 18 min) and using one of the LC-MS methods described above

For example 164, the crude peptide was dissolved in CH₃CN/water (1:1) and purified on a Phenomenex Kinetex column C18 5 μ , 21.5 x 250 mm + 5 μ Cartridge, Flow rate: 20 mL/min, Method: 5-60% ACN in water (each containing 0.10% TFA) over 48 min. The combined fractions were lyophilized to provide

- 20 29.8 mg of example 164 (98.3% pure).

Table 7: Note of materials used and conditions

#	Materials	Coupling reagents	Coupling time
1	Rink Amide ChemMatrix resin, loading 0.5mmol/g (0.1 mmol)	---	
2	5-Fmoc-5-Azaspiro[2.4]heptane-1-carboxylic acid	DIPEA (6 eq)	overnight
3	Fmoc-Ile-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
4	Fmoc-Pen(Trt)-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
5	Fmoc-Ile-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
6	Fmoc-Oic-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
7	Fmoc-Pro-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min

#	Materials	Coupling reagents	Coupling time
8	Fmoc-Leu-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
9	Fmoc-Ser(tBu)-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
10	Fmoc-Arg(Pbf)-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
11	Fmoc-Ser(t-Bu)-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
12	Fmoc-Cys(Trt)-OH (8.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
13	Fmoc-Ile-OH (3.0 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min
14	Fmoc-(N-Me)G-OH (8 eq)	DIC (7.5 eq) Oxyma (7.5 eq)	120 min

General Method for the Manual SPPS of Masp Peptides (Method C)

The synthesis of sequence AIC+SRSLP-(Oic)-I-(Pen)+IPN-OH (example 418) is representative.

Peptide Synthesis:

5 The peptide was synthesized using standard Fmoc chemistry.

- 1) Resin preparation: To the chlorotrityl resin (CTC Resin) (0.3 mmol, 0.3 g, 1.0 mmol/g) was added Fmoc-Asn(Trt)-OH (0.3 mmol, 0.18 g, 1.0 eq) and DIEA (0.21 mL, 1.2 mmol, 4.0 eq) in DCM (6.0 mL). The mixture was agitated with N₂ for 2 h at 20 °C, then added MeOH (0.3 mL) and agitated with N₂ for another 30 min. The resin was washed with DMF (6.0 mL x 3). Then 20% piperidine in DMF (6.0 mL) was added and the mixture was agitated with N₂ for 20 min at 20°C. Then the mixture was filtered to get the resin. The resin was washed with DMF (6.0 mL x 3) and filtered to get the resin.
- 2) Coupling: Fmoc-Pro-OH (0.30 g, 0.9 mmol, 3.0 eq) HBTU (0.32 g, 0.85 mmol, 2.85 eq) and DIEA (1.80 mmol, 0.32 mL, 6.00 eq) in DMF (3.0 mL) was added to the resin and agitated with N₂ for 30 min at 20°C. The resin was then washed with DMF (6.0 mL x 5).
- 3) Deprotection: 20% piperidine in DMF (6.0 mL) was added to the resin and the mixture was agitated with N₂ for 20 min at 20 °C.
- 4) Repeat Step 2 to 3 for all other amino acids:

Table 8: Note of materials used and conditions

#	Materials	Coupling reagents	Coupling time
1	Fmoc-Asn(Trt)-OH (1.0 eq)	DIEA(4.0 eq)	2.0 h
2	Fmoc-Pro-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
3	Fmoc-Ile-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
4	Fmoc-Pen(Trt)-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min

#	Materials	Coupling reagents	Coupling time
5	Fmoc-Ile-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
6	Fmoc-Oic-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
7	Fmoc-Pro-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
8	Fmoc-Leu-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
9	Fmoc-Ser(tBu)-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
10	Fmoc-Arg(Pbf)-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
11	Fmoc-Ser(tBu)-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
12	Fmoc-Cys(Trt)-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
13	Fmoc-Ile-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
14	Fmoc-Ala-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min

20% piperidine in DMF was used for Fmoc deprotection for 30 min. The coupling reaction was monitored by ninhydrin (all amino acids except Pro) and chloranil test (Pro), and the resin was washed with DMF (5.0 mL) for 5 times.

5 Peptide Cleavage and Purification:

- 1) The resin was washed with MeOH (6.0 mL x 5) and dried under vacuum to get 0.62 g peptide resin. Then 6.0 mL of cleavage buffer (92.5%TFA/2.5%EDT/2.5%TIS/2.5%H₂O) was added to the flask containing the side chain protected peptide resin at 20 °C and the mixture was stirred for 2.5 h.
- 2) The peptide was precipitated with cold tert-butyl methyl ether (50 mL) and centrifuged (3 min at 3000 rpm) to get the solid crude. Wash the crude peptide precipitation with tert-butyl methyl ether for two more times (20.0 mL x 3). Dry the crude peptide over vacuum for 2.0 h to give 0.4 g crude peptide.
- 3) The crude (0.4 g) was dissolved in CH₃CN (150 mL) and water (150 mL). Iodine (I₂) (0.1 M in MeOH, 1.5 mL) was added at 20 °C until yellow color persists. Then the mixture was stirred at 20°C for 2 min. Then sodium thiosulfate (0.1 M in water, 0.05 mL) was added dropwise until yellow color disappears.
- 4) The mixture was lyophilized to give the crude powder (0.41 g)
- 5) The crude peptide was purified by prep-HPLC (conditions: A: 0.075% TFA in water B: CH₃CN) and lyophilized to get the desired peptide (example 418) (108.1 mg, 23.0% yield, 97.3% pure by Method W1; 97.4% pure by Method 7) as a white solid and TFA salt. Purification conditions: Peptide was dissolved in TFA/H₂O (7:3); flow rate 20 mL/min; gradient 12-42% over 60 min; Retention time = 42 min; purification over a Luna 25 x 200 mm, C18 10 um, 110 Å column, then again over a Gemini 150 x 30 mm, C18 5 um, 110 Å column to reach the desired purity.

If C-terminal amides were prepared according to this method, the synthesis began with Rink Amide MBHA resin (loading typically around 0.4-0.6 mmol/g, with the above steps being the same.

General Method for the Manual SPPS of Masp Peptides (Method D)

If C-terminal amides were prepared, the synthesis began with Rink Amide MBHA resin (loading typically around 0.4-0.6 mmol/g). The synthesis of sequence PIC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH₂ (example 9) is representative.

- 5 If C-terminal acids were prepared, the synthesis began with 2-chlorotrityl resin, with the first Fmoc amino acid being added according to the method described in Method C.

Peptide Synthesis:

The peptide was synthesized using standard Fmoc chemistry.

- 10 1) Resin preparation: The Rink Amide MBHA resin (0.66 g, 0.30 mmol, loading = 0.45 mmol/g) in DMF (10.0 mL) was agitated with N₂ for 0.5 h at 20°C. Then 20% piperidine in DMF (10 mL) was added and the mixture was agitated with N₂ for 20 min at 20°C. Then the mixture was filtered to get the resin. The resin was washed with DMF (10 x 5 mL) and filtered to get the resin.
- 15 2) Coupling: Fmoc-Asn(Trt)-OH (0.9 mmol, 0.37 g, 3.0 eq) HBTU (0.32 g, 0.85 mmol, 2.95 eq) and DIEA (1.80 mmol, 0.32 mL, 6.00 eq) in DMF (3.0 mL) was added to the resin and agitated with N₂ for 30 min at 20°C. The resin was then washed with DMF (10.0 mL x 3).
- 3) Deprotection: 20% piperidine in DMF (10.0 mL) was added to the resin and the mixture was agitated with N₂ for 20 min at 20 °C.
- 4) Repeat Step 2 to 3 for all other amino acids.

Table 9: Note of materials used and conditions

#	Materials	Coupling reagents	Coupling time
1	Fmoc-Asn(Trt)-OH (1.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
2	Fmoc-Pro-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
3	Fmoc-Ile-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
4	Fmoc-Pen(Trt)-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
5	Fmoc-Ile-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
6	Fmoc-Pro-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
7	Fmoc-Pro-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
8	Fmoc-Ala(t-Bu)-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
9	Fmoc-Ser(tBu)-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
10	Fmoc-Arg(Pbf)-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
11	Fmoc-Ser(tBu)-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
12	Fmoc-Cys(Trt)-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min

#	Materials	Coupling reagents	Coupling time
13	Fmoc-Ile-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min
14	Fmoc-Pro-OH (3.0 eq)	HBTU(2.85 eq) DIEA(6.0 eq)	30 min

20% piperidine in DMF was used for Fmoc deprotection for 30 min. The coupling reaction was monitored by ninhydrin (all amino acids except Pro) and chloranil test (Pro), and the resin was washed with DMF (5.0 mL) for 5 times.

5 Peptide Cleavage and Purification:

- 1) The resin was washed with MeOH (6.0 mL x 5) and dried under vacuum to get 0.66 g peptide resin. Then 6.0 mL of cleavage buffer (90%TFA/5%EDT/2.5%TIS/2.5%H₂O) was added to the flask containing the side chain protected peptide resin at 20 °C and the mixture was stirred for 2.5 h.
- 2) The peptide was precipitated with cold tert-butyl methyl ether (50 mL) and centrifuged (3 min at 3000 rpm) to get the solid crude. The crude peptide precipitation was washed with tert-butyl methyl ether for three more times (20.0 mL x 3). Dry the crude peptide over vacuum for 2.0 h to give 0.4 g crude peptide.
- 3) The crude (0.4 g) was dissolved in CH₃CN (15 mL) and water (15 mL) to achieve a 0.1 mM concentration. NH₄HCO₃ solution (1M) was added to adjust the pH to about 8-9. The solution was allowed to shake at room temperature for about 8 hours. The reaction was monitored by HPLC. After the reaction was complete, the reaction was quenched with acetic acid to adjust the pH to about 6. The reaction mixture was then lyophilized and the resulting solid was purified by reversed-phase HPLC.
- 4) Purified the crude peptide by prep-HPLC (conditions: A: 0.075% TFA in water B: CH₃CN) and lyophilized to get the desired peptide (example 9) (202.40 mg, 41.4.0% yield, 93.7% pure by Method W1; 95.1% pure by Method 7) as a white solid and TFA salt. Purification conditions: Peptide was dissolved in TFA/H₂O (7:3); flow rate 20 mL/min; gradient 12-42% over 60 min; Retention time = 42 min; purification over a Luna 25 x 200 mm, C18 10 µm, 110 Å column.

Alternative General Method for the Manual SPPS of Masp Peptides (Method E)

The synthesis of sequence ((2S)-2[(Amino)-2-(tetrahydro-2H-pyran-4-yl)]acetic acid)-IC+SRSLP-(Oic)-IC+I-OH (example 184) is representative.

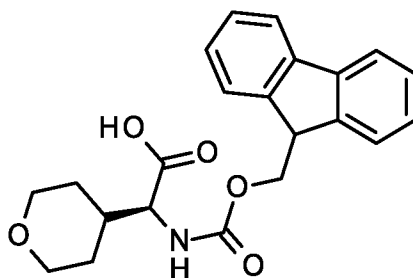
25 Peptide Synthesis:

The peptide was synthesized using standard Fmoc chemistry.

The dark ball in the chemical structures below indicates the solid polymer support used for solid-phase peptide synthesis (SPPS), e.g. 2-chlorotrityl resin, Rink amide resin, etc.

Example 1A

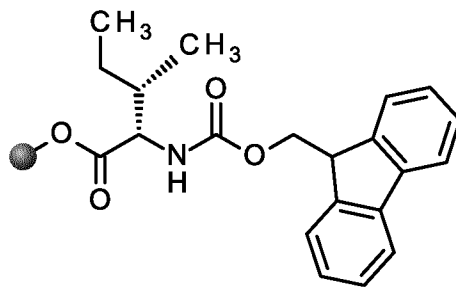
(2S)-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)(oxan-4-yl)acetic acid (Single Stereoisomer)



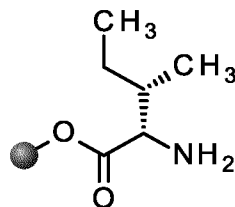
- 5 To (2S)-amino(oxan-4-yl)acetic acid (950 mg, 5.97 mmol) in acetone/water (15 mL / 10 mL) was added sodium bicarbonate (5.01 g, 59.7 mmol) and 1-({[(9H-fluoren-9-yl)methoxy]carbonyl}oxy)pyrrolidine-2,5-dione (2.11 g, 6.27 mmol). The mixture was stirred over the weekend at room temperature. The suspension was treated with water and extracted with MBTE two more times. The aqueous phase was acidified with 1 M aqueous hydrochloric acid and was extracted with dichloromethane three more times. The organic phase was washed with saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtered and evaporated. The residue was purified via silica gel chromatography (Isolera; 50 g SNAP Ultra; gradient: cyclohexane 50% / ethyl acetate 50% + 1% acetic acid to cyclohexane 20% / ethyl acetate 80% + 1% acetic acid). The appropriate fractions were combined and evaporated. The residue was taken up in toluene and evaporated two more times, then the residue was dried under vacuum to give 1.08 g (100%
10 purity, 47% yield) of the target compound.
15

LC-MS (Method 9): $R_t = 0.90$ min; MS (ESIpos): $m/z = 382$ $[M+H]^+$

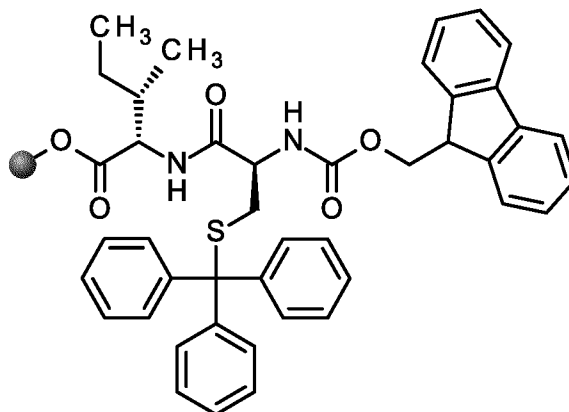
$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: -0.150 (0.43), -0.008 (4.33), 0.008 (3.74), 0.146 (0.43), 1.280 (1.03), 1.300 (2.26), 1.309 (2.36), 1.331 (2.75), 1.341 (2.92), 1.371 (2.77), 1.401 (2.54), 1.411 (2.32), 1.430 (1.49), 1.464 (6.34), 1.496 (3.49), 1.932 (1.76), 1.942 (2.14), 1.951 (1.93), 1.960 (2.08), 2.327 (0.64), 2.366 (0.64), 2.670 (0.64), 2.710 (0.65), 3.169 (2.11), 3.212 (2.67), 3.237 (6.32), 3.265 (6.90), 3.704 (0.49), 3.715 (0.49), 3.840 (4.66), 3.854 (5.62), 3.867 (4.30), 3.879 (6.28), 3.899 (5.14), 3.918 (3.56), 4.201 (2.11), 4.218 (5.83), 4.237 (9.07), 4.263 (8.42), 4.274 (9.80), 4.293 (5.14), 4.318 (1.05), 7.309 (6.35), 7.328 (15.12), 7.347 (9.86), 7.400 (9.65), 7.419 (15.88), 7.437 (7.02), 7.652 (5.77), 7.674 (5.52), 7.735 (8.21), 7.740 (8.28), 7.754 (7.63), 7.883 (16.00), 7.902 (14.80), 12.609 (0.56).

Example 2A

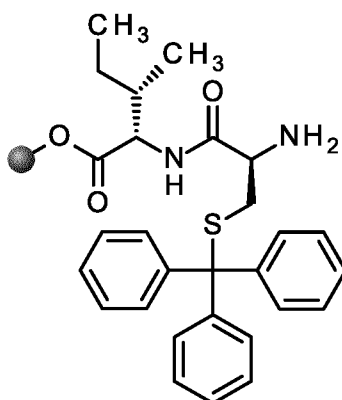
The reaction was carried out under an argon atmosphere. To N-[(9H-fluoren-9-yl)methoxy]carbonyl-L-isoleucine (9.40 g, 26.6 mmol) in 53 mL dichloromethane was added N,N-diisopropylethylamine (19 mL, 110 mmol) and 2-chlorotritylchlorid resin (10.0 g, 13.3 mmol). The reaction mixture was shaken overnight at room temperature. The precipitate was collected by filtration and washed with DMF three times. The collected resin was shaken with dichloromethane/methanol 1:1 for 30 min. The resin was collected by filtration and washed three times in rotation with methanol and dichloromethane. The collected resin was dried under vacuum for the next step.

Example 3A

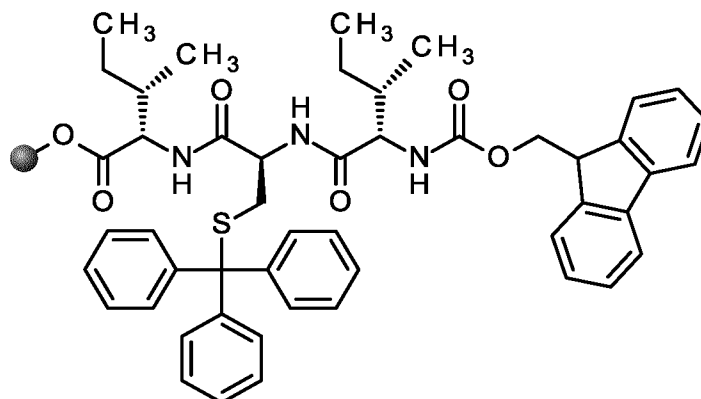
Example 2A (15.2 g, 6.82 mmol) in 200 mL DMF/piperidine 4:1 was shaken for 15 min at room temperature. The resin was collected by filtration and washed with DMF for three times. Both steps were repeated and washed three times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.



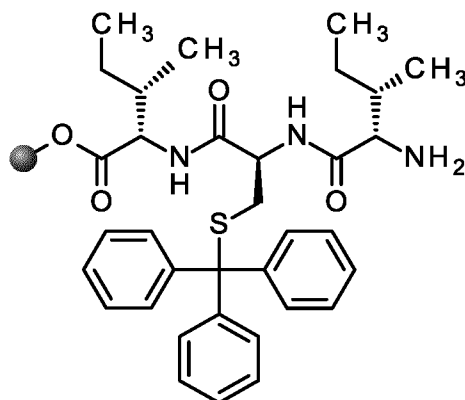
Example 5A



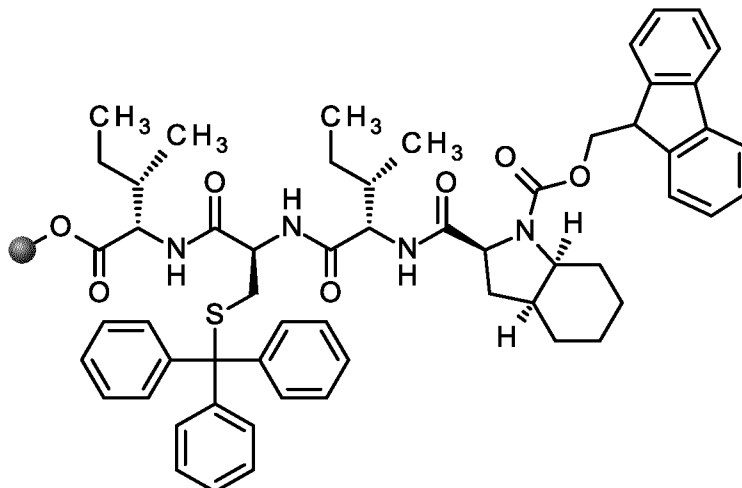
Example 4A (18.9 g, 8.51 mmol) in 200 mL DMF/piperidine 4:1 was shaken for 15 min at room temperature. The resin was collected by filtration and washed with DMF for three times. Both steps were repeated and the collected resin was washed three times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

Example 6A

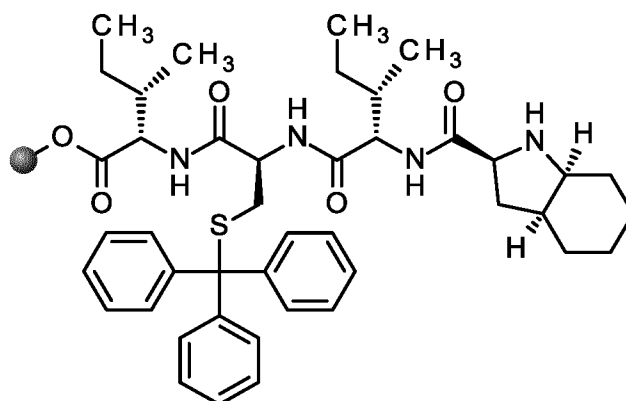
Example 5A (16.3 g, 7.32 mmol) was swelled in 150 mL DMF for 5 min at room temperature. A mixture
5 of N-{[(9H-fluoren-9-yl)methoxy]carbonyl}-L-isoleucine (5.18 g, 14.6 mmol) in 50 mL DMF, DIC (2.2 mL, 14 mmol) and Oxyma (2.03 g, 14.3 mmol) was added and the reaction mixture was shaken for 2 h at room temperature. The resin was collected by filtration and washed with DMF for three times. The coupling step was repeated overnight. The resin was collected by filtration and washed with 200 mL DMF for three times and then three more times in rotation with 200 mL methanol and 200 mL dichloromethane.
10 The collected resin was dried under vacuum for the next step.

Example 7A

Example 6A (20.0 g, 8.98 mmol) in 200 mL DMF/piperidine 4:1 was shaken for 15 min at room temper-
15 ature. The resin was collected by filtration and washed with DMF for three times. Both steps were repeated and the collected resin was washed three times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

Example 8A

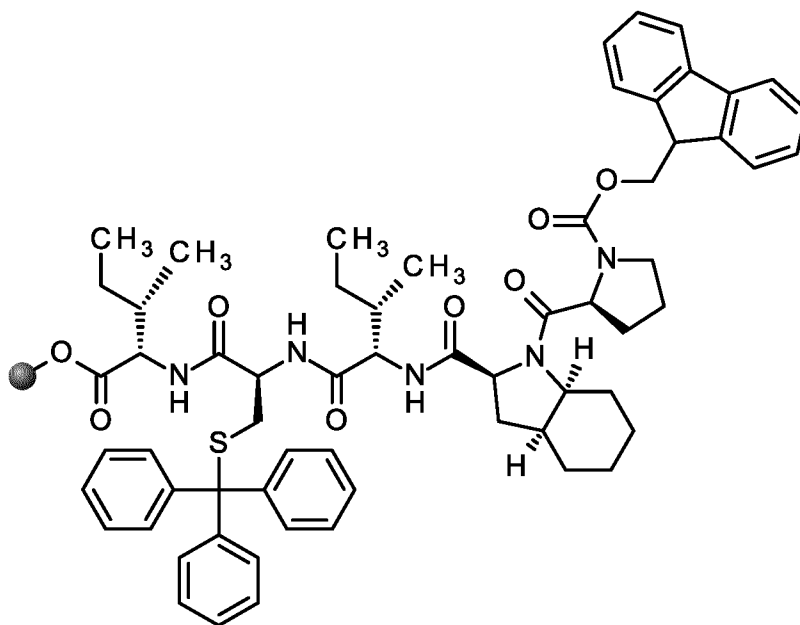
Example 7A (17.8 g, 7.99 mmol) was swelled in 150 mL DMF for 5 min at room temperature. A mixture
5 of (2S,3aS,7aS)-1-1-[(9H-fluoren-9-yl)methoxy]carbonyl}octahydro-1H-indole-2-carboxylic acid (6.25 g,
16.0 mmol) in 50 mL DMF, DIC (2.4 mL, 16 mmol) and Oxyma (2.21 g, 15.6 mmol) was added and the
reaction mixture was shaken for 2 h at room temperature. The resin was collected by filtration and washed
with DMF for three times. The coupling step was repeated over the weekend. The resin was collected by
filtration and washed with 200 mL DMF for three times and then three more times in rotation with 200
10 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

Example 9A

Example 8A (21.5 g, 9.68 mmol) in 200 mL DMF/piperidine 4:1 was shaken for 30 min at room temper-
15 ature. The resin was collected by filtration, washed with DMF for three times and then three more times
in rotation with 200 mL methanol and 200 mL dichloromethane. To the collected resin was added 200 mL
DMF/piperidine 4:1 and shaken for 1 h at room temperature. The resin was collected by filtration, washed

with DMF for three times and then three more times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

Example 10A

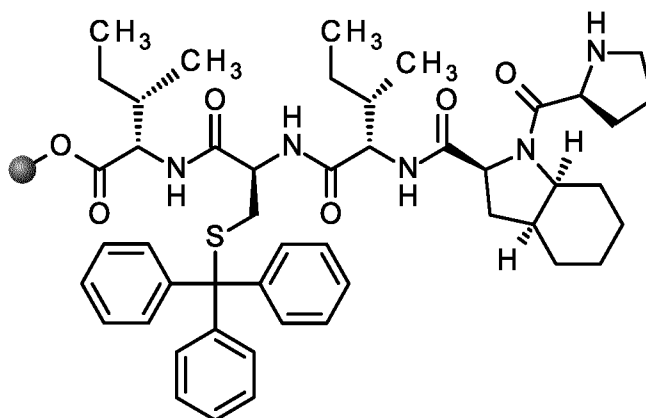


5

Example 9A (18.1 g, 8.13 mmol) was swelled in 150 mL DMF for 5 min at room temperature. A mixture of 1-{[(9H-fluoren-9-yl)methoxy]carbonyl}-L-proline (11.0 g, 32.5 mmol) in 50 mL DMF, DIC (4.9 mL, 32 mmol) and Oxyma (4.51 g, 31.7 mmol) was added and the reaction mixture was shaken over the weekend at room temperature. The resin was collected by filtration, washed with 200 mL DMF for three times and then three more times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

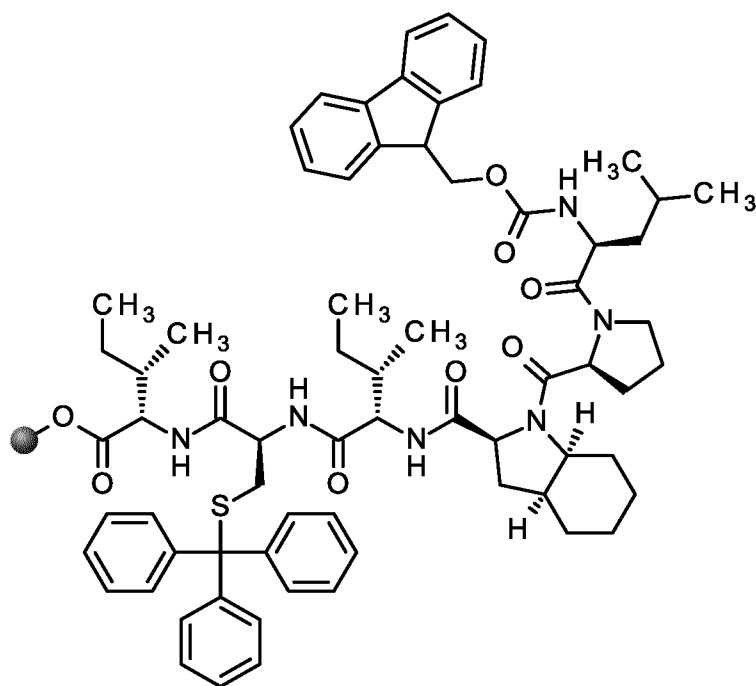
10

Example 11A



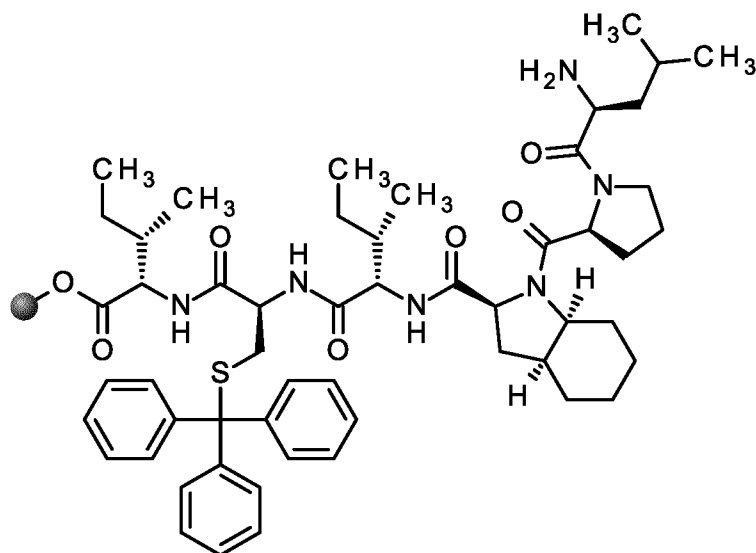
Example 10A (20.4 g, 9.16 mmol) in 150 mL DMF/piperidine 4:1 was shaken for 30 min at room temperature. The resin was collected by filtration and washed with DMF for three times. Both steps were repeated and the collected resin was washed three times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

5 Example 12A



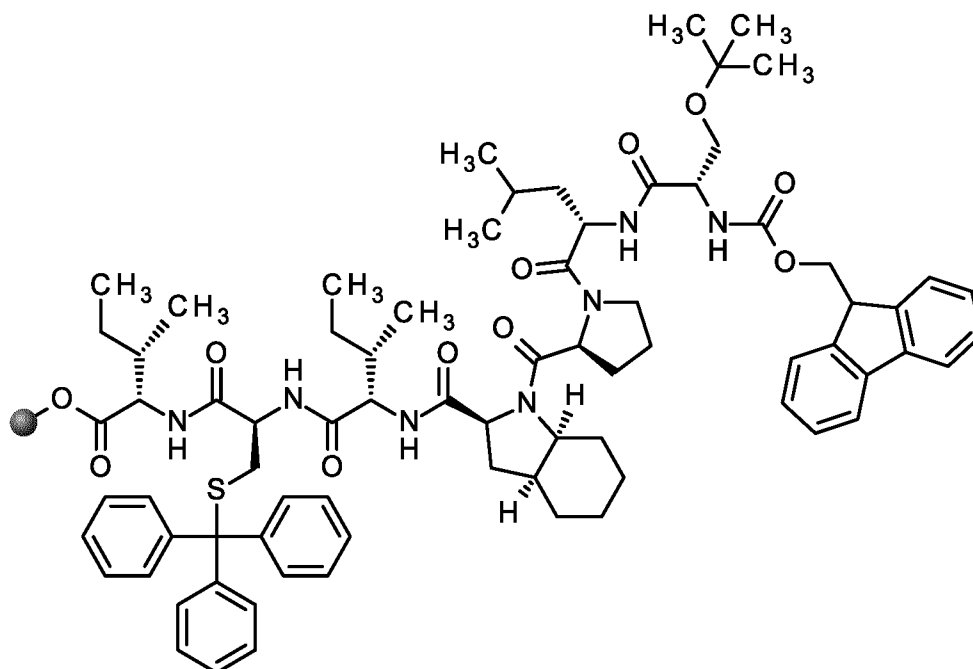
Example 11A (18.2 g, 8.18 mmol) was swelled in 150 mL DMF for 5 min at room temperature. A mixture of N-{[(9H-fluoren-9-yl)methoxy]carbonyl}-L-leucine (11.6 g, 32.7 mmol) in 50 mL DMF, DIC (4.9 mL, 32 mmol) and Oxyma (4.53 g, 31.9 mmol) was added and the reaction mixture was shaken overnight at room temperature. The resin was collected by filtration, washed with 200 mL DMF for three times and then three more times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

Example 13A



Example 12A (20.9 g, 9.42 mmol) in 150 mL DMF/piperidine 4:1 was shaken for 30 min at room temperature. The resin was collected by filtration and washed with DMF for three times. Both steps were repeated and the collected resin was washed three times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

Example 14A

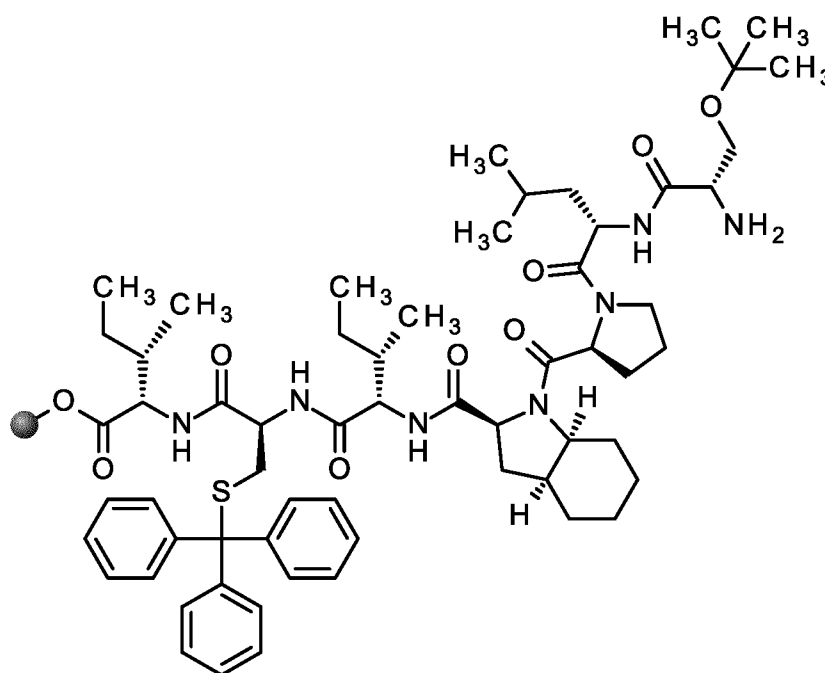


10

Example 13A (18.0 g, 8.11 mmol) was swelled in 150 mL DMF for 5 min at room temperature. A mixture of O-tert-butyl-N-{[(9H-fluoren-9-yl)methoxy]carbonyl}-L-serine (12.4 g, 32.4 mmol) in 50 mL DMF,

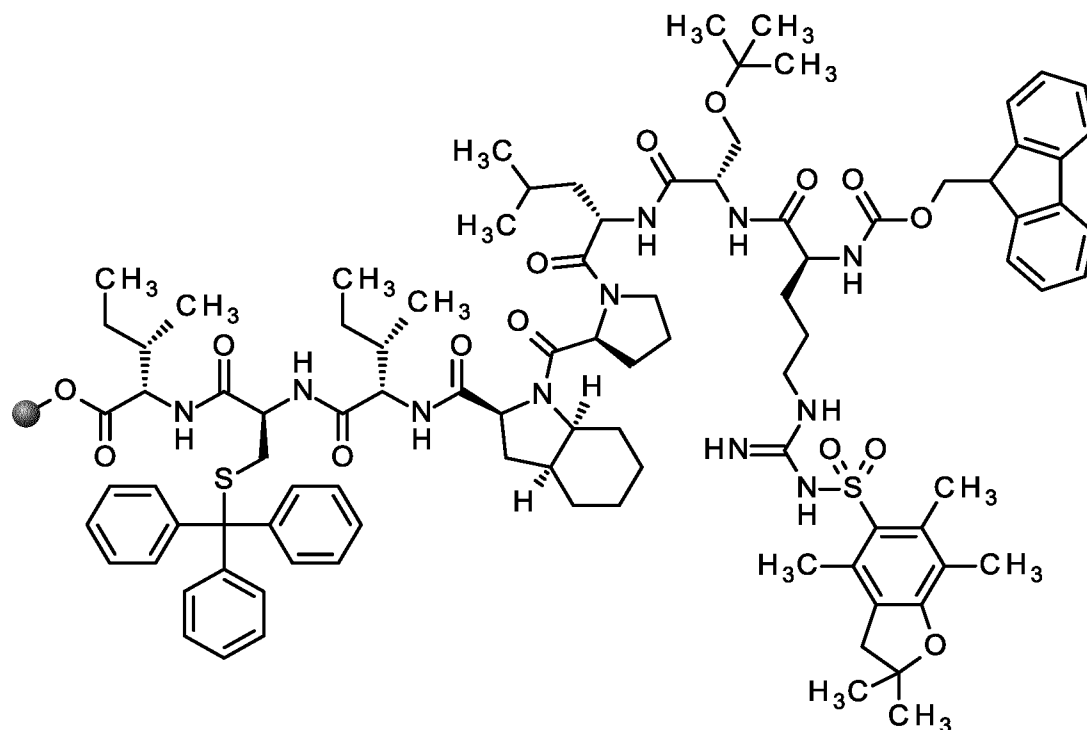
DIC (4.9 mL, 32 mmol) and Oxyma (4.49 g, 31.6 mmol) was added and the reaction mixture was shaken overnight at room temperature. The resin was collected by filtration, washed with 200 mL DMF for three times and then three more times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

5 Example 15A



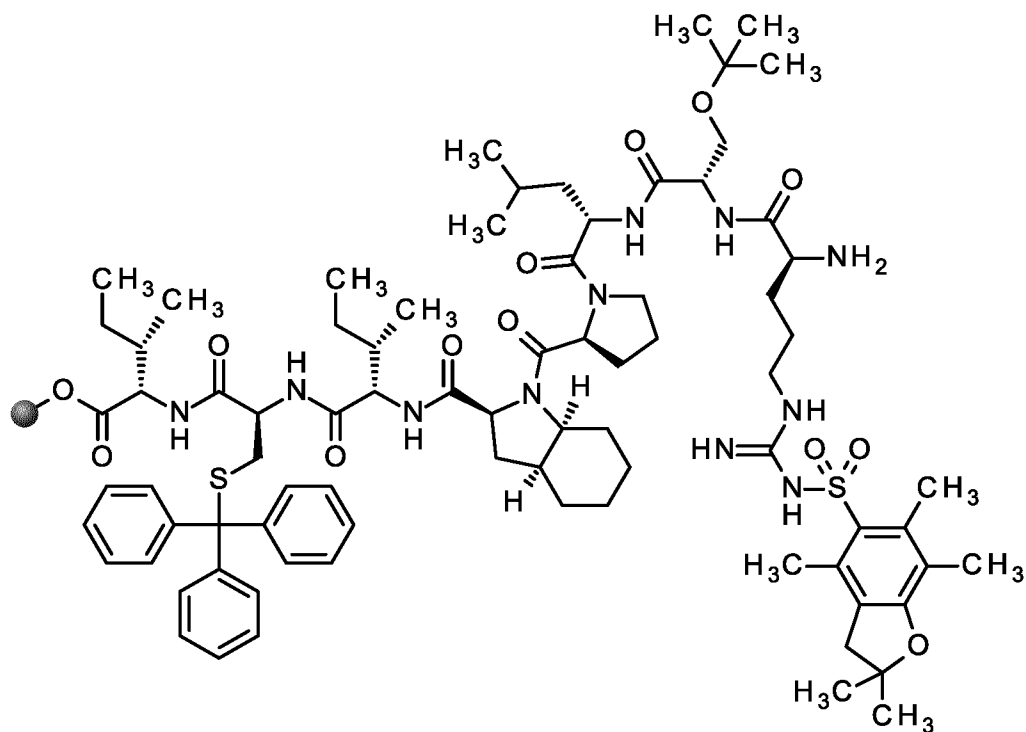
Example 14A (21.4 g, 9.64 mmol) in 200 mL DMF/piperidine 4:1 was shaken for 30 min at room temperature. The resin was collected by filtration and washed with DMF for three times. Both steps were repeated and the collected resin was washed three times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

Example 16A



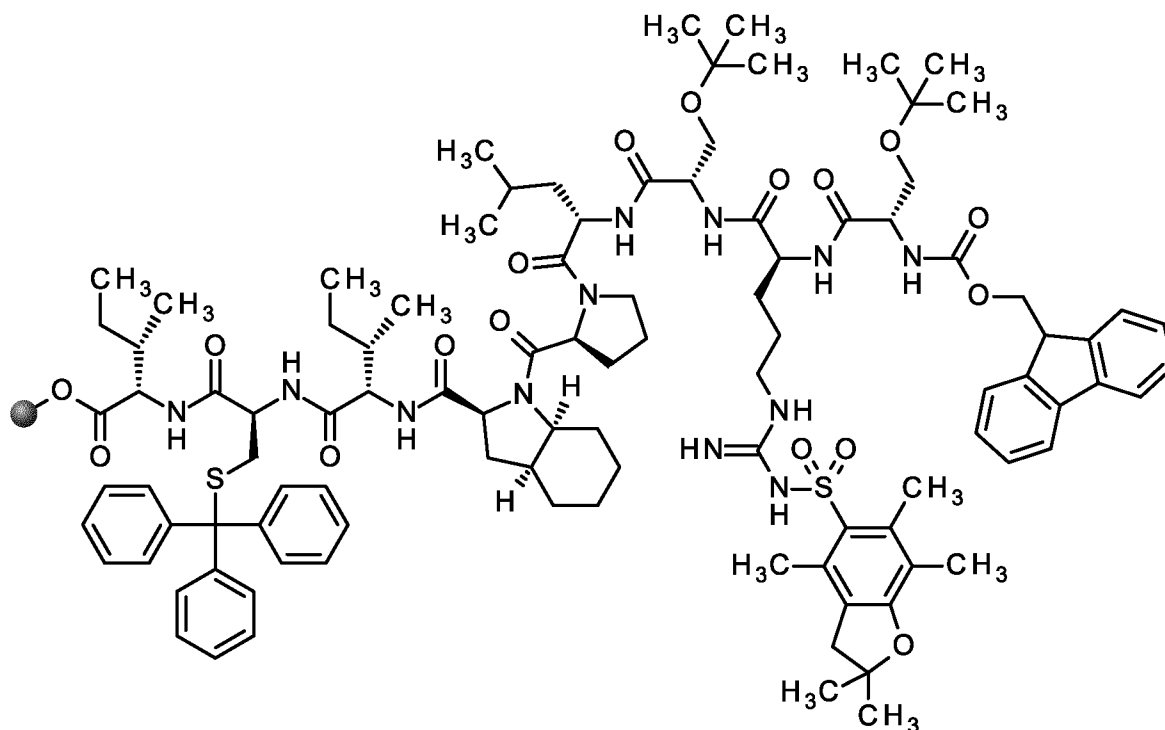
Example 15A (18.9 g, 8.51 mmol) was swelled in 150 mL DMF for 5 min at room temperature. A mixture
 5 of N^2 -{[(9H-fluoren-9-yl)methoxy]carbonyl}- N^5 -[N-(2,2,4,6,7-pentamethyl-2,3-dihydro-1-benzofuran-
 5-sulfonyl)carbamimidoyl]-L-ornithine (22.1 g, 34.0 mmol) in 50 mL DMF, DIC (5.1 mL, 33 mmol) and
 Oxyma (4.72 g, 33.2 mmol) was added and the reaction mixture was shaken overnight at room tempera-
 ture. The resin was collected by filtration, washed with 200 mL DMF for three times and then three more
 10 times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under
 vacuum for the next step.

Example 17A



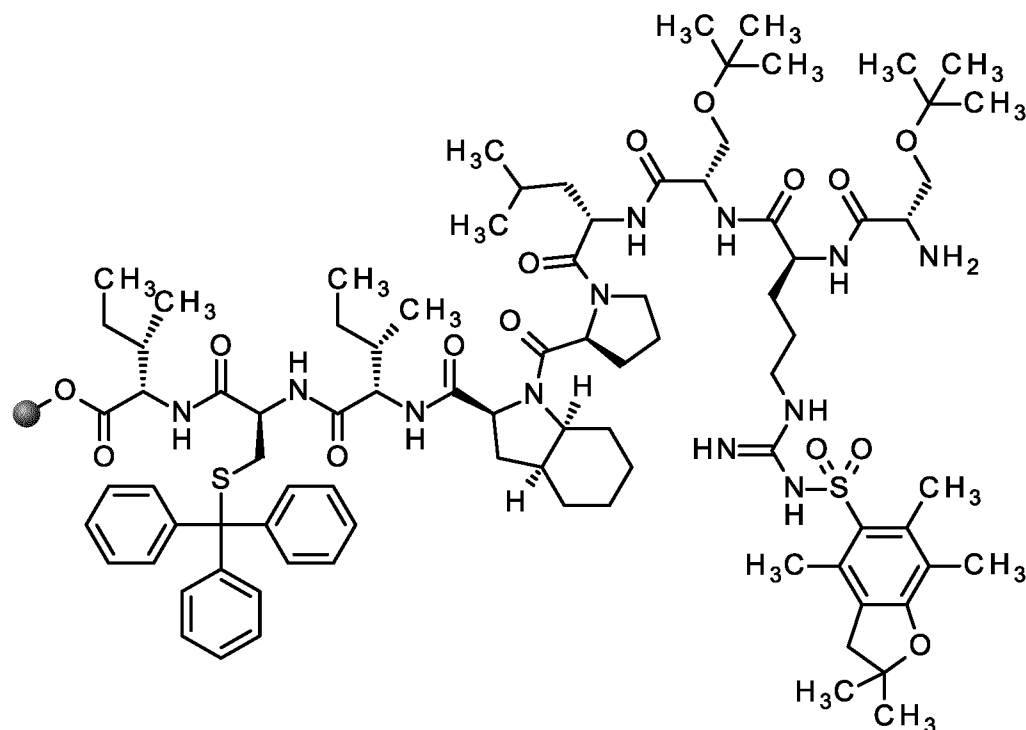
Example 16A (23.8 g, 10.7 mmol) in 200 mL DMF/piperidine 4:1 was shaken for 30 min at room temperature. The resin was collected by filtration and washed with DMF for three times. Both steps were repeated and the collected resin was washed three times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

Example 18 A



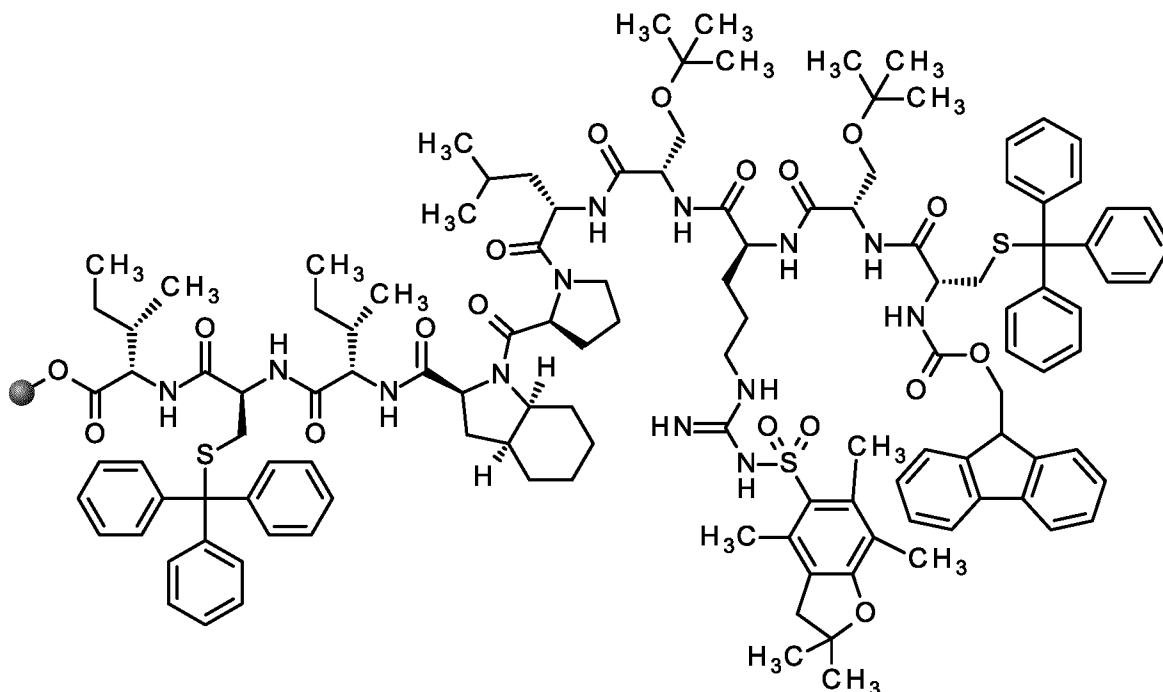
Example 17A (22.1 g, 9.95 mmol) was swelled in 150 mL DMF for 5 min at room temperature. A mixture
 5 of O-tert-butyl-N-[[[(9H-fluoren-9-yl)methoxy]carbonyl]-L-serine (15.3 g, 39.8 mmol) in 50 mL DMF,
 DIC (6.0 mL, 39 mmol) and Oxyma (5.51 g, 38.8 mmol) was added and the reaction mixture was shaken
 overnight at room temperature. The resin was collected by filtration, washed with 200 mL DMF for three
 times and then three more times in rotation with 200 mL methanol and 200 mL dichloromethane. The
 collected resin was dried under vacuum for the next step.

Example 19A



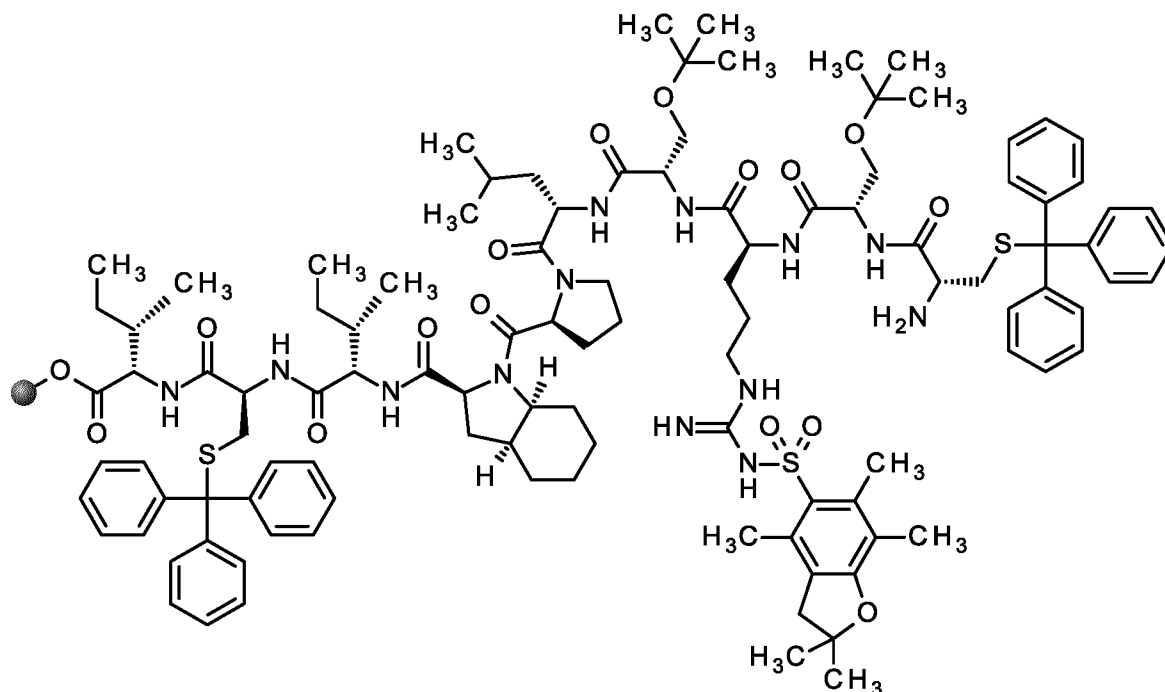
Example 18A (24.7 g, 11.1 mmol) in 200 mL DMF/piperidine 4:1 was shaken for 30 min at room temperature. The resin was collected by filtration and washed with DMF for three times. Both steps were repeated and the collected resin was washed three times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

Example 20A



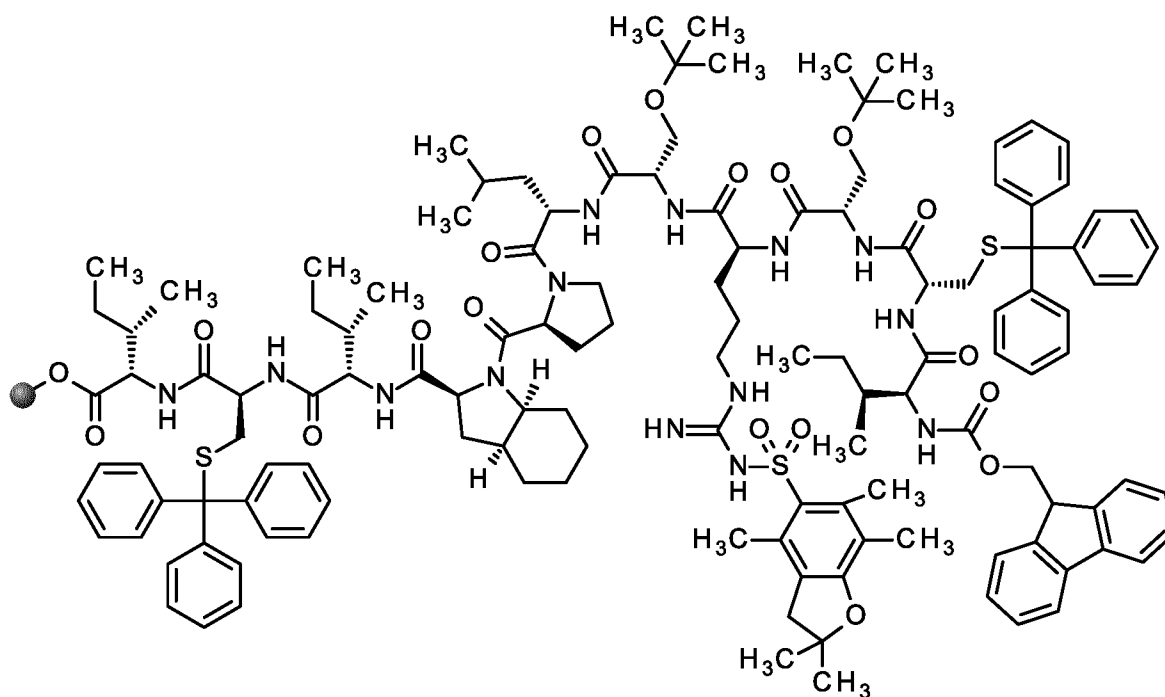
Example 19A (22.2 g, 9.99 mmol) was swelled in 150 mL DMF for 5 min at room temperature. A mixture of N-[[[(9H-fluoren-9-yl)methoxy]carbonyl]-S-(triphenylmethyl)-L-cysteine (23.4 g, 40.0 mmol) in 50 mL DMF, DIC (6.0 mL, 39 mmol) and Oxyma (5.54 g, 39.0 mmol) was added and the reaction mixture was shaken overnight at room temperature. The resin was collected by filtration, washed with 200 mL DMF for three times and then three more times in rotation with 200 mL methanol and 200 mL dichloro-
5 methane. The collected resin was dried under vacuum for the next step.

Example 21 A



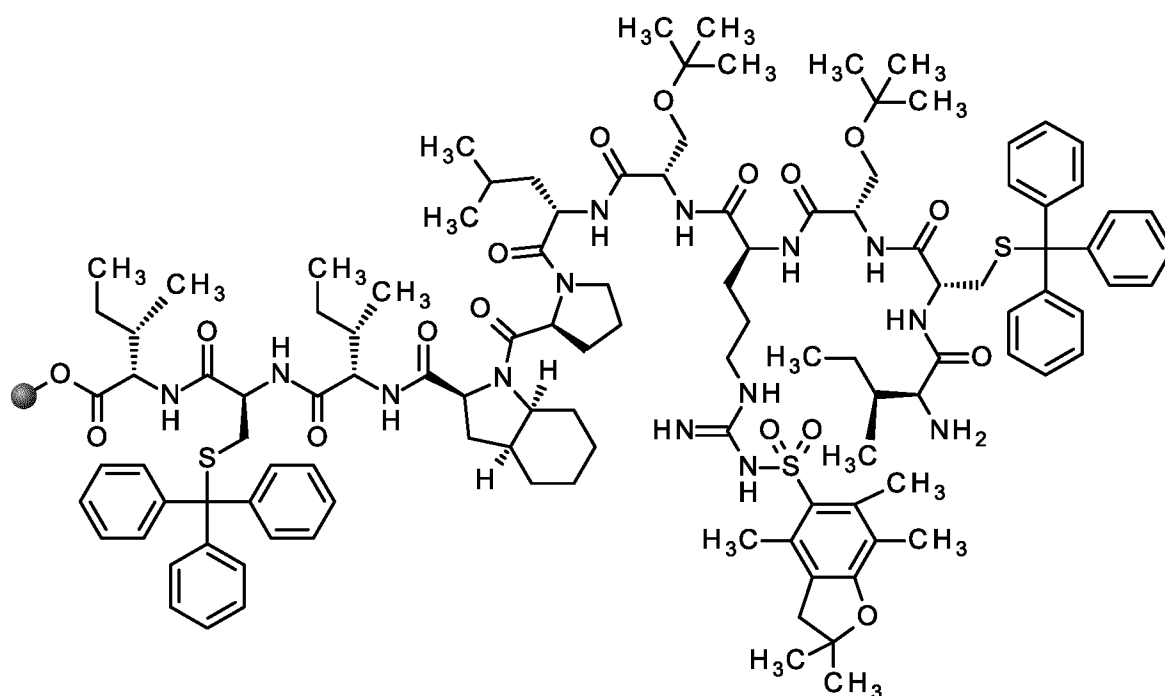
Example 20A (24.5 g, 11.0 mmol) in 200 mL DMF/piperidine 4:1 was shaken for 30 min at room temperature. The resin was collected by filtration and washed with DMF for three times. Both steps were repeated and the collected resin was washed three times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

Example 22A



Example 21A (22.3 g, 10.0 mmol) was swelled in 150 mL DMF for 5 min at room temperature. A mixture of N-[[[9H-fluoren-9-yl)methoxy]carbonyl]-L-isoleucine (14.2 g, 40.1 mmol) in 50 mL DMF, DIC (6.0 mL, 39 mmol) and Oxyma (5.55 g, 39.0 mmol) was added and the reaction mixture was shaken over the weekend at room temperature. The resin was collected by filtration, washed with 200 mL DMF for three times and then three more times in rotation with 200 mL methanol and 200 mL dichloromethane. The collected resin was dried under vacuum for the next step.

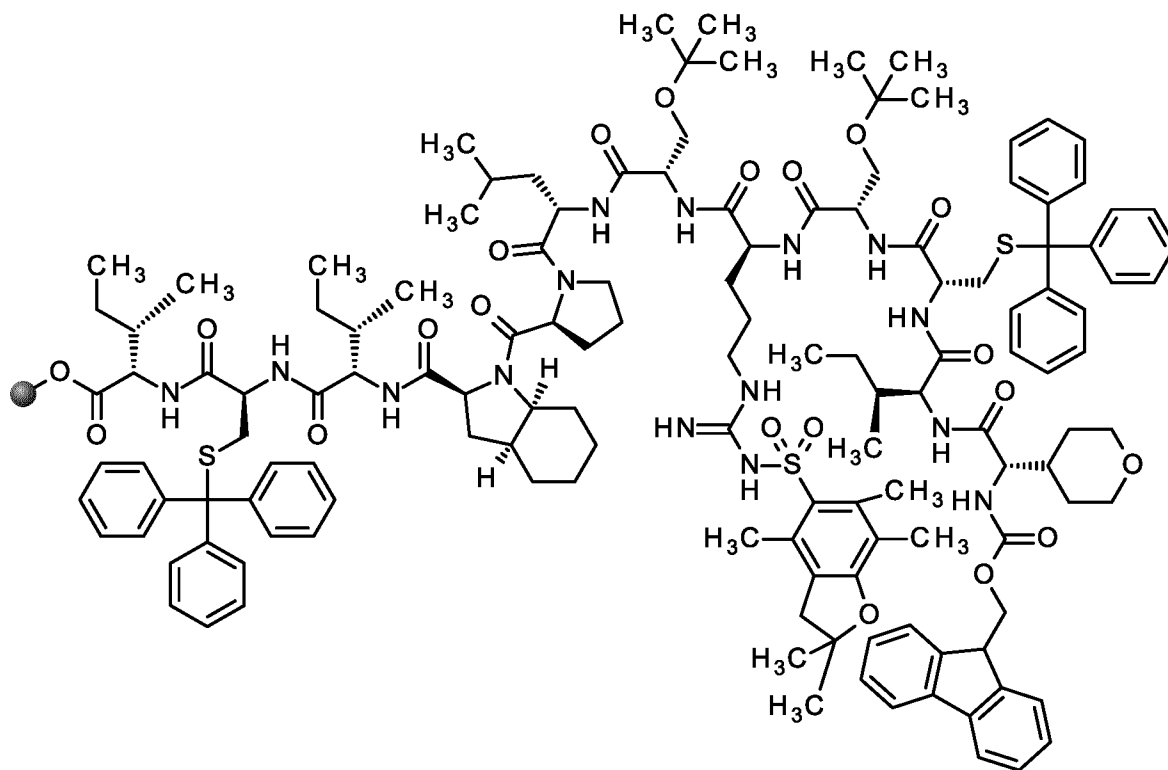
Example 23A



10

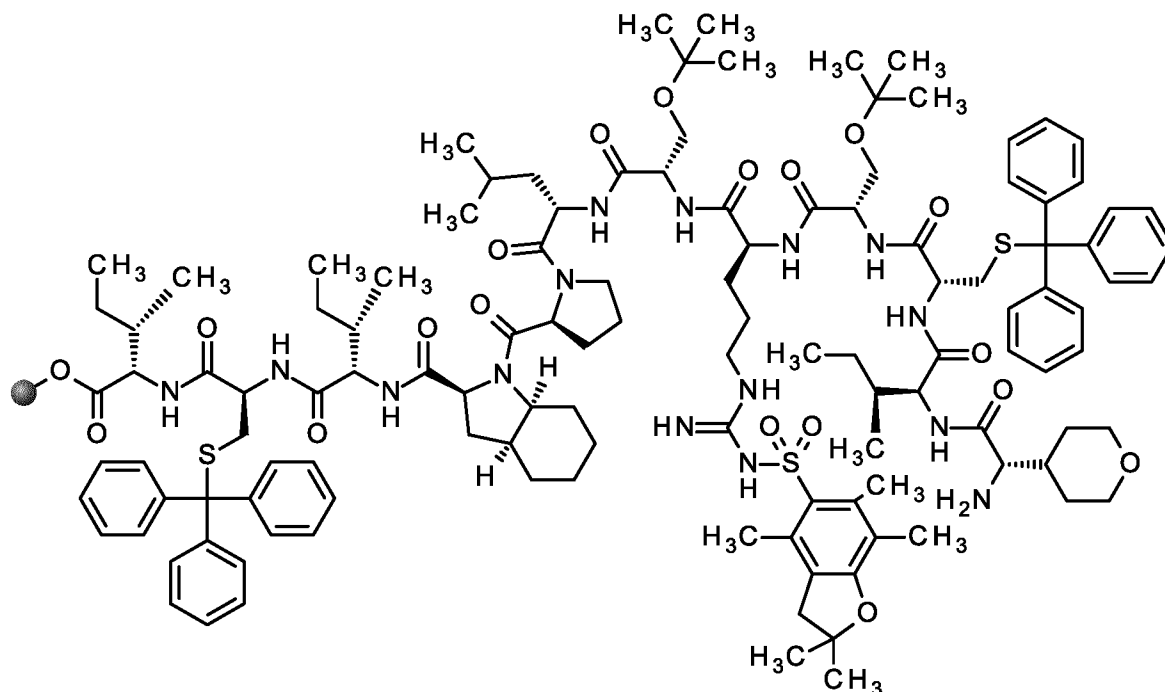
Example 22A (1.00 g, 250 μ mol) in 7.5 mL DMF/piperidine 4:1 was shaken for 30 min at room temperature. The resin was collected by filtration and washed with DMF for three times. Both steps were repeated and the collected resin was washed three times in rotation with methanol and dichloromethane. The collected resin was dried under vacuum for the next step.

Example 24 A



Example 23A (1.00 g, 250 μ mol) was swelled in 5 mL DMF for 5 min at room temperature. A mixture of
5 (2S)-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)(oxan-4-yl)acetic acid (381 mg, 1.00 mmol) in 1 mL DMF, DIC (150 μ L, 980 μ mol) and Oxyma (139 mg, 975 μ mol) was added and the reaction mixture was shaken over the weekend at room temperature. The resin was collected by filtration, washed with DMF for three times and then three more times in rotation with methanol and dichloromethane. The collected resin was dried under vacuum for the next step.

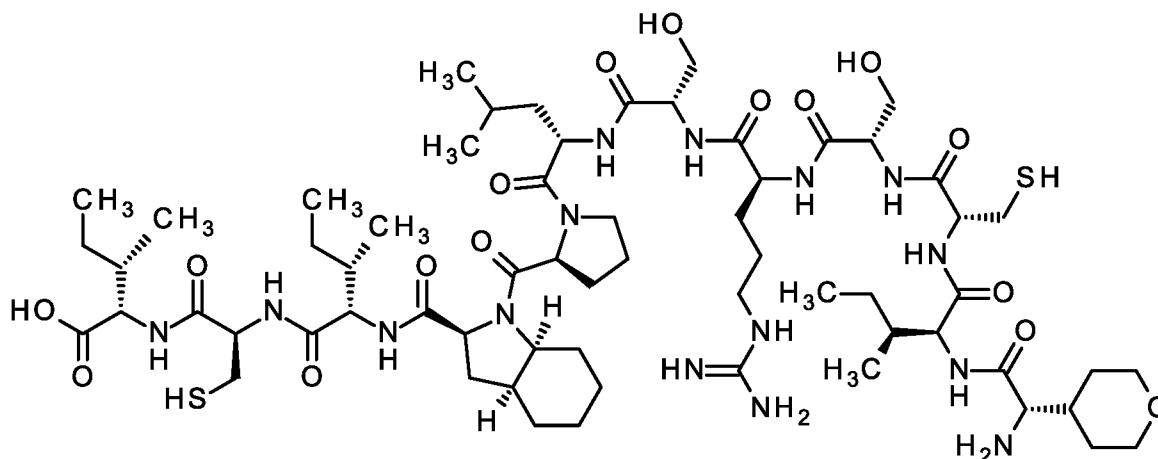
Example 25A



Example 24A (1.00 g, 250 μ mol) in 7.5 mL DMF/piperidine 4:1 was shaken for 30 min at room temperature. The resin was collected by filtration and washed with DMF for three times. Both steps were repeated and the collected resin was washed three times in rotation with methanol and dichloromethane. The collected resin was dried under vacuum for the next step.

Example 26A

(2S,3S)-2-{{[(2R)-2-{{[(2S,3S)-2-{{[(2S,3aS,7aS)-1-{{(2S)-1-[(2S,5S,8S,11S,14R,17S,20S)-20-amino-17-[(2S)-butan-2-yl]-8-(3-carbamimidamidopropyl)-5,11-bis(hydroxymethyl)-2-(2-methylpropyl)-20-(oxan-4-yl)-4,7,10,13,16,19-hexaoxo-14-(sulfanylmethyl)-3,6,9,12,15,18-hexaazaicosanan-1-oyl]pyrrolidine-2-carbonyl}octahydro-1H-indole-2-carbonyl]amino}-3-methylpentanoyl]amino}-3-sulfanylpropanoyl]amino}-3-methylpentanoic acid (Single Stereoisomer)

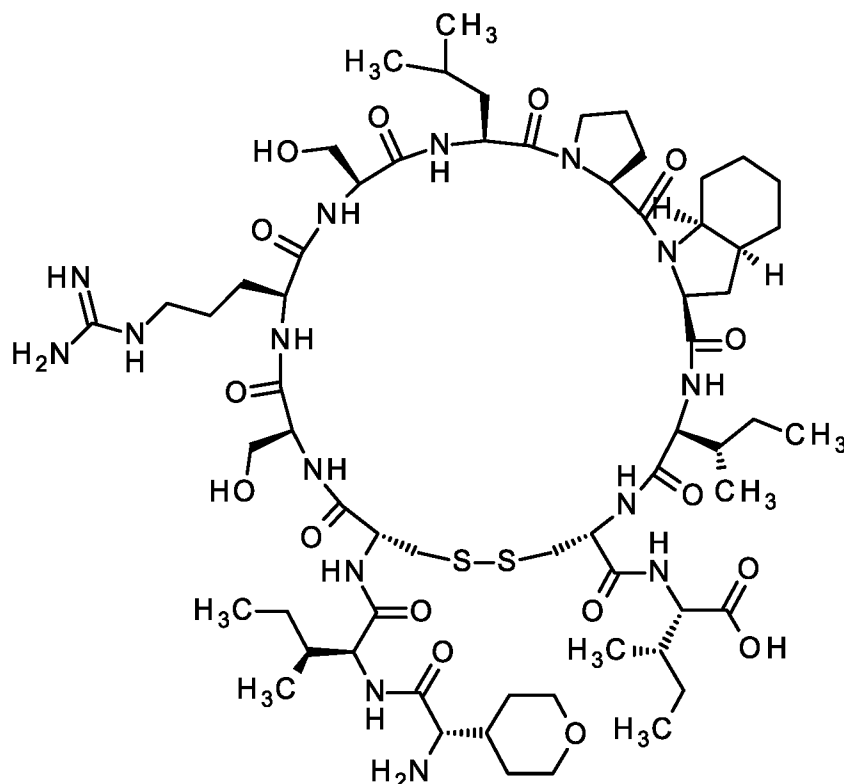


To Example 25A (1.00 g, 250 μ mol) was added the cleavage cocktail of TFA/EDT/Thioanisol (90:3:7) and the mixture was shaken for 2 h at room temperature. The filtrate was collected, and the resin was washed with dichloromethane. The combined filtrate was evaporated. The residue was stirred with diethyl ether and the solid was collected by filtration. The solid was washed several times with diethyl ether and dried under vacuum for the next step.

Example 184

((2S)-2[(Amino)-2-(tetrahydro-2H-pyran-4-yl)]acetic acid)-IC+SRSLP-(Oic)-IC+I-OH (example 184)

- 10 N-[(6S,9S,12S,15S,18R,23R,26S,28aS,29aS,33aS,35aS)-18-({N-[(2S)-2-amino-2-(oxan-4-yl)acetyl]-L-isoleucyl}amino)-26-[(2S)-butan-2-yl]-12-(3-carbamimidamidopropyl)-9,15-bis(hydroxymethyl)-6-(2-methylpropyl)-5,8,11,14,17,25,28,35-octaoxodotriacontahydro-1H,5H,22H-pyrrolo[2',1':13,14][1,2,5,8,11,14,17,20,23,26]dithiaoctaazacyclononacosino[11,10-a]indole-23-carbonyl]-L-isoleucine (Single Stereoisomer)



To Example 26A (425 mg, 304 μ mol) was added 850 mL 0.1 M aqueous ammonium bicarbonate solution (pH 7.86). Air was bubbled through the reaction mixture for 5 min. The reaction mixture was stirred in a
 5 open flask overnight at room temperature. The solvents were lyophilized to give 580 mg of a white solid. Purification using reversed-phase HPLC as described above (Method B) gave three fractions 7.4 mg (> 99%), 24.5 mg (98%) and 15 mg (95%) of the desired example 184.

General Method F: Conversion of Peptide TFA salts to HCl salts

The synthesis of AIC+SRS-((tBu)A)-PPI-((N-Me)C)+IPD-NH₂ (HCl Salt) (example 30) is representa-
 10 tive.

Procedure of Automatic Ion Exchange Station:

Peristaltic pump of the company Hirschmann (Rotarus volume 50), Tubes: Tygon 2001 (ID 0,64mm)

Settings:

Washing with H₂O : run-time 1200 s; 80 min⁻¹; 1 cycle (is 35 mL volume)

15 Sample circulation with peptide: run-time 1200 s; 80 min⁻¹; 1 cycle (is 70 mL volume)

Wash with H₂O (or %ACN in H₂O : run-time 1200 s; 80 min⁻¹; 1 cycle (is 35 mL volume)

Amberlite IRA 410 (HCl form) was used. 1500 mg of the resin was placed into 2 filter cartridges and washed with deionized water (10 times).

The peptide dissolved in 3 mL of a 5% ACN/H₂O solution was loaded onto the column and cycled through the column 10 times. The column was washed with water, and the solution collected into a Falcon tube and lyophilized

72.83 mg of the desired peptide was obtained as the HCl salt: LC-MS (> 99%); Ion Chromatography analysis: 3.7 wt% Cl⁻ (1.57 eq Cl⁻), <1 wt % TFA.

The ion exchange process can also be performed using the following protocol:

Amberlite IRA 410 resin (HCl form) (1-2 g) was placed into a 10 mL frit-syringe (100 mg Peptide needs 1g IRA 410 resin)

- 1) The resin is washed with water (10 times x 3 mL)
- 10 2) The resin is washed with 5% ACN in water (1 time x 3 mL)
- 3) The peptide was dissolved in 5% ACN in water
- 4) The peptide was added to the syringe and the solution was cycled through the column 10 -20 times. The eluent is collected into a Falcon Tube.
- 5) The resin is washed with 5%ACN in water (10 times x 3 mL); this solution is added to the solution
- 15 in the Falkon-tube
- 6) The combined solution is lyophilized.

General Method FA: Conversion of Peptide TFA salts to other salts

Other salt forms:

The chloride counter ion can be exchanged with other counterions by passing a solution of the desired salt form (e.g sodium acetate) repeatedly through the column, then washing the column repeatedly with water. The peptide is then loaded and the above procedures followed. Peptide acetate, tartrate, citrate, and lactate salts have thus been prepared. The following three examples are representative:

Example 249

Sequence: ((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-NH₂ (Acetate salt) (example 249)

25 N-[(6S,9S,12S,15S,18R,23R,26S,28aS,29aS,33aS,35aS)-26-[(2S)-butan-2-yl]-12-(3-carbamimidamidopropyl)-9,15-bis(hydroxymethyl)-22,22-dimethyl-18-[(N-methylglycyl-L-isoleucyl)amino]-6-(2-methylpropyl)-5,8,11,14,17,25,28,35-octaoxodotriacontahydro-1H,5H,22H-pyrrolo[2',1':13,14][1,2,5,8,11,14,17,20,23,26]dithiaoctaazacyclononacosino[11,10-a]indole-23-carbonyl]-L-isoleucyl-L-prolinamide, Acetic Acid salt

IRA 410 chloride resin (12 g) was placed into an empty 20 mL Biotage column and the resin was washed with with a 5% solution of ACN-water (10 times). The cartridge was attached to the Automatic Ion Exchange Station. The resin was washed with 20 column volumes of a 1M NaOH solution (2x 1800s rotation speed 80 min⁻¹). The column was washed with water until the pH of the eluent was < pH 9 (10 column
 5 volumes). The column was then eluted with 1 M acetic acid solution (10 column volumes), then washed with water until the pH of the eluent was > 5 (about 10 column volumes). The peptide (N-Me)GIC+SRSLP-(Oic)-I-Pen+IP-NH₂ (TFA salt) (1200 mg) was loaded onto the column in 12 mL of 5% ACN/water and allowed to cycle through the column 10 times according to the General Method F. The resin was then washed with a 5% ACN/water solution 10 times and the eluent was collected into 50 mL
 10 Falcon tubes. The parameter settings used for this scale were:

Washing with H₂O: run-time 3085 s; 80 min⁻¹; 1 cycle (is 90 mL volume)

Sample circulation with peptide: run-time 3085 s; 80 min⁻¹; 1 cycle (is 180 mL volume)

Wash with H₂O (or with %ACN in H₂O: run-time 3085 s; 80 min⁻¹; 1 cycle (is 90 mL volume)

The combined eluent was lyophilized to give 1120 mg (100 % purity, 97% yield) of the target acetate salt.

15 Ion chromatography (chloride content): < 1% chloride

Ion chromatography (TFA content): < 1% TFA

Ion chromatography (acetate content): 7.1% acetate = 1.84 equiv

LC-MS (MCW-TOF-AQ-YMC-18min): Rt = 7.74 min; MS (ESIpos): m/z = 725 [M+2H]⁺

Example 264

20 Sequence: ((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-NH₂ (L-Tartaric acid salt) (example 264)

N-[(6S,9S,12S,15S,18R,23R,26S,28aS,29aS,33aS,35aS)-26-[(2S)-butan-2-yl]-12-(3-carbamimidamidopropyl)-9,15-bis(hydroxymethyl)-22,22-dimethyl-18-[(N-methylglycyl-L-isoleucyl)amino]-6-(2-methylpropyl)-5,8,11,14,17,25,28,35-octaoxodotriacontahydro-1H,5H,22H-pyr-
 rolo[2',1':13,14][1,2,5,8,11,14,17,20,23,26]dithiaoctaazacyclononacosino[11,10-a]indole-23-carbonyl]-
 25 L-isoleucyl-L-prolinamide (2R,3R)-2,3-dihydroxybutanedioic acid (L-(+) tartaric acid) salt

IRA 410 chloride resin (12.5 g) was placed into an empty 20 mL Biotage column and the resin was washed with with a 5% solution of ACN-water (10 times). The cartridge was attached to the Automatic Ion Exchange Station. The resin was washed with 20 column volumes of a 1M NaOH solution (2x 1800s rotation speed 80 min⁻¹). The column was washed with water until the pH of the eluent was < pH 9 (10 column
 30 volumes). The column was then eluted with 1 M L-tartaric acid solution (10 column volumes), then washed with water until the pH of the eluent was >5 (about 10 column volumes). The peptide ((N-Me)G)-

IC+SRS LP-(Oic)-I-(Pen)+IP-NH₂ (TFA salt) (1200 mg) was loaded onto the column in 12 mL of 5% ACN/water and allowed to cycle through the column 10 times according to the General Method F. The resin was then washed with a 5% ACN/water solution 10 times and the eluent was collected into 50 mL Falcon tubes. The parameter settings used for this scale were:

- 5 Washing with H₂O: run-time 3085 s; 80 min⁻¹; 1 cycle (is 90 mL volume)

Sample circulation with peptide: run-time 3085 s; 80 min⁻¹; 1 cycle (is 180 mL volume)

Wash with H₂O (or %ACN in H₂O: run-time 3085 s; 80 min⁻¹; 1 cycle (is 90 mL volume)

The combined eluent was lyophilized to give 1170 mg (100% purity, 91% yield) of the target L-tartaric acid salt.

- 10 Ion Chromatography (chloride content): <1% chloride

Ion Chromatography (TFA content): <1% TFA

Ion chromatography (acetate content): 13.4% acetate = 1.49 equiv

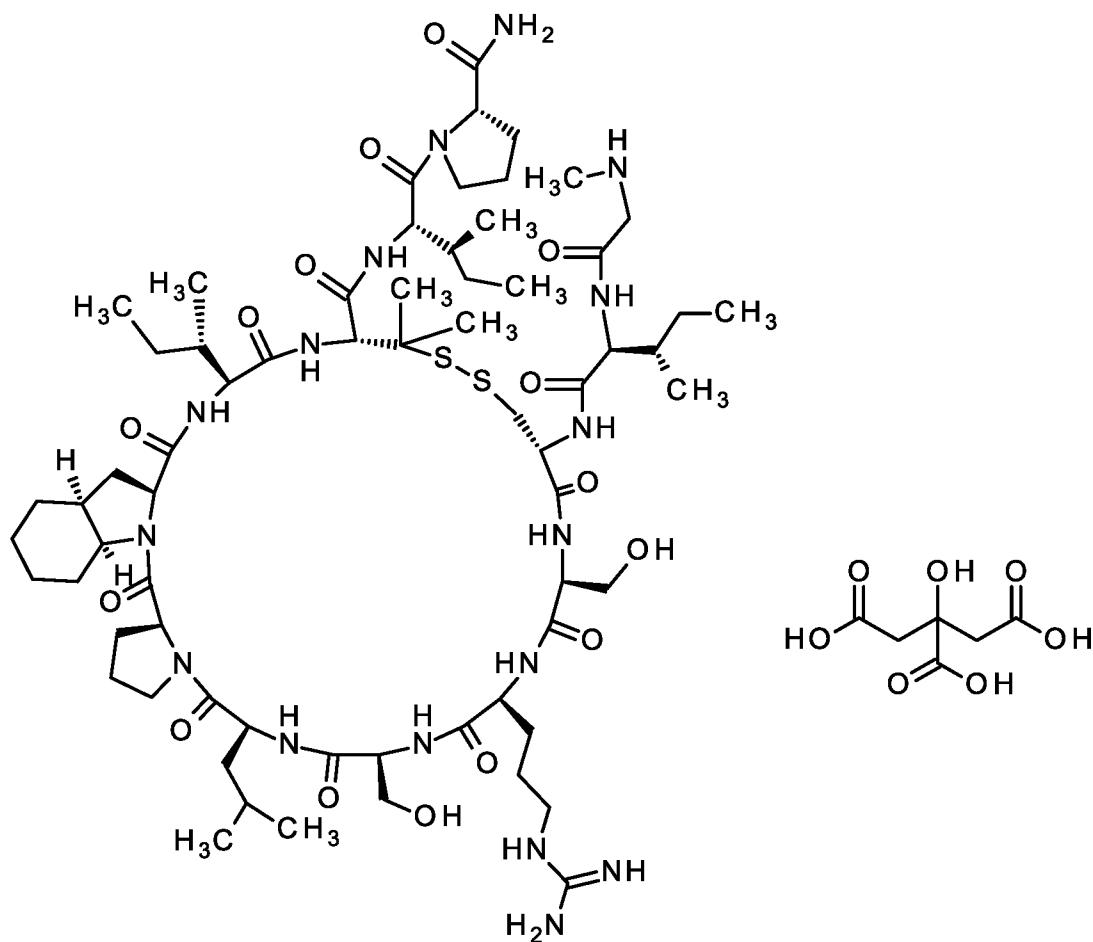
LC-MS (MCW-TOF-AQ-YMC-18min): Rt = 7.62 min; MS (ESIpos): m/z = 725 [M+2H]⁺

Example 376

- 15 Sequence: ((N-Me)G)-IC+SRS LP-(Oic)-I-(Pen)+IP-NH₂ (Citric acid salt) (example 376)

N-[(6S,9S,12S,15S,18R,23R,26S,28aS,29aS,33aS,35aS)-26-[(2S)-butan-2-yl]-12-(3-carbamimidamidopropyl)-9,15-bis(hydroxymethyl)-22,22-dimethyl-18-[(N-methylglycyl-L-isoleucyl)amino]-6-(2-methylpropyl)-5,8,11,14,17,25,28,35-octaoxodotriacontahydro-1H,5H,22H-pyrrolo[2',1':13,14][1,2,5,8,11,14,17,20,23,26]dithiaoctaazacyclononacosino[11,10-a]indole-23-carbonyl]-

- 20 L-isoleucyl-L-prolinamide, 2-hydroxypropane-1,2,3-tricarboxylic acid (citric acid) salt



IRA 410 chloride resin (26 g) was placed into an empty 20 mL Biotage columns and the resin was washed with with a 5% solution of ACN-water (10 times). The cartridge was attached to the Automatic Ion Exchange Station. The resin was washed with 20 column volumes of a 1M NaOH solution (2x 1800s rotation speed 80 min⁻¹). The column was washed with water until the pH of the eluent was < pH 9 (10 column volumes). The column was then eluted with 1 M citric acid solution (10 column volumes), then washed with water until the pH of the eluent was >5 (about 10 column volumes). The peptide (N-Me)GIC+SRS LP-(Oic)-I-Pen+IP-NH₂ (TFA salt) (1200 mg) was loaded onto the column in 12 mL of 5% ACN/water and allowed to cycle through the column 10 times according to the General Method F. The resin was then washed with a 5% ACN/water solution 10 times and the eluent was collected into 50 mL Falcon tubes. The parameter settings used for this scale were:

Washing with H₂O : run-time 3085 s; 80 min⁻¹; 1 cycle (is 90 mL volume)

Sample circulation with peptide: run-time 3085 s; 80 min⁻¹; 1 cycle (is 180 mL volume)

Wash with H₂O (or %ACN in H₂O : run-time 3085 s; 80 min⁻¹; 1 cycle (is 90 mL volume)

The combined eluent was lyophilized to give 1370 mg (100 % purity, 97 % yield) of the target L-tartaric acid salt.

Ion Chromatography (chloride content): <1% chloride

Ion Chromatography (TFA content): <1% TFA

- 5 Ion chromatography (acetate content): 19.0% acetate = 1.77 equiv

LC-MS (MCW-TOF-AQ-YMC-18min): $R_t = 7.6$ min; MS (ESIpos): $m/z = 725$ $[M+2H]^+$

One skilled in the art will recognize that although the stoichiometry by ion chromatography is not always stoichiometric (e.g. 1:2 or 1:1), water content has not been taken into account, which where in a few cases that were measured, can be between 8-15% water content.

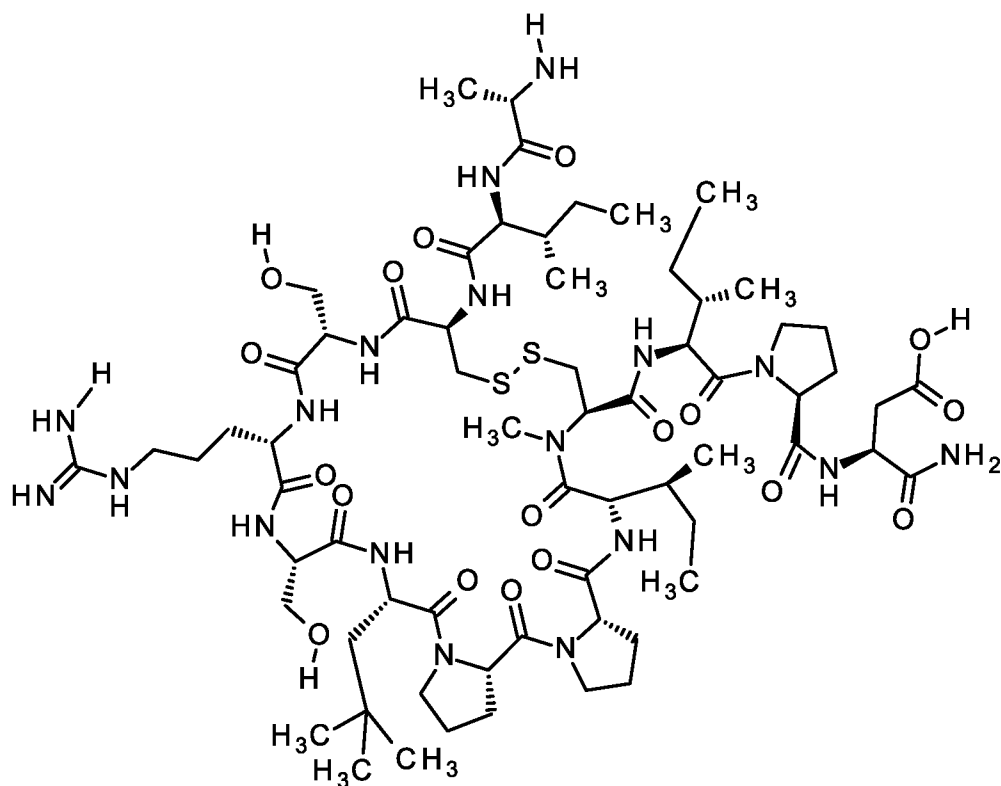
10 General Method G: Preparation of a Salt-Free Form

The preparation of AIC+SRS₂LP-(Oic)-I-(Pen)+IPD-NH₂ (salt free form) (example 27) is representative.

5 Grams of the TFA salt (example 75) was purified by reversed-phase HPLC using an acetonitrile water gradient at 70°C with no acid modifier. The desired fractions were combined and lyophilized. 2.2 grams of the salt-free form (example 27) was obtained; LC-MS (> 99% pure); Ion Chromatography (< 1% TFA)

15 General Method H: Large Scale Synthesis

The synthesis of AIC+SRS-((tBu)A)-PPI-((N-Me)C)+IPD-NH₂ (example 48) is representative



The peptide was synthesized using standard Fmoc chemistry.

- 1) DMF was added to the vessel containing MBHA Resin (180 mmol) and the resin was allowed to swell for 2 hours.
- 5 2) The Fmoc-Rink amide linker was added and mixed for 30 seconds, then the activation buffer was added. Mixing was done using N₂ bubbling for about 1 hour.
- 3) The solution was drained from the resin and the resin was washed with DMF wash (30 sec x 3 times).
- 4) A solution of 20% piperidine/DMF was added and mixed with nitrogen bubbling for 30 min.
- 10 5) The solution was drained and the resin was washed with DMF (5 times).
- 6) The Fmoc-amino acid solution was added and mixed for 30 seconds, then the activation buffer was added. Mixing was done using N₂ bubbling for about 1 hour.
- 7) Steps 3 to 6 were repeated for each amino acid coupling.

Table 10: Note of materials used and conditions

#	Materials	Coupling reagents	Coupling time
1	Fmoc-Asp(OtBu)-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h
2	Fmoc-Pro-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h
3	Fmoc-Ile-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h
4	Fmoc-NMe-Cys(Trt)-OH	HATU (2.0 eq) and DIEA (4.0 eq)	1 h
5	Fmoc-Ile-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h
6	Fmoc-Pro-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h
7	Fmoc-Pro-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h
8	Fmoc-tBuAla-OH	HATU (2.0 eq) and DIEA (4.0 eq)	1 h
9	Fmoc-Ser(tBu)-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h
10	Fmoc-Arg(pbf)-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h
11	Fmoc-Ser(tBu)-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h
12	Fmoc-Cys(Trt)-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h
13	Fmoc-Ile-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h
14	Fmoc-Ala-OH	HBTU (3.0 eq) and DIEA (6.0 eq)	1 h

15

A solution of 20% piperidine in DMF was used for Fmoc deprotection for 30 min. The coupling reaction was monitored by ninhydrin test, and the resin was washed with DMF for 5 times.

Peptide Cleavage and Purification:

- 1) The cleavage buffer (92.5% TFA: 2.5% EDT: 2.5% TIS: 2.5% H₂O) was added to the flask containing the side chain protected peptide at room temperature and the mixture was stirred for 2 hours.
- 5 2) The peptide was precipitated with cold isopropyl ether and centrifuged (2 min at 5000 rpm).
- 3) The peptide was washed twice more with isopropyl ether.
- 4) The crude peptide was dried under vacuum for 2 hours.

Disulfide Formation

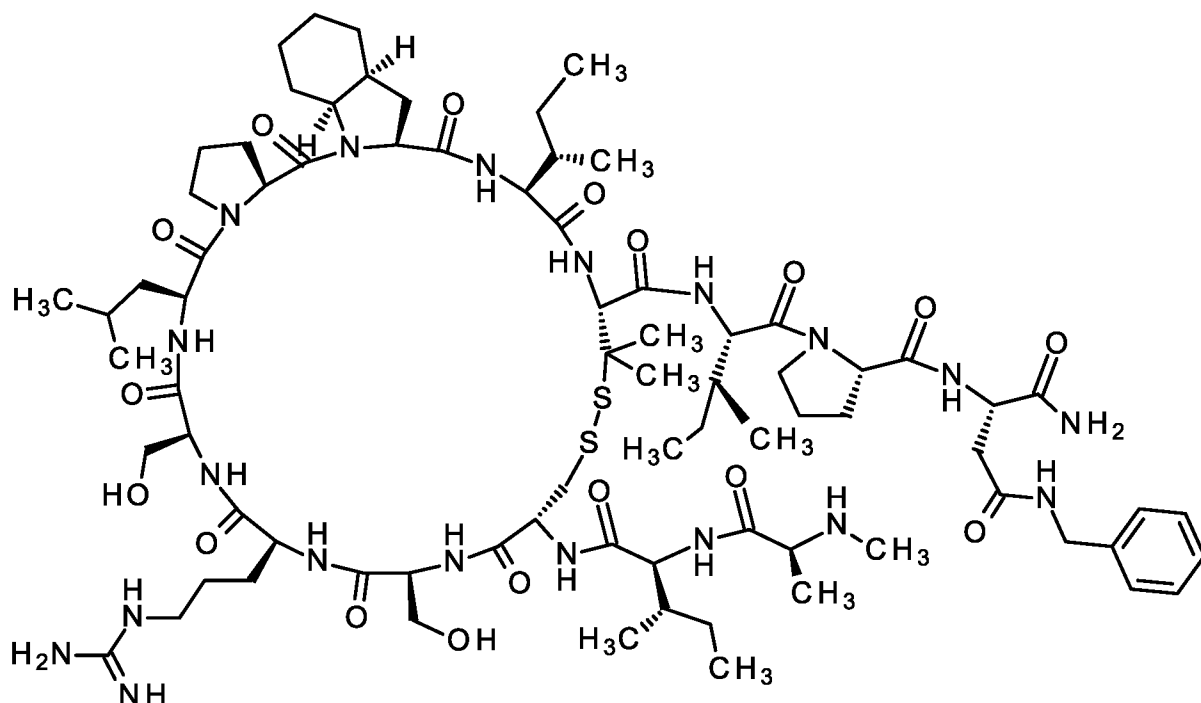
To a solution of the crude linear peptide (15 g, 9.92 mmol, 1.0 eq) (20 batches) in H₂O/I (3 L 2:1) was added NH₄HCO₃ (7.84 g, 99.2 mmol, 10 eq). The mixture was stirred at 30 °C for 16 h, quenched with the addition of HCl to PH~6, and then the mixture was lyophilized. The lyophilizate was purified by Prep-HPLC to give the desired example 48 (41.8 g, 95.8%, 13.3% yield) as a white solid, 156.8 grams total.

Purification conditions

Equipment: Shimadzu 10A; Peptide was dissolved in DMF/H₂O. Mobile Phase: , A (H₂O (0.075% TFA in H₂O), B EtOH; gradient: 10-50%-60 min. Retention time: 47 min; Column: Luna, C18, 10 µm, 100Å, 25 cm x 50 mm; flow rate: 80 mL/min; wavelength: 220/254 nm; oven temp: room temperature.

Method I: Preparation of ((N-Me)A)-IC+SRSLP-(Oic)-I-(Pen)+IP-((N-Benzyl)D)-NH₂ (example 462)

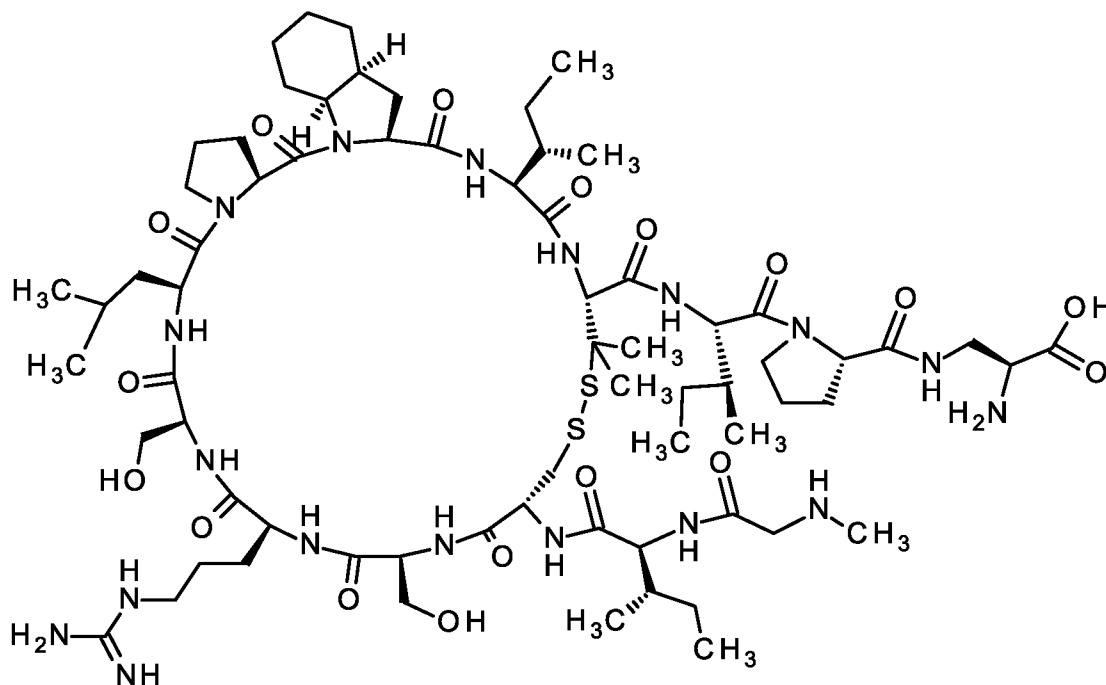
N-[(6S,9S,12S,15S,18R,23R,26S,28aS,29aS,33aS,35aS)-26-[(2S)-butan-2-yl]-12-(3-carbamimidamidopropyl)-9,15-bis(hydroxymethyl)-22,22-dimethyl-18-[(N-methyl-L-alanyl-L-isoleucyl)amino]-6-(2-methylpropyl)-5,8,11,14,17,25,28,35-octaoxodotriacontahydro-1H,5H,22H-pyrrolo[2',1':13,14][1,2,5,8,11,14,17,20,23,26]dithiooctaazacyclononacosino[11,10-a]indole-23-carbonyl]-L-isoleucyl-L-prolyl-N⁴-benzyl-L-aspartamide



The peptide was prepared on Rink Amide MBHA resin (loading typically around 0.4-0.6 mmol/g) on 0.3 mmol scale. The first amino acid Fmoc-Asp(2-Phenylisopropyl ester)-OH (CAS 200336-86-3) was added to the resin using the method described in Method C. The peptide sequence was constructed using the steps described in Method C. Upon completion of the sequence, the 2-OPP protecting group was removed using 1%TFA in DCM, 30 minutes. N-Benzyl amine (3 equiv), DIC (3 equiv) and Oxyma (3 equiv) was added and the mixture was shaken for 2 hours. The peptide was cleaved, the disulfide bond was formed, and the peptide was purified according to Method C.

General Method J. Preparation of ((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(*Dap)-OH (example 467)

N-[(6S,9S,12S,15S,18R,23R,26S,28aS,29aS,33aS,35aS)-26-[(2S)-butan-2-yl]-12-(3-carbamimidamidopropyl)-9,15-bis(hydroxymethyl)-22,22-dimethyl-18-[(N-methylglycyl-L-isoleucyl)amino]-6-(2-methylpropyl)-5,8,11,14,17,25,28,35-octaoxodotriacontahydro-1H,5H,22H-pyrrolo[2',1':13,14][1,2,5,8,11,14,17,20,23,26]dithiooctaazacyclononacosino[11,10-a]indole-23-carbonyl]-L-isoleucyl-N-[(2S)-2-amino-2-carboxyethyl]-L-prolinamide

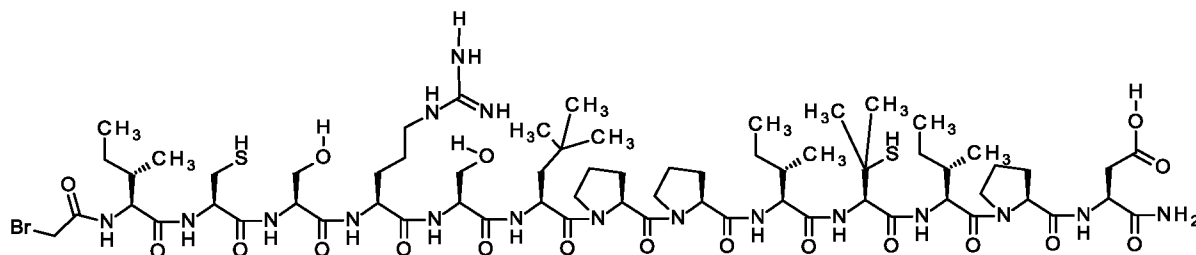


The peptide was prepared on 2-chlorotrityl resin according to Method C. BOC-Dap(Fmoc)-OH (CAS 122235-70-5) was added onto the resin according to Method C. The peptide was constructed and purified according to method C.

General Method K. Preparation of (2-(Morpholine)acetyl)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH2 (example 283)

Example 27A

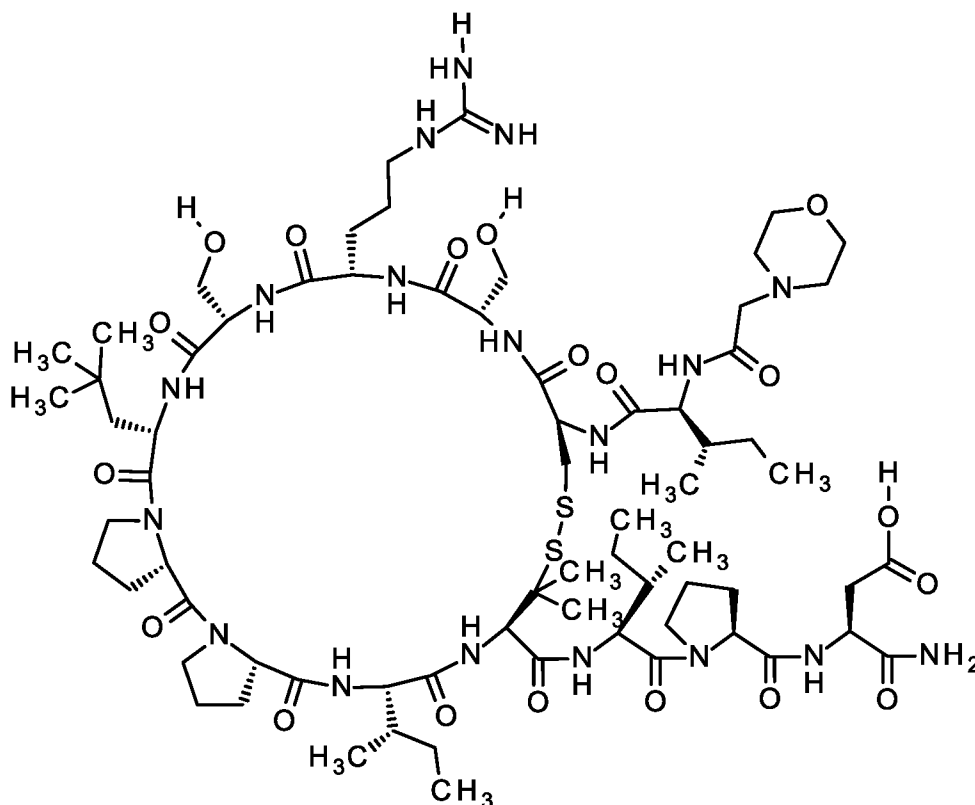
N-(bromoacetyl)-L-isoleucyl-L-cysteinyl-L-seryl-L-arginyl-L-seryl-4-methyl-L-leucyl-L-prolyl-L-prolyl-L-isoleucyl-3-sulfanyl-L-valyl-L-isoleucyl-L-prolyl-L-alpha-asparagine



The sequence IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH₂ was prepared 12 times on Rinkamide ChemMatrix resin at 0.1 mmol scale according to Method A. To the resin-containing peptide (200 mg 100 μmol) was added N,N'-diisopropylcarbodiimide (31 μL, 200 μmol), ethyl(hydroxiimino)cynoacetate (28.4 mg, 200 μmol) and bromoacetic acid (14 μL, 200 μmol) and the mixture was shaken in 5 mL DMF overnight at room temperature on a thermomixer shaker. The resin was filtered, washed with DMF (6 x 5 mL), washed with DCM (6 x 5 mL) and then dried under vacuum for the next step.

Example 283

N-[(5a*S*,11*S*,14*S*,17*S*,20*S*,23*R*,28*R*,31*S*,33a*S*)-31-[(2*S*)-butan-2-yl]-17-(3-carbamimidamidopropyl)-11-(2,2-dimethylpropyl)-14,20-bis(hydroxymethyl)-27,27-dimethyl-23-($\{N-[(\text{morpholin-4-yl})\text{acetyl}]\text{-L-isoleucyl}\}$ amino)-5,10,13,16,19,22,30,33-octaoxo-octacosahydro-1*H*,5*H*,10*H*-dipyrrolo[2,1-*j*:2',1'-m][1,2,5,8,11,14,17,20,23,26]dithiaoctaazacyclononacosine-28-carbonyl]-L-isoleucyl-L-prolyl-L-alpha-asparagine



To example 27a (200 mg, 74% purity, 77.7 μmol) in NMP (10 mL) was added morpholine (34 μL , 390 μmol) and the suspension was shaken at 37 °C in thermomixer. The solid resin was collected by filtration, washed with 10 mL DMF and 10 mL dichloromethane each two times. The residue was treated with TFA/EDT/thionaisol (90:3:7) and shaken 2 h at room temperature. The reaction mixture was treated with cold diethyl ether (-10°C), and then the suspension was centrifuged under nitrogen and washed three times (3 x 30 mL) with cold diethylether. To the residue was added a 0.1 M aqueous ammonium bicarbonate solution (400 mL) and the reaction mixture was shaken overnight in an open-to-the-air round-bottomed flask. The solution was lyophilized and the lyophilized powder was purified according to Method A, to provide 20.4 mg (98% purity, 16% yield) of example 283.

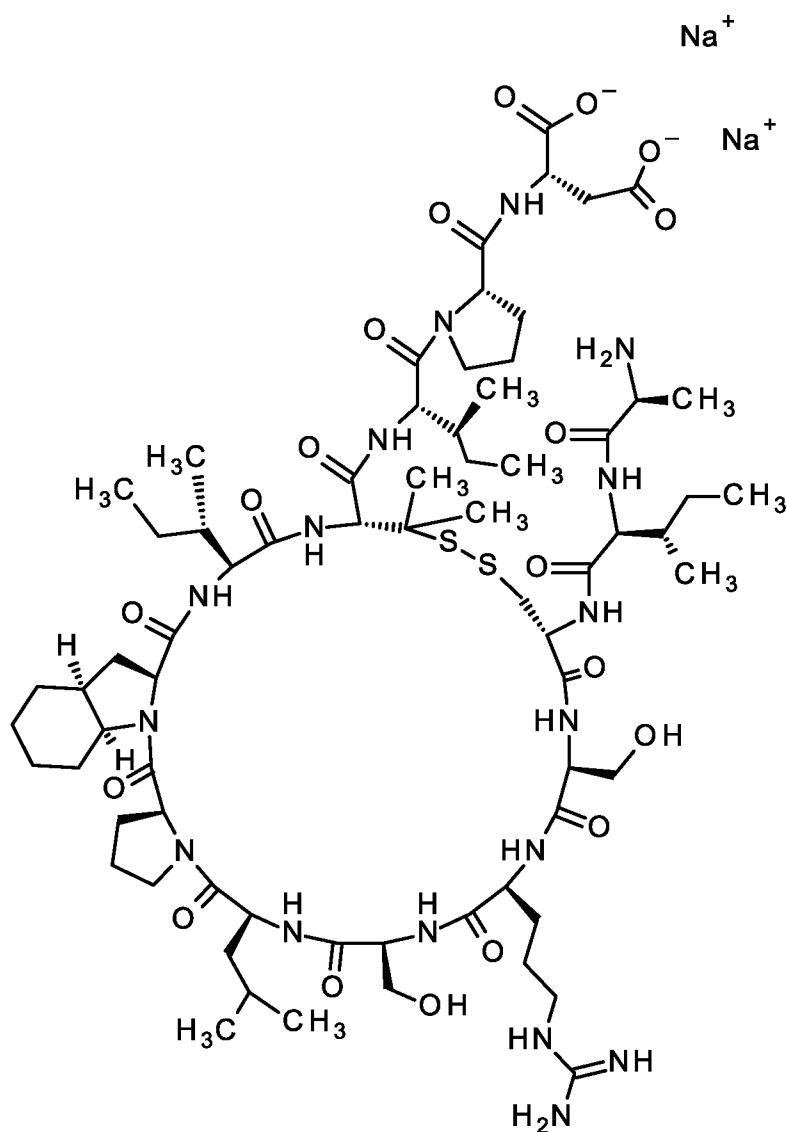
Example 300 (2-Hydroxyacetyl)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH₂ was isolated as a by-product.

General Method L. Preparation of sodium and choline salt forms

These salts were prepared from salt-free form peptides. The two examples below are representative of the general method:

Example 431

- 5 N-[(6S,9S,12S,15S,18R,23R,26S,28aS,29aS,33aS,35aS)-18-[(L-alanyl-L-isoleucyl)amino]-26-[(2S)-butan-2-yl]-12-(3-carbamimidamidopropyl)-9,15-bis(hydroxymethyl)-22,22-dimethyl-6-(2-methylpropyl)-5,8,11,14,17,25,28,35-octaoxodotriacontahydro-1H,5H,22H-pyrrolo[2',1':13,14][1,2,5,8,11,14,17,20,23,26]dithiaoctaazacyclononacosino[11,10-a]indole-23-carbonyl]-L-isoleucyl-N-[(1S)-1,2-dicarboxylatoethyl]-L-prolinamide, Sodium salt



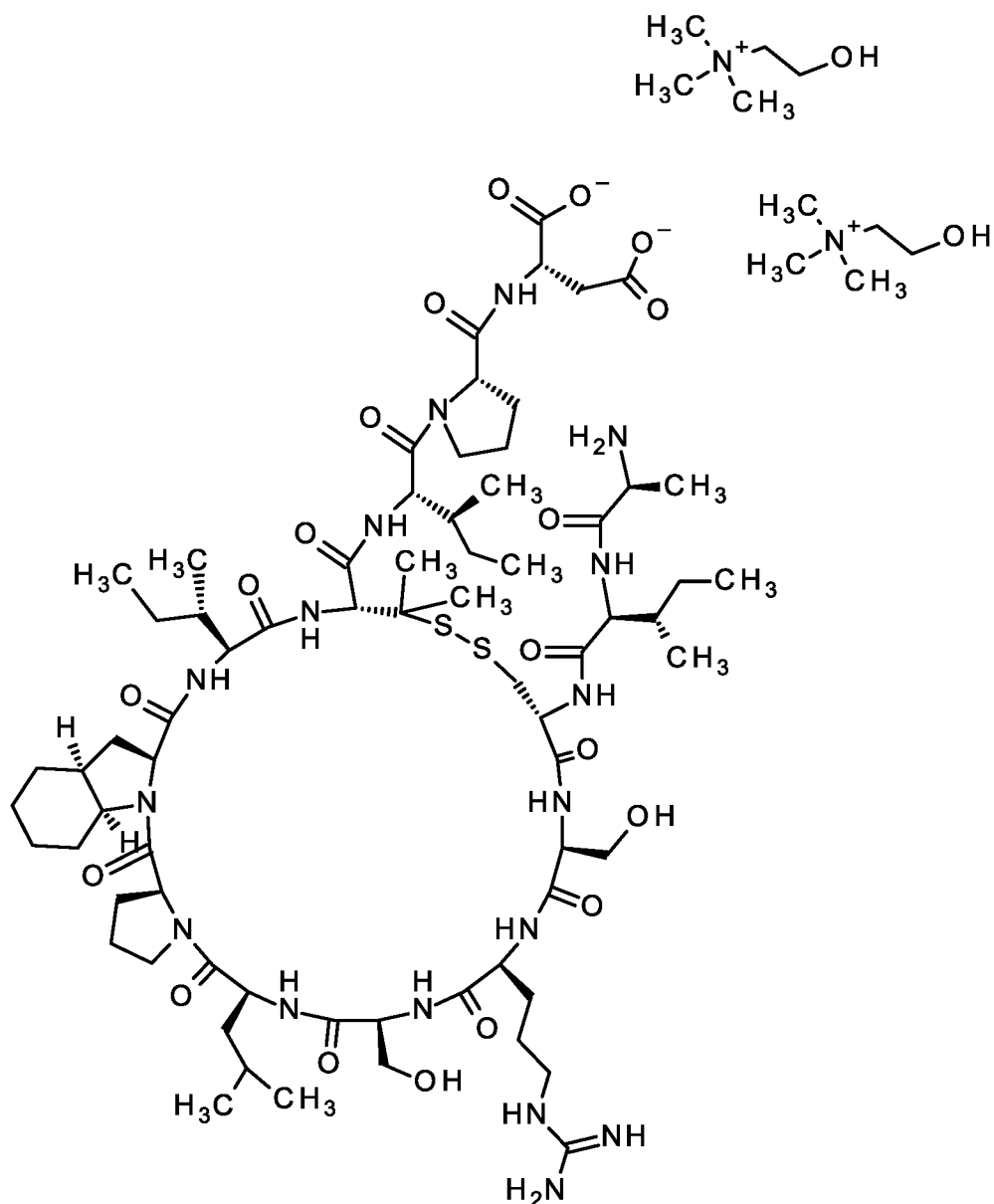
To a solution of AIC+SRS LP-(Oic)-I-(Pen)+IPD-OH (salt-free form) (100 mg) in 20 mL acetonitrile/water 1:1 was added 1.3 mL of a 0.1 M sodium hydroxide solution. The mixture was dried under vacuum to give 109 mg (104% yield, 99% purity) of the target compound.

LC-MS (MCW-TOF-AQ-YMC-18min): $R_t = 8.62$ min; MS (ESIpos): $m/z = 783$ $[M+2H]^{2+}$

- 5 Ion chromatography (sodium content): 2.9% sodium = 2.03 equiv

Example 432

- N-[(6S,9S,12S,15S,18R,23R,26S,28aS,29aS,33aS,35aS)-18-[(L-alanyl-L-isoleucyl)amino]-26-[(2S)-butan-2-yl]-12-(3-carbamimidamidopropyl)-9,15-bis(hydroxymethyl)-22,22-dimethyl-6-(2-methylpropyl)-5,8,11,14,17,25,28,35-octaoxodotriacontahydro-1H,5H,22H-pyr-
 10 rolo[2',1':13,14][1,2,5,8,11,14,17,20,23,26]dithiooctaazacyclononacosino[11,10-a]indole-23-carbonyl]-L-isoleucyl-N-[(1S)-1,2-dicarboxylatoethyl]-L-prolinamide, 2-Hydroxy-N,N,N-trimethylethan-1-ammium) (choline) salt



To a solution of AIC+SRSLP-(Oic)-I-(Pen)+IPD-OH (salt-free form) (100 mg) in 20 mL acetonitrile/water 1:1 was added 511 µL of a 0.25 M choline hydroxide solution. The mixture was dried under vacuum to give 122 mg (105% yield, 99% purity) of the target compound.

LC-MS (MCW-TOF-AQ-YMC-18min): $R_t = 8.65$ min; MS (ESIpos): $m/z = 783$ $[M+2H]^{2+}$

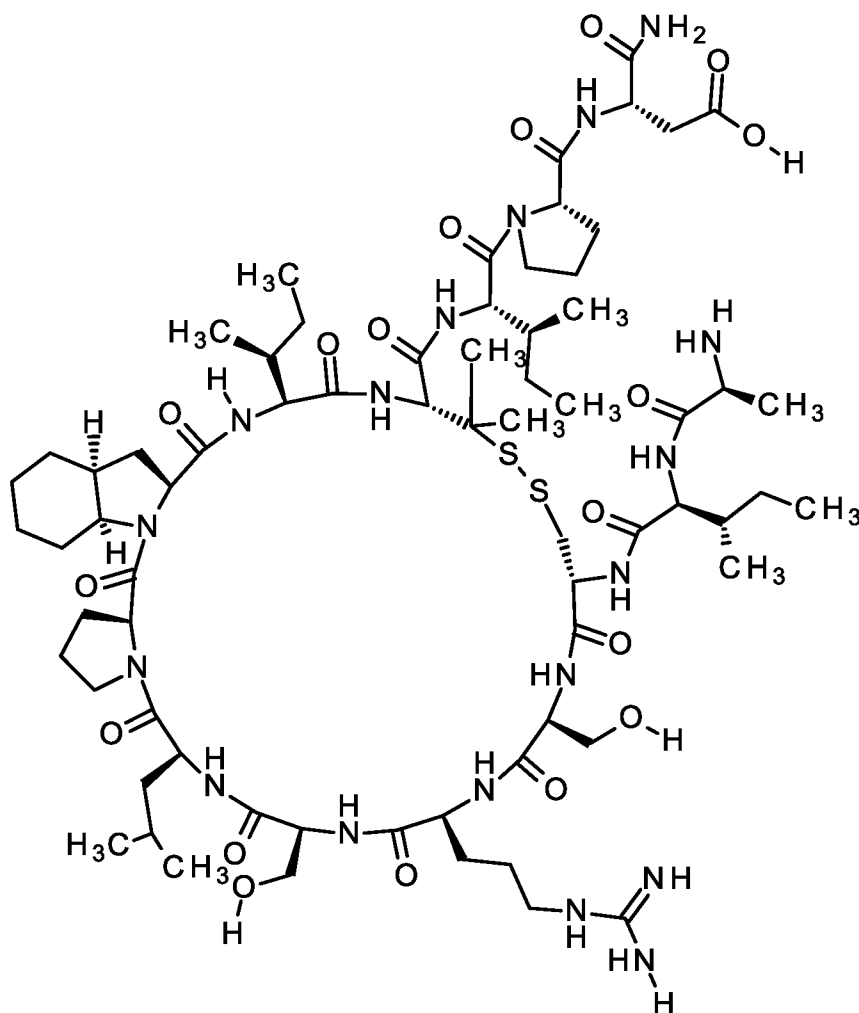
Ion chromatography (choline content): 11.7% choline = 1.97 equiv

Example 13

AIC+SRSLP-(Oic)-I-(Pen)+IPD-OH (example 13)

(3S)-4-amino-3-({(2S)-1-[(2S,3S)-2-{{[(2R)-2-{{[(2S,3S)-2-{{[(2S,3aS,7aS)-1-{{(2S)-1-[(2S,5S,8S,11S,14R,17S,20S)-20-amino-17-[(2S)-butan-2-yl]-8-(3-carbamimidamidopropyl)-5,11-

bis(hydroxymethyl)-2-(2-methylpropyl)-4,7,10,13,16,19-hexaoxo-14-(sulfanylmethyl)-3,6,9,12,15,18-hexaazahenicosanan-1-oyl]pyrrolidine-2-carbonyl}octahydro-1H-indole-2-carbonyl]amino}-3-methylpentanoyl]amino}-3-methyl-3-sulfanylbutanoyl]amino}-3-methylpentanoyl]pyrrolidine-2-carbonyl}amino)-4-oxobutanoic acid



5

Peptide Synthesis

The peptide was synthesized using standard Fmoc chemistry.

- 1) Resin preparation: to the CTC Resin (15.0 mmol, 15.0 g, 1.0 mmol/g) was added Fmoc-Asp(OtBu)-OH (15.0 mmol, 6.17 g, 1.0 eq) and DIEA (10.4 mL, 60.0 mmol, 4.0 eq) in DCM (300 mL). The mixture was agitated with N₂ for 2 h at 20 °C. Methanol was then added (15.0 mL) and the resin agitated with N₂ for another 30 min. The resin was washed with DMF (300 mL x 3). Then a solution of 20% piperidine in DMF (300 mL) was added and the mixture was agitated with N₂ for 20 min at 20°C. Then the mixture was filtered to give the resin. The resin was washed with DMF (300 mL x 3) and filtered to give the resin.

10

15

- 2) Coupling: Fmoc-Pro-OH (15.2 g, 45.0 mmol, 3.0 eq), HBTU (16.2 g, 42.8 mmol, 2.85 eq) and DIEA (90.0 mmol, 15.6 mL, 6.00 eq) in DMF (100 mL) was added to the resin and the resin was agitated with N₂ for 30 min at 20°C. The resin was then washed with DMF (300 mL x 5).
- 3) Deprotection: 20% piperidine in DMF (300 mL) was added to the resin and the mixture was agitated with N₂ for 20 min at 20 °C.
- 4) Steps 2 and 3 were repeated for all other amino acids:

Table 11: Reagents used for example 13

#	Materials	Coupling reagents	Coupling time	Supplier
1	Fmoc-Asp(OtBu)-OH (1.0 eq)	DIEA (4.0 eq)	2.0 h	GL Biochem (shanghai) Ltd
2	Fmoc-Pro-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd
3	Fmoc-Ile-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd
4	Fmoc-Pen(Trt)-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd
5	Fmoc-Ile-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd
6	Fmoc-Oic-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd
7	Fmoc-Pro-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd
8	Fmoc-Leu-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd
9	Fmoc-Ser(tBu)-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd
10	Fmoc-Arg(Pbf)-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	Chengdu aminotp Pharmaceutical Technology Ltd
11	Fmoc-Ser(tBu)-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd
12	Fmoc-Cys(Trt)-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd
13	Fmoc-Ile-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd
14	Fmoc-Ala-OH (3.0 eq)	HBTU (2.85 eq) DIEA (6.0 eq)	30 min	GL Biochem (shanghai) Ltd

20% piperidine in DMF was used for Fmoc deprotection for 30 min. The coupling reaction was monitored by ninhydrin (all amino acids except Pro) and chloranil test (Pro), and the resin was washed with DMF (300 mL) for 5 times.

HBTU, DIEA supplier : Suzhou Highfine Biotech Co.,Ltd

5 Peptide Cleavage and Purification

- 5) The resin was washed with MeOH (300 mL x 5) and dried under vacuum to get 41.5 g of the peptide resin. Then 400 mL of cleavage buffer (92.5%TFA/2.5%3-mercaptopropionic acid/2.5%TIS/2.5%H₂O) was added to the flask containing the side chain protected peptide resin at 20 °C and the mixture was stirred for 2.5 h and then filtered.
- 6) The peptide was precipitated with cold tert-butyl methyl ether (4000 mL) and centrifuged (3 min at 3000 rpm) to get the crude solid peptide. The crude peptide precipitate was washed with tert-butyl methyl ether three more times (1000 mL x 3). The crude peptide was then dried under vacuum for 2.0 h to give 21.8 g of the crude peptide.
- 7) The crude peptide (21.8 g) was dissolved in CH₃CN (6.5 L) and water (6.5 L). I₂ (0.1 M in MeOH, 55 mL) was added at 20 °C until a yellow color persisted. Then the mixture was stirred at 20°C for 2 min. Sodium thiosulfate solution (0.1 M in water, 15 mL) was then added dropwise until the yellow color disappeared. The mixture was then lyophilized to give the crude powder (22.7 g).
- 8) Purification of the crude peptide by preparative HPLC (condition: A: 0.075% TFA in water B: CH₃CN), collection of product-containing fractions, and the lyophilization gave the target peptide (Sequenc ID 13); (4305.1 mg, 18.3% yield, 99.1% by 110 Å C18 RP-HPLC column (Gemini C18 5µm 110 Å 150 x 4.6 mm); 97.8% by 300 Å C18 RP-HPLC column (Discovery BIO Wide Pore C18 5µm 300 Å 150 x 4.6 mm), TFA salt) as a white solid.

Table 12: Purification conditions

Purification condition	
Dissolution condition	TFA/H ₂ O = 7/3
Instrument	Gilson GX-281
Mobile Phase	A: H ₂ O (A:0.075%TFA in H ₂ O, B: ACN)
	B: CH ₃ CN
Gradient	13-43%-60 min Retention time: 40 min

Column	Luna 25 x 200 mm, C18 10 μm , 110 Å + Gemini 150 x 30 mm, C18 5 μm , 110 Å
Flow Rate	20 mL/Min
Wavelength	214/254 nm
Oven Temperature	30 °C

LC-MS: Rt = 1.369 min; 1564 [M+2H]⁺

Rt = 8.72 min (99.1% pure) Gemini C18 5 μm 110 Å 150 x 4.6 mm

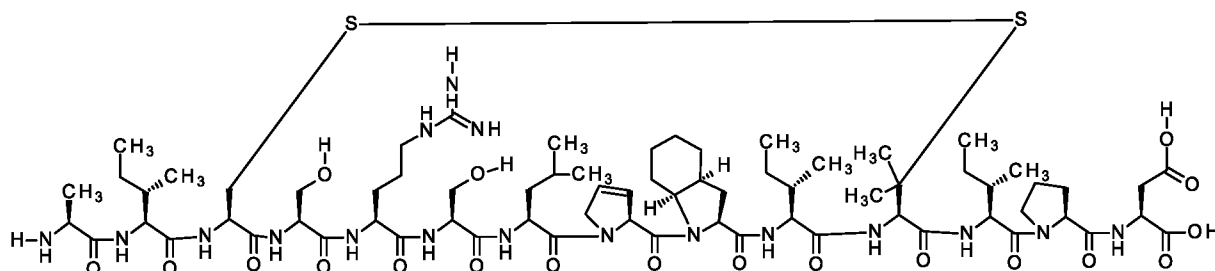
Rt = 9.42 min (97.8%) Discovery BIO Wide Pore C18 5 μm 300 Å 150 x 4.6 mm

- 5 From example 13 (TFA salt), example 29 (HCl salt) can be prepared using General Method F; example 5 (salt-free form) can be prepared using General Method G. The preparations of example 431 (sodium salt) and example 432 (choline salt) from example 5 are shown below as Example 431 and Example 432, respectively.

Example 237

- 10 AIC+SRSL-(L-dehydroproline)-(Oic)-I-(Pen)+IPD-OH (example 237)

N-[(6S,9S,12S,15S,18R,23R,26S,28aS,35aS)-18-[(L-alanyl-L-isoleucyl)amino]-26-[(2S)-butan-2-yl]-12-(3-carbamimidamidopropyl)-9,15-bis(hydroxymethyl)-22,22-dimethyl-6-(2-methylpropyl)-5,8,11,14,17,25,28,35-octaoxo-6,7,8,9,10,11,12,13,14,15,16,17,18,19,23,24,25,26,27,28,28a,29,29a,30,31,32,33,33a,35,35a-tri-acontahydro-3H,5H,22H-pyrrolo[2',1':13,14][1,2,5,8,11,14,17,20,23,26]dithiaoctaazacyclonon-15-acosino[11,10-a]indole-23-carbonyl]-L-isoleucyl-L-prolyl-L-aspartic acid

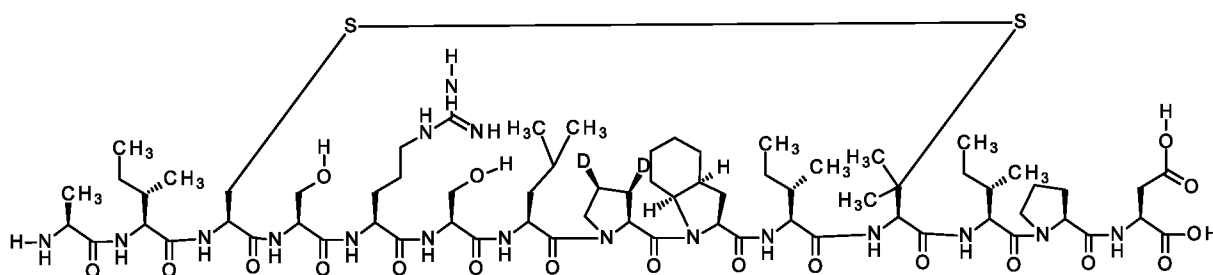


The peptide was prepared according to Method C (3.12 g). The analytical data for this peptide is shown in Table 16 and Table 17.

Example 142

AIC+SRSL-(Pro-D2)-(Oic)-I-(Pen)+IPD-OH (example 142)

N-[(1R,2S,6S,9S,12S,15S,18R,23R,26S,28aS,35aS)-18-[(L-alanyl-L-isoleucyl)amino]-26-[(2S)-butan-2-yl]-12-(3-carbamimidamidopropyl)-9,15-bis(hydroxymethyl)-22,22-dimethyl-6-(2-methylpropyl)-5,8,11,14,17,25,28,35-octaoxo(1,2-²H₂)dotriacontahydro-1H,5H,22H-pyr-
 5 rolo[2',1':13,14][1,2,5,8,11,14,17,20,23,26]dithiaoctaazacyclononacosino[11,10-a]indole-23-carbonyl]-L-isoleucyl-L-prolyl-L-aspartic acid



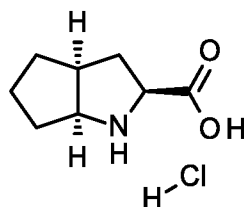
The peptide from Example 237 (30 mg, 19.2 μ mol) was dissolved in methanol-*d*₄ (3 mL) (99.8% purity of D). Rhodium black (CAS 7440-16-6, ACBR, Article No. AB25746) (10 mol%, 1.9 μ mol) was added to the solution under argon in an M-Braun Glovebox. The 8 mL reaction vial mixture was placed into an MTP pressure reactor block that is located on a Chemspeed Swing XL platform and is enclosed inside an M-Braun glovebox (Chemspeed Technologies AG, Wölferstrasse 8, 4414 Füllinsdorf, Switzerland, chemspeed@chemspeed.com). The reaction was shaken with an orbital speed of 650 rpm at room temperature under a pressure of 3 bar of deuterium gas for 16 h overnight. The pressure block was purged with argon and the vial was removed from the reactor block. The methanol-*d*₄ was evaporated with a rotary evaporator and the resulting solid was dissolved in water (2 mL) and stirred for 1 hour to exchange the exchangeable deuterium atoms with hydrogen. After 1 hour, acetonitrile was added and the product was purified by reversed-phase HPLC [column: Phenomenex Aeris Peptide 5 μ XB-C18, AXIA Packed, 21.2 x 250 mm + Cartridge 5 μ , flow: 20 mL/min, focused gradient using H₂O / ACN with 0.1% TFA as acidic modifier. FOK30-40: 0 min 5%ACN, 0-13 min ramp to 30% ACN, 13-39 min flat ramp to 40% ACN, 39-46 min isocratic 40% ACN]. Chromatography fractions containing the product were analyzed by analytical HPLC (focused gradient) and by LC-MS and then pooled accordingly to provide two fractions of the pure product: 11.4 mg (90% purity, 34% yield) and 4.2 mg (99% purity, 14% yield). The analytical data for this peptide is in Table 16.

The product peptide was subjected to trypsin digestion followed by reaction with dithiothreitol and the resulting fragments were analyzed by TOF-MS-MS. Trypsin digestion cleaved the peptide between the arginine and serine (exact mass 1583.8384, found 1583.852 [M+2H]²⁺). Reaction with dithiothreitol produced two fragments, AICSR-OH (exact mass 548.2741, found 548.283 [M+H]⁺) and SL-(Pro-D2)-

(Oic)-I-(Pen)+IPD-OH (exact mass 1037.5800, found 1038.587 [M+H]⁺), with the fragments at 838.47 amu ((Pro-D2)-(Oic)-I-(Pen)+IPD-OH) and at 738.40 amu SL-((Oic)-I-(Pen)+IPD-OH) verifying the position of the deuterium atoms.

Example 28A

- 5 (2S,3aS,6aS)-Octahydrocyclopenta[b]pyrrole-2-carboxylic acid—hydrogen chloride (1/1) (single stereoisomer)

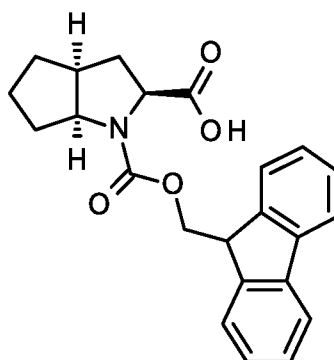


The reaction was performed under argon atmosphere. To palladium on carbon (200 mg, 10%) was added
 10 20 mL methanol and benzyl (2S,3aS,6aS)-octahydrocyclopenta[b]pyrrole-2-carboxylate—hydrogen chloride (1/1) (1.00 g, 3.55 mmol). The reaction flask was charged with hydrogen gas (1 atm) and the reaction mixture was stirred under hydrogen atmosphere overnight at room temperature. The reaction mixture was filtered over diatomaceous earth and the filter cake was washed with methanol. The filtrate was evaporated and dried under vacuum to give 698 mg of the target compound.

- 15 (2S,3aS,6aS)-octahydrocyclopenta[b]pyrrole-2-carboxylic acid is also commercially available as a salt free form.

Example 29A

(2S,3aS,6aS)-1-({[(9H-Fluoren-9-yl)methoxy]carbonyl}octahydrocyclopenta[b]pyrrole-2-carboxylic acid (single stereoisomer)



To (2S,3aS,6aS)-octahydrocyclopenta[b]pyrrole-2-carboxylic acid—hydrogen chloride (1/1) (680 mg, 3.55 mmol) in 12 mL water was added sodium bicarbonate (2.98 g, 35.5 mmol) and a solution of 1-({[(9H-fluoren-9-yl)methoxy]carbonyl}oxy)pyrrolidine-2,5-dione (Fmoc-OSu) (1.26 g, 3.73 mmol) in 18 mL

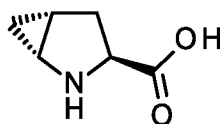
acetone. The reaction mixture was stirred overnight at room temperature. The reaction mixture was treated with water, acidified with 1 M hydrochloric acid and extracted with ethyl acetate two times. The organic phase was washed with saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtered and then evaporated. The residue was separated via silica gel chromatography (Biotage Isolera, column: 50 g SNAP Ultra; eluent: dichloromethane/methanol 100:2 – 100:5). The appropriate fractions were combined, evaporated and dried under vacuum to give 1.27 g (99% purity, 94% yield) of the target compound.

LC-MS (Method 10): $R_t = 2.08$ min; MS (ESI pos): $m/z = 378$ $[M+H]^+$

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: 1.260 (1.76), 1.274 (1.85), 1.299 (1.78), 1.321 (2.09), 1.335 (2.38), 1.440 (0.92), 1.455 (1.10), 1.473 (0.87), 1.521 (3.61), 1.537 (3.86), 1.551 (2.28), 1.607 (4.53), 1.620 (3.68), 1.640 (3.08), 1.653 (1.66), 1.712 (2.02), 1.781 (1.38), 1.816 (2.30), 1.827 (2.26), 1.839 (2.37), 1.855 (1.68), 2.073 (1.20), 2.305 (0.83), 2.328 (1.99), 2.351 (1.35), 2.360 (1.64), 2.384 (0.92), 2.468 (0.60), 2.606 (1.50), 2.670 (1.11), 3.860 (0.94), 3.874 (1.94), 3.893 (1.95), 3.907 (0.92), 4.143 (2.61), 4.156 (3.68), 4.167 (5.16), 4.186 (5.89), 4.266 (1.43), 4.280 (3.20), 4.294 (2.11), 4.345 (1.31), 4.371 (3.19), 4.386 (3.83), 4.396 (4.39), 4.411 (3.74), 4.422 (2.40), 4.437 (0.98), 7.292 (0.97), 7.311 (3.89), 7.329 (6.99), 7.346 (6.08), 7.363 (2.49), 7.395 (9.91), 7.414 (16.00), 7.432 (6.98), 7.663 (9.73), 7.680 (6.46), 7.879 (12.31), 7.897 (11.49), 12.586 (0.80).

Example 30A

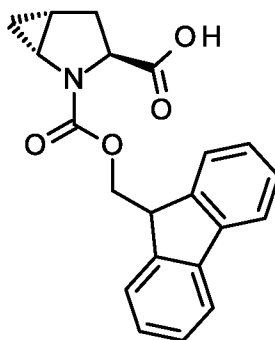
(1R,3S,5R)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid (single stereoisomer)



(1R,3S,5R)-2-(tert-butoxycarbonyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid (CAS RN: 197142-34-0, 500 mg) was dissolved in 4M HCl-dioxane solution (3 mL) and stirred for 8 h at room temperature. The solvent was evaporated and the crude product was used directly for the next step

Example 31A

(1R,3S,5R)-2-{[(9H-fluoren-9-yl)methoxy]carbonyl}-2-azabicyclo[3.1.0]hexane-3-carboxylic acid (single stereoisomer)



To a solution of (1R,3S,5R)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid (Example 30A, 280 mg, 2.20 mmol) in 3 mL water was added sodium bicarbonate (1.85 g, 22.0 mmol) and a solution of 1-({(9H-fluoren-9-yl)methoxy}carbonyl)oxy)pyrrolidine-2,5-dione (0.78 g, 2.31 mmol) in 4.5 mL acetone. The reaction mixture was stirred overnight at room temperature. The reaction mixture was treated with water and extracted with twice with ethyl acetate. The organic phase was washed with saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtrated, and then concentrated. The crude product was purified by preparative reversed-phase HPLC (Column: Reprosil; C18; 10 μ m; 125 x 30 mm; eluant A: CAN, eluant B: water with 0.1% formic), and the product-containing fractions were combined and lyophilized to give 88.0 mg (96% purity, 11% yield) of the target compound.

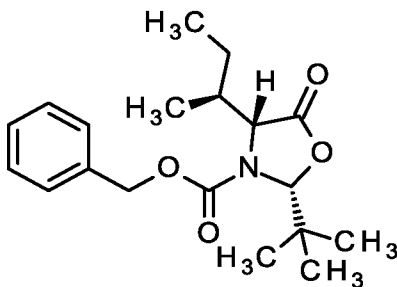
LC-MS (Method 11): R_t = 1.32 min; MS (ESI pos): m/z = 372 $[M+Na]^+$

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: -0.149 (1.15), -0.008 (10.73), 0.008 (9.13), 0.146 (1.21), 0.468 (1.54), 0.534 (3.66), 0.803 (4.38), 1.682 (2.68), 2.072 (0.75), 2.128 (2.19), 2.142 (2.32), 2.223 (1.05), 2.293 (2.19), 2.327 (4.45), 2.349 (2.68), 2.366 (2.75), 2.523 (3.89), 2.670 (1.44), 2.710 (1.31), 2.816 (0.82), 3.413 (3.93), 3.757 (1.83), 4.010 (2.91), 4.140 (2.39), 4.211 (3.11), 4.285 (12.30), 6.929 (0.43), 6.950 (0.43), 7.340 (9.91), 7.358 (6.02), 7.406 (10.08), 7.425 (16.00), 7.443 (7.10), 7.650 (2.85), 7.702 (4.52), 7.724 (5.53), 7.746 (3.40), 7.892 (12.40), 7.910 (9.85), 12.680 (0.65).

This amino acid was used in Fmoc SPPS. This fmoc amino acid is also commercially available.

20 Example 32A

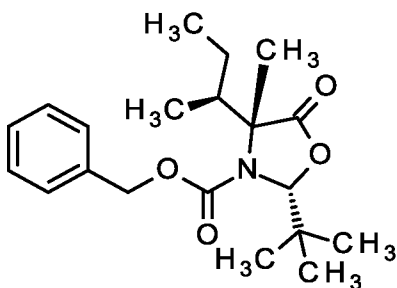
Benzyl (2R,4R)-4-[(2S)-butan-2-yl]-2-tert-butyl-5-oxo-1,3-oxazolidine-3-carboxylate (single stereoisomer)



To a suspension of D-alloisoleucine (8.06 g, 61.4 mmol) in 120 mL ethanol, was added a sodium hydroxide solution made from 2.45 g sodium hydroxide in 11 mL water. The reaction mixture was stirred 90 min at room temperature and then evaporated. The residue was treated with 195 mL pentane and then 2,2-dimethylpropanal (10 mL, 92 mmol) was added. The reaction mixture was stirred 24 h at 45 °C with a Dean-Stark trap attached to the round-bottomed flask. After cooling, the solvents were evaporated and the residue was dried several times with toluene (azeotropic distillation). The residue was treated with 165 mL dichloromethane and cooled to 0°C. Benzyl carbonochloridate (13 mL, 92 mmol) was added and the reaction mixture was stirred for one week at 0-4 °C. 4-Dimethylaminopyridine (375 mg, 3.1 mmol) and 65 mL water were added to the reaction mixture, the reaction mixture was allowed to come to room temperature and then it was stirred overnight at room temperature. The reaction mixture was treated with ethyl acetate several times and extracted. The combined organic phase was washed twice with 10% aqueous citric acid and then twice with saturated aqueous sodium bicarbonate solution. The aqueous phase was reextracted with ethyl acetate. The combined organic phases were washed with saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtrated and then evaporated. The residue was purified via silica gel chromatography (Isolera; eluent: cyclohexane/ethyl acetate 100:3 – 100:7). The product-containing fractions were combined, evaporated and then dried under vacuum to give 8.71 g of the target compound.

Example 33A

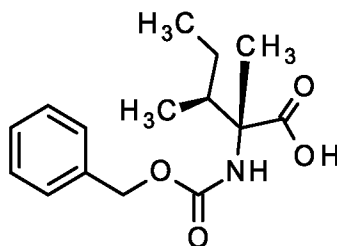
Benzyl (2R,4R)-4-[(2S)-butan-2-yl]-2-tert-butyl-4-methyl-5-oxo-1,3-oxazolidine-3-carboxylate (single stereoisomer)



The reaction was carried out under argon atmosphere. Benzyl (2R,4R)-4-[(2S)-butan-2-yl]-2-tert-butyl-5-oxo-1,3-oxazolidine-3-carboxylate (Example 32A, 8.12 g, 24.4 mmol) in 100 mL dry THF was cooled to -78 °C. Potassium hexamethyldisilazide (44 mL, 29 mmol) was added dropwise and the reaction mixture stirred for 2 h at -78 °C. Iodomethane (2.3 mL, 37 mmol) was added dropwise to the reaction mixture and the reaction mixture was stirred for 2.5 h at room temperature. Saturated aqueous ammonium chloride solution (20 mL) was added to the reaction mixture, the reaction mixture allowed to room temperature, and then the product was extracted into ethyl acetate. The combined organic phases were washed with saturated aqueous sodium bicarbonate solution, dried over magnesium sulfate, filtrated, and then concentrated. The residue was purified via silica gel chromatography (Isolera, column: 100 g SNAP Ultra; eluent: cyclohexane/ethyl acetate 100:3 – 100:7). The product-containing fractions were combined, evaporated and then dried under vacuum to give 3.2 g of the target compound.

Example 34A

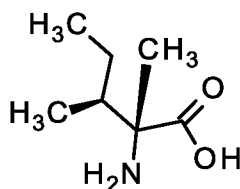
N-[(Benzyloxy)carbonyl]-2-methyl-D-alloisoleucine (single stereoisomer)



To a solution of benzyl (2R,4R)-4-[(2S)-butan-2-yl]-2-tert-butyl-4-methyl-5-oxo-1,3-oxazolidine-3-carboxylate (Example 33A, 3.20 g, 9.21 mmol) in methanol (40 mL) was added 1 M sodium hydroxide solution (40 mL). The reaction mixture was stirred for 2 h at reflux. After cooling, the methanol was evaporated. The residue was treated with 10% citric acid solution and the product was extracted into ethyl acetate. The aqueous phase was extracted with ethyl acetate two more times. The combined organic phases were washed with saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtrated, evaporated and then dried under vacuum to give 2.77 g of the target compound.

Example 35A

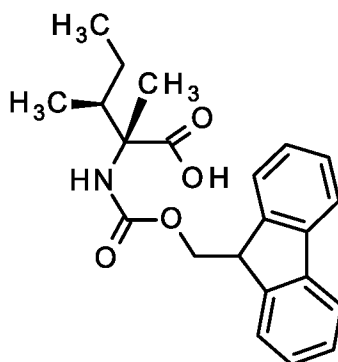
2-Methyl-D-alloisoleucine (single stereoisomer)



The reaction was carried out under argon atmosphere. To N-[(benzyloxy)carbonyl]-2-methyl-D-alloisoleucine (Example 34A, 2.57 g, 9.20 mmol) in 190 mL methanol was added palladium on carbon (390 mg, 10%). The reaction mixture was stirred under a hydrogen atmosphere for 4 h at room temperature and normal pressure. The reaction mixture was filtrated over diatomaceous earth and the filtrate was concentrated, then dried under vacuum to give 1.32 g (98% yield) of the target compound.

Example 36A

N-{[(9H-Fluoren-9-yl)methoxy]carbonyl}-2-methyl-D-alloisoleucine (single stereoisomer)



To a solution of 2-methyl-D-alloisoleucine (Example 35 A, 1.32 g, 9.09 mmol) in a mixture of water (20 mL) and acetone (29 mL) was added sodium bicarbonate (7.64 g, 90.9 mmol) and then a solution of 1-({[(9H-fluoren-9-yl)methoxy]carbonyl}oxy)pyrrolidine-2,5-dione (3.22 g, 9.55 mmol) in acetone (20 mL) was added. The reaction mixture was stirred overnight at room temperature. The reaction mixture was treated with MTBE and extracted. The aqueous phase was acidified with conc. hydrochloric acid (pH 1) and extracted with ethyl acetate three more times. The combined organic phases were dried over magnesium sulfate, filtrated and evaporated. The residue was separated via silica gel chromatography (Isolera, column: 50 g SNAP Ultra; eluent: dichloromethane/methanol 100:3 – 100:5). The appropriate fractions were combined, evaporated and then dried under vacuum.

The MTBE phase was subsequently treated with a 5% sodium bicarbonate solution and extracted with ethyl acetate (600 mL). The aqueous phase was acidified with conc. hydrochloric acid (pH 1) and extracted with ethyl acetate each time. The combined organic phases were dried over magnesium sulfate, filtrated, evaporated and then dried under vacuum. The combined crude product yield was 2.53 g.

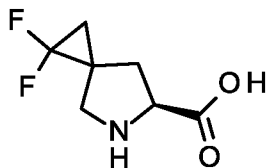
The residue was purified via silica gel chromatography (Isolera, column: 50 g SNAP Ultra; eluent: dichloromethane/methanol 97:3 – 97:5). The product-containing fractions were combined, evaporated and then dried under vacuum to give 967.4 mg of the target compound. The product was further dissolved in water/acetonitrile and lyophilized prior to use.

LC-MS (Method 10): R_t = 2.10 min; MS (ESI pos): m/z = 368.1857 $[M+H]^+$

This amino acid was used in Fmoc SPPS.

Example 37A

(6S)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (mixture of stereoisomers)

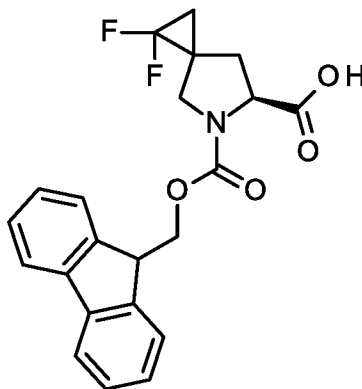


5

To (6S)-5-(tert-butoxycarbonyl)-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (Omega, CAS 1357482-03-1) (2.50 g, 9.02 mmol) in 85 mL dichloromethane was added trifluoroacetic acid (15 mL, 250 mmol). The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated and dried under vacuum for the next step.

10 **Example 38A**

(6S)-5-{[(9H-Fluoren-9-yl)methoxy]carbonyl}-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (mixture of stereoisomers)



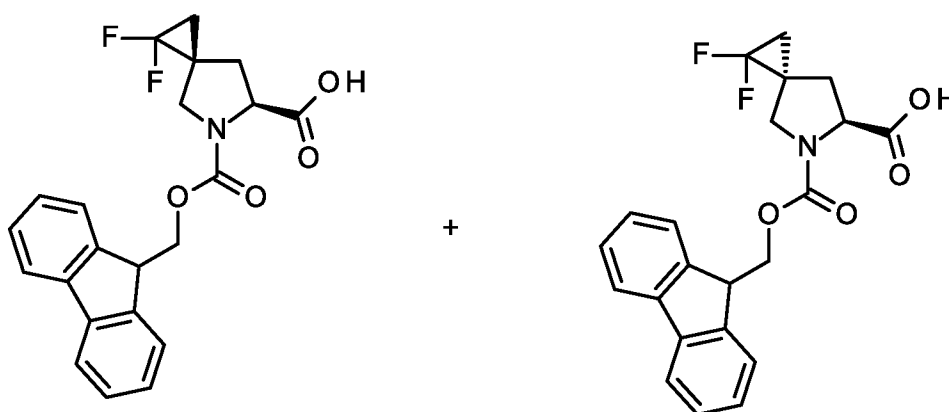
15 To a solution of (6S)-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (mixture of stereoisomers) (Example 37A, 1.60 g, 9.03 mmol) in water (13 mL) was added sodium bicarbonate (7.59 g, 90.3 mmol) and then a solution of 1-({[(9H-fluoren-9-yl)methoxy]carbonyl}oxy)pyrrolidine-2,5-dione (3.20 g, 9.48 mmol) in acetone (20 mL). The reaction mixture was stirred overnight at room temperature. The reaction mixture was treated with water and extracted twice with MTBE. The aqueous phase was acidified with
20 hydrochloric acid to pH3 and extracted with dichloromethane three times. The organic phase was washed with saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtrated, evaporated and dried under vacuum to give 3.31 g (92% yield) of the target compound.

LC-MS (Method 10): R_t = 1.99 min; MS (ESI pos): m/z = 400 $[M+H]^+$

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 1.106 (16.00), 1.589 (0.92), 1.605 (1.39), 1.619 (1.06), 1.627 (1.11), 1.655 (0.62), 1.682 (1.25), 1.703 (1.21), 1.916 (0.49), 1.949 (0.86), 1.978 (0.91), 2.012 (0.85), 2.042 (0.52), 2.086 (11.35), 2.558 (0.83), 2.568 (0.67), 2.591 (0.67), 2.645 (0.52), 2.670 (0.56), 3.077 (5.29), 3.442 (0.43), 3.452 (0.50), 3.471 (1.17), 3.479 (1.13), 3.500 (1.39), 3.527 (0.51), 3.562 (1.21), 3.627 (1.20), 3.652 (1.65), 3.677 (0.78), 4.186 (1.02), 4.200 (2.72), 4.223 (2.09), 4.238 (2.18), 4.257 (1.63), 4.279 (2.63), 4.294 (2.46), 4.306 (1.36), 4.322 (1.56), 4.348 (2.13), 4.359 (1.17), 4.372 (1.33), 4.382 (0.84), 4.477 (0.57), 4.499 (0.61), 4.510 (0.69), 4.518 (0.72), 4.533 (0.73), 4.541 (0.64), 5.754 (0.94), 7.300 (0.77), 7.316 (3.26), 7.334 (5.65), 7.353 (3.32), 7.406 (4.71), 7.425 (7.78), 7.443 (3.45), 7.640 (4.69), 7.659 (4.35), 7.683 (0.73), 7.884 (5.08), 7.903 (4.79).

10 Example 39A

(6S)-5-[[[(9H-Fluoren-9-yl)methoxy]carbonyl]-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1) and (enantiomer 2)



- 15 The diastereomeric mixture was separated using a THAR supercritical fluid chromatography Prep 200 instrument using a Chiralcel AD-H (SFC) 5μm, 250 x 30 mm column; eluent: CO₂/iso-propanol (83:17); pressure: 135 bar; temperature eluent: 38°C; temperatur zyklon: 40°C; pressure zyklon: 24 bar; flow rate: 100 g/min; detection: UV 210nm; injection volume 0.4 mL/injection; under general operating conditions 1g/min of CO₂ is approximately equivalent to 1 mL/min.
- 20 Sequence Settings: cycle time = 11,2 min

Fraction 1 was collected between 5.85 min and 8.0 min (enantiomer 1)

Fraction 2 was collected between 8.4 min and 10.25 min (enantiomer 2)

The fractions were concentrated.

The fractions were analyzed using analytical SFC-MS (Agilent, column: Chiralpak AD-3 3 μ m 100 x 4,6 mm, eluent: CO₂/iso-Propanol (85:15); BPR pressure: 130 bar; BPR temperature: 60°C; column temperature: 40°C; flow rate: 3 mL/min; UV 210 nm).

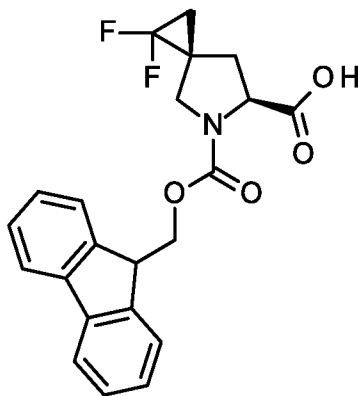
Fraction 1 (rt = 2.081 min) was assigned the addition “(enantiomer 1)”.

- 5 Fraction 2 (rt = 2.838 min) was assigned the addition “(enantiomer 2)”.

The stereochemistry of the two enantiomers was not assigned.

Example 40A

(3R*,6S)-5-[(9H-Fluoren-9-yl)methoxy]carbonyl]-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (single enantiomer, enantiomer 1, stereochemistry at 3 position not defined)



10

From the chiral SFC preparative purification, 2,015 g of the title compound was obtained, 100% ee

LC-MS (Method 10): R_t = 1.99 min; MS (ESI pos): m/z = 400 [M+H]⁺

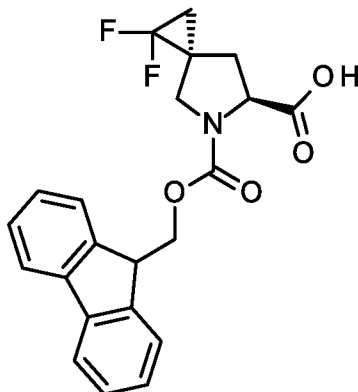
¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: -0.008 (0.70), 0.008 (0.74), 1.032 (15.85), 1.047 (16.00), 1.285 (1.59), 1.568 (0.69), 1.589 (1.52), 1.617 (1.57), 1.635 (0.96), 1.656 (0.63), 1.686 (1.36), 1.702 (1.37), 1.941 (0.90), 1.974 (1.05), 2.008 (0.87), 2.041 (0.90), 2.073 (1.11), 2.557 (1.08), 2.566 (0.95), 2.591 (0.80), 2.645 (0.71), 2.670 (0.95), 2.701 (0.65), 3.451 (0.87), 3.468 (1.64), 3.479 (1.88), 3.495 (1.12), 3.505 (1.17), 3.626 (2.11), 3.651 (2.91), 3.677 (1.38), 3.770 (0.41), 3.778 (0.42), 4.169 (0.43), 4.186 (1.17), 4.200 (3.11), 4.221 (2.05), 4.238 (1.40), 4.256 (1.57), 4.279 (3.79), 4.293 (3.74), 4.304 (1.62), 4.322 (2.70), 4.336 (1.12), 4.348 (2.93), 4.358 (1.92), 4.371 (1.64), 4.381 (1.52), 4.509 (1.14), 4.517 (1.25), 4.531 (1.28), 4.540 (1.12), 7.300 (0.91), 7.315 (3.87), 7.334 (7.04), 7.350 (3.82), 7.352 (4.21), 7.406 (5.88), 7.424 (9.76), 7.443 (4.35), 7.640 (7.54), 7.658 (6.74), 7.885 (6.80), 7.904 (6.34), 10.194 (0.55), 10.991 (0.42).

20

This amino acid was used in Fmoc SPPS.

Example 41A

(3S*,6S)-5-{[(9H-Fluoren-9-yl)methoxy]carbonyl}-1,1-difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (single enantiomer, enantiomer 2, stereochemistry at cyclopropyl center not assigned)



5

From chiral SFC preparative purification, 1.041 g of the title compound was obtained, 94.58% ee

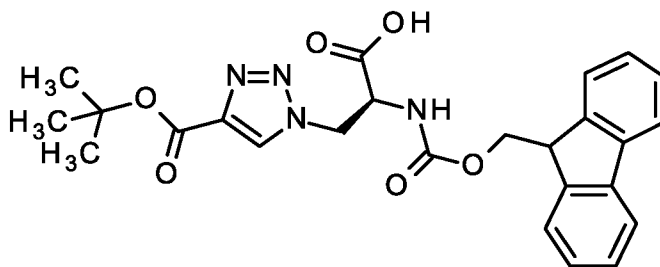
LC-MS (Method 10): R_t = 1.93 min; MS (ESI pos): m/z = 400 $[M+H]^+$

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: -0.008 (1.19), 0.008 (1.31), 1.031 (0.55), 1.047 (0.56), 1.285 (0.60), 1.583 (1.53), 1.604 (4.87), 1.627 (5.45), 1.650 (3.25), 1.673 (2.78), 1.682 (3.76), 1.704 (3.11),
10 1.718 (0.95), 1.915 (3.17), 1.949 (3.55), 1.979 (3.13), 2.012 (3.33), 2.073 (3.85), 2.526 (1.60), 2.590 (1.44), 2.626 (1.61), 2.649 (0.89), 2.659 (0.80), 3.471 (2.04), 3.498 (5.50), 3.527 (3.26), 3.561 (7.96), 3.570 (4.68), 3.598 (0.84), 4.167 (0.94), 4.183 (2.75), 4.199 (6.46), 4.223 (7.75), 4.238 (9.60), 4.258 (5.95), 4.277 (4.06), 4.290 (3.98), 4.306 (3.80), 4.319 (4.63), 4.340 (4.17), 4.346 (4.47), 4.354 (2.34), 4.367 (3.96), 4.477 (3.66), 4.497 (3.66), 7.300 (1.97), 7.318 (8.34), 7.334 (11.70), 7.337 (12.42), 7.352
15 (6.35), 7.355 (6.41), 7.407 (9.62), 7.425 (16.00), 7.444 (7.11), 7.633 (6.46), 7.651 (10.65), 7.666 (6.62), 7.683 (4.36), 7.883 (9.71), 7.893 (10.32), 7.901 (9.57), 7.912 (8.99), 12.802 (0.79), 12.984 (0.67).

This amino acid was used in Fmoc SPPS.

Example 42A

3-[4-(tert-Butoxycarbonyl)-1H-1,2,3-triazol-1-yl]-N-{[(9H-fluoren-9-yl)methoxy]carbonyl}-L-alanine
20 (single stereoisomer)



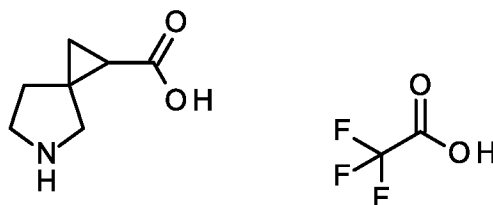
To 3-azido-N-[(9H-fluoren-9-yl)methoxy]carbonyl-L-alanine (1.00 g, 2.84 mmol) in a mixture of 27 mL water/tert. butanol/dichloromethane (1:1:1) was added tert-butyl prop-2-ynoate (580 μ L, 4.3 mmol),
 5 copper(II) sulfate pentahydrate (709 mg, 2.84 mmol) and a solution of sodium ascorbate (1.41 g, 7.10 mmol) in 14 mL water. The reaction mixture was stirred overnight at room temperature. The reaction mixture was treated with water, acidified with diluted citric acid and extracted with dichloromethane three times. The organic phase was dried over sodium sulfate, filtrated and then evaporated. The residue was taken up in acetonitrile/water and separated in 2 portions via preparative HPLC (column: Chromatorex
 10 C18 10 μ m 125 x 40mm; water-acetonitrile-gradient with 0.05% TFA: 0-2 min 25% acetonitrile, 2-22 min 25-75% acetonitrile, 23-26min 75% acetonitrile, 26-28 min back to 25% acetonitrile; flow 100 mL/min). The product-containing fractions were combined, evaporated and dried under vacuum to give 1.02 g (100% purity, 75% yield) of the target compound.

LC-MS (Method 11): R_t = 1.30 min; MS (ESI neg): m/z = 477 $[M-H]^-$

15 This amino acid was used in Fmoc SPPS.

Example 43A

5-Azaspiro[2.4]heptane-1-carboxylic acid—trifluoroacetic acid (1/1)

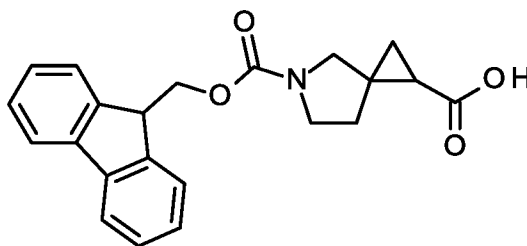


20 To a solution of 5-(tert-butoxycarbonyl)-5-azaspiro[2.4]heptane-1-carboxylic acid (1.00 g, 4.14 mmol) in 25 mL dichloromethane was added TFA (960 μ L, 12 mmol). The reaction mixture stirred 2 h at room temperature. The reaction mixture was evaporated and dried under vacuum to give 0.80 g (76% yield) of the target compound.

LC-MS (Method 9): R_t = 0.18 min; MS (ESI pos): m/z = 142 $[M+H]^+$

Example 44A

5-{{[(9H-Fluoren-9-yl)methoxy]carbonyl}-5-azaspiro[2.4]heptane-1-carboxylic acid



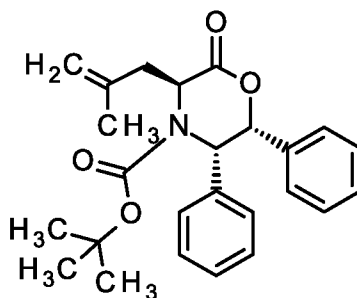
- 5 To a solution of 5-azaspiro[2.4]heptane-1-carboxylic acid—trifluoroacetic acid (1/1) (Example 43A, 994 mg, 3.90 mmol) in a mixture of 5 mL acetonitrile and 5 mL water was added 1-({[(9H-fluoren-9-yl)methoxy]carbonyl}oxy)pyrrolidine-2,5-dione (2.63 g, 7.79 mmol) and triethylamine (2.2 mL, 16 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was treated with water, acidified with 1 M hydrochloric acid to pH 4 and then extracted with dichloromethane three times. The combined organic phases were dried over sodium sulfate, filtrated and evaporated. The residue was triturated with acetonitrile, then the precipitate was collected by filtration and washed with acetonitrile. The filtrate was purified in 3 portions via preparative HPLC (column: Chromatorex C18 10 μ m 125x40mm; water-acetonitrile-gradient with 0.05% TFA: 0-2 min 25% acetonitrile, 2-22 min 25-75% acetonitrile, 23-26 min 75% acetonitrile, 26-28min back to 25% acetonitrile; flow 100 mL/min). The appropriate fractions were combined, evaporated and dried under vacuum to give 1.16 g (95% purity, 78% yield) of the target compound.

LC-MS (Method 9): R_t = 0.99 min; MS (ESI pos): m/z = 364 $[M+H]^+$

This amino acid was used in Fmoc SPPS.

Example 45A

- 20 tert-Butyl (3S,5S,6R)-3-(2-methylallyl)-2-oxo-5,6-diphenyl-morpholine-4-carboxylate



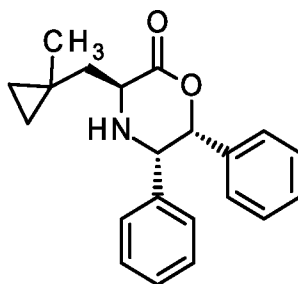
Two batches were run in parallel: To a solution of tert-butyl (2R,3S)-6-oxo-2,3-diphenyl-morpholine-4-carboxylate (90.0 g, 255 mmol) and 3-bromo-2-methyl-prop-1-ene (37.0 g, 27.6 mL, 274 mmol) in THF (1.8 L) and HMPA (180 mL) was added sodium bis(trimethylsilyl)amide (279 mL, 1 M in THF) dropwise at -70°C over a period of 60 mins under nitrogen atmosphere. Then the solution was stirred at -70°C for 30 mins. The reaction was combined and quenched by saturated aqueous ammonium chloride solution (2.5 L), then extracted with ethyl acetate (2.0 L x 2). The combined organic phase was washed with saturated aqueous sodium chloride solution (1.5 L x 2), dried over sodium sulfate, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate= 1/0, 10/1) to give the residue, the mixture of residue and Petroleum ether/EtOAc (2.0 L/0.2 L) was stirred for 0.5 h at 25°C, then filtered to give 145 g (89% purity, 62% yield) of the target compound.

LC-MS: Rt = 1.027 min, MS+Na = 430.2

¹H NMR: CDCl₃: 7.35–7.00 (m, 8H), 6.52 (m, 2H), 6.35–6.28 (m, 1H), 5.15–4.82 (m, 4H), 2.95–2.70 (m, 2H), 1.87 (s, 3H), 1.41 (s, 3H), 1.03 (s, 6H)

Example 46A

(3S,5S,6R)-3-[(1-Methylcyclopropyl)methyl]-5,6-diphenyl-morpholin-2-one

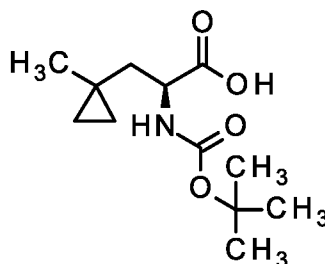


Three batches were run in parallel. To a solution of tert-butyl (3S,5S,6R)-3-(2-methylallyl)-2-oxo-5,6-diphenyl-morpholine-4-carboxylate (Example 45A, 35.0 g, 76 mmol) in 1.3 L dichloromethane was added diethylzinc (400 mL, 1M in toluene) at 0 °C over 30 min, then chloro(iodo)methane (140 g, 793 mmol, 57.6 mL) was added at 0°C over 30 min. The reaction was stirred for 1.5 h at 0°C. The reaction was combined and poured into 2.0 L saturated aqueous sodium bicarbonate solution, the solution was filtered, and then the filtrate was extracted with dichloromethane (1.0 L x 2). The combined organic phase was washed with 1.5 L saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered through a short piece of silica. Then the filtrate was concentrated to give residue. The mixture of residue and petroleum ether/ethyl acetate/dichloromethane (10/1/1, 400 mL) was stirred for 0.5 h at 25 °C and filtered. The filtrate was concentrated to about 120 mL, then filtered to give 20 g (92% purity, 25% yield) of the target compound.

LC/MS: Rt = 0.909 min, M+H⁺ = 322.2

Example 47A

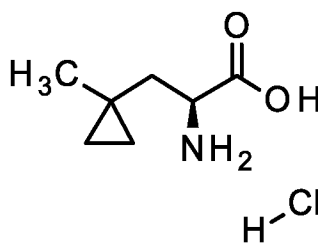
(2S)-2-(tert-Butoxycarbonylamino)-3-(1-methylcyclopropyl)propanoic acid



- 5 To a stirred solution containing ethanol (50 mL), liquid ammonia (600 mL), THF (500 mL) and (3S,5S,6R)-3-[(1-methylcyclopropyl)methyl]-5,6-diphenyl-morpholin-2-one (Example 46A, 57.4 g) that was cooled to -70 °C was added lithium (8 g, 1.15 mol) in small pieces over 0.5 h at -70 °C until a deep blue color persisted for 10 min at -70 °C. The reaction was quenched by careful addition of 50 mL 20% aqueous ammonium chloride solution, the cold bath was removed, and the reaction was warmed to 25 °C.
- 10 The reaction mixture was diluted with 300 mL water and extracted with MTBE (200 mL x 2). The aqueous phase was cooled in ice, acidified to pH= 1 with 1 M aqueous hydrochloric acid, and immediately extracted with ethyl acetate (800 mL x 2). The combined ethyl acetate phases were washed with 300 mL saturated aqueous sodium chloride solution, dried over sodium sulfate and concentrated at reduced pressure to give 24 g (99 mmol) of the target compound.

Example 48A

(2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid hydrochloride

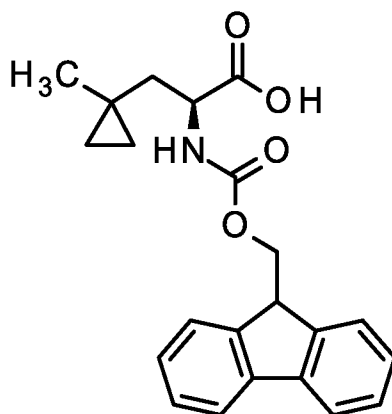


- To the solution of (2S)-2-(tert-butoxycarbonylamino)-3-(1-methylcyclopropyl)propanoic acid (Example 47A, 24.0 g, 99 mmol) in dichloromethane (60 mL) was added hydrochloric acid/dioxane (200 mL, 4M) over 15 min at 25 °C, then the solution was stirred for 0.5 h at 25 °C. The reaction was concentrated to give a residue. The residue was treated with 150 mL of MTBE and stirred for 15 min at 25 °C, then filtered to give 16 g (90% yield) of the target compound.

- ¹H NMR: DMSO-d₆: 0.21-0.33 (3 H, m), 0.37-0.51 (1 H, m), 1.64-1.81 (2 H, d), 3.88-3.92 (1 H, t), 8.41 (3 H, s), 13.73 (1 H, s)

Example 49A

(2S)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-(1-methylcyclopropyl)propanoic acid



- 5 To a solution of ((2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid hydrochloride (Example 48A, 14 g, 77.9 mmol) and sodium carbonate (25 g, 236 mmol) in THF (200 mL) and water (200 mL) was added 1-({[(9H-fluoren-9-yl)methoxy]carbonyl}oxy)pyrrolidine-2,5-dione (25 g, 74.1 mmol) over 30 min at 0 °C, then the solution was stirred for 2 h at 0 °C. 100 mL water was added. The solution was extracted with MTBE (300 mL x 2) before the the pH of aqueous phase was adjusted to 2 with 2 M aqueous hydrochloric acid. The combined organic phase was washed with 150 mL saturated aqueous sodium chloride solution, dried over sodium sulfate, and filtered through a small column of silica. The filtrate was concentrated to give a residue. The combined residue (from previous runs) was purified by silica gel chromatography (petroleum ether / ethyl acetate (10:1, 3:1) to afford a semi-pure residue. The residue was further purified by preparative HPLC (column: Phenomenex Luna C18 250 x 50mm, 10 µm; mobile phase: [water (0.05% HCl)-ACN]; B%: 38% - 68%, 31min, 50% min) to give 24.4 g (97% purity, 74% yield) of the target compound.

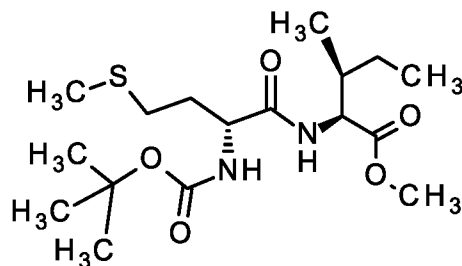
LC-MS: Rt = 0.914 min, [M+H]⁺ = 366.1

¹H NMR: CDCl₃: δ ppm 0.23- 0.40 (4 H, m), 1.02- 1.20 (3 H, m), 1.48- 1.95 (2 H, m), 4.22- 4.33 (1 H, m), 4.39 - 4.61 (3 H, m), 5.26- 5.33 (1 H, m), 7.31- 7.49 (4 H, m), 7.56- 7.81 (4 H, m).

- 20 This amino acid was used in Fmoc SPPS.

Example 50A

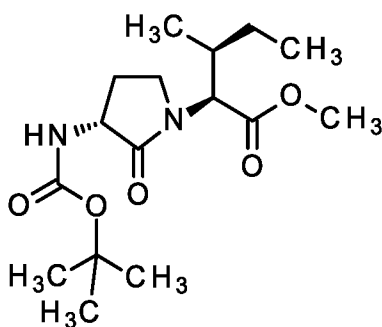
Methyl *N*-(*tert*-butoxycarbonyl)-*D*-methionyl-*L*-isoleucinate



- 5 *N*-(*tert*-Butoxycarbonyl)-*D*-methionine (2.42 g, 9.71 mmol), methyl *L*-isoleucinate—hydrogen chloride (1/1) (1.76 g, 9.71 mmol), 1*H*-benzotriazol-1-ol (1.78 g, 11.6 mmol), 3-[(Ethylimino)methylene]amino}-*N,N*-dimethylpropan-1-amine hydrochloride (1:1) (2.23 g, 11.6 mmol) and *N,N*-diisopropylethylamine (3.5 mL, 20 mmol) were dissolved in DMF (30 mL). The mixture was stirred at ambient temperature for 16 h. Water (100 mL) was then added, the mixture was extracted with two portions of ethyl acetate. The combined organic extract was washed sequentially with hydrochloric acid, water (10 mL), saturated aqueous sodium bicarbonate solution (10 mL) and brine, and then dried over anhydrous sodium sulfate. The organic layer was filtered and the filtrate was concentrated. The residue was dissolved in ether and the product crystallized. The solid was filtered and washed sequentially with ether and pentane. 3.13 g (99 % purity, 86 % yield) of the title compound were obtained.

Example 51A

Methyl (2*S*, 3*S*)-2-[(3*R*)-3-[(*tert*-butoxycarbonyl)amino]-2-oxopyrrolidin-1-yl]-3-methylpentanoate

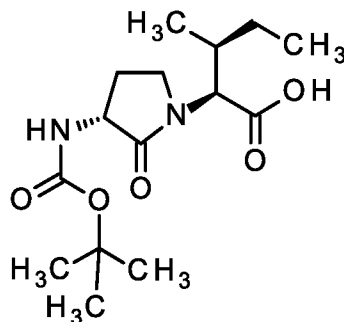


- 20 Methyl *N*-(*tert*-butoxycarbonyl)-*D*-methionyl-*L*-isoleucinate (Example 50A, 2.85 g, 7.57 mmol) was dissolved in dichloromethane (40 mL) and the solution was cooled to 0°C. Trimethyloxonium tetrafluoroborate (1.12 g, 7.57 mmol) was added and the mixture was stirred at room temperature for 3 h. Potassium carbonate (3.13 g, 22.71 mmol) was added. Then the mixture was heated to reflux for 16 h. The reaction mixture was poured into 50 mL dichloromethane and washed with five portions of water. The organic

layer was washed with brine, dried over anhydrous magnesium sulfate, and then filtered. The filtrate was concentrated and crystallized from hexane yielding 2.01 g (100 % purity, 81 % yield) of the title compound.

Example 52A

(2*S*, 3*S*)-2-[(3*R*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxopyrrolidin-1-yl]-3-methylpentanoic acid



5

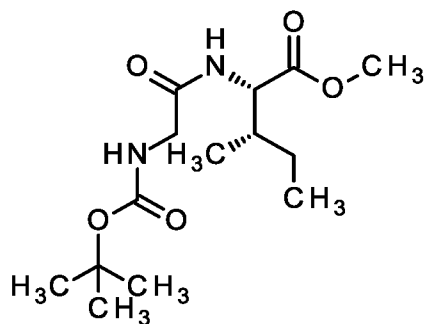
Methyl (2*S*,3*S*)-2-[(3*R*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxopyrrolidin-1-yl]-3-methylpentanoate (example 51A, 1.96 g, 5.97 mmol) was dissolved in THF (7.8 mL) and methanol (7.8 mL). A solution of lithium hydroxide (300 mg, 12.5 mmol) in 8 mL of water was added and the mixture was stirred for 1 h at room temperature. After the reaction mixture was concentrated, the white residue was dissolved in water (40 mL), washed with DCM (6 mL), and then acidified with 1 M hydrochloric acid (12 mL). The mixture was extracted with three portions of ethyl acetate. The combined organic extract was dried over anhydrous sodium sulfate, filtered, and then concentrated. The residue was crystallized from ethyl acetate and hexane to provide 1.3 g (100 % purity, 67% yield) of the title compound.

15 LC-MS (Method 11): R_t = 1.09 min; MS (ESI neg): m/z = 313 $[M-H]^-$

^1H -NMR (400 MHz, DMSO- d_6) δ [ppm]: 0.810 (0.96), 0.829 (2.41), 0.847 (1.25), 0.889 (2.53), 0.906 (2.59), 1.387 (16.00), 3.260 (0.71), 3.274 (0.56), 3.283 (0.75), 3.298 (0.64), 4.200 (0.97), 4.225 (0.94).

Example 53A

Methyl *N*-(*tert*-butoxycarbonyl)glycyl-*L*-isoleucinate



20

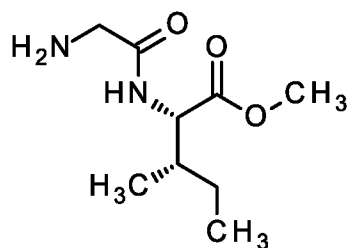
Methyl *L*-isoleucinate (3.00 g, 20.7 mmol) and *N*-(*tert*-butoxycarbonyl)glycine (3.98 g, 22.7 mmol) were dissolved in DMF (20 mL). This solution was cooled to 0-5°C in an ice bath. *N,N*-diisopropylethylamine (3.6 mL, 21 mmol), 1*H*-benzotriazol-1-ol hydrate (316 mg, 2.07 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimidhydrochloride (4.36 g, 22.7 mmol) were added and the mixture was stirred at room temperature. The reaction mixture was quenched with concentrated aqueous ammonium chloride and extracted with three portions of ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated. The crude product was purified by chromatography on silica gel (cyclohexane / ethyl acetate 0 % to 10 %) to give the title compound, 5.40 g (90 % purity, 78 % yield).

LC-MS (Method 11): R_t = 1.13 min; MS (ESI pos): m/z = 247 $[M-tBu]^+$

^1H -NMR (400 MHz, DMSO- d_6) δ [ppm]: 0.819 (4.90), 0.835 (8.09), 0.853 (2.63), 1.152 (0.40), 1.174 (0.45), 1.354 (1.52), 1.375 (16.00), 1.459 (0.46), 3.561 (0.84), 3.571 (0.98), 3.577 (1.45), 3.594 (0.81), 3.630 (11.35), 4.220 (0.63), 4.237 (0.78), 4.240 (0.82), 4.257

Example 54A

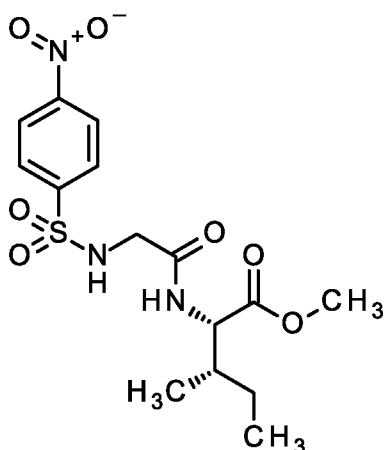
Methyl glycy-*L*-isoleucinate



Methyl *N*-(*tert*-butoxycarbonyl)glycyl-*L*-isoleucinate (example 53A, 1.50 g, 4.96 mmol) was dissolved in DCM (27 mL). Trifluoroacetic acid (3.0 mL, 39 mmol) was added and the reaction mixture was stirred for 30 min at room temperature. The reaction mixture was concentrated to afford the crude product, which was used directly for the next step.

Example 55A

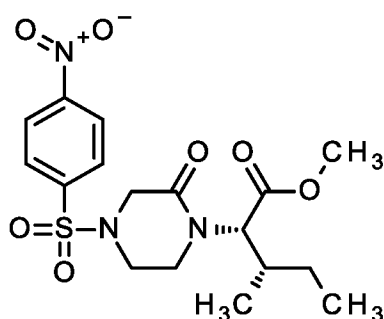
Methyl *N*-(4-nitrobenzene-1-sulfonyl)glycyl-*L*-isoleucinate



Methyl glyceryl-*L*-isoleucinate (Example 54A, 1.00 g, 4.96 mmol) was dissolved in dichloromethane (40 mL, 620 mmol), triethylamine (2.4 mL, 17 mmol) was added, and then the solution was cooled to 0°C. 4-Nitrobenzene-1-sulfonyl chloride (1.21 g, 5.46 mmol) was added and the reaction mixture was stirred for 1 h. The reaction was then quenched with concentrated aqueous ammonium chloride and extracted with three portions of ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and then concentrated, to give 1.70 g (71 % yield) of the title compound. The product was used directly for the next step.

Example 56A

Methyl (2*S*, 3*S*)-3-methyl-2-[4-(4-nitrobenzene-1-sulfonyl)-2-oxopiperazin-1-yl]pentanoate

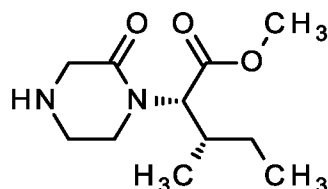


Methyl *N*-(4-nitrobenzene-1-sulfonyl)glycyl-*L*-isoleucinate (Example 55A, 1.92 g, 4.96 mmol) and 1,2-dibromoethane (4.3 mL, 50 mmol) were dissolved in DMF. Potassium carbonate (6.86 g, 49.6 mmol) was added and the mixture was heated to 60°C for 3 h. The reaction was then quenched with concentrated aqueous ammonium chloride and extracted with three portions of ether. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and then evaporated. The crude product was purified by column chromatography on silica gel (cyclohexane / ethyl acetate 6:1) to give title compound 1.40 g (97 % purity, 66 % yield).

LC-MS (Method 11): R_t = 1.30 min; MS (ESI pos): m/z = 414 $[M+H]^+$

Example 57A

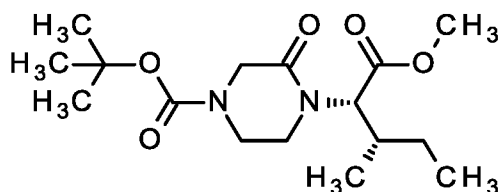
Methyl (2*S*, 3*S*)-3-methyl-2-(2-oxopiperazin-1-yl)pentanoate



- 5 Methyl (2*S*, 3*S*)-3-methyl-2-[4-(4-nitrobenzene-1-sulfonyl)-2-oxopiperazin-1-yl]pentanoate (Example 56A, 1.40 g, 3.39 mmol) and benzenethiol (1.0 mL, 10 mmol) were dissolved in acetonitrile (20 mL). Potassium carbonate (936 mg, 6.77 mmol) was added and the mixture was stirred at room temperature. The mixture was filtered and evaporated. The residue was purified by flash chromatography on silica gel (gradient dichloromethane / methanol 100:0 to 95:5) to afford 310 mg (40 % yield) of the title compound,
10 that was used directly for the next step.

Example 58A

tert-Butyl 4-[(2*S*,3*S*)-1-methoxy-3-methyl-1-oxopentan-2-yl]-3-oxopiperazine-1-carboxylate



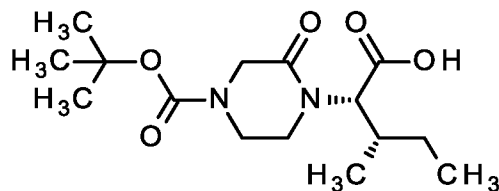
- 15 Methyl (2*S*, 3*S*)-3-methyl-2-(2-oxopiperazin-1-yl)pentanoate (Example 57A, 310 mg, 1.36 mmol) was dissolved in dichloromethane (1.7 mL, 26 mmol). Di-*tert*-butyl dicarbonate (370 μ L, 1.6 mmol) was added and the mixture was stirred at room temperature for 1 h. The reaction was then quenched with concentrated aqueous ammonium chloride and then extracted with three portions of DCM (20 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered and then concentrated, to afford
20 440 mg (99 % purity, 98 % yield) of the title compound.

LC-MS (§Method 11): R_t = 1.27 min; MS (ESI pos): m/z = 273 $[M+H-tBu]^+$

^1H -NMR (400 MHz, DMSO- d_6) δ [ppm]: 0.807 (0.86), 0.826 (2.27), 0.844 (1.20), 0.870 (2.25), 0.887 (2.30), 1.410 (16.00), 3.645 (6.00), 3.963 (0.63), 3.970 (0.62), 4.766 (0.82), 4.792 (0.80), 5.754 (0.87).

Example 59A

- 25 (2*S*, 3*S*)-2-[4-(*tert*-butoxycarbonyl)-2-oxopiperazin-1-yl]-3-methylpentanoic acid



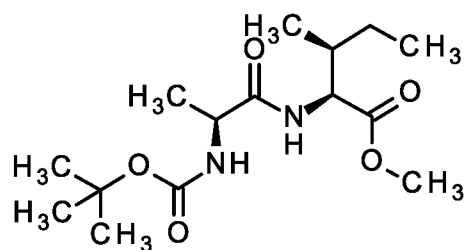
tert-Butyl 4-[(2*S*, 3*S*)-1-methoxy-3-methyl-1-oxopentan-2-yl]-3-oxopiperazine-1-carboxylate (Example 58A, 440 mg, 80 % purity, 1.07 mmol) was dissolved in THF (1.4 mL) and methanol (1.4 mL). A solution of lithium hydroxide (53.9 mg, 2.25 mmol) in water (1.4 mL) was added and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated and the residue was dissolved in water and then washed with DCM. The aqueous layer was separated, acidified to pH 3-4 with 1M hydrochloric acid, and then extracted three times with ethyl acetate. The combined organic layer was dried over anhydrous magnesium sulfate, filtered, and then concentrated under reduced pressure to give 310 mg (95% purity, 87% yield) of the title compound as a colorless oil.

LC-MS (Method 11): R_t = 1.11 min; MS (ESI neg): m/z = 313 $[M-H]^-$

^1H -NMR (400 MHz, DMSO- d_6) δ [ppm]: 0.809 (0.81), 0.827 (2.06), 0.845 (1.12), 0.908 (2.11), 0.924 (2.09), 1.411 (16.00), 3.438 (0.43), 3.968 (0.78), 4.688 (0.79), 4.713 (0.77).

Example 60A

Methyl *N*-(*tert*-butoxycarbonyl)-*L*-alanyl-*L*-isoleucinate



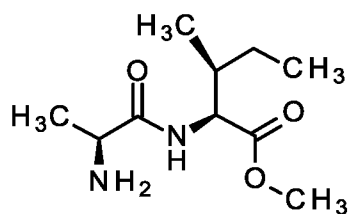
N-(*tert*-Butoxycarbonyl)-*L*-alanine (1.50 g, 7.93 mmol) and methyl *L*-isoleucinate—hydrogen chloride (1/1) (1.58 g, 8.72 mmol) were dissolved in DMF (7.7 mL). This solution was cooled to 0-5°C in an ice bath. *N,N*-diisopropylethylamine (1.4 mL, 7.9 mmol), 1*H*-benzotriazol-1-ol hydrate (121 mg, 793 μ mol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.67 g, 8.72 mmol) were added and the mixture was stirred at room temperature. The reaction was then quenched with concentrated aqueous ammonium chloride and extracted with three portions of ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and then concentrated. The crude product was purified by chromatography on silica gel (cyclohexane / ethyl acetate 0% to 15%) to give 2.12 g (100 % purity, 84 % yield) of the title compound.

LC LC-MS (Method 10): $R_t = 1.70$ min; MS (ESI pos): $m/z = 317$ $[M+H]^+$

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: -0.008 (1.00), 0.008 (0.77), 0.818 (3.17), 0.825 (4.25), 0.836 (7.51), 0.842 (4.72), 0.855 (3.30), 1.144 (4.07), 1.161 (4.10), 1.179 (0.54), 1.200 (0.53), 1.371 (16.00), 1.398 (13.44), 1.775 (0.49), 2.523 (0.85), 3.621 (15.73), 4.021 (0.46), 4.039 (0.60), 4.207 (1.06), 4.223 (1.19), 4.228 (1.22), 4.244 (1.06), 6.908 (0.61), 6.928 (0.58), 7.886 (0.57), 7.907 (0.60).

Example 61A

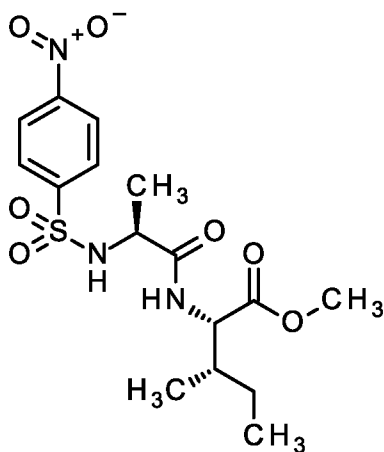
Methyl *L*-alanyl-*L*-isoleucinate



- 10 Methyl *N*-(*tert*-butoxycarbonyl)-*L*-alanyl-*L*-isoleucinate (Example 60A, 2.10 g, 6.64 mmol) was dissolved in DCM (20 mL). Trifluoroacetic acid (20 mL, 260 mmol) was added and the reaction mixture was stirred for 30 min at room temperature. The reaction mixture was concentrated and the crude dipeptide was used directly for the next step.

Example 62A

- 15 Methyl *N*-(4-nitrobenzene-1-sulfonyl)-*L*-alanyl-*L*-isoleucinate



- 20 Methyl *L*-alanyl-*L*-isoleucinate (Example 61A, 1.44 g, 6.64 mmol) was dissolved in dichloromethane (54 mL), triethylamine (3.2 mL, 23 mmol) was added, and then the solution was cooled to 0°C. 4-Nitrobenzene-1-sulfonyl chloride (1.62 g, 7.30 mmol) was added and the reaction mixture was stirred. The reaction was then quenched with concentrated aqueous ammonium chloride and extracted with two portions of dichloromethane. The combined organic extracts were washed with saturated sodium chloride aqueous

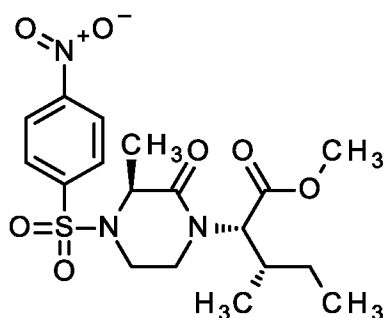
solution, dried over anhydrous magnesium sulfate, filtered, and the concentrated to afford 1.65g (95 % purity, 59 % yield) of the title compound.

LC-MS (Method 11): $R_t = 1.18$ min; MS (ESI neg): $m/z = 400$ $[M-H]^-$

^1H -NMR (400 MHz, DMSO- d_6) δ [ppm]: 0.008 (0.40), 0.676 (6.59), 0.693 (6.80), 0.740 (2.79), 0.758 (6.99), 0.777 (3.46), 1.013 (0.52), 1.025 (0.62), 1.047 (0.69), 1.066 (0.50), 1.117 (6.09), 1.135 (6.12), 1.175 (0.51), 1.192 (0.64), 1.203 (0.60), 1.210 (0.56), 1.222 (0.67), 1.237 (0.48), 1.243 (0.42), 1.551 (0.53), 1.568 (0.61), 1.578 (0.45), 1.584 (0.47), 1.988 (0.64), 3.577 (16.00), 3.933 (1.14), 3.948 (1.32), 3.953 (1.31), 3.968 (1.12), 4.056 (0.61), 4.073 (0.82), 4.091 (0.54), 7.994 (4.08), 7.999 (1.44), 8.012 (1.63), 8.016 (4.58), 8.135 (1.41), 8.155 (1.39), 8.365 (4.39), 8.383 (1.48), 8.388 (3.90), 8.463 (1.04), 8.482 (0.99).

Example 63A

Methyl (2*S*, 3*S*)-3-methyl-2-[(3*S*)-3-methyl-4-(4-nitrobenzene-1-sulfonyl)-2-oxopiperazin-1-yl]pentanoate

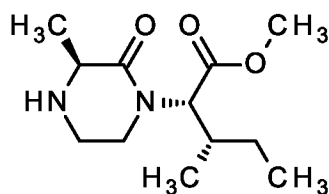


Methyl *N*-(4-nitrobenzene-1-sulfonyl)-*L*-alanyl-*L*-isoleucinate (Example 62A, 1.65 g, 4.11 mmol) and 1,2-dibromoethane (3.5 mL, 41 mmol) were dissolved in DMF (35 mL). Potassium carbonate (5.68 g, 41.1 mmol) was added and the reaction mixture was heated to 60°C for 3 h. The reaction was then quenched with concentrated aqueous ammonium chloride and extracted with three portions of ether. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and then concentrated. The crude product was purified by chromatography on silica gel (cyclohexane / ethyl acetate 6:1) to give 1.60 g (70 % purity, 64 % yield) of the title compound.

LC-MS (Method 11): $R_t = 1.30$ min; MS (ESI pos): $m/z = 428$ $[M+H]^+$

Example 64A

Methyl (2*S*,3*S*)-3-methyl-2-[(3*S*)-3-methyl-2-oxopiperazin-1-yl]pentanoate

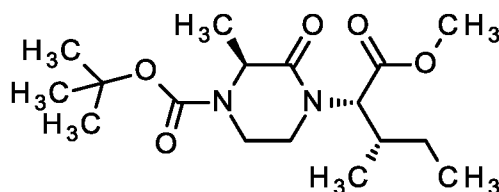


Methyl (2*S*, 3*S*)-3-methyl-2-[(3*S*)-3-methyl-4-(4-nitrobenzene-1-sulfonyl)-2-oxopiperazin-1-yl]pentanoate (Example 63A, 900 mg, 2.11 mmol) and benzenethiol (650 μ L, 6.3 mmol) were dissolved in acetonitrile (12 mL). Potassium carbonate (582 mg, 4.21 mmol) was added and the reaction mixture was stirred at room temperature overnight. The mixture was filtered and concentrated. The residue was purified by flash chromatography on silica gel (gradient dichloromethane / methanol 100:0 to 95:5) to afford 336mg (100 % purity, 66 % yield) of the title compound.

LC-MS (Method 13): R_t = 1.09 min; MS (ESI pos): m/z = 243 $[M+H]^+$

10 Example 65A

tert-Butyl (2*S*)-4-[(2*S*, 3*S*)-1-methoxy-3-methyl-1-oxopentane-2-yl]-2-methyl-3-oxopiperazine-1-carboxylate



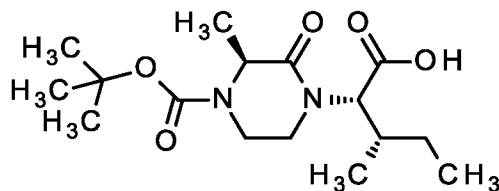
Methyl (2*S*, 3*S*)-3-methyl-2-[(3*S*)-3-methyl-2-oxopiperazin-1-yl]pentanoate (Example 64A, 736 mg, 3.04 mmol) was dissolved in dichloromethane (8 mL). Di-*tert*-butyl dicarbonate (1.0 mL, 4.6 mmol) was added, and the reaction mixture was stirred at room temperature over the weekend. The reaction was then quenched with concentrated aqueous ammonium chloride and extracted with two portions of DCM. The combined organic extracts were washed with sodium chloride solution, dried over anhydrous magnesium sulfate, filtered and then concentrated. The crude product was purified by chromatography on silica gel (cyclohexane / ethyl acetate 3:1) to afford 1.08 g (100 % purity, 104 % yield) of the title compound.

LC-MS (Method 10): R_t = 1.98 min; MS (ESI pos): m/z = 343 $[M+H]^+$

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6 , δ [ppm]: 0.800 (0.95), 0.818 (2.41), 0.837 (1.25), 0.875 (2.38), 0.892 (2.43), 1.305 (2.02), 1.323 (2.06), 1.414 (16.00), 3.645 (6.08), 4.769 (0.88), 4.795 (0.86).

25 Example 66A

(2*S*, 3*S*)-2-[(3*S*)-4-(*tert*-Butoxycarbonyl)-3-methyl-2-oxopiperazin-1-yl]-3-methylpentanoic acid



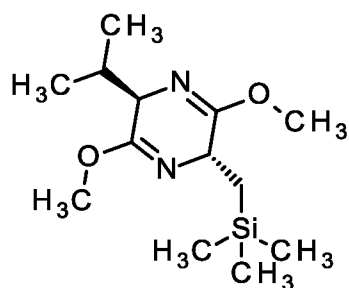
tert-Butyl (2*S*)-4-[(2*S*,3*S*)-1-methoxy-3-methyl-1-oxopentane-2-yl]-2-methyl-3-oxopiperazine-1-carboxylate (Example 65A, 1.08 g, 3.15 mmol) was dissolved in a mixture of THF (4.5 mL) and methanol (4.5 mL). A solution of lithium hydroxide (159 mg, 6.62 mmol) in water (4.5 mL) was added and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated and the residue was dissolved in concentrated aqueous ammonium chloride. The aqueous layer was used acidified to pH 3-4 with 1M hydrochloric and extracted three times with DCM. The combined organic layer was dried over anhydrous magnesium sulfate, filtered, and then concentrated to afford 952 mg (100 % purity, 92 % yield) of the title compound.

LC-MS (Method 9): R_t = 0.87 min; MS (ESI pos): m/z = 329 $[M+H]^+$

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: 0.802 (1.09), 0.820 (2.24), 0.838 (1.18), 0.911 (2.22), 0.927 (2.28), 1.295 (0.50), 1.308 (2.03), 1.326 (1.99), 1.415 (16.00), 3.342 (0.65), 4.691 (0.84), 4.717 (0.82).

Example 67A

(2*R*, 5*R*)-3,6-Dimethoxy-2-(propan-2-yl)-5-[(trimethylsilyl)methyl]-2,5-dihydropyrazine



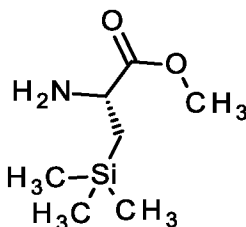
(2*R*)-3,6-Dimethoxy-2-(propan-2-yl)-2,5-dihydropyrazine (2.9 mL, 16 mmol) was dissolved in THF (58 mL) and the solution cooled to -78°C . *n*-Butyl lithium (10 mL, 1.6 M, 16 mmol) was added dropwise with stirring. Stirring was continued for 15 min at -78°C . (Chloromethyl)(trimethyl)silane (5.0 mL, 36 mmol) was added and the resulting mixture was stirred at ambient temperature overnight. The reaction mixture was diluted with water and extracted with diethyl ether. The combined organic layer was dried over anhydrous sodium sulfate, filtered, and then concentrated. The crude product was purified by chromatography on silica gel (cyclohexane / ethyl acetate 2%) to afford 3.0 g (87 % purity, 60 % yield) of the title compound.

LC-MS (Method 9): $R_t = 1.47$ min; MS (ESI pos): $m/z = 271$ $[M+H]^+$

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: -0.036 (0.45), -0.028 (9.14), -0.020 (0.46), -0.008 (1.38), 0.580 (3.69), 0.597 (3.75), 0.737 (0.61), 0.762 (0.63), 0.773 (0.73), 0.798 (0.73), 0.959 (3.60), 0.977 (3.69), 1.107 (0.73), 1.119 (0.75), 1.143 (0.66), 1.148 (0.47), 1.155 (0.63), 1.370 (1.30), 2.512 (16.00), 3.574 (8.95), 3.582 (8.85), 3.885 (0.64), 3.894 (1.26), 3.903 (0.72), 3.999 (0.49), 4.023 (0.47).

Example 68A

Methyl 3-(trimethylsilyl)-*L*-alaninate

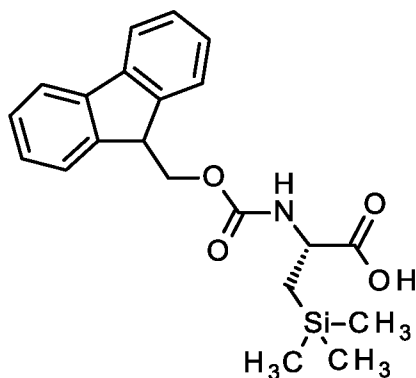


(2*R*, 5*R*)-3,6-Dimethoxy-2-(propan-2-yl)-5-[(trimethylsilyl)methyl]-2,5-dihydropyrazine (Example 67A, 3.00 g, 11.1 mmol) was dissolved in methanol (30 mL, 740 mmol) and the solution was cooled to 0°C. Hydrochloric acid (10 mL, 10%) was added and the solution was stirred for 2 h. The solution was evaporated and the residue was dissolved in DCM and sodium carbonate solution (100 mL, 2.0 M, 200 mmol). The aqueous layer was extracted with two portions of DCM. The DCM extract was dried over anhydrous magnesium sulfate, filtered, and then concentrated. The crude product was purified by chromatography on silica gel (2% methanol: (1% *N,N*-diethylethanamine in DCM)) to give 2.83g (68 % purity, 99 % yield) of the title compound.

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: -0.010 (3.10), 0.008 (1.81), 0.736 (0.85), 0.759 (0.89), 0.772 (1.48), 0.795 (1.56), 0.803 (1.89), 0.820 (1.90), 0.841 (1.84), 0.858 (2.43), 0.875 (1.64), 0.900 (4.19), 0.911 (1.07), 0.918 (8.65), 0.936 (4.28), 1.682 (1.42), 2.384 (1.35), 2.402 (4.11), 2.420 (4.02), 2.438 (1.25), 3.088 (0.55), 3.102 (0.54), 3.302 (1.19), 3.306 (1.81), 3.321 (1.27), 3.330 (1.15), 3.345 (1.03), 3.583 (16.00), 3.591 (0.48), 3.605 (4.33), 5.742 (4.37).

Example 69A

N-{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}-3-(trimethylsilyl)-*L*-alanine



Methyl 3-(trimethylsilyl)-L-alaninate (Example 68A, 2.83 g, 68 % purity, 11.0 mmol) was dissolved in methanol (44 mL) and the solution cooled to 0°C. A sodium hydroxide solution (11 mL, 1.0 M, 11 mmol) was added and the reaction mixture was stirred over night at room temperature. Water (40 mL), sodium hydrogen carbonate (922 mg, 11.0 mmol) and sodium carbonate (1.53 g, 14.4 mmol) were added. The methanol was removed under reduced pressure and then 1,4-dioxane (32 mL) was added to the aqueous solution. This solution was cooled to 0°C in an ice bath. (9H-Fluoren-9-yl)methyl carbonochloridate (Fmoc-Cl) (4.26 g, 16.5 mmol) was then added and the reaction mixture was stirred for 5 h at room temperature. Additional Fmoc-Cl (1.42 g, 5.5 mmol) was added and the reaction mixture was stirred for an additional 2 h. Equal portions of water and *tert*-butyl methyl ether were added to the reaction mixture. The aqueous phase was separated and washed further with *tert*-Butyl methyl ether, then acidified with 1 M hydrochloric acid (pH 3-4). The acidified mixture was extracted with three portions of MTBE. The combined organic layer was dried over anhydrous magnesium sulfate, filtered, and then concentrated. The crude product was purified by preparative HPLC (column: Reprosil; C18; 10 µm; 125 x 40 mm; eluant: AcCN/H₂O with 0,1% HCOOH; flow rate: 100 mL/min; gradient: 0 - 5.50 min 10 : 90; sample injection at 3.00 min; 5.50 - 17.65 min to 95 : 5; 17.65 - 19.48 min 95 : 5; 19.48 - 19.66 min to 10 : 90; 19.66 - 20.72 min 10 : 90;) to give 1.65 g (99 % purity, 39 % yield) of the title compound.

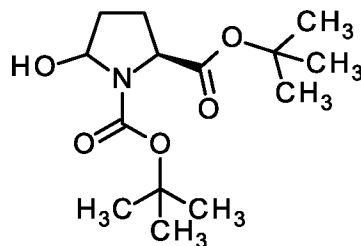
LC-MS (Method 10): R_t = 2.20 min; MS (ESI pos): m/z = 384 [M+H]⁺

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: -0.006 (16.00), 0.987 (3.81), 1.006 (3.64), 3.309 (2.95), 3.950 (0.67), 3.970 (1.71), 3.991 (1.59), 4.010 (0.53), 4.182 (0.67), 4.199 (1.66), 4.217 (1.67), 4.228 (1.08), 4.253 (2.79), 4.268 (3.86), 4.287 (1.60), 4.293 (0.85), 4.313 (0.41), 7.286 (1.24), 7.291 (1.27), 7.293 (1.31), 7.302 (2.68), 7.304 (2.70), 7.309 (2.55), 7.321 (1.75), 7.323 (1.70), 7.328 (1.57), 7.389 (2.90), 7.408 (4.73), 7.426 (2.11), 7.566 (1.81), 7.587 (1.74), 7.697 (2.14), 7.714 (3.62), 7.731 (1.81), 7.872 (4.43), 7.891 (4.02), 12.451 (0.54).

This amino acid was used in Fmoc SPPS.

Example 70A

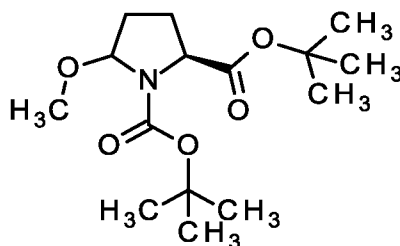
Di-*tert*-butyl (2*S*)-5-hydroxypyrrolidine-1,2-dicarboxylate (mixture of isomers)



- 5 Di-*tert*-butyl (2*S*)-5-oxopyrrolidine-1,2-dicarboxylate (CAS-RN: 91229-91-3, 21.0 g, 73.6 mmol) was dissolved in 250 mL THF and the solution was cooled to -78°C. Diisobutyl aluminum hydride (160 mL, 1.0 M, 160 mmol) was added dropwise and the mixture was stirred for 1 h at -78 °C. The reaction was then quenched with 2-propanol (25 mL). Potassium sodium (2*R*,3*R*)-2,3-dihydroxybutanedioate solution (200 mL) was added, the mixture was stirred at room temperature, and then the reaction mixture was
- 10 diluted with MTBE and water. The water layer was separated and extracted further with two portions of MTBE. The combined organic extract was dried over anhydrous magnesium sulfate, filtered, and the residue concentrated under reduced pressure to give 19.53 g (79% purity, 73% yield) of the title compound as a colorless oil.

Example 71A

- 15 Di-*tert*-butyl (2*S*,-)-5-methoxypyrrolidine-1,2-dicarboxylate (mixture of isomers)

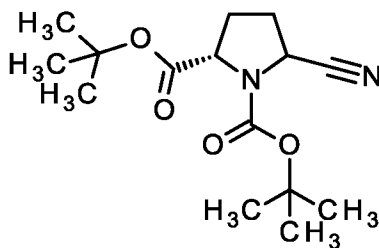


- Di-*tert*-butyl (2*S*)-5-hydroxypyrrolidine-1,2-dicarboxylate (Example 70A ,19.5 g, 67.9 mmol) was dissolved in methanol (160 mL). Pyridinium para-toluenesulfonate (341 mg, 1.36 mmol) was added to the
- 20 solution and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated and the crude product was purified by flash chromatography on silica gel (gradient cyclohexane - cyclohexane ethyl acetate 7:3) to afford 14.4 g (90 % purity, 64 % yield) of the title compound.

- ¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 1.363 (9.51), 1.375 (8.10), 1.393 (2.67), 1.419 (16.00), 1.756 (0.58), 1.775 (0.93), 1.781 (0.88), 1.807 (0.46), 3.225 (3.77), 3.240 (1.93), 3.261 (2.02), 3.307 (4.01),
- 25 4.059 (0.64), 4.081 (0.82), 5.128 (0.56).

Example 72A

Di-*tert*-butyl (2*S*)-5-cyanopyrrolidine-1,2-dicarboxylate (mixture of isomers)

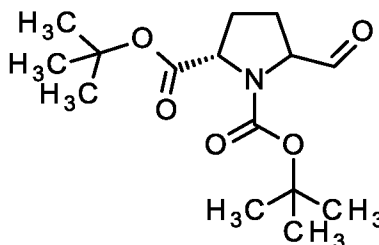


- 5 Di-*tert*-butyl (2*S*)-5-methoxypyrrolidine-1,2-dicarboxylate (Example 71A, 14.4 g, 47.8 mmol) was dissolved in DCM (125 mL) and the solution was cooled to -40°C. Trimethylsilyl trifluoromethanesulfonate (1.3 mL) (1 vol%) was added, then trimethylsilanecarbonitrile (7.0 mL, 53 mmol) was added dropwise over a 40 min period. The mixture was subsequently stirred for an additional 1.5 h at -40°C. The reaction mixture was quenched with methanol (19 mL) and then concentrated to afford 14.87 g (86 % yield) of the
- 10 title compound were isolated.

GC-MS (GC-MS Method 1): 195.2 (M⁺-Boc), 139.1 (-Boc, -*t*-Bu)

Example 73A

Di-*tert*-butyl (2*S*)-5-formylpyrrolidine-1,2-dicarboxylate (mixture of isomers)

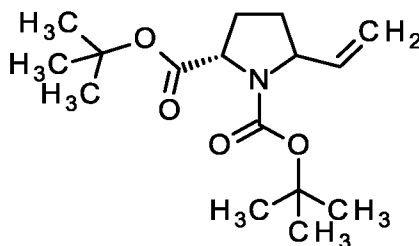


- 15 Di-*tert*-butyl (2*S*)-5-cyanopyrrolidine-1,2-dicarboxylate (Example 72A, 14.0 g, 47.2 mmol) was dissolved in a mixture of pyridine (250 mL), acetic acid (130 mL) and water (130 mL). A Raney-Nickel-water suspension (70.0 g, 50 % purity) was added and the mixture was hydrogenated over 7 h at ambient pressure at 80°C. The catalyst was then removed by filtration over a layer of diatomaceous earth and the solvent
- 20 was washed with 250 mL of water. The aqueous phase was extracted three times with MTBE (650 mL). The combined organic layer was washed with water three times, washed with brine, dried over anhydrous magnesium sulfate and then filtered. The filtrate was evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel (gradient cyclohexane – cyclohexane/ethyl acetate 7:3) to afford 7.40 g (85 % purity, 44 % yield) of a viscous oil.

- 25 GC-MS (GC-MS Method 1): (M⁺ - CO); (M⁺ - CO, - Boc)

Example 74A

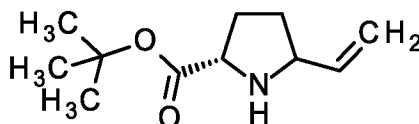
Di-*tert*-butyl (2*S*)-5-ethenylpyrrolidine-1,2-dicarboxylate (mixture of isomers)



- 5 Methyl(triphenyl)phosphonium bromide (17.7 g, 49.4 mmol) was suspended in 180 mL of THF. Potassium bis(trimethylsilyl)amide solution (1M in toluene, 75 mL, 15 % solution, 49 mmol) was added at dropwise at room temperature and stirred for 1 h. The mixture was cooled to -78°C. Di-*tert*-butyl (2*S*)-5-formylpyrrolidine-1,2-dicarboxylate (Example 73A, 7.40 g, 24.7 mmol) was dissolved in THF (60 mL). The solution was added and stirred for 2 h at room temperature. The reaction mixture was poured into a
- 10 solution of potassium sodium (2*R*,3*R*)-2,3-dihydroxybutanedioate (140 mL) and water (85 mL) and extracted with three portions of MTBE (210 mL). The combined organic extract was washed with sodium chloride solution, dried over anhydrous sodium sulfate, filtered and then concentrated. The crude product was purified by flash chromatography on silica gel (gradient cyclohexane - cyclohexane ethyl acetate (9:1)) to afford 4.25 g (100 % purity, 58 % yield) of the title compound were isolated.
- 15 GC-MS (GC-MS Method 1): Rt = 5.07 min, 5.17 min; 197.3 (M⁺-Boc)

Example 75A

tert-Butyl 5-vinyl-L-prolinate (mixture of isomers)

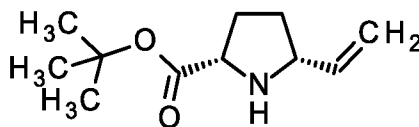


- 20 Di-*tert*-butyl (2*S*)-5-ethenylpyrrolidine-1,2-dicarboxylate (Example 74A, 4.25 g, 14.3 mmol) was dissolved in 45 mL DCM and the solution was cooled to 0°C. Trimethylsilyl trifluoromethanesulfonate (2.6 mL, 14 mmol) was added dropwise and the resulting mixture was stirred for 5 min at 0 °C. The reaction was then quenched with concentrated aqueous sodium hydrogen carbonate solution (21 mL) and extracted with two portions of DCM. The combined organic layers were dried over magnesium sulfate, filtered and
- 25 then concentrated. The crude product was purified by flash chromatography on silica gel (gradient methanol - dichloromethane (100:2)) to afford 2.09 g (100 % purity, 74 % yield) of the title compound.

GC-MS (GC-MS Method 1): Rt = 3.37 min, 3.50 min; 197.3 (M⁺)

Example 76A

tert-Butyl (5*R*)-5-ethenyl-*L*-prolinate (single stereoisomer)



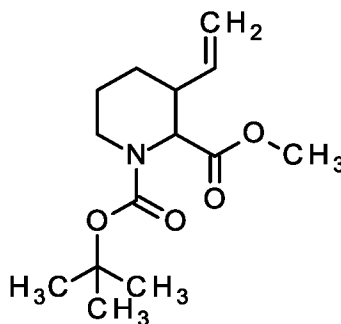
- 5 Isomeric separation of *tert*-Butyl-5-ethenyl-*L*-prolinate (diastomeric mixture) (Example 75A, 2.55 g, 12.9 mmol) by flash chromatography on silica gel (gradient cyclohexane - ethyl acetate - trimethylamine (70:30:1)) afforded 1.35 g (100% purity, 53 % yield) of the title compound.

GC-MS (GC-MS Method 1): R_t = 3.50 min; 197.3 (M^+)

- ¹H-NMR (400 MHz, DMSO-*d*₆) δ [ppm]: 1.3-1.4 (1H), 1.40 (9H), 2.4 (1H), 3.5 (2H), 4.9 (1H), 5.15 (1H),
10 5.8 (1H).

Example 77A

1-*tert*-Butyl 2-methyl-3-ethenylpiperidine-1,2-dicarboxylate (mixture of isomers)

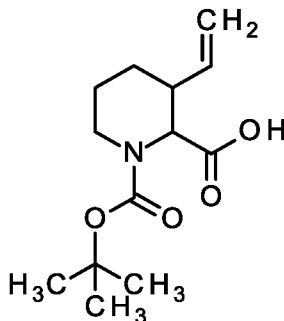


- 15 A solution of ethenylmagnesium bromide (39 mL, 0.70 M, 27 mmol) in anhydrous THF (20 mL) was cooled to -35°C and then a solution of copper (I) bromide – dimethyl sulfide (1:1) (741 mg, 3.61 mmol) was added. The reaction mixture was stirred for 1 h at -35°C. A solution of 1-*tert*-Butyl 2-methyl 5,6-dihydropyridine-1,2(4*H*)-dicarboxylate (CAS-RN: 155905-80-9, 4.35 g, 18.0 mmol) in THF (10 mL) was added to the mixture, and then the reaction mixture was stirred for 6 h at -35°C. The mixture was quenched
20 with a mixture of aqueous ammonium chloride (85 mL) and aqueous ammonia (85 mL) and extracted with two portions of MTBE (285 mL). The combined organic layers were washed sequentially with aqueous ammonium chloride (570 mL) and brine (570 mL), dried over magnesium sulfate, filtered, and then concentrated. The crude product was purified by chromatography on silica gel (gradient: cyclohexane- ethyl acetate (2%-20%)) to give 1.59 g (100% purity, 33 % yield) of the title compound.
- 25 LC-MS (Method 12): R_t = 3.33 min; MS (ESI pos): m/z = 270 [$M+H$]⁺

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 1.038 (0.51), 1.087 (0.77), 1.382 (16.00), 1.419 (0.83), 1.429 (0.59), 1.448 (0.73), 1.459 (0.80), 1.466 (0.54), 1.479 (1.01), 1.485 (1.09), 1.496 (0.73), 1.502 (0.66), 1.506 (0.59), 1.514 (0.47), 1.532 (0.47), 1.544 (0.42), 1.600 (0.64), 1.627 (0.61), 2.899 (0.88), 3.690 (13.11), 3.817 (0.47), 3.849 (0.45), 5.120 (2.03), 5.148 (0.90), 5.164 (1.49), 5.869 (0.57).

5 Example 78A

1-(*tert*-Butoxycarbonyl)-3-ethenylpiperidine-2-carboxylic acid (mixture of isomers)

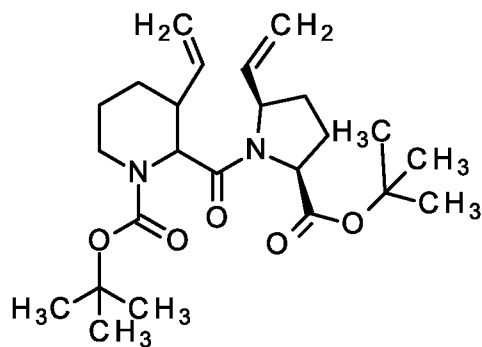


1-*tert*-Butyl 2-methyl 3-ethenylpiperidine-1,2-dicarboxylate (Example 77A, 1.59 g, 5.90 mmol) was dissolved in a mixture of methanol (5.9 mL) and THF (17.7 mL). A lithium hydroxide solution (5.9 mL, 2.0 M, 12 mmol) was added and the mixture was stirred for 5 h at 50°C, then cooled to room temperature. The reaction mixture was washed with MTBE (7.5 mL), cooled to 0°C, acidified with 1 M hydrochloric acid (14.5 mL), then extracted five times with DCM (15 mL). The combined organic extract was dried over magnesium sulfate, filtered, and then concentrated to afford 1.39 g (100 % purity, 92% yield) of the title compound.

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 1.381 (16.00), 1.423 (0.70), 1.434 (0.57), 1.455 (0.77), 1.465 (1.32), 1.473 (0.93), 1.488 (1.66), 1.495 (1.29), 1.519 (0.46), 1.530 (0.40), 1.608 (0.80), 1.632 (0.75), 2.911 (0.96), 3.815 (0.50), 3.842 (0.47), 5.113 (1.95), 5.140 (0.73), 5.156 (1.59), 5.753 (0.50), 5.874 (0.51), 12.910 (0.69).

20 Example 79A

tert-Butyl 2-{[(2*S*, 5*R*)-2-(*tert*-butoxycarbonyl)-5-vinylpyrrolidin-1-yl]carbonyl}-3-vinylpiperidine-carboxylate (mixture of isomers)

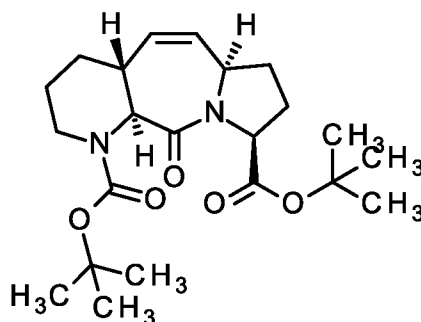


1-(*tert*-Butoxycarbonyl)-3-ethenylpiperidine-2-carboxylic acid (racemate) (Example 78A, 1.35 g, 5.30 mmol) and *tert*-butyl (5*R*)-5-ethenyl-*L*-prolinate (Example 76A, 950 mg, 4.82 mmol) were dissolved in acetonitrile (60 mL). *N,N*-diisopropylethylamine (2.5 mL, 14 mmol) was added and the reaction mixture was cooled to 0°C. A solution of 1*H*-benzotriazol-1-yloxy)(tripyrrolidin-1-yl)phosphonium hexafluorophosphate (3.26 g, 6.26 mmol) in acetonitrile (30 mL) was added to the mixture, and the reaction mixture was stirred for 20 h at room temperature. The reaction mixture was concentrated, redissolved in MTBE and washed with water. The aqueous phase re-extracted with MTBE. The combined organic layer was washed with brine, dried over magnesium sulfate, filtered, and then concentrated. The crude product was purified by chromatography on silica gel (cyclohexane / ethyl acetate (85:15)) to give 2.03 g (88 % yield) of the title compound.

¹H-NMR (400 MHz, DMSO-*d*₆) δ [ppm]: -0.008 (0.60), 1.373 (10.15), 1.392 (16.00), 1.396 (14.53), 1.458 (0.57), 1.489 (0.46), 1.763 (0.46), 1.780 (0.56), 2.105 (0.42), 2.122 (0.42), 2.524 (0.41), 5.050 (0.44), 5.090 (0.70), 5.116 (0.79), 5.754 (1.04).

Example 80A

di-tert-Butyl (4*aR*,6*aR*,9*S*,11*aS*)-11-oxo-2,3,4,4*a*,6*a*,7,8,9,11,11*a*-decahydro-1*H*-pyrido[3,2-*e*]pyrrolo[1,2-*a*]azepine-1,9-dicarboxylate (single stereoisomer)



tert-Butyl 2-{[(2*S*,5*R*)-2-(*tert*-butoxycarbonyl)-5-vinylpyrrolidin-1-yl]carbonyl}-3-vinylpiperidine-carboxylate (Example 79A, 2.00 g, 4.60 mmol) was dissolved in DCM (100 mL) and [1,3-bis(2,4,6-trimethylphenyl)imidazolidin-2-ylidene](dichloro)(phenylmethyldene)ruthenium-tricyclohexylphosphane

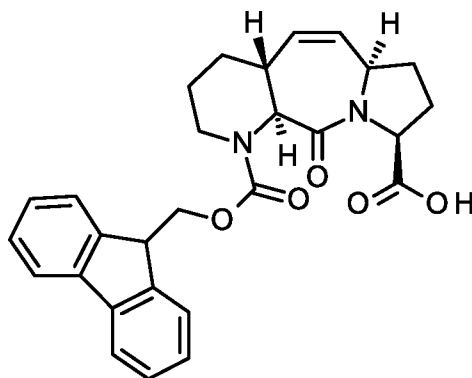
(1:1) (781 mg, 920 μ mol) was added. The reaction mixture was stirred for 48 h under reflux, then it was cooled to room temperature. DMSO (2.0 mL) was added and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated and the crude product was purified by chromatography on silica gel (cyclohexane / ethyl acetate (25%-50%)) to afford title 805 mg (99 % purity, 42 % yield) of the title compound.

LC-MS (Method 9): R_t = 1.12 min; MS (ESI pos): m/z = 407 $[M+H]^+$

^1H -NMR (400 MHz, DMSO- d_6) δ [ppm]: 1.296 (5.30), 1.361 (16.00), 1.378 (2.93), 5.540 (0.72), 5.562 (0.51).

Example 81A

- 10 (4*aR*,6*aR*,9*S*,11*aS*)-1- $\{[(9H$ -fluoren-9-yl)methoxy]carbonyl $\}$ -11-oxo-2,3,4,4*a*,6*a*,7,8,9,11,11*a*-decahydro-1*H*-pyrido[3,2-*e*]pyrrolo[1,2-*a*]azepine-9-carboxylic acid (single stereoisomer)



- Di-*tert*-Butyl-(4*aR*,6*aR*,9*S*,11*aS*)-11-oxo-2,3,4,4*a*,6*a*,7,8,9,11,11*a*-decahydro-1*H*-pyrido[3,2-*e*]pyrrolo[1,2-*a*]azepine-1,9-dicarboxylate (Example 80A, 800 mg, 1.97 mmol) was dissolved in 8 mL DCM and the solution cooled to 0°C. Trifluoroacetic acid (8.0 mL, 100 mmol) was added and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was concentrated and the residue was diluted with sodium hydrogen carbonate solution (20 mL, pH=8). A solution of (9*H*-fluoren-9-yl)methyl carbonochloridate (CAS-RN: 28920-43-6, 764 mg, 2.95 mmol) in THF (24 mL) was added and the reaction mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with DCM (100 mL) and acidified with 1 M hydrochloric acid (40 mL) (pH=1). The solution was extracted two times with DCM (50 mL) and the combined organic extract was dried over magnesium sulfate, filtered and then concentrated. Purification of the crude product by column chromatography on silica gel (DCM/MeOH 95/5) gave 676 mg (100% purity, 73 % yield) of the title compound.

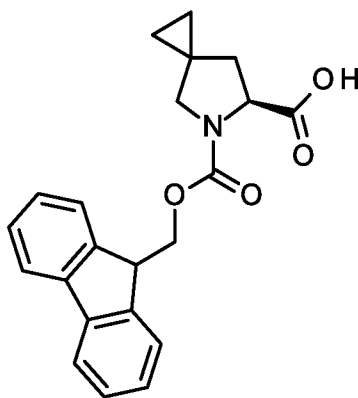
- LC-MS (Method 10): R_t = 1.86 min; MS (ESI pos): m/z = 473 $[M+H]^+$

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: -0.008 (2.87), 0.008 (2.54), 0.778 (0.66), 0.800 (1.51), 0.808 (1.58), 0.832 (2.85), 0.856 (1.93), 0.865 (2.01), 0.889 (0.79), 1.125 (0.77), 1.157 (1.38), 1.182 (0.90), 1.215 (0.50), 1.235 (1.06), 1.517 (2.61), 1.538 (3.44), 1.563 (6.49), 1.593 (6.17), 1.610 (5.67), 1.636 (4.98), 1.660 (3.92), 1.684 (2.46), 1.719 (2.25), 1.747 (2.01), 1.846 (4.49), 1.858 (7.62), 1.868 (6.96), 1.901 (3.19), 2.010 (0.57), 2.073 (9.83), 2.189 (2.95), 2.200 (3.74), 2.241 (3.30), 2.276 (2.71), 2.307 (1.21), 2.327 (1.10), 2.366 (0.52), 2.398 (0.80), 2.428 (1.41), 2.456 (0.84), 2.670 (0.63), 2.710 (0.50), 2.816 (1.60), 2.833 (1.66), 2.849 (2.51), 2.862 (2.28), 2.877 (2.34), 2.895 (1.47), 3.040 (0.69), 3.075 (1.10), 3.086 (1.04), 3.101 (1.03), 3.121 (0.69), 3.690 (1.24), 3.724 (4.64), 3.744 (6.62), 3.756 (6.47), 3.777 (4.44), 3.823 (8.20), 3.852 (7.84), 4.120 (4.36), 4.137 (4.86), 4.148 (4.24), 4.191 (3.57), 4.202 (7.64), 4.212 (4.19), 4.261 (1.02), 4.276 (2.33), 4.291 (4.52), 4.316 (4.20), 4.328 (5.37), 4.346 (4.61), 4.370 (3.31), 4.383 (5.08), 4.395 (4.54), 4.411 (5.53), 4.422 (5.83), 4.456 (3.22), 4.595 (1.63), 4.761 (4.95), 4.773 (5.04), 4.788 (4.59), 4.800 (4.19), 5.364 (2.72), 5.393 (7.67), 5.422 (7.48), 5.451 (2.73), 5.510 (0.98), 5.540 (4.23), 5.553 (4.14), 5.585 (0.91), 7.282 (3.12), 7.300 (7.84), 7.319 (10.54), 7.337 (13.57), 7.355 (9.71), 7.366 (2.95), 7.377 (5.06), 7.399 (12.17), 7.420 (16.00), 7.438 (6.68), 7.521 (8.10), 7.539 (6.98), 7.613 (12.53), 7.631 (11.16), 7.646 (3.71), 7.665 (3.16), 7.847 (8.31), 7.865 (15.04), 7.884 (10.42), 7.908 (4.87), 12.429 (2.52).

This amino acid was used in Fmoc SPPS.

Example 82A

(6S)-5-[[[(9H-Fluoren-9-yl)methoxy]carbonyl]-5-azaspiro[2.4]heptane-6-carboxylic acid



20

(6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid (CAS-RN:152723-57-4, 1.38 g, 9.79 mmol) was dissolved in water (14 mL) and sodium hydrogen carbonate (8.23 g, 97.9 mmol) was added. Then a solution from 1-([[(9H-fluoren-9-yl)methoxy]carbonyl]oxy)pyrrolidine-2,5-dione (CAS-RN:82911-69-1, 3.47 g, 10.3 mmol) in acetone (21 mL) was added and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with water and extracted two times with MTBE. The aqueous layer was acidified to pH 3-4 with 1M hydrochloric and extracted three times with DCM. The combined organic

25

phase was dried over anhydrous magnesium sulfate, filtered, and then concentrated, to afford 3.3 g (100 % purity, 93 % yield) of the title compound.

LC-MS (Method 9): $R_t = 1.04$ min; MS (ESI pos): $m/z = 364$ $[M+H]^+$

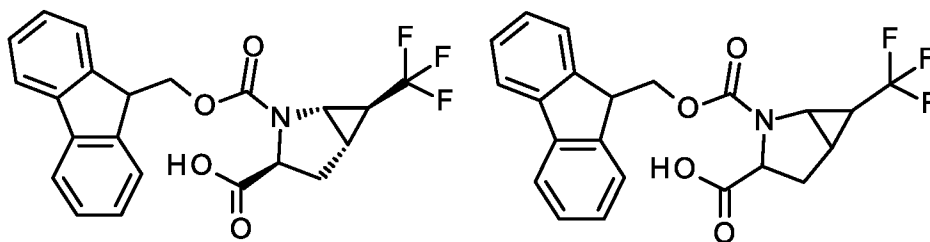
^1H -NMR (400 MHz, DMSO- d_6) δ [ppm]: 0.497 (1.67), 0.510 (2.34), 0.527 (5.55), 0.559 (3.82), 0.567 (5.51), 0.590 (9.33), 0.598 (11.21), 0.619 (1.87), 0.636 (0.58), 1.106 (9.23), 1.714 (2.14), 1.723 (2.15), 1.745 (2.40), 1.755 (2.50), 1.764 (1.94), 1.772 (1.85), 1.796 (2.02), 1.804 (1.93), 2.086 (7.36), 2.280 (2.12), 2.301 (2.47), 2.311 (2.21), 2.333 (2.24), 2.390 (1.75), 2.412 (2.07), 2.421 (1.87), 2.443 (1.71), 2.524 (0.88), 2.594 (3.10), 3.076 (2.73), 3.219 (3.24), 3.245 (4.13), 3.283 (3.41), 3.309 (5.19), 3.385 (5.05), 3.403 (4.32), 3.410 (3.56), 3.429 (3.16), 3.747 (1.01), 4.160 (0.68), 4.175 (1.57), 4.187 (3.09), 4.192 (3.69), 4.209 (6.08), 4.218 (4.18), 4.224 (3.09), 4.237 (2.43), 4.247 (1.22), 4.254 (2.15), 4.272 (16.00), 4.284 (3.83), 4.297 (4.75), 4.306 (2.72), 4.427 (2.10), 4.435 (2.31), 4.449 (2.34), 4.457 (2.02), 5.754 (0.45), 7.301 (1.47), 7.318 (5.18), 7.320 (5.43), 7.325 (2.74), 7.336 (7.24), 7.339 (7.37), 7.344 (4.81), 7.348 (3.25), 7.352 (3.50), 7.354 (3.84), 7.357 (3.67), 7.403 (9.03), 7.422 (14.80), 7.440 (6.64), 7.640 (8.10), 7.647 (7.82), 7.658 (7.43), 7.666 (6.87), 7.884 (10.09), 7.902 (9.58).

This amino acid was used in Fmoc SPPS.

This Fmoc amino acid was also prepared from (6*S*)-5-azaspiro[2.4]heptane-6-carboxylic acid prepared in Example 124A.

Example 83A

(1*S*,3*S*,5*S*,6*S*)-2-({(9*H*-Fluoren-9-yl)methoxy}carbonyl)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid and rel-(1*R*,3*R*,5*R*,6*R*)-2-({(9*H*-Fluoren-9-yl)methoxy}carbonyl)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid



(1*S*, 3*S*, 5*S*, 6*S*)-6-(Trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid (450 mg, 2.31 mmol) was dissolved in water (6 mL) and sodium hydrogen carbonate (1.94 g, 23.1 mmol) was added. Then a solution from 1-({(9*H*-fluoren-9-yl)methoxy}carbonyl)oxy)pyrrolidine-2,5-dione (CAS-RN: 82911-69-1, 817 mg, 2.42 mmol) in acetone (9 mL) was added and the resulting suspension was stirred overnight at room temperature. The reaction mixture was diluted with water and extracted two times with MTBE. The aqueous layer was acidified to pH 3-4 with 1M hydrochloric and extracted three times with DCM. The

combined organic phase was dried over anhydrous magnesium sulfate, filtered and then concentrated. The crude product was purified by preparative HPLC (column: Reprosil; C18; 10 μ m; 125 x 40 mm; solvent: AcCN/H₂O containing 0,1% HCOOH; flow rate: 100 mL/min ; gradient: 0 - 5.50 min 10 : 90; sample injection at 3.00 min; 5.50 - 17.65 min gradient to 95 : 5; 17.65 - 19.48 min at 95 : 5; 19.48 - 19.66 min to 10 : 90; 19.66 - 20.72 min 10 : 90;), affording 571 mg (96 % purity, 57 % yield) of the title compound.

LC-MS (Method 9): R_t = 1.06 min; MS (ESI pos): m/z = 418 $[M+H]^+$

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 2.073 (0.99), 2.203 (4.88), 2.345 (2.00), 2.366 (1.94), 3.755 (2.94), 4.078 (1.74), 4.221 (1.50), 4.253 (4.04), 4.270 (5.55), 4.290 (4.95), 4.337 (2.21), 7.293 (3.93), 7.301 (4.07), 7.312 (9.32), 7.319 (8.88), 7.330 (6.19), 7.337 (5.57), 7.407 (9.40), 7.425 (16.00), 7.444 (7.29), 7.695 (5.70), 7.887 (13.98), 7.906 (12.87), 12.866 (0.63).

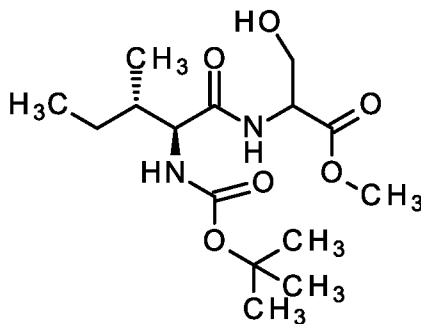
The substance is also commercially available (CAS RN: 1986905-54-7)

The racemic amino acid using rel-(1*R*, 3*R*, 5*R*, 6*R*)-6-(Trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid (Enamine) was prepared in a similar manner.

These amino acids were used in SPPS.

Example 84A

Methyl *N*-(*tert*-butoxycarbonyl)-*L*-isoleucylserinate (mixture of diastereomers)



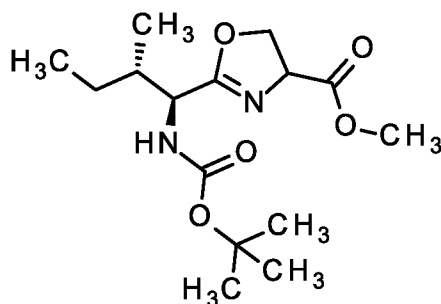
N-(*tert*-Butoxycarbonyl)-*L*-isoleucine (7.50 g, 32.4 mmol) and Methyl *DL*-serinate—hydrogen chloride (1/1) (5.05 g, 32.4 mmol) were dissolved in dichloromethane (65 mL). (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (14.9 g, 33.6 mmol) and *N,N*-diisopropylethylamine (11 mL, 65 mmol) were added and the reaction mixture was stirred overnight at room temperature. Dichloromethane (250 mL) was added and the reaction mixture was washed sequentially with water, aqueous hydrochloric acid (1M) and aqueous sodium hydrogen carbonate solution. The combined organic extract was dried over magnesium sulfate, filtered and then concentrated. The crude product was dissolved in DCM and thoroughly washed with water, two times again with aqueous hydrochloric acid (1M) and then two more times with aqueous sodium hydrogen carbonate. Subsequently the combined organic phase was

dried over magnesium sulfate, filtered, and then concentrated. The crude product was used directly for the next step.

LC-MS (Method 11): 355.2 (M^+Na^+)

Example 85A

- 5 Methyl 2-{(1*S*,2*S*)-1-[(*tert*-butoxycarbonyl)amino]-2-methylbutyl}-4,5-dihydro-1,3-oxazole-4-carboxylate (mixture of diastereomers)

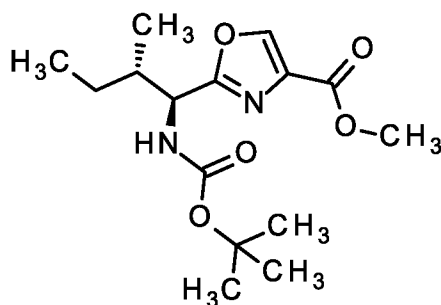


- Methyl *N*-(*tert*-butoxycarbonyl)-*L*-isoleucylserinate (Example 84A, 4.62 g, 13.9 mmol) was dissolved in dichloromethane (51 mL) and the solution cooled to -78°C . *N*-Ethyl-*N*-(trifluoro- λ^4 -sulfanyl)ethanamine (2.2 mL, 17 mmol) was added and the reaction mixture was stirred at -78°C for 1.5 h. The reaction was quenched with potassium carbonate (7.68 g, 55.6 mmol) and then allowed to cool to room temperature. The solution was stirred for 1 h at room temperature. Then the solution was diluted with DCM (50 mL) and washed three times with saturated aqueous sodium hydrogen carbonate solution. The aqueous phase was further extracted with DCM. The combined organic phase was washed with brine, dried over anhydrous magnesium sulfate, filtered, and then concentrated to afford 5.75 g of the desired product.

LC-MS (Method 11): 315.2 (M^+H^+), 337.2 (M^+Na^+)

Example 86A

Methyl 2-{(1*S*,2*S*)-1-[(*tert*-butoxycarbonyl)amino]-2-methylbutyl}-1,3-oxazole-4-carboxylate

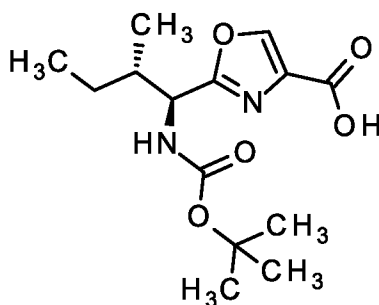


Methyl 2-{(1*S*,2*S*)-1-[(*tert*-butoxycarbonyl)amino]-2-methylbutyl}-4,5-dihydro-1,3-oxazole-4-carboxylate (Example 85A, 4.37 g, 13.9 mmol) was dissolved in dichloromethane (35 mL, 540 mmol) and the solution was cooled at -40°C. Bromo(trichloro)methane (2.7 mL, 27 mmol) was added, then 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (4.6 mL, 31 mmol) was also added. The reaction solution was stirred for 2.5 h at 0°C and then diluted with DCM. The reaction mixture was washed five times with a 10% citric acid solution. The combined aqueous layer was extracted with DCM. The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated. The crude product was purified by chromatography on silica gel (gradient: cyclohexane / ethyl acetate (4:1)) to give 2.2 g (50.7% yield) of the title compound.

10 LC-MS (Method 10): 335.15 (M^+Na^+)

Example 87A

2-{(1*S*,2*S*)-1-[(*tert*-Butoxycarbonyl)amino]-2-methylbutyl}-1,3-oxazole-4-carboxylic acid

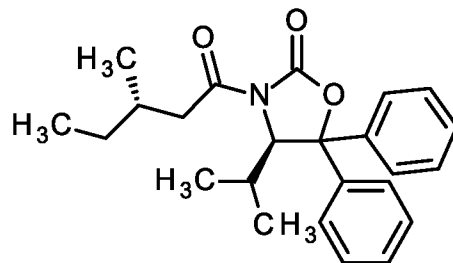


15 Methyl 2-{(1*S*,2*S*)-1-[(*tert*-butoxycarbonyl)amino]-2-methylbutyl}-1,3-oxazole-4-carboxylate (Example 86A, 2.21 g, 7.06 mmol) was dissolved in methanol (13.5 mL). Aqueous sodium hydroxide solution (4.3 mL, 3M) was added and the reaction mixture was stirred for 40 min and then concentrated to remove the methanol. The aqueous solution was acidified with aqueous 1M hydrochloric acid (14 mL) and extracted with ethyl acetate. The combined organic layer was dried over anhydrous magnesium sulfate, filtered and then concentrated under reduced pressure to give 12.0 g (95% purity, 51% yield) of the title compound.

LC-MS (MCW_OA_S1): R_t = 1.13 min; MS (ESI neg): m/z = 297 [$M+H$]⁻

Example 88A

(4*R*)-3-[(3*S*)-3-methylpentanoyl]-5,5-diphenyl-4-(propan-2-yl)-1,3-oxazolidin-2-one



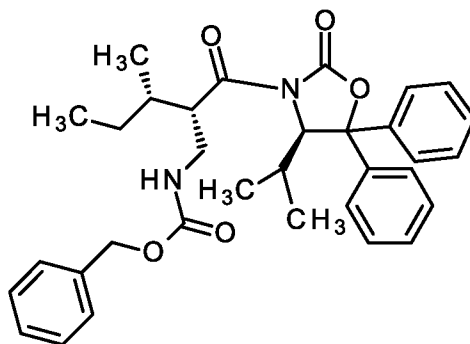
A solution of (3S)-3-methylpentanoic acid (867 mg, 7.46 mmol) in anhydrous THF (50 mL) was cooled to -30°C. 2,2-Dimethylpropanoyl chloride (920 µl, 7.5 mmol) was added dropwise and the reaction mixture was stirred for 1.5 h at -30°C. Dry lithium chloride (347 mg, 8.17 mmol) and (4R)-5,5-diphenyl-4-(propan-2-yl)-1,3-oxazolidin-2-one (2.00 g, 7.11 mmol) ((R)-DIOZ) were sequentially added and the reaction mixture was slowly allowed to come to room temperature and stirred overnight. The reaction mixture was diluted with ethyl acetate and water. The water layer was separated and extracted with ethyl acetate. The combined ethyl acetate layer was washed with aqueous sodium chloride solution, dried over magnesium sulfate, filtered, and then concentrated, to give 2.87 g of a white solid. The crude product was dissolved in DCM with a small amount of methanol and was purified by column chromatography over silica gel (Biotage Isolera, 50 g cartridge) with cyclohexane / ethyl acetate (80:20) as eluant. The product-containing fractions were combined, concentrated and dried to give 2.25 g (98 % purity, 82 % yield) of the target compound.

LC-MS (Method 10): R_t = 2.69 min; MS (ESI pos): m/z = 380 $[M+H]^+$

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: 0.616 (13.33), 0.633 (13.58), 0.650 (7.84), 0.660 (16.00), 0.668 (15.61), 0.676 (15.27), 0.686 (7.40), 0.719 (0.40), 0.875 (12.87), 0.893 (12.59), 0.940 (0.92), 0.958 (1.50), 0.975 (2.08), 0.993 (2.23), 1.011 (1.38), 1.028 (0.84), 1.045 (1.41), 1.061 (1.93), 1.079 (1.89), 1.095 (1.16), 1.112 (0.62), 1.541 (0.55), 1.557 (1.29), 1.574 (1.91), 1.590 (1.82), 1.607 (1.10), 1.623 (0.40), 2.020 (0.71), 2.036 (1.44), 2.049 (1.78), 2.065 (1.28), 2.082 (0.50), 2.449 (2.60), 2.468 (2.84), 2.755 (2.63), 2.771 (2.50), 2.793 (2.05), 2.808 (1.92), 5.557 (5.98), 5.563 (5.64), 7.271 (2.61), 7.289 (6.37), 7.307 (5.02), 7.352 (5.04), 7.368 (10.74), 7.385 (9.60), 7.403 (3.47), 7.557 (8.45), 7.577 (6.54), 7.639 (8.45), 7.659 (6.92).

Example 89A

Benzyl {(2S,3S)-3-methyl-2-[(4R)-2-oxo-5,5-diphenyl-4-(propan-2-yl)-1,3-oxazolidine-3-carbonyl]pentyl}carbamate



The literature procedure (Sebesta and Seebach, *Helv. Chim. Acta* **2003**, 86, 4061-4072) was followed. (4R)-3-[(3S)-3-methylpentanoyl]-5,5-diphenyl-4-(propan-2-yl)-1,3-oxazolidin-2-one (Example 88A,
 5 2.20 g, 5.80 mmol) was dissolved in DCM (20 mL) and the solution was cooled to -15°C. A solution of Titanium (IV) chloride (6.1 mL, 1.0 M in DCM, 6.1 mmol) was added dropwise while maintaining the temperature at -15°C. Triethylamine (890 µl, 6.4 mmol) was then added and the reaction mixture was stirred for 30 min at -15°C.

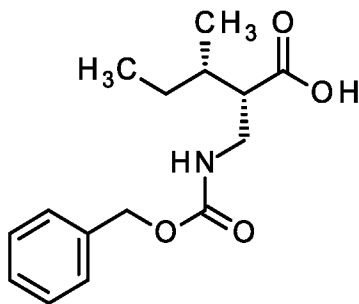
In a separate round-bottomed flask a solution of benzyl (methoxymethyl)carbamate (1.24 g, 6.38 mmol,
 10 CAS RN: 94471-35-9) in DCM (10 mL) was cooled to 0°C. A solution of Titanium (IV) chloride (6.4 mL, 1.0 M in DCM, 6.4 mmol) was added dropwise, and then the entire solution was added dropwise to the flask containing (4R)-3-[(3S)-3-methylpentanoyl]-5,5-diphenyl-4-(propan-2-yl)-1,3-oxazolidin-2-one. The reaction mixture was stirred for 4 h at 0°C, then it was allowed to stir at room temperature overnight. The reaction was quenched by adding an aqueous ammonium chloride solution and then diluted with
 15 additional DCM. The separated organic layer was washed with aqueous 1M HCl solution, aqueous 1M NaOH solution, and then with aqueous sodium chloride solution. The organic layer was filtered and then concentrated, to give 3.39 g of a yellow oil. The crude product was purified by column chromatography over silica gel (Biotage Isolera 100g cartridge) with cyclohexane/ethyl acetate (85:15) eluant. The product-containing fractions were combined, concentrated and then dried to give 1.50 g (96 % purity, 46 %
 20 yield, selectivity) of the title compound with a diastereoselectivity of 96,3% (lit. 96%).

LC-MS (Method 10): R_t = 2.63 min; MS (ESI pos): m/z = 543 $[M+H]^+$

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 0.429 (3.22), 0.446 (3.32), 0.480 (1.44), 0.498 (3.18), 0.516 (1.79), 0.595 (4.27), 0.611 (4.65), 0.643 (0.49), 0.663 (0.46), 0.768 (0.45), 0.786 (0.46), 0.874 (3.86), 0.891 (4.01), 1.098 (0.48), 1.397 (16.00), 1.993 (0.45), 2.010 (0.57), 2.023 (0.45), 3.146 (0.44), 3.169 (0.66), 3.180 (1.02), 3.192 (0.63), 3.206 (0.44), 3.222 (0.53), 3.243 (0.48), 3.816 (0.57), 4.920 (0.59), 4.952 (1.85), 4.978 (2.04), 5.010 (0.65), 5.539 (1.70), 5.544 (1.73), 7.187 (0.53), 7.201 (0.87), 7.282 (3.41), 7.289 (5.28), 7.306 (4.14), 7.322 (1.84), 7.342 (2.69), 7.362 (3.73), 7.385 (3.47), 7.405 (1.52), 7.566 (3.08), 7.584 (2.46), 7.662 (2.88), 7.681 (2.48).

Example 90A

(2S,3S)-2-({[(Benzyloxy)carbonyl]amino}methyl)-3-methylpentanoic acid



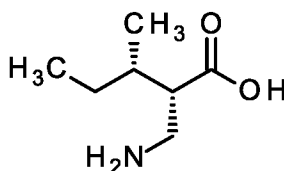
- 5 A solution of benzyl {(2S,3S)-3-methyl-2-[(4R)-2-oxo-5,5-diphenyl-4-(propan-2-yl)-1,3-oxazolidine-3-carbonyl]pentyl}carbamate (Example 89A, 1.49 g, 2.75 mmol) in THF (15 mL) was prepared. A solution of LiOH (105 mg, 4.39 mmol) in water (5 mL) was added and the reaction mixture was stirred at room temperature for 24 h. HPLC analysis indicated that the reaction was not completed, so an additional equiv-
- 10 alent of LiOH (65.8 mg, 2.75 mmol) in water (3 mL) was added. The reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with diethyl ether, the mixture was stirred for 5 minutes, and then filtered. The filter cake was washed with water and with ether, and then the layers were separated. The aqueous layer was acidified with aqueous 1M HCl solution to pH 1 and extracted three times with diethyl ether. The combined ether phase was washed with sodium chloride solution, dried over magnesium sulfate, filtered, and then concentrated. After drying under high vacuum, 715 mg (100 %
- 15 purity, 93 % yield) of the title compound was obtained.

LC-MS (Method 10): $R_t = 1.71$ min; MS (ESI pos): $m/z = 280$ $[M+H]^+$

- ¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 0.837 (6.34), 0.856 (14.23), 0.863 (13.72), 0.880 (12.65), 1.098 (0.91), 1.117 (1.35), 1.133 (1.47), 1.151 (1.49), 1.170 (0.95), 1.348 (0.42), 1.367 (1.02), 1.380 (1.33), 1.398 (1.53), 1.414 (1.09), 1.431 (0.72), 1.596 (0.82), 1.611 (1.42), 1.627 (1.61), 1.644 (1.16), 1.660 (0.53), 1.908 (3.41), 2.396 (1.17), 2.413 (2.79), 2.429 (2.67), 2.445 (1.07), 2.501 (16.00), 3.139 (4.27), 3.155 (7.41), 3.171 (4.60), 5.000 (15.52), 7.221 (1.46), 7.234 (2.66), 7.248 (1.41), 7.283 (0.89), 7.290 (0.97), 7.300 (2.98), 7.318 (5.22), 7.332 (11.34), 7.339 (12.29), 7.356 (5.30), 7.375 (1.45), 12.135 (3.58).
- 20

Example 91A

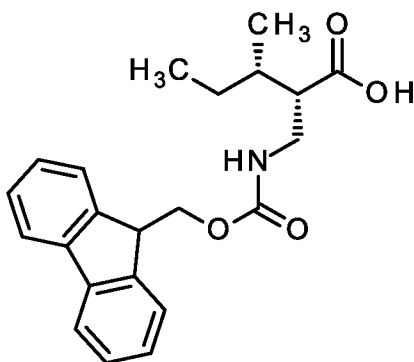
(2S,3S)-2-(Aminomethyl)-3-methylpentanoic acid



10% Palladium on carbon (100 mg) was weighed out into a round-bottomed flask and the flask was flushed with Argon. A solution of (2*S*,3*S*)-2-({[(benzyloxy)carbonyl]amino}methyl)-3-methylpentanoic acid (Example 90A, 710 mg, 2.54 mmol) in methanol (40 mL) was added and the reaction mixture was stirred at room temperature under a normal pressure of H₂ gas. HPLC analysis indicated that after 2 hours at room temperature, the reaction was completed. The reaction mixture was filtered through a plug of silica gel and the filter cake was washed several times with methanol. The combined filtrate was concentrated under reduced pressure to provide 345 mg (93%) of the title compound. The compound was used directly for the next step.

Example 92A

10 (2*S*,3*S*)-2-[(9*H*-Fluoren-9-yl)methoxy]carbonyl]amino)methyl]-3-methylpentanoic acid



(2*S*,3*S*)-2-(Aminomethyl)-3-methylpentanoic acid (Example 91A, 344 mg, 2.37 mmol) was dissolved in aqueous sodium carbonate solution (32 mL, 0.15 M, 4.7 mmol). 1-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}oxy)pyrrolidine-2,5-dione (959 mg, 2.84 mmol) was dissolved in acetone (30 mL) and this solution was added to the previous solution and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated to remove the acetone. The aqueous solution was diluted with water and extracted with ethyl acetate. The aqueous layer was acidified to pH 3-4 with 1M aqueous hydrochloric acid and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and then concentrated. The crude product was purified by preparative HPLC (column: Reprosil; C18; 10 μm; 125 x 30 mm; solvents: AcCN/H₂O containing 0.1% HCOOH; flow rate: 75 mL/min; gradient: 0 - 5.50 min (10:90); sample injection at 3.00 min; 5.50 - 17.65 min gradient to 95:5; 17.65 - 19.48 min (95:5); 19.48 - 19.66 min gradient to 10:90; 19.66 - 20.72 min (10:90) to give 795 mg (96 % purity, 88 % yield) of the title compound.

25 LC-MS (Method 9): R_t = 1.06 min; MS (ESI pos): m/z = 368 [M+H]⁺

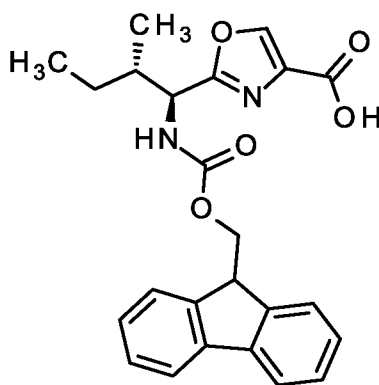
¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: -0.008 (1.04), 0.008 (0.54), 0.752 (0.73), 0.841 (7.69), 0.860 (16.00), 0.870 (15.28), 0.878 (10.54), 0.887 (13.22), 1.085 (0.41), 1.103 (0.99), 1.123 (1.45), 1.139 (1.58),

1.157 (1.60), 1.176 (1.00), 1.371 (1.16), 1.384 (1.50), 1.403 (1.71), 1.418 (1.23), 1.435 (0.83), 1.604 (0.88), 1.619 (1.50), 1.636 (1.70), 1.651 (1.23), 2.408 (1.27), 2.425 (2.91), 2.441 (3.13), 2.458 (1.88), 2.524 (0.87), 2.749 (0.82), 3.143 (4.18), 3.158 (6.08), 3.174 (3.40), 4.176 (1.25), 4.194 (3.04), 4.211 (4.04), 4.241 (8.74), 4.255 (4.27), 4.263 (3.35), 7.303 (5.42), 7.322 (12.94), 7.340 (10.81), 7.353 (1.98),
5 7.394 (7.49), 7.413 (11.83), 7.431 (5.34), 7.673 (5.88), 7.683 (5.84), 7.691 (5.19), 7.701 (4.30), 7.876 (12.54), 7.895 (11.30), 12.176 (1.21).

This amino acid was used in SPPS.

Example 93A

10 2-[(1*S*,2*S*)-1-({[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid



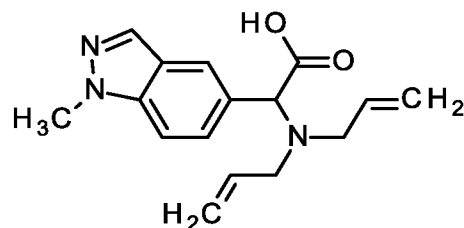
2-[(1*S*,2*S*)-1-[(*tert*-Butoxycarbonyl)amino]-2-methylbutyl]-1,3-oxazole-4-carboxylic acid (Example 87A, 1.85 g, 6.21 mmol) was dissolved in trifluoroacetic acid (7.6 mL, 99 mmol) and the solution was stirred for 15 min. Then the solution was diluted with toluene and evaporated. This step was repeated again. The crude product was dissolved in 1,4-dioxane (20 mL, 230 mmol) and water (20 mL). Potassium carbonate (1.60 g, 11.6 mmol) and 1-[(9*H*-fluoren-9-ylmethoxy)carbonyl]oxy}pyrrolidine-2,5-dione (2.35 g, 6.96 mmol) was added and the reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with water (65 mL) and acidified to pH 2-3 with a 10% aqueous citric acid solution. The reaction mixture was extracted with ethyl acetate (three times), and the combined organic layer was washed with brine, dried over magnesium sulfate, filtered, and then concentrated under reduced pressure. The residue was purified by chromatography on silica gel (dichloromethane - methanol (3-7% + 1% acetic acid), and the product-containing fractions were combined, diluted with toluene, and then concentrated. Toluene was then added again and then the solution was concentrated, to provide 2.09 g
20 (100% purity, 80 % yield) of the title compound.

LC-MS (MCW_FT_MS_M1): $R_t = 2.01$ min; MS (ESI pos): $m/z = 421$ $[M+H]^+$

This amino acid was used in SPPS.

Example 94A

di(Prop-2-en-1-yl)amino](1-methyl-1*H*-indazol-5-yl)acetic acid (racemate)



5

(1-Methyl-1*H*-indazol-5-yl)boronic acid (CAS-RN: 590418-08-9, 500 mg, 2.84 mmol), oxoacetic acid (CAS-RN: 298-12-4, 310 μ l, 50 % purity, 2.8 mmol) and *N*-(Prop-2-en-1-yl)prop-2-en-1-amine (CAS-RN:124-02-7, 350 μ l, 2.8 mmol) were dissolved in acetonitrile (7.5 mL) and the reaction mixture was stirred for 40 h at 60°C. After cooling, the resulting solid was filtered, washed with ethyl acetate and dried to give 740 mg (100 % purity, 91 % yield) of the title compound.

10

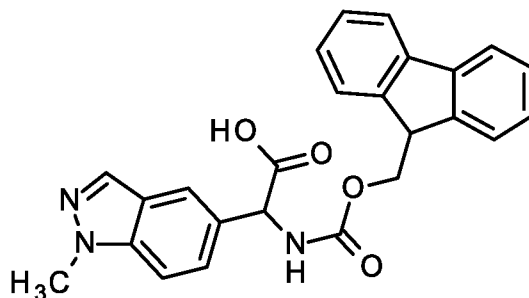
LC-MS (Method 9): R_t = 0.44 min; MS (ESI neg): m/z = 284 $[M-H]^-$

¹H-NMR (400 MHz, DMSO-*d*₆) δ [ppm]: 0.008 (1.87), 2.073 (0.51), 3.110 (0.68), 3.125 (0.77), 3.147 (2.14), 3.161 (2.16), 3.176 (2.32), 3.193 (2.28), 3.213 (0.90), 3.230 (0.96), 4.030 (16.00), 4.531 (3.61), 5.110 (2.47), 5.134 (4.58), 5.174 (2.56), 5.752 (0.60), 5.768 (1.07), 5.778 (0.73), 5.783 (0.73), 5.794 (1.49), 5.810 (1.35), 5.820 (0.63), 5.826 (0.67), 5.837 (0.87), 5.852 (0.49), 6.514 (1.52), 7.432 (1.39), 7.435 (1.47), 7.454 (1.77), 7.457 (1.87), 7.602 (2.26), 7.624 (1.75), 7.699 (3.10), 8.041 (4.18).

15

Example 95A

((9*H*-Fluoren-9-yl)methoxy]carbonyl)amino)(1-methyl-1*H*-indazol-5-yl)acetic acid (racemate)



20

1,3-dimethyl-1,3-diazinane-2,4,6-trione (CAS-RN: 769-42-6, 1.97 g, 12.6 mmol) and tetrakis(triphenylphosphine)palladium(0) (48.6 mg, 42.1 μ mol) were placed into a round-bottomed flask under Argon. A solution of [di(Prop-2-en-1-yl)amino](1-methyl-1*H*-indazol-5-yl)acetic acid (Example 94A, 600 mg, 2.10 mmol) in dry DCM was then added, and the reaction mixture was stirred for 2 h at 35°C. The

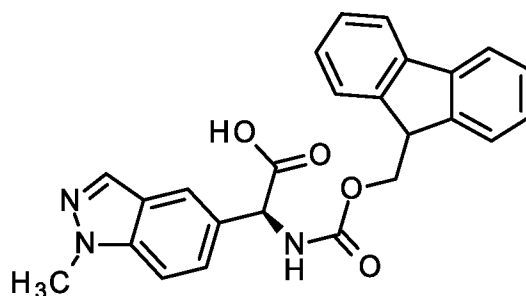
suspension was diluted with water acetone and water and then the reaction mixture was concentrated to remove the DCM. Sodium hydrogen carbonate (2.65 g, 31.5 mmol) was added (attention gas formation). 1-((9H-fluoren-9-yl)methoxy)carbonylpyrrolidine-2,5-dione (780 mg, 2.31 mmol) was added to the suspension and the reaction mixture was stirred overnight at room temperature. Water and an aqueous solution of sodium carbonate (10%) were added and the solution was extracted two times with MTBE. The aqueous phase was acidified with aqueous citric acid solution and then extracted three times with DCM. The combined organic layers were washed with brine, dried of magnesium sulfate, filtered, and then concentrated. The residue was purified by preparative HPLC (column: Reprosil; C18; 10 μ m; 125 x 40 mm; solvent: AcCN/H₂O containing 0.1% HCOOH; flow rate: 100 mL/min; gradient: 0 - 5.50 min (10:90); sample injection at 3.00 min; 5.50 - 17.65 min gradient to 95 : 5; 17.65 - 19.48 min (95:5); 19.48 - 19.66 min gradient to 10:90; 19.66 - 20.72 min (10:90) to give 422 mg (100 % purity, 47 % yield) of the title compound.

LC-MS (Method 9): R_t = 0.94 min; MS (ESI pos): m/z = 428 [M+H]⁺

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 2.073 (0.59), 4.041 (16.00), 4.198 (0.63), 4.215 (1.87), 4.231 (3.51), 4.253 (1.84), 4.269 (1.50), 4.280 (1.92), 4.298 (1.70), 4.319 (0.59), 5.256 (1.81), 5.276 (1.80), 7.275 (0.90), 7.293 (2.03), 7.320 (2.08), 7.339 (1.26), 7.388 (1.98), 7.406 (3.38), 7.424 (1.73), 7.445 (1.60), 7.467 (1.96), 7.621 (2.30), 7.643 (1.76), 7.744 (3.56), 7.763 (3.31), 7.792 (3.19), 7.875 (4.81), 7.893 (4.48), 8.063 (4.53), 8.213 (1.70), 8.232 (1.65), 12.835 (1.03).

Example 96A

((9H-Fluoren-9-yl)methoxy)carbonyl(1-methyl-1H-indazol-5-yl)acetic acid (enantiomer 1)



The diastereomeric mixture (Example 95A, 600 mg) was dissolved in methanol / acetonitrile (60 mL) and separated using a THAR supercritical fluid chromatography Prep 200 instrument using a Chiralpak AD-H (SFC) 5 μ m, 250 x 30 mm column; eluent: CO₂/iso-propanol (80:20); pressure: 135 bar; temperature eluent: 38°C; flow rate: 100 g/min; detection: UV 210 nm; injection volume 0.4 mL/injection; under general operating conditions 1g/min of CO₂ is approximately equivalent to 1 mL/min.

Sequence Settings: cycle time = 11.2 min

Fraction 1 was collected at 5.8 min (enantiomer 1); Fraction 2 was collected between 7.7 min (enantiomer 2). The fractions were concentrated.

The fractions were analyzed using analytical SFC-MS (Agilent, column: Daicel AD-3 3 μ m 100 x 4,6 mm, eluent: CO₂/methanol (80:20); BPR pressure: 130 bar; BPR temperature: 60°C; column temperature: 40°C; flow rate: 3 mL/min; UV 210 nm.

The stereochemistry of the two enantiomers was not assigned.

185 mg of enantiomer 1 (100% purity, 99.5% ee) were obtained (stereochemistry not determined)

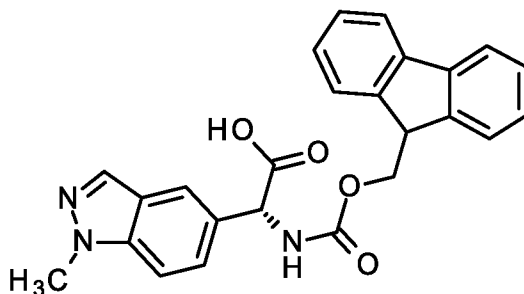
LC-MS (Method 10): R_t = 1.80 min; MS (ESI pos): m/z = 428 [M+H]⁺

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 4.040 (16.00), 4.197 (0.54), 4.214 (1.44), 4.230 (2.80), 4.251 (1.59), 4.268 (1.38), 4.278 (1.68), 4.295 (1.46), 4.316 (0.48), 5.241 (1.20), 5.261 (1.19), 7.275 (0.78), 7.294 (1.73), 7.321 (1.78), 7.339 (1.11), 7.386 (1.77), 7.394 (1.54), 7.405 (2.97), 7.412 (2.26), 7.423 (1.63), 7.444 (1.36), 7.466 (1.53), 7.617 (1.85), 7.638 (1.42), 7.742 (3.01), 7.761 (2.84), 7.787 (2.53), 7.874 (4.50), 7.893 (4.19), 8.058 (4.56), 8.183 (0.93), 8.201 (0.92).

This amino acid was used in SPPS.

Example 97A

{{[(9H-Fluoren-9-yl)methoxy]carbonyl}amino}(1-methyl-1H-indazol-5-yl)acetic acid (enantiomer 2)



Using the chiral separation method described in Example 96A, 145 mg (100 % purity, 94.3%) of the enantiomer 2 were obtained (stereochemistry not determined).

LC-MS (Method 10): R_t = 1.80 min; MS (ESI pos): m/z = 428 [M+H]⁺

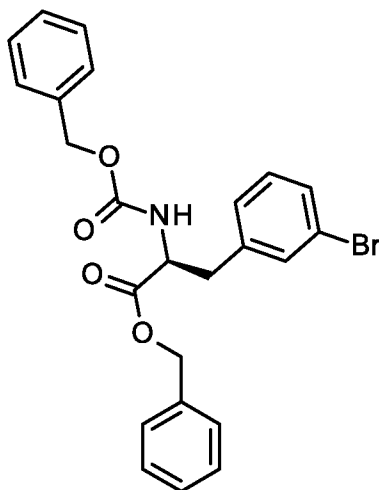
¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: -0.008 (0.78), 0.008 (0.70), 4.040 (16.00), 4.198 (0.41), 4.214 (1.16), 4.230 (2.33), 4.252 (1.27), 4.268 (1.03), 4.278 (1.36), 4.295 (1.14), 4.300 (0.99), 5.243 (0.98), 5.262 (0.98), 7.276 (0.59), 7.294 (1.34), 7.313 (1.11), 7.321 (1.38), 7.340 (0.86), 7.387 (1.36), 7.394 (1.12), 7.406 (2.32), 7.413 (1.70), 7.424 (1.22), 7.431 (0.89), 7.444 (1.06), 7.466 (1.21), 7.618 (1.52),

7.640 (1.16), 7.743 (2.38), 7.762 (2.23), 7.787 (2.02), 7.875 (3.68), 7.894 (3.45), 8.059 (3.94), 8.188 (0.73), 8.207 (0.72).

This amino acid was used in SPPS.

Example 98A

5 Benzyl *N*-[(benzyloxy)carbonyl]-3-bromo-*L*-phenylalaninate



N-[(Benzyloxy)carbonyl]-3-bromo-*L*-phenylalanine (15.3 g, 40.5 mmol) was dissolved in methanol (113 mL). Caesium carbonate (6.59 g, 20.2 mmol) was added and the mixture was stirred until gas no evolution
10 ceased. The solution was evaporated to dryness. The residue was dissolved in DMF (140 mL, 1.8 mol) and (bromomethyl)benzene (5.3 mL, 44 mmol) was added dropwise. After a short time a solid precipitated. The suspension was diluted with ether and water. The organic phase was separated, and the aqueous phase was extracted two times with diethyl ether. The combined organic layers were washed with water, washed with brine, and then dried over magnesium sulfate. The crystalline residue was dissolved in ether
15 and after the addition of pentane, a precipitate formed. The solid was filtered and dried. The ether/pentane solution was evaporated and dissolved in ether; after the addition of pentane a precipitate formed. The solid was filtered and dried, to give 16.0 g (100 % purity, 84 % yield) of the title compound.

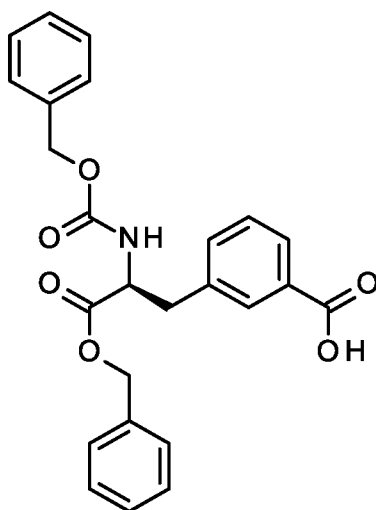
LC-MS (Method 10): R_t = 2.42 min; MS (ESI pos): m/z = 468 $[M+H]^+$

¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm]: 2.874 (2.41), 2.895 (2.86), 2.902 (3.10), 2.923 (2.76), 3.071 (2.84), 3.082 (2.96), 3.099 (2.26), 3.109 (2.09), 3.636 (0.82), 4.336 (1.73), 4.347 (2.20), 4.353 (2.43), 4.357 (2.48), 4.363 (2.48), 4.373 (1.83), 4.383 (1.37), 4.921 (0.55), 4.963 (1.81), 4.989 (12.84), 4.994 (11.01), 5.020 (1.18), 5.043 (0.96), 5.093 (1.11), 5.119 (12.74), 5.139 (0.55), 5.147 (0.97), 7.174 (0.85), 7.211 (3.13), 7.226 (9.23), 7.241 (11.35), 7.248 (16.00), 7.263 (11.82), 7.298 (10.22), 7.313 (14.37), 7.316

(14.35), 7.321 (12.62), 7.332 (10.08), 7.336 (11.10), 7.344 (8.08), 7.351 (11.64), 7.366 (7.19), 7.379 (2.35), 7.414 (5.80), 7.429 (4.73), 7.499 (7.87), 7.880 (4.47), 7.896 (4.30).

Example 99A

3-[(2*S*)-3-(Benzyloxy)-2-{[(benzyloxy)carbonyl]amino}-3-oxopropyl]benzoic acid



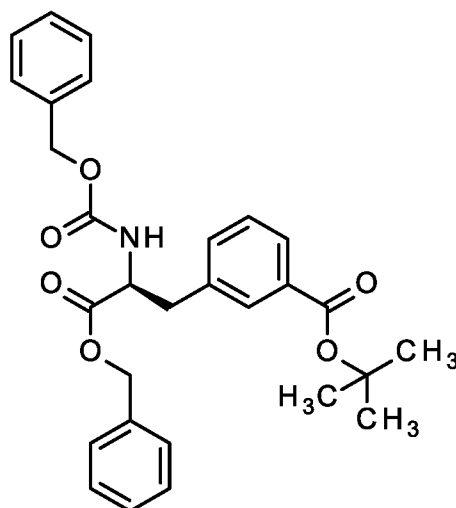
5

The reaction was set up under argon. In a 2L autoclave (stirring speed 750 rpm) are added palladium(II)acetate (2.16 g, 9.61 mmol), 1,1'-Bis(diphenylphosphino)ferrocene (10.63 g, 19.22 mmol) and potassium acetate (47.1 g, 480 mmol). The autoclave is flushed with argon and the reagents are dissolved by adding dry DMF (450 mL). A solution of benzyl *N*-[(benzyloxy)carbonyl]-3-bromo-*L*-phenylalaninate (Example 98A, 45.0 g, 96.1 mmol) in dry DMF (930 mL) was added to the autoclave, followed the addition of water (18 mL). The Autoclave was sealed, purged repeatedly with nitrogen and allowed to stir briefly. The autoclave was pressurized with carbon monoxide (3 bar) and then heated to 80°C and 3bar for 960 min. After the reaction was completed, the autoclave was purged, opened, and then the contents were dissolved in water (13 L). The reaction mixture was extracted 3 times with ethyl acetate (7 L) and the combined organic layer was washed with brine, and then concentrated. The crude product was purified by preparative normal phase chromatography (10 kg silica gel) with DCM/MeOH (95/5) and then further purified by preparative chromatography (DCM/MeOH gradient, Biotage Isolera) to provide 10.7 g (92% purity) of the title compound.

20 LC-MS (Method 10): R_t = 1.92 min; MS (ESI neg): m/z = 432.14 $[M-H]^-$

Example 100A

Benzyl *N*-[(benzyloxy)carbonyl]-3-(*tert*-butoxycarbonyl)-*L*-phenylalaninate

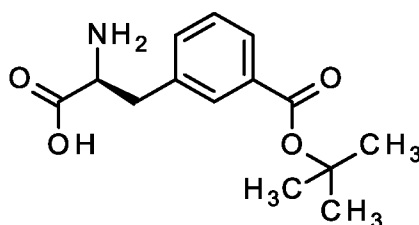


To 3-[(2*S*)-3-(Benzyloxy)-2-[(benzyloxy)carbonyl]amino]-3-oxopropyl]benzoic acid (Example 99A, 18.0 g, 41.5 mmol) was added dichloromethane (280 mL, 4.4 mol). Cyclohexane (140 mL, 1.3 mol), *tert*-butyl 2,2,2-trichloroethanimidate (36.3 g, 166 mmol) and (diethyl ether)(trifluoro)boron (1.6 mL, 12 mmol) were added and the reaction mixture was stirred at room temperature. After 1 h, additional diethyl ether)(trifluoro)boron (1.6 mL, 12 mmol) was added and the reaction mixture was stirred overnight at room temperature. (diethyl ether)(trifluoro)boron (1 mL, 7.5 mmol) was added and reaction mixture was stirred for 2 h at room temperature. Additional *tert*-Butyl 2,2,2-trichloroethanimidate (9.0 g, 40.5 mmol) and additional (diethyl ether)(trifluoro)boron (1 mL, 7.5 mmol) were added and the reaction mixture was stirred overnight at room temperature. The suspension was filtered and the precipitate was washed with DCM : cyclohexane (2:1). The solution was diluted with concentrated aqueous sodium hydrogen carbonate solution. The combined water layers were extracted two times with DCM. The combined organic layers were then dried over magnesium sulfate, filtered and then evaporated. The resulting black oil was stirred with cyclohexane and ethyl acetate (2%), the precipitate was filtered, and the filtrate was evaporated. The resulting black oil was purified by column chromatography on silica gel (cyclohexane – ethyl acetate (9 : 1)) to yield 12.1 g (100 % purity, 60 % yield) of the title compound.

LC-MS (Method 9): $R_t = 1.34$ min; MS (ESI pos): $m/z = [M+H]^+ 490$

Example 101A

3-(*tert*-Butoxycarbonyl)-*L*-phenylalanine



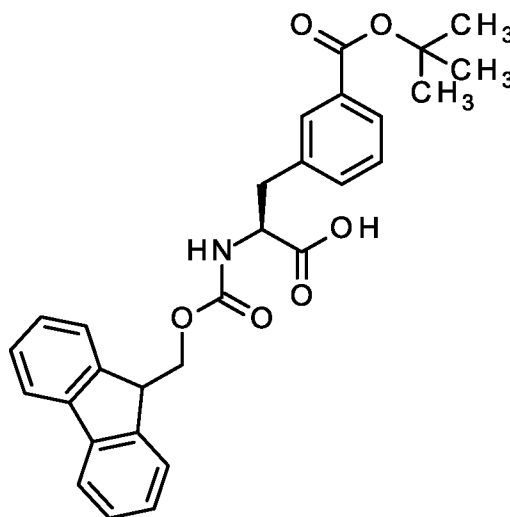
Benzyl *N*-[(benzyloxy)carbonyl]-3-(*tert*-butoxycarbonyl)-*L*-phenylalaninate (Example 100A, 13.0 g, 26.6 mmol) was dissolved in methanol (250 mL). 10% Palladium on charcoal (1.54 g) was added and the mixture was hydrogenated at ambient pressure for 4 hours at room temperature. A precipitate was formed. The suspension was dissolved in methanol and was stirred at reflux. The warm solution was filtered over a layer of diatomaceous earth and the filter cake was washed with methanol (ca. 1L). The combined filtrate was concentrated and dried, affording 6.10 g (94% purity, 81% yield) of the title compound.

LC-MS (Method 9): R_t = 0.59 min; MS (ESI neg): m/z = 264 $[M-H]^-$

^1H -NMR (400 MHz, DMSO- d_6) δ [ppm]: 1.525 (5.74), 1.546 (16.00), 2.326 (0.50), 2.366 (0.48), 2.670 (0.50), 2.709 (0.48), 3.169 (0.57), 7.400 (0.89), 7.419 (0.66), 7.501 (0.76), 7.739 (0.84), 7.759 (0.85), 7.796 (1.02).

Example 102A

3-(*tert*-Butoxycarbonyl)-*N*-{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-*L*-phenylalanine



3-(*tert*-Butoxycarbonyl)-*L*-phenylalanine (Example 101A, 6.10 g, 23.0 mmol) was dissolved in water (100 mL) and acetone (100 mL). Sodium hydrogen carbonate (19.3 g, 230 mmol) was added. A solution of 1-([(9*H*-fluoren-9-yl)methoxy]carbonyl)oxy)pyrrolidine-2,5-dione (8.14 g, 24.1 mmol) was dissolved in acetone (100 mL) was added and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with water and extracted with one portion of MTBE. The aqueous layer was acidified with 1M hydrochloric and extracted with DCM. The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and then concentrated to afford 8.90 g (100% purity, 79% yield) of the title compound. Product was also present in the organic layer. The organic layer was concentrated. DCM and 1M hydrochloric were added to the residue and the mixture was stirred for 5 min. The residue was filtered and washed with water and DCM. The layers were separated, and the aqueous layer was extracted with DCM. The combined organic layer was then dried over anhydrous magnesium sulfate,

filtered, and then concentrated. The crude product was purified by flash chromatography on silica gel (gradient DCM : methanol (0-10%)). The residue was taken up in acetonitrile and purified by preparative HPLC (column: Reprosil; C18; 10 μ m; 125 x 40 mm; solvent: AcCN/H₂O containing 0,1% HCOOH; flow rate: 100 mL/min ; gradient: 0 - 5.50 min (10 : 90); sample injection at 3.00 min; 5.50 - 17.65 min gradient to 95 : 5; 17.65 - 19.48 min (95 : 5); 19.48 - 19.66 min gradient to 10 : 90; 19.66 - 20.72 min (10 : 90). In total, 1.58 g (100 % purity, 14 % yield) of the title compound was obtained.

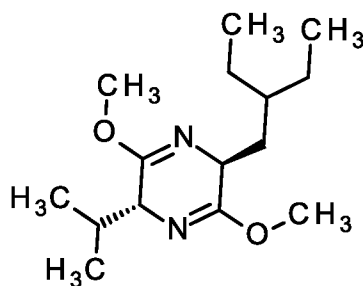
LC-MS (Method 10): R_t = 2.27 min; MS (ESI neg): m/z = 486 [M-H]⁻

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 1.106 (1.34), 1.144 (4.63), 1.519 (16.00), 1.544 (0.64), 2.086 (1.34), 2.119 (1.97), 2.483 (1.16), 2.595 (0.68), 3.077 (0.43), 3.319 (1.89), 4.158 (0.42), 4.169 (0.81), 4.181 (2.03), 4.195 (0.76), 4.203 (0.48), 4.541 (0.43), 7.259 (0.80), 7.277 (0.76), 7.293 (0.68), 7.312 (0.44), 7.378 (0.62), 7.384 (0.86), 7.397 (1.11), 7.403 (1.62), 7.415 (0.62), 7.422 (0.90), 7.521 (0.63), 7.540 (0.45), 7.583 (0.68), 7.605 (0.86), 7.626 (0.62), 7.747 (0.59), 7.766 (0.57), 7.784 (0.68), 7.806 (0.63), 7.847 (1.02), 7.864 (1.58), 7.883 (1.42).

This amino acid was used in SPPS.

Example 103A

(2*S*,5*R*)-2-(2-Ethylbutyl)-3,6-dimethoxy-5-(propan-2-yl)-2,5-dihydropyrazine



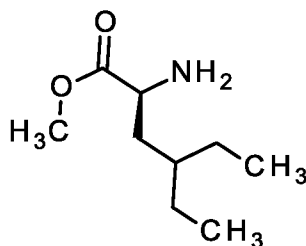
(2*R*)-3,6-Dimethoxy-2-(propan-2-yl)-2,5-dihydropyrazine (CAS-RN: 109838-859, 2.9 mL, 16 mmol) was dissolved in anhydrous THF (60 mL, 740 mmol) and the solution was cooled to -78°C. *n*-Butyllithium (10 mL, 1.6 M, 16 mmol) was added dropwise slowly and dropwise over a 15 minute period while stirring at -78°C. 3-(Bromomethyl)pentane (5.91 g, 35.8 mmol) was added and the reaction mixture was stirred for 2 h at -78°C, then allowed to come to room temperature overnight. The solution was cooled to 0°C and slowly quenched with water. Diethyl ether was added and after stirring several minutes, the organic layer was separated. The aqueous phase was extracted two times with diethyl ether. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and evaporated. The crude product was purified by chromatography on silica gel (cyclohexane / ethyl acetate 2%) to give 2.60 g (95 % purity, 57 % yield) of the title compound.

LC-MS (Method 10): $R_t = 2.90$ min; MS (ESI pos): $m/z = 269$ $[M+H]^+$

^1H -NMR (400 MHz, DMSO- d_6) δ [ppm]: -0.008 (0.51), 0.008 (0.45), 0.608 (6.77), 0.624 (6.91), 0.771 (3.04), 0.790 (7.57), 0.796 (3.51), 0.808 (4.20), 0.815 (7.45), 0.833 (3.54), 0.999 (6.56), 1.016 (6.71), 1.179 (0.65), 1.197 (0.97), 1.215 (0.95), 1.223 (0.51), 1.232 (0.73), 1.240 (0.81), 1.257 (1.26), 1.275 (1.64), 1.294 (1.28), 1.310 (0.57), 1.343 (0.55), 1.356 (1.67), 1.364 (0.66), 1.369 (0.83), 1.376 (1.52), 1.388 (1.40), 1.398 (0.66), 1.403 (0.63), 1.409 (1.04), 1.422 (0.43), 1.459 (0.56), 1.474 (0.63), 1.489 (0.45), 1.637 (0.66), 1.647 (0.68), 1.656 (0.56), 1.667 (0.69), 1.670 (0.69), 1.681 (0.54), 1.690 (0.48), 1.700 (0.44), 2.191 (0.54), 2.199 (0.55), 2.208 (0.71), 2.216 (0.71), 2.225 (0.52), 2.233 (0.51), 3.597 (16.00), 3.616 (15.85), 3.916 (1.08), 3.925 (2.24), 3.933 (1.46), 3.959 (0.67), 3.969 (1.06), 3.980 (0.99), 3.990 (1.00), 4.000 (0.49).

Example 104A

Methyl 4-ethyl-*L*-norleucinate

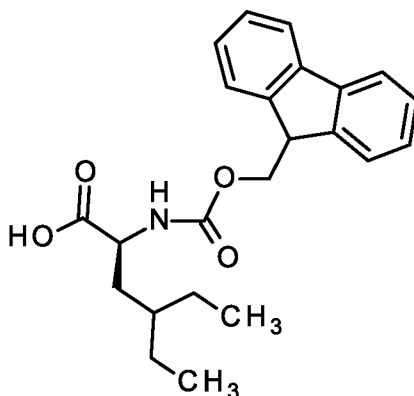


A solution (2*S*,5*R*)-2-(2-Ethylbutyl)-3,6-dimethoxy-5-(propan-2-yl)-2,5-dihydropyrazine (Example 103A, 2.60 g, 9.69 mmol) in methanol (30 mL) was cooled to 0°C and a 10% aqueous solution of hydrochloric acid (10 mL) was added and then the reaction mixture was stirred for 2 h. The solution was evaporated and the residue was diluted with DCM and sodium carbonate solution (100 mL, 2.0 M). The separated aqueous layer was extracted two times with DCM. The combined organic layer was dried over magnesium sulfate, filtered and then concentrated to give 3.49 g (48% purity, 100% yield) of the title compound.

GC-MS (GC-MS Method 1): $R_t = 3.18$ min, $m/z = 173$ $[M^+]$

Example 105A

4-Ethyl-*N*-{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-*L*-norleucine



To methyl 4-ethyl-*L*-norleucinate (Example 104A, 3.97 g, 48% purity, 11.0 mmol) was added methanol (100 mL) and the resulting solution was cooled to 0°C. Sodium hydroxide solution (24 mL, 1.0 M) was added and the reaction mixture was stirred for 1 h at 0°C, then warmed to room temperature and stirred further for 4 h. Water (80 mL), sodium hydrogen carbonate (2.03 g, 24.2 mmol) and sodium carbonate (4.00 g, 37.7 mmol) were sequentially added to the solution. The methanol was removed from the reaction mixture by rotary evaporation and the aqueous solution was diluted with 1,4-dioxane (60 mL). The suspension was cooled to 0°C and (9*H*-fluoren-9-yl)methyl carbonochloridate (CAS-RN: 28920-43-6, 8.54 g, 33.0 mmol) was added. The reaction mixture was stirred overnight at room temperature. Water and MTBE were added, stirred briefly, and then the organic layer was separated. The aqueous layer was extracted with MTBE and then the combined organic layer was washed with 5% sodium carbonate solution. The combined aqueous layer was acidified to pH 1 with potassium hydrogen sulfate and the aqueous suspension was extracted three times with DCM. The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and then concentrated. The crude product was purified by preparative HPLC (column: Reprosil; C18; 10 µm; 125 x 40 mm; solvent: AcCN/H₂O containing 0.1% HCOOH; flow rate: 100 mL/min ; gradient: 0 - 5.50 min (10 : 90); sample injection at 3.00 min; 5.50 - 17.65 min gradient to 95 : 5; 17.65 - 19.48 min (95 : 5); 19.48 - 19.66 min gradient to 10 : 90; 19.66 - 20.72 min (10 : 90), affording 1.57 g (100 % purity, 37 % yield) of the title compound.

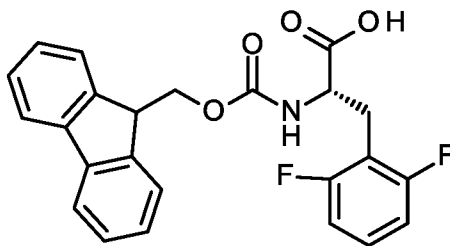
LC-MS (Method 11): *R*_t = 1.49 min; MS (ESI pos): *m/z* = 404 [M+Na]⁺

¹H-NMR (400 MHz, DMSO-*d*₆) δ [ppm]: -0.008 (3.19), 0.008 (3.01), 0.774 (6.73), 0.792 (16.00), 0.809 (13.36), 0.826 (14.04), 0.844 (7.32), 1.181 (0.92), 1.197 (1.90), 1.216 (3.12), 1.234 (3.84), 1.253 (2.82), 1.273 (1.79), 1.293 (2.23), 1.308 (3.41), 1.331 (4.60), 1.339 (4.12), 1.505 (0.45), 1.557 (3.55), 1.575 (5.01), 1.590 (3.09), 3.950 (1.22), 3.970 (2.67), 3.989 (2.69), 4.009 (1.11), 4.204 (1.35), 4.220 (3.90), 4.238 (7.46), 4.263 (5.22), 4.279 (3.57), 4.286 (5.10), 4.304 (3.43), 4.310 (2.32), 4.328 (1.06), 7.299 (2.60), 7.306 (2.93), 7.317 (5.73), 7.322 (5.59), 7.336 (3.69), 7.341 (3.48), 7.400 (6.17), 7.419 (10.33), 7.437 (4.62), 7.626 (4.21), 7.647 (3.80), 7.711 (8.98), 7.729 (8.16), 7.884 (9.94), 7.903 (9.15), 12.540 (0.98).

This amino acid was used in SPPS.

Example 106A

N-{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}-2,6-difluoro-*L*-phenylalanine



5

To methyl 2,6-difluoro-*L*-phenylalaninate (CAS-RN: 1192057-28-5, 1.03 g, 4.79 mmol) was added methanol (18.5 mL) and the solution was cooled to 0°C. Sodium hydroxide solution (4.8 mL, 1.0 M) was added and the reaction mixture was stirred for 1 h at room temperature. Water (17 mL), sodium hydrogen carbonate (402 mg, 4.79 mmol) and sodium carbonate (667 mg, 6.29 mmol) were added to the solution. The methanol was removed by rotary evaporation and the aqueous layer was diluted with 1,4-dioxane (14 mL). The suspension was cooled to 0°C and (9*H*-fluoren-9-yl)methyl carbonochloridate (CAS-RN: 28920-43-6, 1.86 g, 7.18 mmol) was added. The mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with water and washed with MTBE. The aqueous layer was acidified to pH 1 with 1 M hydrochloric acid and extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and the concentrated, to afford 1.74 g (100% purity, 86% yield) of the title compound.

10

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LC-MS (Method 10): R_t = 2.00 min; MS (ESI pos): m/z = 424 $[M+H]^+$

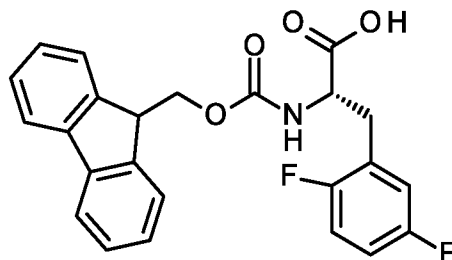
¹H-NMR (400 MHz, DMSO-*d*₆) δ [ppm]: 1.157 (0.74), 1.175 (1.50), 1.193 (0.76), 1.988 (2.79), 2.992 (0.74), 3.014 (0.87), 3.025 (1.22), 3.048 (1.17), 3.118 (1.24), 3.134 (1.32), 3.153 (0.81), 3.169 (0.76), 3.316 (16.00), 4.021 (0.71), 4.039 (0.70), 4.131 (0.95), 4.149 (1.39), 4.166 (2.72), 4.194 (4.15), 4.213 (2.65), 4.235 (0.72), 7.013 (1.71), 7.033 (3.28), 7.053 (2.07), 7.290 (1.33), 7.307 (3.44), 7.323 (4.18), 7.341 (2.36), 7.357 (0.48), 7.397 (2.41), 7.415 (4.00), 7.434 (1.83), 7.639 (2.22), 7.659 (3.87), 7.679 (1.99), 7.818 (1.97), 7.839 (1.95), 7.876 (4.80), 7.894 (4.40), 12.793 (0.81).

20

This amino acid was used in SPPS.

25 Example 107A

N-{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}-2,5-difluoro-*L*-phenylalanine



To methyl 2,5-difluoro-*L*-phenylalaninate (CAS-RN: 1213491-95-2) (2.27 g, 10.5 mmol) was added in methanol (40 mL) and the resulting solution was cooled to 0°C. Sodium hydroxide solution (11 mL, 1.0 M) was added and the reaction mixture was stirred for 1.5 h at room temperature. Water (35 mL), sodium hydrogen carbonate (886 mg, 10.5 mmol) and sodium carbonate (1.47 g, 13.9 mmol) were added to the solution. The methanol was removed by rotary evaporation and the aqueous layer was diluted with 1,4-dioxane (30 mL). The suspension was cooled to 0°C and (9*H*-fluoren-9-yl)methyl carbonochloridate (CAS-RN: 28920-43-6, 4.09 g, 15.8 mmol) was added, and the reaction mixture was stirred for 2 h at room temperature. The mixture was diluted with water and washed with MTBE. The aqueous layer was acidified to pH 1 with 1 M hydrochloric acid solution and extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and the concentrated. The crude product was purified by flash chromatography on silica gel (gradient DCM : methanol 0-5%), then the residue was taken up in acetonitrile and purified again by preparative HPLC (column: Reprosil; C18; 10 µm; 125 x 40 mm; solvent: AcCN/H₂O containing 0,1% HCOOH; flow rate: 100 mL/min ; gradient: 0 - 5.50 min (10 : 90); sample injection at 3.00 min; 5.50 - 17.65 min gradient to 95 : 5; 17.65 - 19.48 min (95 : 5); 19.48 - 19.66 min bis 10 : 90; 19.66 - 20.72 min (10 : 90) to give 1.82 g (100 % purity, 41 % yield) of the title compound.

LC-MS (Method 9): R_t = 1.04 min; MS (ESI pos): m/z = 424 [M+H]⁺

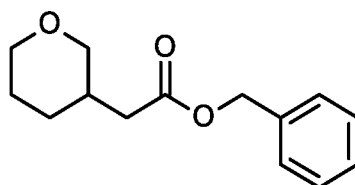
¹H-NMR (400 MHz, DMSO-*d*₆) δ [ppm]: -0.008 (2.88), 0.008 (2.75), 1.234 (0.68), 2.329 (0.47), 2.671 (0.46), 2.843 (2.48), 2.870 (3.13), 2.877 (3.47), 2.904 (3.10), 3.166 (3.44), 3.177 (3.74), 3.200 (2.97), 3.212 (2.73), 4.141 (1.90), 4.158 (5.16), 4.173 (9.08), 4.196 (12.08), 4.201 (12.22), 4.211 (8.55), 4.221 (7.38), 4.233 (3.76), 4.246 (2.97), 4.259 (2.15), 7.084 (0.87), 7.093 (1.61), 7.106 (2.33), 7.114 (3.81), 7.124 (3.21), 7.135 (2.97), 7.144 (2.17), 7.175 (3.13), 7.187 (4.77), 7.197 (6.58), 7.210 (7.69), 7.220 (4.45), 7.232 (3.61), 7.263 (4.47), 7.282 (9.79), 7.301 (7.22), 7.305 (7.95), 7.325 (4.82), 7.349 (0.45), 7.389 (6.78), 7.408 (11.73), 7.426 (5.46), 7.489 (0.48), 7.509 (0.67), 7.532 (0.46), 7.610 (7.40), 7.628 (12.92), 7.646 (6.48), 7.791 (6.23), 7.812 (6.10), 7.871 (16.00), 7.890 (14.50), 12.862 (3.43).

This amino acid was used in SPPS.

This amino acid was also prepared from methyl 2,5-difluoro-*L*-phenylalaninate prepared in Example 126A. The amino acid can also be prepared from 2,5-difluoro-*L*-phenylalaninate (CAS RN: 31105-92-7) using the procedure described in Example 106A.

Example 108A

5 Benzyl tetrahydro-2*H*-pyran-3-ylacetate (racemate)



Tetrahydro-2*H*-pyran-3-ylacetic acid (1.00 g, 6.94 mmol) was dissolved in methanol (10 mL, 250 mmol). Cesium carbonate (1.13 g, 3.47 mmol) was added and the reaction mixture was stirred at room temperature until the evolution of gas ceased. The solution was then evaporated. The residue was dissolved in DMF (10 mL), (bromomethyl)benzene (1.3 mL, 11 mmol) was added, and the reaction mixture was stirred overnight at room temperature. Additional (bromomethyl)benzene (325 μ L, 2.75 mmol) was added and the reaction mixture was stirred for 3 h at room temperature. The suspension was then diluted with water and ether. After stirring for a few minutes, the organic layer was separated. The aqueous layer was extracted three times with diethyl ether. The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and then concentrated. The crude product was purified by chromatography on silica gel (cyclohexane / ethyl acetate (85/15)) to give 1.50 g (100 % purity, 92 % yield) of the title compound.

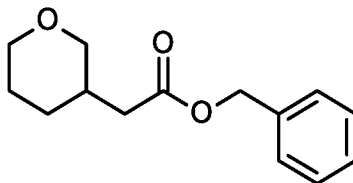
LC-MS (Method 10): R_t = 1.84 min; MS (ESI pos): m/z = 235 $[M+H]^+$

¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm]: 1.161 (0.45), 1.170 (0.51), 1.183 (0.89), 1.187 (0.68), 1.191 (0.98), 1.196 (0.60), 1.204 (0.68), 1.208 (1.03), 1.212 (0.77), 1.217 (1.00), 1.229 (0.62), 1.238 (0.63), 1.428 (0.47), 1.447 (0.62), 1.449 (0.74), 1.455 (0.95), 1.463 (0.63), 1.468 (0.83), 1.476 (1.42), 1.484 (0.80), 1.489 (0.56), 1.498 (0.98), 1.501 (0.53), 1.506 (1.11), 1.509 (1.00), 1.516 (1.65), 1.523 (1.08), 1.532 (0.56), 1.543 (0.70), 1.550 (0.40), 1.747 (0.90), 1.751 (0.77), 1.755 (0.89), 1.764 (0.62), 1.773 (0.82), 1.777 (0.69), 1.781 (0.77), 1.887 (0.41), 1.894 (0.68), 1.901 (0.68), 1.908 (0.76), 1.914 (0.90), 1.921 (0.74), 1.927 (0.68), 1.934 (0.63), 2.194 (1.30), 2.208 (1.18), 2.225 (3.67), 2.239 (3.41), 2.255 (3.55), 2.270 (3.23), 2.286 (1.23), 2.301 (1.12), 3.018 (2.02), 3.038 (2.30), 3.041 (2.34), 3.059 (2.06), 3.246 (1.05), 3.252 (1.07), 3.267 (1.61), 3.273 (1.61), 3.289 (1.21), 3.295 (1.19), 3.685 (0.75), 3.692 (1.34), 3.705 (1.77), 3.708 (1.89), 3.713 (2.24), 3.716 (2.16), 3.726 (1.17), 3.730 (1.14), 3.734 (1.08), 3.738 (0.97), 5.089 (16.00), 7.310 (0.41), 7.315 (0.73), 7.320 (0.71), 7.327 (2.21), 7.334 (1.16), 7.339

(1.36), 7.342 (1.95), 7.345 (2.49), 7.348 (2.36), 7.351 (1.63), 7.360 (9.48), 7.364 (11.33), 7.371 (1.08), 7.376 (3.55), 7.378 (3.74), 7.382 (1.62), 7.390 (0.55), 7.393 (1.20), 7.395 (0.74).

Example 109A

Benzyl tetrahydro-2*H*-pyran-3-yl acetate



5

The diastereomeric mixture (Example 108A, 1.5 g) was dissolved in methanol / acetonitrile (30 mL) and separated using a THAR supercritical fluid chromatography Prep 200 instrument using a Chiralpak AZ-H (SFC) 5 μ m, 250 x 30 mm column; eluent: CO₂/ethanol (90:10); pressure: 135 bar; temperature eluant: 35°C; flow rate: 100 g/min; detection: UV 210 nm; injection volume 0.4 mL/injection; under general operating conditions 1g/min of CO₂ is approximately equivalent to 1 mL/min.

Sequence Settings: cycle time = 11,2 min

Fraction 1 was collected at 1.48 min (enantiomer 1)

Fraction 2 was collected between 1.89 min (enantiomer 2)

15 The product-containing fractions combined and were concentrated.

The fractions were analyzed using analytical SFC-MS (Agilent, column: Daicel AZ-3 3 μ m 100 x 4,6 mm, eluent: CO₂/ethanol (90:10); BPR pressure: 130 bar; BPR temperature: 60°C; column temperature: 40°C; flow rate: 3 mL/min; UV 210 nm.

The stereochemistry of the two enantiomers was not assigned.

20 Benzyl tetrahydro-2*H*-pyran-3-ylacetate (enantiomer 1) was obtained with 99.5% ee (641 mg (100 % purity).

LC-MS (Method 9): *R*_t = 0.98 min; MS (ESI pos): *m/z* = 235 [M+H]⁺

¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm]: 1.161 (0.45), 1.170 (0.51), 1.183 (0.89), 1.187 (0.67), 1.191 (0.98), 1.196 (0.61), 1.204 (0.67), 1.209 (1.03), 1.213 (0.77), 1.217 (1.00), 1.230 (0.61), 1.238 (0.62), 1.428 (0.48), 1.447 (0.61), 1.449 (0.75), 1.455 (0.95), 1.463 (0.64), 1.468 (0.82), 1.476 (1.41), 1.484 (0.79), 1.489 (0.56), 1.498 (0.98), 1.501 (0.52), 1.507 (1.05), 1.510 (1.00), 1.516 (1.65), 1.523 (1.07), 1.532 (0.55), 1.543 (0.70), 1.747 (0.90), 1.751 (0.78), 1.755 (0.89), 1.764 (0.63), 1.773 (0.81), 1.777

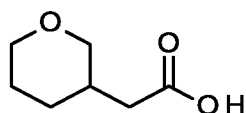
25

(0.69), 1.781 (0.78), 1.887 (0.40), 1.894 (0.69), 1.901 (0.68), 1.908 (0.76), 1.914 (0.91), 1.921 (0.74), 1.927 (0.67), 1.934 (0.64), 2.194 (1.30), 2.208 (1.18), 2.225 (3.69), 2.239 (3.42), 2.255 (3.56), 2.270 (3.24), 2.286 (1.23), 2.301 (1.12), 3.019 (2.02), 3.038 (2.29), 3.041 (2.36), 3.060 (2.07), 3.246 (1.05), 3.252 (1.08), 3.268 (1.63), 3.273 (1.63), 3.289 (1.24), 3.295 (1.23), 3.686 (0.75), 3.692 (1.34), 3.705 (1.73), 3.708 (1.90), 3.713 (2.19), 3.715 (2.20), 3.726 (1.19), 3.730 (1.13), 3.734 (1.08), 3.738 (0.97), 5.089 (16.00), 7.310 (0.40), 7.315 (0.74), 7.321 (0.69), 7.328 (2.23), 7.334 (1.13), 7.339 (1.36), 7.342 (1.99), 7.345 (2.52), 7.347 (2.34), 7.350 (1.66), 7.360 (9.33), 7.364 (11.16), 7.371 (1.09), 7.376 (3.58), 7.378 (3.72), 7.382 (1.63), 7.390 (0.54), 7.393 (1.20), 7.396 (0.75).

Benzyl tetrahydro-2*H*-pyran-3-ylacetate (enantiomer 2) was also obtained with 99.5% ee (611 mg (100 % purity)).

Example 110A

Tetrahydro-2*H*-pyran-3-ylacetic acid (enantiomer 1)



10% Palladium on charcoal (84.0 mg) was humidified with methanol under argon. A solution of benzyl tetrahydro-2*H*-pyran-3-ylacetate (Example 109A, 641 mg, 2.74 mmol) added. The reaction mixture was then hydrogenated for 2 h at ambient pressure at room temperature. The catalyst was removed by filtration over a layer of diatomaceous earth, the filter cake was washed with methanol, and the solvent was removed by rotary distillation, to provide 364 mg (100 % purity, 92 % yield) of the title compound.

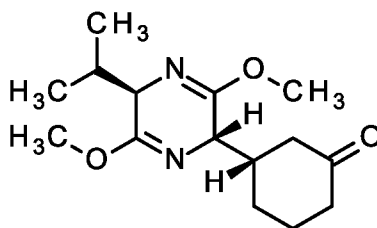
GC-MS (GC-MS Method 1): R_t = 3.57 min, m/z = 144 [M^+]

¹H-NMR (500 MHz, DMSO-*d*₆) δ [ppm]: 1.145 (1.98), 1.154 (2.21), 1.166 (4.03), 1.171 (3.11), 1.175 (4.45), 1.180 (2.85), 1.188 (3.11), 1.192 (4.69), 1.196 (3.55), 1.201 (4.57), 1.213 (2.68), 1.222 (2.75), 1.428 (1.03), 1.436 (1.93), 1.445 (1.35), 1.449 (1.65), 1.455 (2.72), 1.458 (3.25), 1.463 (4.18), 1.471 (2.92), 1.476 (3.82), 1.484 (6.50), 1.493 (3.67), 1.498 (2.58), 1.506 (5.73), 1.514 (6.62), 1.521 (7.99), 1.528 (5.13), 1.537 (2.67), 1.548 (3.18), 1.554 (1.80), 1.563 (0.64), 1.759 (1.78), 1.767 (4.22), 1.771 (3.85), 1.775 (4.26), 1.784 (2.89), 1.793 (3.85), 1.797 (3.49), 1.801 (3.85), 1.809 (1.56), 1.824 (0.81), 1.832 (1.31), 1.839 (1.87), 1.846 (3.17), 1.853 (3.16), 1.859 (3.72), 1.866 (4.34), 1.873 (3.58), 1.879 (3.34), 1.886 (3.00), 1.893 (1.65), 1.900 (1.44), 1.908 (0.69), 2.023 (6.18), 2.036 (5.11), 2.054 (16.00), 2.068 (14.33), 2.087 (15.54), 2.101 (13.64), 2.118 (5.69), 2.133 (5.09), 2.999 (9.23), 3.018 (10.90), 3.021 (11.02), 3.040 (9.47), 3.134 (0.64), 3.247 (6.14), 3.253 (6.29), 3.269 (9.57), 3.275 (9.49), 3.291 (7.06), 3.297 (6.90), 3.586 (0.59), 3.696 (3.77), 3.703 (6.42), 3.709 (4.14), 3.717 (8.63), 3.725 (11.12), 3.739 (5.37), 3.743 (5.40), 3.747 (5.21), 3.750 (4.68).

This substance was used in SPPS.

Example 111A

(3*S*)-3-[(2*S*,5*R*)-3,6-Dimethoxy-5-(propan-2-yl)-2,5-dihydropyrazin-2-yl]cyclohexan-1-one



5

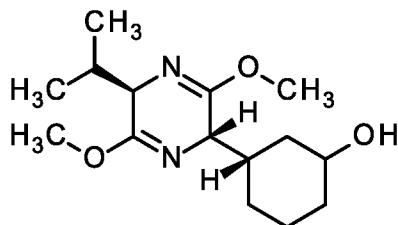
(2*R*)-3,6-Dimethoxy-2-(propan-2-yl)-2,5-dihydropyrazine (4.61 g, 25.0 mmol) was dissolved in THF (20 mL) and cooled to -78°C. Butyllithium (16 mL, 1.6 M, 25 mmol) was added dropwise with stirring. Stirring was continued for 15 min at -78°C. Lithium (azanidylidenemethylidene)(thiophen-2-yl)copper (100 mL, 0.25 M, 25 mmol) was added and the mixture was stirred for 15 min at -78°C. A solution from
 10 cyclohex-2-en-1-one (2.4 mL, 25 mmol) in THF (10 mL) was added and the reaction mixture was stirred for 10 min at -78°C, then it was further stirred at ambient temperature for 2 h. The reaction was quenched with water and extracted two times with ethyl acetate. The combined organic layer was washed with sodium hydrogen carbonate solution and brine, dried over anhydrous magnesium sulfate, filtered, and then the residue was concentrated under reduced pressure to give 6.8 g of a colorless oil. The crude product
 15 was purified by flash chromatography on silica gel (gradient cyclohexane - ethyl acetate 85-15) to give 5.7 g (90 % purity, 64 % yield) of the title compound.

LC-MS (Method 12): R_t = 3.14 min; MS (ESI pos): m/z = 281.2 $[M+H]^+$

¹H-NMR (400 MHz, DMSO-*d*₆) δ [ppm]: 0.587 (0.94), 0.604 (1.17), 0.617 (6.04), 0.634 (6.04), 0.971 (0.51), 0.988 (5.87), 0.995 (1.32), 1.005 (5.67), 1.012 (0.98), 1.032 (0.88), 1.049 (0.91), 1.575 (0.55),
 20 1.596 (0.42), 1.607 (0.61), 1.750 (0.61), 1.760 (0.78), 1.784 (1.05), 1.794 (1.28), 1.816 (0.50), 1.837 (0.50), 1.843 (0.57), 1.874 (0.43), 1.979 (0.82), 2.012 (1.26), 2.024 (0.52), 2.032 (0.55), 2.044 (0.95), 2.057 (0.40), 2.122 (0.46), 2.148 (0.80), 2.156 (1.00), 2.164 (1.00), 2.173 (0.67), 2.181 (0.70), 2.190 (0.69), 2.199 (0.54), 2.207 (0.59), 2.225 (0.71), 2.242 (0.64), 2.259 (0.74), 2.275 (0.64), 2.294 (0.45), 2.310 (0.65), 2.319 (0.47), 2.329 (0.48), 2.338 (0.58), 2.348 (0.54), 3.602 (0.89), 3.631 (16.00), 3.642
 25 (4.12), 3.652 (14.26), 3.902 (1.14), 3.911 (2.10), 3.920 (1.33), 3.943 (0.45), 4.069 (1.14), 4.078 (1.82), 4.086 (0.98), 4.492 (0.55), 5.674 (0.91).

Example 112A

(1*R*,3*S*)-3-[(2*S*,5*R*)-3,6-Dimethoxy-5-(propan-2-yl)-2,5-dihydropyrazin-2-yl]cyclohexan-1-ol (2 stereoisomers present)



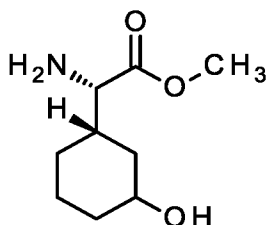
(3*S*)-3-[(2*S*,5*R*)-3,6-dimethoxy-5-(propan-2-yl)-2,5-dihydropyrazin-2-yl]cyclohexan-1-one (Example 111A, 5.70 g, 20.3 mmol) was dissolved in methanol (50 mL) and cooled to 0°C. Sodium tetrahydroborate (769 mg, 20.3 mmol) was added and the reaction mixture was stirred overnight at room temperature. Additional sodium tetrahydroborate (769 mg, 20.3 mmol) was added and stirring was continued at room temperature. This step is repeated 2 more times with 0.5 eq sodium tetrahydroborate. The methanol was evaporated and the residue was diluted with water and ethyl acetate. After stirring for several minutes, the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and the residue concentrated under reduced pressure to give 5.7 g of a yellow oil. The crude product was purified by flash chromatography on silica gel (gradient cyclohexane - cyclohexane ethyl acetate 5%-30%) to give 4.95 g (86 % yield) of the title compound.

LC-MS (Method 10): $R_t = 1.87$ min; MS (ESI pos): $m/z = 283.2$ $[M+H]^+$; $R_t = 1.91$ min; MS (ESI pos): $m/z = 283.2$ $[M+H]^+$

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: 0.589 (1.83), 0.600 (6.10), 0.605 (2.52), 0.617 (6.02), 0.739 (0.91), 0.768 (0.96), 0.798 (0.42), 0.898 (0.47), 0.934 (0.50), 0.997 (6.80), 1.014 (7.00), 1.157 (0.53), 1.175 (0.92), 1.193 (0.73), 1.203 (0.72), 1.211 (0.59), 1.227 (0.47), 1.235 (0.72), 1.243 (0.51), 1.313 (0.52), 1.321 (0.63), 1.352 (0.99), 1.398 (2.43), 1.485 (0.59), 1.515 (0.56), 1.668 (0.44), 1.676 (0.56), 1.684 (0.44), 1.708 (0.51), 1.745 (0.57), 1.774 (0.54), 1.879 (0.58), 1.887 (0.56), 1.988 (1.39), 2.185 (0.54), 2.193 (0.57), 2.202 (0.73), 2.210 (0.75), 2.219 (0.55), 2.227 (0.55), 3.611 (7.06), 3.617 (3.03), 3.622 (16.00), 3.629 (14.28), 3.845 (0.55), 3.854 (0.50), 3.862 (1.17), 3.871 (2.39), 3.880 (1.79), 3.933 (1.11), 3.942 (1.74), 3.951 (0.86), 4.215 (0.59), 4.223 (0.57), 4.431 (2.16), 4.443 (2.10).

Example 113A

Methyl (2*S*)-amino[(1*S*)-3-hydroxycyclohexyl]acetate (2 stereoisomers present)

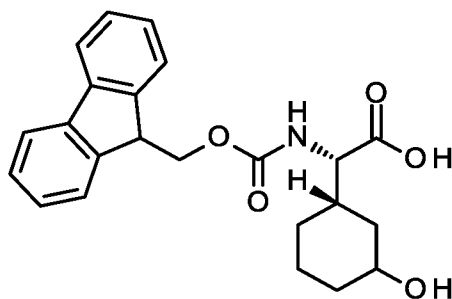


(3*S*)-3-[(2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl]cyclohexanol (Example 112A, 4.95 g, 17.5 mmol) was dissolved in methanol (45 mL) and the solution was cooled to 0°C. A 10% aqueous hydrochloric acid solution (15 mL) was added and the reaction mixture was stirred for a short time at 0°C, then it was allowed to stir at room temperature for 3 h. The reaction mixture was concentrated, the residue was diluted with DCM and then an aqueous sodium carbonate solution (150 mL, 2.0 M, 300 mmol) was added. The mixture was stirred and then the layers were separated. The aqueous layer was extracted two times with DCM. The combined organic layer was dried over anhydrous magnesium sulfate, filtered, and then concentrated. The crude product was purified by flash chromatography on silica gel (DCM: methanol 95+5) to give 1.92 g (58 % yield) of the title compound.

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: -0.008 (1.04), 0.008 (0.93), 0.760 (0.69), 0.777 (0.71), 0.834 (0.61), 0.867 (1.92), 0.875 (1.16), 0.885 (1.54), 0.892 (1.18), 0.910 (0.45), 0.922 (0.61), 0.942 (1.05), 0.951 (0.95), 0.973 (1.17), 0.981 (1.06), 1.003 (0.54), 1.012 (0.50), 1.157 (0.67), 1.165 (0.41), 1.190 (0.65), 1.198 (0.44), 1.223 (0.42), 1.375 (0.69), 1.407 (0.79), 1.498 (0.52), 1.513 (0.89), 1.519 (0.95), 1.527 (0.74), 1.549 (0.69), 1.633 (2.72), 1.671 (0.69), 1.680 (0.78), 1.753 (1.26), 1.783 (1.67), 1.959 (0.49), 2.086 (0.85), 3.096 (0.46), 3.110 (0.48), 3.138 (1.12), 3.151 (1.10), 3.162 (1.07), 3.175 (1.02), 3.276 (0.43), 3.288 (0.55), 3.593 (0.71), 3.603 (4.85), 3.612 (16.00), 3.626 (1.87), 3.633 (0.96), 3.637 (1.57), 4.232 (0.60), 4.240 (0.55), 4.481 (1.83), 4.492 (1.73), 5.754 (0.68).

Example 114A

(2*S*)-2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-2-[(1*S*)-3-hydroxycyclohexyl]acetic acid (2 stereoisomers present)



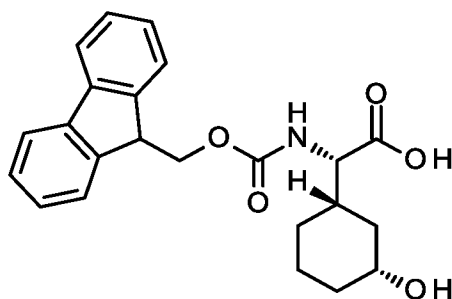
Methyl (2*S*)-amino[(1*S*)-3-hydroxycyclohexyl]acetate (Example 113A, 1.92 g, 10.3 mmol) was added to methanol (40 mL) and the solution was cooled to 0°C. Sodium hydroxide solution (10 mL, 1.0 M, 10 mmol) was added and the reaction mixture was stirred for 1.5 h at room temperature and then for 1 h at 50°C. Water (37 mL), sodium hydrogen carbonate (861 mg, 10.3 mmol), and sodium carbonate (1.43 g, 13.5 mmol) were added to the solution. The methanol was evaporated and the aqueous layer was diluted with 1,4-dioxane (30 mL). The suspension was cooled to 0°C and (9*H*-fluoren-9-yl)methyl carbonochloridate (CAS-RN: 28920-43-6, 3.98 g, 15.4 mmol) was added. The mixture was stirred over night at room temperature. Water and MTBE were added and the organic layer was separated. The aqueous layer

was acidified and extracted three times with DCM. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, and filtered. The crude product was purified by flash chromatography on silica gel (DCM: methanol/HOAc 95+5+1) to give 2.22 g (55 % yield) of the title compound.

LC-MS (Method 10): $R_t = 1.62$ min; MS (ESI pos): $m/z = 396.18$ $[M+H]^+$; $R_t = 1.67$ min; MS (ESI pos): $m/z = 396.18$ $[M+H]^+$

Example 115A

(2*S*)-2-({[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino)-2-[(1*S*,3*R*)-3-hydroxycyclohexyl]acetic acid (diastereomer 1)



Diastereomer 1

(2*S*)-{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino}[(1*S*)-3-hydroxycyclohexyl]acetic acid (Example 114A, 2.22 g) was purified by chiral chromatography using the method described in Example 96 A to provide 890 mg (100 % purity, 40 % yield, 99% ee) of diastereomer 1.

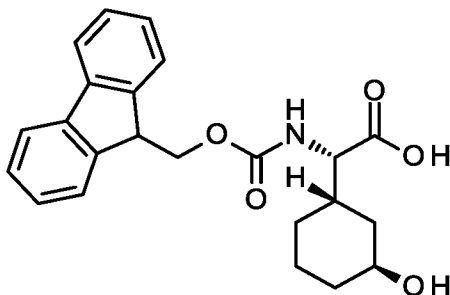
LC-MS (Method 10): $R_t = 1.62$ min; MS (ESI pos): $m/z = 396.2$ $[M+H]^+$

¹H-NMR (400 MHz, DMSO-*d*₆) δ [ppm]: 0.878 (0.88), 0.909 (1.80), 0.940 (2.23), 0.962 (1.87), 0.986 (2.99), 1.025 (4.63), 1.053 (3.69), 1.083 (1.14), 1.151 (1.18), 1.184 (2.16), 1.217 (1.71), 1.249 (0.53), 1.492 (2.55), 1.523 (2.32), 1.668 (2.86), 1.701 (2.42), 1.779 (6.54), 1.802 (4.90), 2.073 (4.22), 3.875 (0.46), 3.900 (3.08), 3.916 (3.51), 3.921 (3.52), 3.936 (2.67), 4.197 (1.31), 4.211 (3.35), 4.223 (9.23), 4.238 (12.01), 4.242 (10.59), 4.253 (6.34), 4.270 (3.91), 4.296 (0.72), 4.563 (2.04), 7.312 (6.21), 7.331 (14.61), 7.349 (9.50), 7.402 (9.30), 7.420 (15.31), 7.439 (6.83), 7.603 (4.81), 7.624 (4.95), 7.752 (11.74), 7.770 (10.65), 7.884 (16.00), 7.903 (14.82), 8.155 (0.69).

This amino acid was used in SPPS.

Example 116A

(2*S*)-2-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)-2-[(1*S*,3*S*)-3-hydroxycyclohexyl]acetic acid (diastereomer 2)



5

Also isolated during the chiral chromatography of the diastereomeric mixture of (2*S*)-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)-2-[(1*S*,3*S*)-3-hydroxycyclohexyl]acetic acid (Example 114A) was 280 mg (95 % purity, 12 % yield) of diastereomer 2.

LC-MS (Method 10): $R_t = 1.67$ min; MS (ESI pos): $m/z = 396.2$ $[M+H]^+$

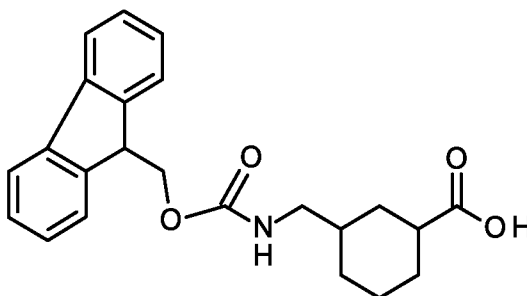
10 $^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: 0.862 (0.81), 0.879 (0.71), 1.024 (0.76), 1.055 (1.68), 1.084 (1.84), 1.113 (1.00), 1.274 (1.39), 1.308 (2.53), 1.339 (1.79), 1.404 (4.60), 1.430 (3.90), 1.498 (3.23), 1.531 (6.31), 1.566 (4.20), 1.609 (2.04), 1.640 (1.55), 2.179 (1.80), 2.328 (0.51), 2.670 (0.47), 3.820 (0.62), 3.839 (0.86), 3.858 (0.76), 3.904 (4.71), 3.920 (3.46), 3.925 (3.34), 3.941 (2.46), 4.195 (1.38), 4.210 (3.69), 4.223 (9.20), 4.238 (10.72), 4.244 (8.66), 4.254 (7.18), 4.272 (6.16), 7.309 (5.80), 7.328 (13.47), 7.346 (8.67), 7.400 (9.17), 7.419 (15.07), 7.437 (6.77), 7.487 (3.68), 7.508 (3.60), 7.546 (0.67), 7.637 (0.61), 7.748 (11.33), 7.767 (10.18), 7.883 (16.00), 7.901 (14.74).

15

This amino acid was used in SPPS.

Example 117A

3-({[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}methyl)cyclohexanecarboxylic acid (racemate)



20

3-({[(*tert*-Butoxycarbonyl)amino]methyl}cyclohexanecarboxylic acid (racemate) (CAS RN: 145149-55-9, 1.00 g, 3.89 mmol) was dissolved in dichloromethane (20 mL, 310 mmol), trifluoroacetic acid (20 mL, 260 mmol) was added, and the reaction mixture was stirred for 30 min at room temperature. The solution was evaporated and the residue was dissolved in acetone (15 mL). Water (10 mL), sodium hydrogen carbonate (6.53 g, 77.7 mmol) and 1-({[(9H-fluoren-9-yl)methoxy]carbonyl}oxy)pyrrolidine-2,5-dione (CAS-RN: 82911-69-11.38 g, 4.08 mmol) were added. The suspension was stirred overnight at room temperature. Sodium hydrogen carbonate (6.53 g, 77.7 mmol) was added and the reaction mixture was allowed to stir for 1 h at room temperature. The suspension was diluted with water and extracted with MTBE. The aqueous layer was acidified with aqueous hydrochloric acid (1M) and then extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and then concentrated and evaporated, to provide three product-containing fractions: 130 mg (96 % purity, 8 % yield), 127 mg (96 % purity, 8 % yield), 51.0 mg (3 % yield).

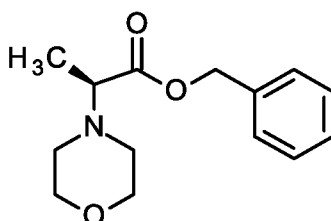
LC-MS (Method 9): $R_t = 1.01$ min; MS (ESI pos): $m/z = 380$ $[M+H]^+$

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: 0.736 (0.49), 0.759 (1.44), 0.790 (1.73), 0.821 (0.80), 0.851 (0.96), 0.882 (2.75), 0.913 (2.99), 0.944 (1.23), 1.083 (0.45), 1.120 (0.52), 1.153 (1.38), 1.182 (3.81), 1.206 (3.18), 1.235 (1.28), 1.418 (1.48), 1.598 (1.97), 1.630 (2.23), 1.717 (2.24), 1.739 (1.48), 1.747 (1.53), 1.835 (1.93), 1.862 (3.95), 1.897 (2.04), 2.074 (2.00), 2.123 (1.19), 2.130 (1.10), 2.152 (2.02), 2.174 (0.85), 2.181 (0.95), 2.524 (1.06), 2.785 (0.60), 2.801 (1.06), 2.818 (2.22), 2.835 (3.08), 2.849 (2.89), 2.862 (3.29), 2.878 (2.21), 2.895 (1.16), 2.911 (0.57), 4.188 (1.46), 4.206 (3.84), 4.223 (3.22), 4.238 (0.88), 4.253 (0.91), 4.287 (11.07), 4.304 (6.87), 4.333 (0.64), 4.351 (0.40), 4.451 (0.68), 7.306 (6.09), 7.324 (16.00), 7.341 (10.19), 7.392 (6.85), 7.410 (10.96), 7.429 (4.86), 7.459 (0.43), 7.626 (0.73), 7.643 (0.75), 7.683 (9.67), 7.702 (8.66), 7.875 (11.34), 7.894 (10.10), 12.028 (1.95).

This amino acid was used in SPPS.

Example 118A

Benzyl (2S)-2-(morpholin-4-yl)propanoate



L-alanine (589 mg, 6.61 mmol), 1-bromo-2-(2-bromoethoxy)ethane (910 μL , 7.3 mmol) and sodium carbonate were dissolved in ethanol and the solution was refluxed for 2 days. The suspension was allowed to come to room temperature and the suspension was filtered. The filtrate was evaporated, suspended in

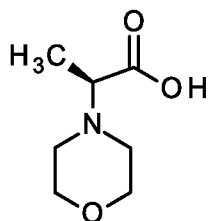
ethanol (30 mL) and 3M hydrochloric acid was added to adjust the pH (to pH = 3). The suspension was filtered, and the filtrate was evaporated. The residue was dissolved in DMF (20 mL). Cesium carbonate (1.08 g, 3.30 mmol) and (bromomethyl)benzene (1.6 mL, 13 mmol) were added and the mixture was stirred for 2 days at room temperature. Then water was added and then the solution was purified by reversed-phase HPLC (column: Reprosil; C18; 10 μ m; 125 x 30 mm; eluent ACN/H₂O with 0.1% HCOOH modifier; flow: 75 mL/min ; gradient: 0-5.50 min 10/ 90; Sample injection until 3.00 min; 5.50-17.65 min gradient to 95/5; 17.65-19.48 min 95/5; 19.48-19.66 min gradient to 10/90; 19.66-20.72 min 10/90;). The desired fractions were combined and lyophilized to provide 800 mg (98 % purity, 48 % yield) of the title compound as a colorless oil; ee >99%.

LC-MS (Method 9): R_t = 0.51 min; MS (ESI pos): m/z = 250 [M+H]⁺

¹H-NMR (500 MHz, DMSO-d₆) δ [ppm]: 1.191 (10.46), 1.205 (10.52), 2.462 (0.66), 2.469 (0.77), 2.473 (0.76), 2.485 (1.55), 2.521 (1.43), 2.562 (0.63), 3.330 (0.93), 3.344 (2.66), 3.358 (2.62), 3.372 (0.86), 3.499 (0.46), 3.506 (0.63), 3.511 (0.45), 3.521 (2.34), 3.530 (3.52), 3.532 (3.50), 3.538 (3.54), 3.549 (2.18), 3.564 (0.56), 5.101 (0.60), 5.126 (5.99), 5.131 (5.75), 5.156 (0.57), 7.323 (0.69), 7.327 (0.52), 7.332 (1.27), 7.341 (1.23), 7.343 (0.94), 7.350 (1.14), 7.358 (0.50), 7.369 (1.15), 7.377 (16.00), 7.386 (6.48), 7.392 (0.46).

Example 119A

(2S)-2-(Morpholin-4-yl)propanoic acid



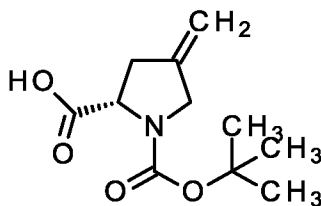
Palladium (10% on activated carbon) (100 mg) was moistened with methanol under argon. After that, benzyl (2S)-2-(morpholin-4-yl)propanoate (Example 118A, 800 mg, 3.21 mmol) was dissolved in methanol (100 mL) and the moistened palladium on activated carbon was added to the solution under argon. The suspension then was hydrogenated for 2 hours at ambient pressure under a hydrogen atmosphere. The vessel was purged of hydrogen with argon and the suspension was filtered over celite. The filtrate was evaporated, and the residue was dissolved in MeCN/H₂O and lyophilized to provide 501 mg (>99 % purity, 98 % yield) of the title compound as a light-brown solid.

LC-MS (Method 9): R_t = 0.16 min; MS (ESI pos): m/z = 160 [M+H]⁺

¹H-NMR (500 MHz, deuterium oxide) δ [ppm]: 1.519 (16.00), 1.534 (15.96), 3.695 (1.39), 3.709 (4.02), 3.724 (3.89), 3.738 (1.30).

Example 120A

1-(Tert-butoxycarbonyl)-4-ethylidene-L-proline

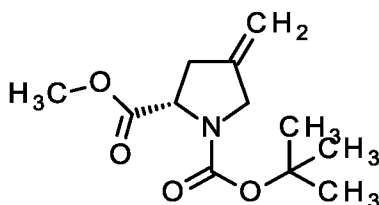


5

Methyl(triphenyl)phosphonium bromide (34.7 g, 97.1 mmol) was suspended in 400 mL THF. A solution of KHMDS solution in toluene (146.6 mL, 15% w/v, 97.1 mmol) was added dropwise at room temperature and stirred for one hour at room temperature. After that, the solution was cooled to 0°C, and 1-(tert-butoxycarbonyl)-4-oxo-L-proline (11.1 g, 48.6 mmol) was added in portions. The mixture was stirred 2.5 hours at room temperature. Water was added to the reaction mixture. The mixture was made slightly acidic with 10% citric acid solution and the extracted three times with ethyl acetate. The combined ethyl acetate layer was washed with a saturated aqueous sodium chloride solution, dried over magnesium sulfate, and then dried under vacuum to give a thick orange resin. The crude product was used for the next step without further purification.

Example 121A

1-Tert-butyl 2-methyl (2S)-4-methylidenepyrrolidine-1,2-dicarboxylate



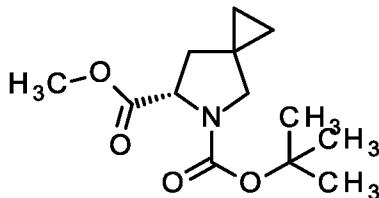
The crude 1-(tert-butoxycarbonyl)-4-ethylidene-L-proline (example 120A) (11.0 g, 48.6 mmol) was dissolved in DMF (60 mL). Potassium carbonate (10.1 g, 72.9 mmol) was added, then iodomethane (4.5 mL, 73 mmol) was added, and then the mixture was stirred overnight at room temperature. The DMF was removed with a rotary evaporator, ethyl acetate was added, and then the mixture was washed one time with water. The aqueous phase was one time re-extracted with ethyl acetate. The combined organic phases were washed with a saturated aqueous sodium chloride solution, dried over magnesium sulfate, and then dried under vacuum to give the crude product. The crude product was dissolved in DCM and purified by normal-phase chromatography using a Biotage Isolara flash chromatography system, using an eluant of cyclohexane – ethyl acetate (85/15) to give 10.2 g (80%) of the title compound as a yellow oil.

LC-MS (Method 10): $R_t = 1.77$ min; MS (ESI pos): $m/z = 186$ M-C₄H₈⁺

¹H-NMR (500 MHz, DMSO-d₆) δ [ppm]: 1.339 (16.00), 1.405 (10.53), 3.313 (10.02), 3.627 (3.90), 3.938 (2.21), 3.967 (0.60), 4.331 (0.51), 4.337 (0.54), 4.350 (0.57), 4.356 (0.59), 4.363 (0.47), 4.367 (0.42), 4.992 (1.33), 5.019 (1.17).

5 Example 122A

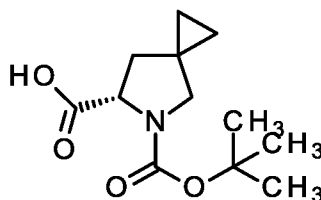
5-Tert-butyl 6-methyl (6S)-5-azaspiro[2.4]heptane-5,6-dicarboxylate



- Under argon, diethylzinc (22 mL, 1.0 M in hexane, 22 mmol) was dissolved in anhydrous DCM (27 mL).
- 10 The solution was cooled to 5°C and then a solution of TFA (1.7 mL, 22 mmol) in DCM (17 mL) was added dropwise over a 1 hour period. The solution was stirred at 5°C for an additional 30 min. A solution of diiodomethane (1.8 mL, 22 mmol) in anhydrous DCM (5 mL) was added dropwise quickly, and the resulting solution was stirred at 5°C for 30 min. After that, a solution of 1-tert-butyl 2-methyl (2S)-4-methylidenepyrrolidine-1,2-dicarboxylate (example 121A) (2.70 g, 11.2 mmol) in anhydrous DCM (5 mL)
- 15 was added to the reaction mixture. The reaction mixture was stirred in a thawing ice-bath for 90 hours, then quenched with saturated, aqueous ammonium chloride solution. The phases were separated, and the aqueous phase was re-extracted with DCM one time. The organic phases were combined, dried over magnesium sulfate, and then evaporated to provide 1.57 g the crude product as a yellow oil. The oil was dissolved in ethyl acetate (54 mL), di-tert-butyl dicarbonate (2.6 mL, 11 mmol) and triethylamine (3.1 mL,
- 20 22 mmol) were sequentially added, and then the reaction mixture was stirred overnight at room temperature. The reaction was controlled using TLC (petroleum ether/ethyl acetate 10/1; colored with ninhydrin). The crude product was purified by normal-phase chromatography using a Biotage Isolara flash chromatography system (100 g Biotage cartridge Ultra), using an eluant gradient of petroleum ether – ethyl acetate (EA) (2% to 18% EA) to provide 1.02 g of the title compound as a colorless oil.

25 Example 123A

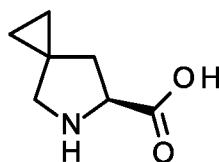
(6S)-5-(Tert-butoxycarbonyl)-5-azaspiro[2.4]heptane-6-carboxylic acid



5-Tert-butyl 6-methyl (6S)-5-azaspiro[2.4]heptane-5,6-dicarboxylate (example 122A) (2.50 g, 9.79 mmol) was dissolved in 50% THF/water (40 mL). Lithium hydroxide (703 mg, 29.4 mmol) was added, and the reaction mixture was stirred overnight at room temperature. The THF was removed using a rotary evaporator, and the aqueous solution was diluted with additional water (20 mL) water and washed once with MTBE. The separated aqueous phase was acidified with 1M hydrochloric acid (30 mL) and then extracted two times with DCM. The combined organic phase was dried over magnesium sulfate, filtered, and then concentrated to give 2.4 g of the title compound as a colorless oil.

Example 124A

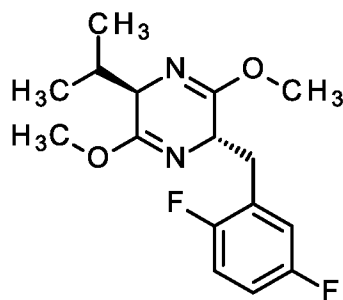
(6S)-5-azaspiro[2.4]heptane-6-carboxylic acid



(6S)-5-(tert-butoxycarbonyl)-5-azaspiro[2.4]heptane-6-carboxylic acid (example 123A) (2.36 g, 9.79 mmol) was dissolved in DCM (50 mL). Trifluoroacetic acid (10 mL, 250 mmol) was added, and the reaction mixture was stirred 2 hours at room temperature. The reaction control was made using TLC (DCM/MeOH 9/1). The solution was evaporated and the crude product was dried under vacuum to provide the title compound as a brown residue. This was used directly for the next step (Fmoc protection, see Example 82A).

Example 15A

(2S,5R)-2-[(2,5-difluorophenyl)methyl]-3,6-dimethoxy-5-(propan-2-yl)-2,5-dihydropyrazine



The reaction was performed under argon. (2R)-3,6-dimethoxy-2-(propan-2-yl)-2,5-dihydropyrazine (CAS-RN: 109838-85-9) (2.9 mL, 16 mmol) was dissolved in anhydrous THF (40 mL) and the solution was cooled to -78°C. At this temperature, an n-butyl lithium solution (10 mL, 1.6 M in hexane, 16 mmol) was slowly added dropwise, and the resulting solution was stirred 15 min at -78°C. Then a solution of 2-(bromomethyl)-1,4-difluorobenzene (4.05 g, 19.5 mmol) in anhydrous THF (15 mL) was added and stirring was continued until the reaction came to room temperature. The reaction mixture was stirred for an

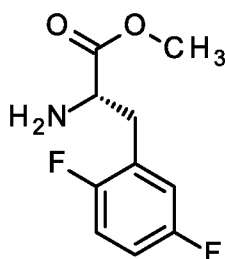
additional hour at room temperature, while monitoring the reaction progress by HPLC. The mixture was quenched with water and extracted two times with ethyl acetate. The combined organic phases were washed one time with a saturated aqueous sodium chloride solution, dried over magnesium sulfate, and then concentrated to give 5.46 g of a crude yellow oil. The oil was purified by normal phase chromatography using a Biotage Isolara flash chromatography system (100 g Biotage cartridge Ultra), using an eluant of cyclohexane – ethyl acetate (EA) (95/5) to afford 4.16 g (82%) of the title compound as an oil.

LC-MS (Method 10): $R_t = 2.44$ min; MS (ESI pos): $m/z = 311$ $[M+H]^+$

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ [ppm]: 0.563 (6.79), 0.580 (6.97), 0.933 (6.65), 0.951 (6.88), 2.103 (0.58), 2.111 (0.61), 2.120 (0.78), 2.129 (0.79), 2.137 (0.59), 2.145 (0.57), 2.864 (0.79), 2.882 (0.83), 2.898 (1.08), 2.915 (1.09), 3.089 (1.06), 3.101 (1.10), 3.123 (0.82), 3.134 (0.81), 3.313 (7.20), 3.518 (1.28), 3.527 (2.46), 3.535 (1.37), 3.566 (16.00), 4.293 (0.58), 4.304 (1.07), 4.313 (1.08), 4.321 (1.01), 4.330 (0.58), 6.992 (0.47), 7.000 (0.66), 7.005 (0.63), 7.015 (1.06), 7.023 (0.71), 7.028 (0.64), 7.037 (0.63), 7.075 (0.63), 7.084 (0.91), 7.095 (0.74), 7.105 (0.66), 7.131 (0.68), 7.143 (0.73), 7.154 (1.05), 7.166 (1.04), 7.177 (0.45), 7.189 (0.40).

Example 126A

Methyl 2,5-difluoro-L-phenylalaninate



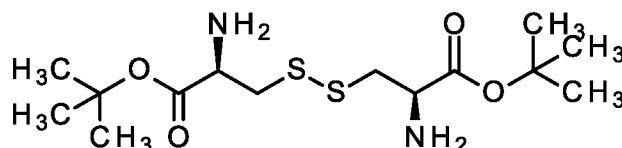
(2S,5R)-2-[(2,5-difluorophenyl)methyl]-3,6-dimethoxy-5-(propan-2-yl)-2,5-dihydropyrazine (example 125A) (4.15 g, 13.4 mmol) was dissolved in methanol (35 mL) and the solution was cooled to 0°C. A solution of a aqueous 10% hydrochloric acid (12 mL) was added and the solution was stirred 0°C for some minutes. Then the reaction solution was allowed to come to room temperature and then stirred three hours at this temperature. The methanol was evaporated. The residue was diluted with DCM, 2M aqueous sodium carbonate solution (120 mL) was added and the resulting mixture was stirred vigorously for a few minutes. The layers were separated, and the aqueous phase was extracted two times with DCM. The combined organic phases were dried over magnesium sulfate, filtered, and then concentrated to give 3.97 g of a colorless liquid. The crude liquid was purified by normal phase chromatography using a Biotage Isolara flash chromatography system (100 g Biotage cartridge Ultra), using a gradient eluant of cyclohexane – ethyl acetate (EA) (10% to 75% EA) to provide 2.27 g (79%) of the title compound as a colorless liquid.

LC-MS (Method 13): $R_t = 1.20$ min; MS (ESI pos): $m/z = 216$ $[M+H]^+$

¹H-NMR (400 MHz, DMSO-d₆) δ [ppm]: 1.833 (16.00), 2.733 (3.07), 2.753 (3.26), 2.767 (4.81), 2.787 (5.01), 2.889 (4.71), 2.904 (4.91), 2.923 (3.10), 2.938 (3.19), 3.315 (15.20), 3.401 (0.42), 3.553 (5.22), 3.568 (6.29), 3.572 (6.60), 3.768 (0.41), 7.060 (0.89), 7.069 (1.71), 7.081 (2.21), 7.090 (4.07), 7.101 (3.20), 7.111 (2.84), 7.120 (1.89), 7.151 (5.97), 7.163 (5.82), 7.174 (9.84), 7.186 (7.52), 7.196 (4.42),
 5 7.208 (2.10).

Example 127A

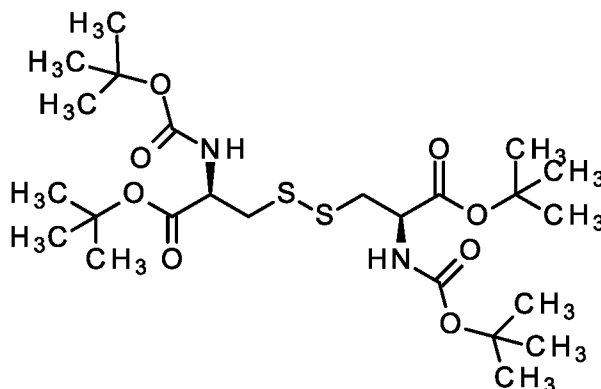
tert-Butyl (2R)-2-amino-3-[[[(2R)-2-amino-3-tert-butoxy-3-oxo-propyl]disulfanyl]propanoate



- 10 To the suspension of L-Cystine (CAS RN: 56-89-3) (10.00 g, 41.61 mmol) in tert-butyl acetate (63.22 g, 544.24 mmol, 73 mL) was added HClO₄ (18.26 g, 127.23 mmol, 11 mL, 70% purity) (70% in H₂O) drop-wise at 0 °C over 0.5 h, then the solution was stirred for 17 h at 20 °C. Ice was added and the solution was used for the next step without further purification (14.67 g in theory).

Example 128A

- 15 tert-Butyl (2R)-3-[[[(2R)-3-tert-butoxy-2-(tert-butoxycarbonylamino)-3-oxo-propyl]disulfanyl]-2-(tert-butoxycarbonylamino)propanoate



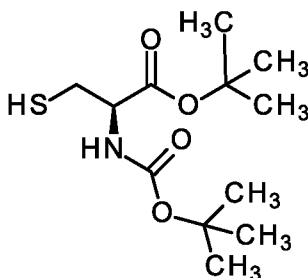
- 20 To the mixture of tert-butyl (2R)-2-amino-3-[[[(2R)-2-amino-3-tert-butoxy-3-oxo-propyl]disulfanyl]propanoate (14.67 g in theory, from Example 127A) in water (200 mL) and EA (200 mL) was added Na₂CO₃ (30.00 g, 283.08 mmol) and Boc₂O (27.25 g, 124.85 mmol) at 0°C, and the mixture (pH was 8-9) was stirred for 17 h at 20°C. The mixture was diluted with EA (100 mL) and water (200 mL). The organic layer was separated, and aqueous layer was extracted with EA (200 mL). The combined organic

layer was washed with brine (200 mL), concentrated to give a residue, the residue was purified by silical gel chromatography (PE/EA=1/0 to 1/1) to give two batches of the title compound: 8.5 g crude as a light brown oil and 6.5 g as a light brown oil.

¹H-NMR (400 MHz, CDCl₃) δ [ppm]: 1.51, 1.61, 1.62, 1.64, 3.11, 3.12, 3.14, 3.16, 3.19, 3.203, 3.23, 3.24, 4.44, 4.46, 4.48, 4.49, 5.35, 5.37.

Example 129A

tert-Butyl (2R)-2-(tert-butoxycarbonylamino)-3-sulfanyl-propanoate

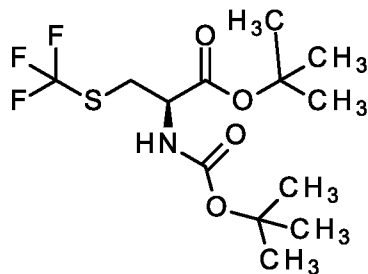


To the solution of tert-butyl (2R)-3-[[[(2R)-3-tert-butoxy-2-(tert-butoxycarbonylamino)-3-oxo-propyl]di-sulfanyl]-2-(tert-butoxycarbonylamino)propanoate (8.5 g, crude, from example 128A) in THF (150 mL) and water (15 mL) was added tributylphosphane (3.77 g, 4.6 mL) at 0 °C over 0.5 h, then the solution was stirred for 2 h at 20 °C. The mixture was worked up with another batch, prepared from 6.5 g of rare material (from Example 128A). Both reaction mixtures were poured into water (300 mL) and extracted with ethyl acetate (150 mL x 2). The combined organic phase was washed with brine (300 mL), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (PE/EA = 40/1 to 10/1) to afford 12.5 g of the title compound as a colorless oil, which was used as pure. 6.5 g of an impure batch containing residual tributylphosphine was also obtained but discarded.

¹H-NMR (400 MHz, CDCl₃) δ [ppm]: 1.45, 2.94, 2.95, 2.96, 2.97, 4.46, 4.47, 4.48, 4.49, 5.39, 5.41

Example 130A

tert-Butyl (2R)-2-(tert-butoxycarbonylamino)-3-(trifluoromethylsulfanyl)propanoate

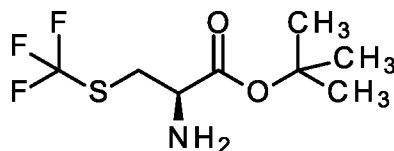


To a solution of 3,3-dimethyl-1-(trifluoromethyl)-1,2-benziodoxole (18 g, 54.53 mmol) in MeOH (150 mL) was added a solution of tert-butyl (2R)-2-(tert-butoxycarbonylamino)-3-sulfanyl-propanoate (14 g, 50.47 mmol, from Example 129A) in MeOH (20 mL) at -78°C under N₂ over 30 min, and the reaction was stirred for 1 h at this temperature, upon which it becomes colorless solution. The reaction mixture was warmed to 20°C, stirred for 1 h, and concentrated to give a residue, which was purified by silica gel chromatography (PE/EA=1/0 to 40/1) twice to give 13.2 g of the title compound as a colorless oil.

¹H-NMR (400 MHz, CDCl₃) δ [ppm]: 1.47, 3.26, 3.28, 3.30, 3.31, 3.45, 3.46, 3.48, 3.50, 4.47, 4.48, 5.34, 5.35.

Example 131A

tert-Butyl (2R)-2-amino-3-(trifluoromethylsulfanyl)propanoate hydrochloride

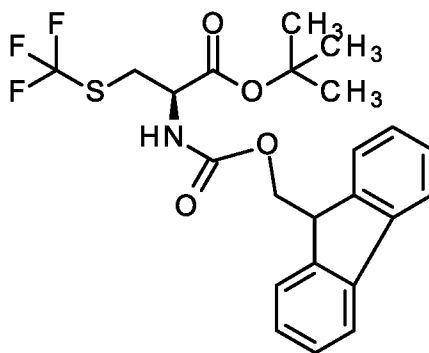


To a solution of tert-butyl (2R)-2-(tert-butoxycarbonylamino)-3-(trifluoromethylsulfanyl)-propanoate (12.20 g, 35.32 mmol, from example 130A) in DCM (10 mL) was added a solution of HCl/dioxane (4 M, 50 mL) and the reaction was stirred for 3 h at 10°C. The mixture was concentrated to give a white glassy solid, which was used for next step directly.

Example 132A

tert-Butyl (2R)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(trifluoromethylsulfanyl)-propanoate

- 226 -



To the mixture of tert-butyl (2R)-2-amino-3-(trifluoromethylsulfanyl)propanoate hydrochloride (crude from Example 131A) in dioxane (80 mL) was added saturated NaHCO_3 (80 mL), and Fmoc-OSu (19 g, 56.33 mmol), and the reaction (pH ~7-8) was stirred for 17 h at 10°C. The mixture was concentrated, and the residue was diluted with water (100 mL), then extracted with EA (100 mL x 2). The organic layer was washed with brine (100 mL), concentrated, and then purified by silica gel chromatography (PE/EA=40/1~10/1) to give 13 g of the title compound as a colorless oil.

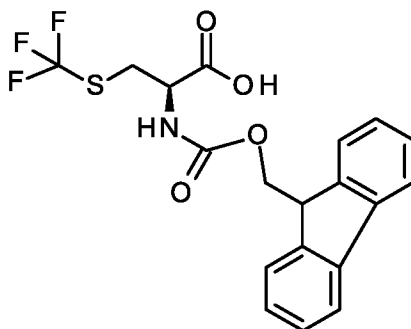
LC-MS (Method 15): $R_t = 1.028$ min; MS (ESI pos): $m/z = 490.2$ $[\text{M}+\text{Na}]^+$

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ [ppm]: 1.45, 3.32, 3.47, 3.36, 3.49, 3.50, 4.16, 4.22, 4.24, 4.26, 4.37, 4.44, 4.46, 4.58, 5.62, 5.64, 7.33, 7.35, 7.40, 7.42, 7.44, 7.56, 7.61, 7.77, 7.79.

$^{19}\text{F-NMR}$: (376 MHz, CDCl_3) δ [ppm]: -40.85.

Example 133A

(2R)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(trifluoromethylsulfanyl)propanoic acid



To a solution of tert-butyl (2R)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(trifluoromethylsulfanyl)propanoate (13 g, 27.81 mmol) in DCM (15 mL) was added TFA (30 mL) and thioanisole (15.75 g, 126.81 mmol, 15 mL), and the reaction mixture was stirred for 17 h at 10°C. The mixture was concentrated to give a residue, which was diluted with saturated NaHCO_3 solution (100 mL), and then extracted with EA (50 mL x 2). The organic layer was washed with 1M HCl (50 mL), concentrated, and then purified by silica gel chromatography (PE/EA=10/1, then with DCM/MeOH=40/1 to 5/1) to give 10 g of the title

compound as a white glassy solid purity: 98.8%. ¹H-NMR indicated that the product contained ethyl acetate, so the solid was re-dissolved in DCM (80 mL), concentrated and then dried under high vacuum to give 9.67 g (23.22 mmol, 83.5% yield, 98.8% purity) of the title compound as a white glassy solid.

LC-MS (Method 15): R_t = 0.895 min; MS (ESI pos): m/z = 434.1 [M+Na]⁺

- 5 ¹H-NMR (400 MHz, CDCl₃) δ [ppm]: 3.25, 3.26, 3.32, 3.33, 3.93, 3.95, 4.22, 4.24, 4.26, 4.28, 4.30, 4.31, 6.95, 6.98, 7.31, 7.32, 7.33, 7.34, 7.39, 7.41, 7.42, 7.68, 7.70, 7.87, 7.89.

¹⁹F-NMR: (376 MHz, CDCl₃) δ [ppm]: -40.89

Chiral SFC: R_t = 2.49 min, 99% ee

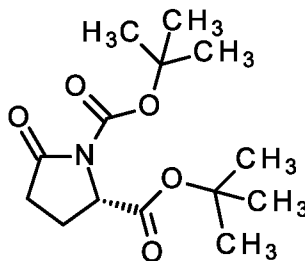
Chiral SFC condition: AD-3_5CM_IPA (DEA)_10_20_3ML_T35 Column: Chiralpak AD-3 50 x 4.6 mm

- 10 I.D., 3 μm Mobile phase: iso-propanol (0.05% DEA) in CO₂ from 10% to 20% Flow rate: 3 mL/min Wavelength: 220 nm.

This amino acid was used in SPPS. This amino acid is also commercially available (CAS-RN: 1994331-25-7).

Example 134A

- 15 Di-tert-butyl (2S)-5-oxopyrrolidine-1,2-dicarboxylate



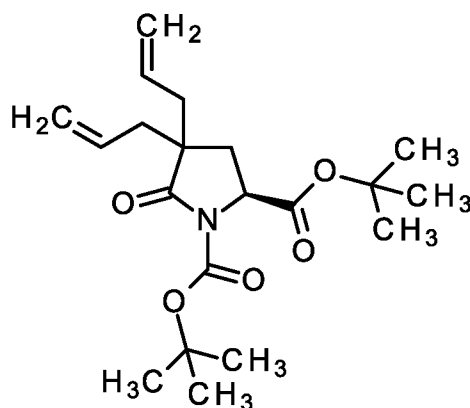
- To a solution of compound tert-butyl (2S)-5-oxopyrrolidine-1,2-dicarboxylate (CAS-RN: 35418-16-7) (290 g, 1.57 mol, 1.00 eq) and DMAP (1.91 g, 15.66 mmol, 0.01 eq) in CH₃CN (2.00 L), was added Boc₂O (341 g, 1.57 mol, 359 mL, 1.00 eq) at 5 °C. After the addition, the mixture was stirred at 20 °C for 12 h. TLC showed that the starting material was completely consumed, and two new spots (TLC) were formed (PE/EA = 3/1, R_f = 0.43, R_f = 0.9). The reaction mixture was concentrated under reduced pressure to remove CH₃CN and the residue was partitioned between DCM (1000 mL) and water (1000 mL). The organic layer was washed with brine (300 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give the crude product. The residue was purified by column chromatography (SiO₂, PE/EA=100/1 to 10/1) to afford the title compound (260.00 g, 874.75 mmol, 55.72% yield, 96% purity) as off-white solid.

LC-MS (Method 15): $R_t = 0.873$ min; MS (ESI pos): $m/z = 308.0$ $[M+Na]^+$, 331 $[M+2Na]^+$

^1H -NMR (400 MHz, $CDCl_3$) δ [ppm]: 1.44-1.42 (d, $J=8.4$, 8H), 1.97-1.92 (m, 1H), 2.22-2.19 (m, 1H), 2.43-2.40 (m, 1H), 2.58-2.49 (m, 1H), 4.42-4.39 (m, 1H).

Example 135A

5 Di-tert-butyl (2S)-4,4-diallyl-5-oxo-pyrrolidine-1,2-dicarboxylate



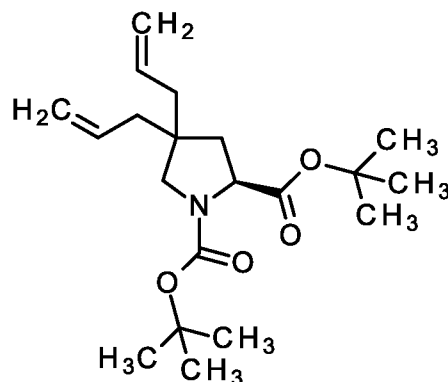
To a solution of di-tert-butyl (2S)-5-oxopyrrolidine-1,2-dicarboxylate (61.9 g, 208 mmol, 1.00 eq, from Example 134A) in THF (40.0 mL) was added LiHMDS (1 M, 437 mL, 2.10 eq) dropwise at -73 °C and then stirred at -73 °C for 1 h. To the mixture was added compound allyl bromide (50.4 g, 416 mmol, 2.00 eq) dropwise. After the addition, the mixture was stirred at 15 °C for 2 h. TLC showed compound that the starting material was completely consumed, and four new spots were formed ($PE/EA = 3/1$, $R_f = 0.9$, $R_f = 0.8$, $R_f = 0.7$, $R_f = 0.1$). The reaction mixture was quenched with saturated aqueous ammonium chloride (1000 mL), extracted with DCM (500 mL x 3), dried over sodium sulfate, filtered, and then concentrated under reduced pressure to give a crude residue. The residue was purified by column chromatography (SiO_2 , $PE/EA = 100/1$ to $30/1$) to afford the title compound (100 g, 38% yield, 86% purity) as a colorless oil.

LC-MS (Method 15): $R_t = 1.044$ min; MS (ESI pos): $m/z = 411.4$ $[M+2Na]^+$

^1H -NMR (400 MHz, $CDCl_3$) δ [ppm]: 1.49-1.43 (d, $J=8.4$, 8H), 1.87-1.82 (m, 1H), 2.22-2.18 (m, 3H),

20 **Example 136A**

Di-tert-butyl (2S)-4,4-diallylpyrrolidine-1,2-dicarboxylate



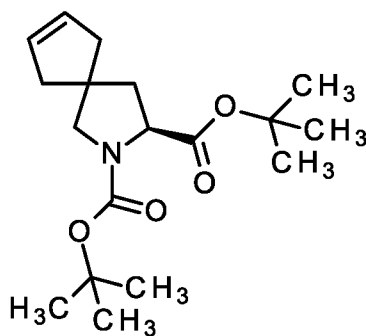
To a solution of di-tert-butyl (2S)-4,4-diallyl-5-oxo-pyrrolidine-1,2-dicarboxylate (80.0 g, 188 mmol, 1.00 eq, from Example 135A) in THF (300 mL) was added LiBHET₃ (1 M, 225 mL, 1.20 eq) at -78 °C. After the addition, the mixture was stirred at -78 °C for 3 h. LCMS showed the desired MS was detected. TLC showed two new spots were formed (PE/EA = 3/1, R_f = 0.65, R_f = 0.8). The reaction mixture was quenched with saturated aqueous sodium carbonate (50 mL) at 0 °C and then 30% H₂O₂ (1 mL) was added dropwise at 0 °C. The mixture was concentrated under reduced pressure to remove the THF and then the residue was diluted with water (1000 mL) and DCM (1000 mL). The water phase was extracted with DCM (300 mL x 3). The combined organic layer was dried over sodium sulfate, filtered, and then concentrated under reduced pressure to give the crude product. The crude residue was purified by column chromatography (SiO₂, PE/EA = 80/1 to 30/1) to afford the title compound (56.0 g, 69% yield, 85% purity) as a colorless oil.

LC-MS (Method 15): R_t = 1.192 min; MS (ESI pos): m/z = 390.3 [M+K]⁺

¹H-NMR (400 MHz, CDCl₃) δ [ppm]: 1.53-1.47 (m, 18H), 2.13-1.98 (m, 2H), 2.22-2.15 (m, 3H), 2.39-2.24 (m, 1H), 2.92-2.91 (m, 1H), 4.22-4.08 (m, 1H), 5.18-5.06 (m, 5H), 5.85-5.77 (m, 2H).

Example 137A

Di-tert-Butyl (3S)-2-azaspiro[4.4]non-7-ene-2,3-dicarboxylate

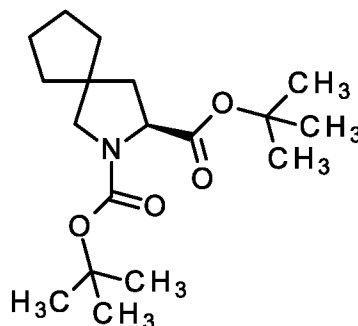


To a solution of the Grubb's 1st generation catalyst (CAS RN: 172222-30-9) (2.85 g, 3.47 mmol, 0.05 eq) in DCM (400.00 mL) was added di-tert-butyl (2S)-4,4-diallylpyrrolidine-1,2-dicarboxylate (25.0 g, 69.4 mmol, 1.00 eq, from Example 136A) dropwise at 34 °C. After the addition was completed, the mixture was stirred at 34 °C under N₂ for 1 h. TLC showed two new spots were formed (PE/EA = 5/1, R_f = 0.6, R_f = 0.7). The reaction mixture was filtered and concentrated under reduced pressure to give the crude product. The residue was purified by column chromatography (SiO₂, PE/EA = 1/0 to 80/1) to afford the title compound (12.0 g) as a colorless oil.

¹H-NMR (400 MHz, CDCl₃) δ [ppm]: 1.46-1.43 (m, 18H), 1.92-1.87 (m, 1H), 2.45-2.22 (m, 5H), 3.49-3.29 (m, 2H), 4.21-4.19 (m, 1H), 5.64 (s, 2H).

10 Example 138A

Di-tert-butyl (3S)-2-azaspiro[4.4]nonane-2,3-dicarboxylate

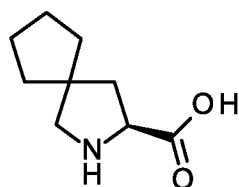


To a solution of di-tert-butyl (3S)-2-azaspiro[4.4]non-7-ene-2,3-dicarboxylate (12.0 g, 37.1 mmol, 1.0 eq, from Example 137A) in EtOH (100 mL) was added Pd/C (10%, 0.1 g) under a N₂ atmosphere. The suspension was degassed and purged 3 times with H₂ gas. The mixture was stirred under H₂ (15 Psi) at 25 °C for 16 h. Part of suspension was filtered, and the filtrate was concentrated, and proton NMR was taken, which showed that the desired product was formed. The reaction mixture was filtered, and the filtrate was concentrated to give 10.0 g (crude) of the title compound as a white solid.

¹H-NMR (400 MHz, CDCl₃) δ [ppm]: 4.12-4.02 (m, 1H), 3.65-3.60 (m, 2H), 3.12-3.38 (m, 5H), 2.11-2.06 (m, 1H), 1.80-1.77 (m, 1H), 1.57-1.56 (m, 6H), 1.40-1.36 (m, 18H).

Example 139A

(3S)-2-Azaspiro[4.4]nonane-3-carboxylic acid

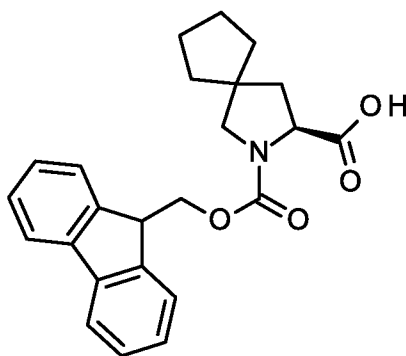


The compound di-tert-butyl (3S)-2-azaspiro[4.4]nonane-2,3-dicarboxylate (12 g, 36.8 mmol, 1 eq, from Example 138A) was added into a solution of HCl/EtOAc (150 mL), and then the mixture was stirred at 15 °C for 1 h. LC-MS analysis showed that the starting material was consumed, and the desired target mass was found. The reaction mixture was concentrated in vacuum to remove the solvent, affording 7.5 g (36.4 mmol, 99% yield) of the crude title compound as a white solid as a hydrochloride salt.

¹H-NMR (400 MHz, DMSO) δ [ppm]: 10.07 (s, 1H), 8.81 (s, 1H), 4.38-4.27 (m, 1H), 3.16-3.02 (m, 2H), 2.22-2.21 (m, 1H), 1.96-1.90 (m, 1H), 1.63-1.56 (m, 8H).

10 **Example 140A**

(3S)-2-[(9H-Fluoren-9-yl)methoxy]carbonyl}-2-azaspiro[4.4]nonane-3-carboxylic acid



To a solution of (3S)-2-azaspiro[4.4]nonane-3-carboxylic acid (5.1 g, 24.8 mmol, from Example 139A) and Fmoc-OSu (10.0 g, 29.7 mmol, 1.2 eq) in THF (70 mL) and H₂O (70 mL) was added NaHCO₃ (8.5 g, 101 mmol, 3.94, 4.08 eq). The mixture was stirred at 15 °C for 1 h. LCMS analysis showed that the starting material was consumed, and the desired target mass was obtained. Water (200 ml) was added, and then the mixture was extracted with DCM (200 mL x 2). The combined organic layer was washed with brine (100 mL), dried over Na₂SO₄, filtered, and then concentrated. The crude product was purified for reversed-phase MPLC (EA) to afford 6.9 g (17.5 mmol, 70 % yield, 99% purity) of the title compound as a yellow solid.

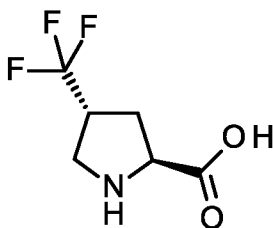
LC-MS (Method 15): R_t = 2.501 min; MS (ESI pos): m/z = 392.2 [M+H]⁺

¹H-NMR (400 MHz, CDCl₃) δ [ppm]: 7.80-7.79 (d, *J* = 7.2, 2H), 7.59-7.54 (m, 2H), 7.44-7.42 (m, 2H), 7.36-7.35 (m, 2H), 4.55-4.52 (m, 2H), 4.42-4.38 (m, 1H), 4.31-4.20 (m, 1H), 3.34-3.17 (m, 2H), 2.33-2.28 (m, 1H), 2.13-2.08 (m, 1H), 1.73-1.50 (m, 8H).

This amino acid was used in SPPS. This amino acid is also commercially available (CAS-RN: 394734-78-2).

Example 141A

(2S,4R)-4-(Trifluoromethyl)pyrrolidine-2-carboxylic acid



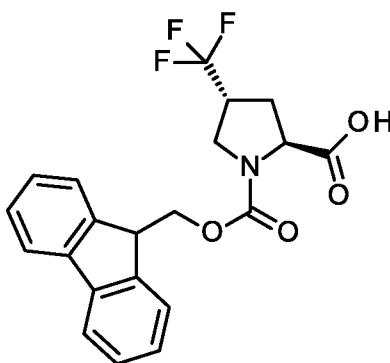
To a solution of (2S,4R)-1-tert-butoxycarbonyl-4-(trifluoromethyl)pyrrolidine-2-carboxylic acid (7 g, 24.7 mmol, 1 eq) in DCM (70 mL) was added TFA (8.00 mL, 108 mmol, 4.37 eq). The mixture was stirred at 10 °C for 4 h. LCMS analysis showed that the starting material was consumed and that the desired target mass was found. The reaction mixture was concentrated under vacuum and the crude product was used directly for the next step. The title compound (7.34 g, 24.7 mmol, 100% yield, TFA salt) was obtained as a white solid.

LC-MS (Method 15): *R*_t = 0.18 min; MS (ESI pos): *m/z* = 184.1 [M+H]⁺

¹H-NMR (400 MHz, CDCl₃) δ [ppm]: 4.51-4.48 (m, 1H), 3.82 (m, 1H), 3.57-3.55 (m, 1H), 2.65-2.62 (m, 2H), 2.53-2.49 (m, 1H).

Example 142A

(2S,4R)-1-(9H-Fluoren-9-ylmethoxycarbonyl)-4-(trifluoromethyl)pyrrolidine-2-carboxylic acid



To a solution of the crude (2S,4R)-4-(trifluoromethyl)pyrrolidine-2-carboxylic acid (2 g, 6.73 mmol, 1 eq, TFA salt, from Example 141A) in H₂O (70 mL) and acetone (70 mL) was added Na₂CO₃ (2.85 g, 26.9 mmol, 4 eq) and Fmoc-OSu (2.72 g, 8.08 mmol, 1.2 eq). The mixture was stirred at 10 °C for 12 h. LCMS analysis showed only one peak and that the desired mass was found. TLC analysis (DCM/MeOH = 10/1) showed that the starting material was consumed, and that a main spot (R_f = 0.4) appeared. HCl solution (1M, 30 mL) was added to the mixture until the pH = 2. The mixture was extracted with DCM (70 mL x 2), and the combined organic layer was washed with brine (70 ml), dried over Na₂SO₄, filtered, and then concentrated. The residue was purified by column chromatography (SiO₂, PE/ EA = 10/1 to 5/1), providing 1.7g (61%, 97% purity) of the title compound as a yellow solid.

LC-MS (Method 15): R_t = 2.425 min; MS (ESI pos): m/z = 406.2 [M+H]⁺, 406.2 [M+Na]⁺

¹H-NMR (400 MHz, DMSO) δ [ppm]: 7.92-7.89 (m, 2H), 7.66-7.64 (m, 2H), 7.42-7.41 (m, 2H), 7.35-7.32 (m, 2H), 4.16-4.19 (m, 4H), 3.72-3.50 (m, 3H), 2.38-2.30 (m, 2H), 2.18 (s, 1H).

¹⁹F-NMR (400 MHz, DMSO) δ [ppm]: -70.128, -70.275.

Chiral SFC: R_t = 2.33 min, 99% ee

Chiral SFC condition: AD-3_5CM_IPA (DEA)_10_20_3ML_T35 Column: Chiralpak AD-3 50 x 4.6 mm I.D., 3 μ m Mobile phase: iso-propanol (0.05% DEA) in CO₂ from 10% to 20% Flow rate: 3 mL/min Wavelength: 220 nm.

This amino acid was used for SPPS.

Table 13: Reference peptides

Reference No	Identifier	Sequence	Method of Preparation
1	SFMI-1	GIC+SRSLPPIC+IPD-OH	Method B
2	SFMI-1 HCl	GIC+SRSLPPIC+IPD-OH (HCl Salt)	Method B, F
3	SFMI-1 amide	GIC+SRSLPPIC+IPD-NH ₂	Method A
A	Ala-1 SFMI-1	AIC+SRSLPPIC+IPD-OH	Method B
B	Ala-1 SFMI-1 amide	AIC+SRSLPPIC+IPD-NH ₂	Method A
C	Ala-1 SFMI-1 HCl amide	AIC+SRSLPPIC+IPD-NH ₂ (HCl Salt)	Method A, F

Table 14: Peptides according to the invention

Example No	Sequence	Method of Preparation
4	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG1(10 atoms))-NH ₂	Method A
5	AIC+SRSLP-(Oic)-I-(Pen)+IPD-OH (salt free form)	Method B, C, G
6	(N ^{iva})-IC+SRS-(tBu)A)-PPI-(((N-Me)C))+I-NH ₂	Method D
7	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(PEG2(13 atoms))-NH ₂	Method A
8	(beta-P)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-NH ₂	Method A
9	PIC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
10	(Aib)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
11	A-((N-Me)I)-C+SRS-(tBu)A)-PPI-((N-Me)C)+IPD-NH ₂	Method A

12	AIC+SRS-((tBu)A)-PPI-(Pen)+IPD-OH	Method B
13	AIC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method B, C, D
14	AIC+SRS-((tBu)A)-P-(Oic)-I-(Pen)+IPD-OH	Method B
15	(2-(Pyrrolidine)acetyl)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method K
16	((N-Me)G)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-(PEG3(16atoms))-NH ₂	Method A
17	AIC+SRSLP-((6S)-5-Azaspino[2.4]heptane-6-carboxylic acid)-I-(Pen)+IPD-NH ₂	Method A
18	(Om)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-OH	Method B
19	RIC+SRS-((tBu)A)-PPI-((N-Me)C)+IPD-NH ₂	Method A
20	AIC+SRS-((tBu)A)-PPI-((N-Me)C)+(2-[(1S,2S)-1-(Amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid)-D-NH ₂	Method A
21	AIC+SRS-((tBu)A)-PPI-(Pen)+IPe-NH ₂	Method D
22	AIC+SRS-((tBu)A)-PPI-(Pen)+IPh-NH ₂	Method D
23	((N-Me)A)-IC+SRS-((tBu)A)-P-(Oic)-I-(Pen)+IP-NH ₂	Method C
24	((N-Me)A)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
25	AIC+SRS-((tBu)A)-PPI-((N-Me)C)+I-NH ₂	Method A
26	AIC+SRSLP-(Oic)-I-(Pen)+IPD-NH ₂ (HCl salt)	Method A, F
27	AIC+SRSLP-(Oic)-I-(Pen)+IPD-NH ₂ (salt free form)	Method A, G
28	(Nle)-IC+SRS-((tBu)A)-PPI-((N-Me)C)+I-NH ₂	Method D
29	AIC+SRSLP-(Oic)-I-(Pen)+IPD-OH (HCl salt)	Method B, C, F
30	AIC+SRS-((tBu)A)-PPI-((N-Me)C)+IPD-NH ₂ (HCl salt)	Method A, F
31	AIC+SRS-((tBu)A)-P-((6S)-5-Azaspino[2.4]heptane-6-carboxylic acid)-I-(Pen)+IPD-OH	Method B
32	(ACMP)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
33	((1R,3S,4S)-2-azabicyclo[2.2.1]heptane-3-carboxylic acid)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-NH ₂	Method A

34	(N-Me)G)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-(PEG7(25 atoms))-NH ₂	Method A
35	KIC+SRS-((tBu)A)-PPI-((N-Me)C)+IPD-NH ₂	Method A
36	AIC+SRSLP-((6S)-5-Azapiro[2.4]heptane-6-carboxylic acid)-I-(Pen)+IPD-OH	Method B
37	AIC+SRS-((tBu)A)-PPIC+I-NH ₂	Method A
38	RIC+SRS-((tBu)A)-PPI-((N-Me)C)+I-NH ₂	Method A
39	((N-Me)G)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-(PEG1(10 atoms))-NH ₂ (HCl salt)	Method A, F
40	(Cit)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
41	AIC+SRS-((tBu)A)-P-((6S)-5-Azapiro[2.4]heptane-6-carboxylic acid)-I-((N-Me)C)+IPD-NH ₂	Method A
42	((N-Me)G)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-(S)-3-(2H-tetrazol-5-yl)propanoic acid)-NH ₂	Method D
43	((N-Me)A)-IC+SRS-((tBu)A)-P-(Oic)-I-(Pen)+I-NH ₂	Method C
44	((N-Me)G)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-(PEG9(31 atoms))-NH ₂	Method A
45	AIC+SRS-((tBu)A)-P-((6S)-5-Azapiro[2.4]heptane-6-carboxylic acid)-I-(Pen)+IPD-NH ₂	Method A
46	(Pip)-IC+SRS-((tBu)A)-PPI-((N-Me)C)+IPD-NH ₂	Method A
47	(3-Amino-3-methylbutyric acid)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
48	AIC+SRS-((tBu)A)-PPI-((N-Me)C)+IPD-NH ₂	Method A
49	(Om)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method A
50	(Om)-IC+SRSLP-(Oic)-I-(Pen)+IPD-NH ₂	Method A
51	aIC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
52	A-(Cba)-C+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
53	TIC+SRS-((tBu)A)-PPI-((N-Me)C)+I-NH ₂	Method D
54	VIC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
55	((N-Me)G)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-(PEG5-CH ₂ CO ₂ H (18 atoms))-NH ₂	Method A

56	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-(Orn)-(Orn)-(Orn)-NH ₂	Method A
57	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(N-Me)G)-(N-Me)G)-(N-Me)G)-NH ₂	Method A
58	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(3-Carboxy)F)-NH ₂	Method A
59	Ac-G-(N-Me)I)-C+SRS+PPIC+IPD-OH	Method B
60	IIC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
61	AIC+SRS+PPI-(Oic)-I-(Pen)+I-NH ₂	Method D
62	((N-Me)G)-IC+SRS+PPI-(Oic)-I-(Pen)+IPD-(PEG2(13 atoms))-NH ₂	Method A
63	((N-Me)G)-IC+SRS+PPI-(Oic)-I-(Pen)+IPD-(PEG5-CH ₂ CO ₂ H (18 atoms))-NH ₂	Method A
64	((N-Me)G)-IC+SRS+PPI-(Oic)-I-(Pen)+IPD-(PEG5-CH ₂ CO ₂ H (18 atoms))-NH ₂	Method A
65	LIC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
66	(Abu)-IC+SRS-(tBu)A)-PPIC+IPD-NH ₂	Method A
67	((N-Me)G)-IC+SRS+PPI-(Oic)-I-(Pen)+IP-NH ₂ (HCl salt)	Method A, F
68	LIC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
69	(Nva)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
70	(Cit)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-OH	Method D
71	AIC+SRS+PPI-(Oic)-I-(Pen)+IP-NH ₂	Method D
72	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG2(13 atoms))-NH ₂	Method A
73	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG4-CH ₂ CO ₂ H (15 atoms))-NH ₂	Method A
74	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG8(28 atoms))-NH ₂	Method A
75	AIC+SRS+PPI-(Oic)-I-(Pen)+IPD-NH ₂	Method A, H
76	((N-Me)G)-IC+SRS+PPI-(Oic)-I-(Pen)+IP-(PEG1(10 atoms))-NH ₂	Method A
77	(Orn)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+IPD-NH ₂	Method A

78	(Om)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+IPD-NH ₂ (HCl salt)	Method A, F
79	KIC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
80	((N-Isopropyl-N-methylamino)acetyl)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method K
81	WIC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
82	(Azetidine-2-carboxylic acid)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
83	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-(PEG5-CH ₂ CO ₂ H (18 atoms))-NH ₂	Method A
84	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IPD-(PEG4(19 atoms))-NH ₂	Method A
85	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(TTDS)-NH ₂	Method A
86	AIC+SRS-(tBu)A)-PPI-(N-Me)C)+IPD-OH	Method B
87	AIC+SRS-(tBu)A)-P-(Oic)-IC+IPD-NH ₂ (HCl salt)	Method A, F
88	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IPD-OH	Method D
89	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG5(22 atoms))-NH ₂	Method A
90	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-(PEG5(22 atoms))-NH ₂	Method A
91	(Om)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
92	(ACMP)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
93	(3-Pal)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
94	(beta-2-thienylalanine)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
95	(Nle)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
96	aIC+SRS-IP-(Oic)-I-(Pen)+I-OH	Method D
97	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-(PEG4(19 atoms))-NH ₂	Method A
98	AIC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method A
99	AIC+SRS-(tBu)A)-P-(Oic)-IC+I-NH ₂	Method A

100	(N-Me)G)-(N-Me)I)-C+SRS-(tBu)A)-PPI-(Pen)+I-NH ₂	Method D
101	(N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IPD-(PEG3(16 atoms))-NH ₂	Method A
102	AIC+SRS-IP-(rel-1(R,3R,5R,6R)-6-(Trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid)-IC+IPD-NH ₂	Method A
103	SIC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
104	AIC+SRS-(2-BromoF)-PPI-(Pen)+IPD-NH ₂	Method A
105	AIC+SRS-IP-(Oic)-I-(Pen)+IPE-NH ₂	Method D
106	(N-Me)A)-IC+SRS-IP-(Oic)-I-(Pen)+IPD-NH ₂	Method A
107	(N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IPD-(PEG7(25 atoms))-NH ₂	Method A
108	AIC+SRS-(tBu)A)-P-(Oic)-I-(Pen)+IPD-NH ₂	Method A
109	(N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-NH ₂	Method A, H
110	(N-Me)A)-IC+SRS-IP-(Oic)-I-(Pen)+IP-OH	Method D
111	AIC+SRS-IP-(Oic)-I-(Pen)+IP-OH	Method D
112	AIC+SRS-IP-(Oic)-I-(Pen)+IPE-OH	Method D
113	(N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-(PEG4-CH ₂ CO ₂ H (15 atoms))-NH ₂	Method A
114	AIC+SRS-(tBu)A)-PP-(2S)-2-(Amino)-2-[1S,3R)-3-hydroxycyclohexyl]acetic acid)-((N-Me)C)+IPD-NH ₂	Method A
115	(Hoo)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
116	(Gamma-Abu)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
117	AIC+SRS-IP-(Oic)-I-(Pen)+I-OH	Method D
118	AIC+SRS-IP-(Oic)-I-(Pen)+IPQ-OH	Method D
119	(N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IPD-(TTDS)-NH ₂	Method A
120	A-(N-Me)I)-C+SRS-(tBu)A)-P-(Oic)-I-(N-Me)C)+IPD-NH ₂	Method A
121	AIC+SRS-(t-Bu)A)-PPIC-ID-OH	Method B

122	AIC+SRSLP-(Oic)-I-(Pen)+IPE-NH ₂	Method D
123	AIC+SRSLP-(Oic)-I-(N-Me)C)+IP-NH ₂	Method A
124	HIC+SRSLP-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
125	(Om)-IC+SRSLP-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
126	((N-Me)A)-IC+SRSLP-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
127	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(PEG3(16 atoms))-NH ₂	Method A
128	AIC+SRSLP-(tBu)A)-P-(4-CF ₃)P)-I-(N-Me)C)+IPD-NH ₂	Method A
129	AIC+SRSLP-(tBu)A)-P-(Oic)-IC+I-NH ₂	Method A
130	(2-(Piperidin)acetyl)-IC+SRSLP-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method K
131	((N-Me)A)-IC+SRSLP-(Oic)-I-(Pen)+IPE-NH ₂	Method D
132	AIC+SRSLP-(Oic)-I-(Pen)+IPQ-NH ₂	Method D
133	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG4-CH ₂ CO ₂ H (15 atoms))-NH ₂	Method A
134	((N-Me)A)-IC+SRSLP-(Oic)-I-(Pen)+I-OH	Method D
135	((N-Me)A)-IC+SRSLP-(Oic)-I-(Pen)+IP-NH ₂	Method A
136	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(TTDS)-NH ₂	Method A
137	(2-(Diethylamino)acetyl)-IC+SRSLP-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method K
138	AIC+SRSLP-(Oic)-I-(Pen)+IPE-OH	Method D
139	((N-Me)A)-IC+SRSLP-(Oic)-I-(Pen)+IPQ-NH ₂	Method D
140	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(PEG5(22 atoms))-NH ₂	Method A
141	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG8(28 atoms))-NH ₂	Method A
142	AIC+SRSLP-(Pro-D2)-(Oic)-I-(Pen)+IPD-OH	Deuterium
143	TIC+SRSLP-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D

144	(Om)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-OH	Method D
145	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG4(19 atoms))-NH ₂	Method A
146	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IPD-(PEG5(22 atoms))-NH ₂	Method A
147	A-(Abu)-C+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
148	TIC+SRS-(tBu)A)-PPI-(Pen)+IPD-OH	Method D
149	((N-Me)G)-((N-Me)I)-C+SRS-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
150	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-(PEG7(25 atoms))-NH ₂	Method A
151	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-(PEG9(31 atoms))-NH ₂	Method A
152	((N-Me)A)-IC+SRS-IP-(Oic)-I-(Pen)+IP-NH ₂	Method A
153	AIC+SRS-IP-(Oic)-IC+IPD-OH	Method B
154	HIC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
155	AIC+SRS-(tBu)A)-P-(Oic)-I-((N-Me)C)+IPD-OH (HCl salt)	Method B, F
156	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
157	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IPP-NH ₂	Method D
158	AIC+SRS-(tBu)A)-P-(4-CF ₃)P)-IC+IPD-NH ₂	Method A, C
159	AIC+SRS-IP-(Oic)-I-((N-Me)C)+I-NH ₂	Method A
160	aIC+SRS-IP-(Oic)-I-(Pen)+I-NH ₂	Method D
161	((N-Me)A)-IC+SRS-IP-(Oic)-I-(Pen)+IPE-OH	Method D
162	((N-Me)A)-IC+SRS-IP-(Oic)-I-(Pen)+IPD-OH	Method D
163	(Dab)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method A
164	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+I-(5-Azapiro[2.4]heptane-1-carboxylic acid)-OH	Method B
165	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IPD-NH ₂	Method A, D

166	(3-Amino-2,2-dimethylpropionic acid)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method A
167	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-OH	Method D
168	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-(PEG8(28 atoms))-NH ₂	Method A
169	(Abu)-IC+SRS-IP-(Chg)-C+IPD-NH ₂	Method A
170	AIC+SRS-IP-(Pen)+IPD-NH ₂	Method A
171	AIC+SRS-(tBu)A)-PPI-(Pen)+IP-(Tranexamic)-NH ₂	Method A
172	aIC+SRS-IP-(Oic)-I-(Pen)+IP-NH ₂	Method A
173	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IPE-NH ₂	Method D
174	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG3(16 atoms))-NH ₂	Method A, D
175	((N-Me)A)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+IPD-NH ₂	Method A
176	AIC+SRS-(tBu)A)-P-(2S,3aS,6aS)-Octahydrocyclopenta[b]pyrrole-2-carboxylic acid)-I-((N-Me)C)+IPD-NH ₂	Method A
177	AIC+SRS-(tBu)A)-P-(Oic)-I-((N-Me)C)+I-OH	Method B
178	(2-Pal)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
179	((N-Me)A)-IC+SRS-IP-(Oic)-I-(Pen)+IPQ-OH	Method D
180	AIC+SRS-IP-(Oic)-I-((N-Me)C)+IPD-OH	Method B
181	FIC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
182	YIC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
183	AIC+SRS-(tBu)A)-PP-(2S)-2-(Amino)-2-[(1S,3S)-3-hydroxycyclohexyl]acetic acid)-((N-Me)C)+IPD-NH ₂	Method A
184	((2S)-2-[(Amino)-2-(tetrahydro-2H-pyran-4-yl)]acetic acid)-IC+SRS-IP-(Oic)-IC+I-OH	Method E
185	AIC+SRS-IP-(Oic)-I-((N-Me)C)+IPD-NH ₂	Method A
186	(Om)-IC+SRS-(tBu)A)-PPIC+I-NH ₂	Method A
187	(4-(Aminomethyl)benzoic acid)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D

188	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+I-OH	Method D
189	aIC+SRSLP-(Oic)-I-(Pen)+IPQ-OH	Method D
190	aIC+SRSLP-(Oic)-I-(Pen)+IP-OH	Method D
191	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPE-NH ₂	Method D
192	aIC+SRSLP-(Oic)-I-(Pen)+IPE-NH ₂	Method D
193	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(3-Carboxy)F)-NH ₂	Method A
194	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+I-(5-Azaspiro[2.4]heptane-1-carboxylic acid)-OH	Method B
195	(Dap)-IC+SRSLP-(tBu)A)-PPI-(Pen)+I-NH ₂	Method D
196	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPE-OH	Method D
197	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPE-OH	Method A
198	AIC+SRSLP-(tBu)A)-P-(Oic)-I-(N-Me)C)+I-NH ₂	Method A
199	A-(Nva)-C+SRSLP-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
200	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPQ-NH ₂	Method D
201	aIC+SRSLP-(Oic)-I-(Pen)+IPE-OH	Method D
202	aIC+SRSLP-(Oic)-I-(Pen)+IPQ-NH ₂	Method D
203	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPQ-OH	Method D
204	(3-(Aminomethyl)benzoic acid)-IC+SRSLP-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
205	G-(N-Me)I)-C+SRSLP+IPD-NH ₂ (HCl salt)	Method A, F
206	AIC+SRSLP-(Chg)-(Pen)+IPD-NH ₂	Method D
207	((N-Me)G)-IC+SRSLP-(tBu)A)-PPI-(Pen)+IP-(PEG1(10 atoms))-NH ₂	Method D
208	(ACBA)-IC+SRSLP-(tBu)A)-PPI-(Pen)+I-NH ₂	Method D
209	AIC+SRSLP-(S)-2-amino-3-ethyl-pentanoic acid)-C+IPD-NH ₂	Method A

210	AIC+SRS-((tBu)A)-PPIC+IDD-OH	Method B
211	FIC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
212	GIC+SRS-((tBu)A)-P-(Oic)-I-((N-Me)C)+IPD-NH ₂	Method A
213	((N-Me)G)-GIC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
214	((N-Me)G)-IC+SRS-((S)-(trifluoromethyl))-L-cysteine)-PPI-(Pen)+IP-NH ₂	Method A
215	((N-Me)G)-IC+SRS-((Oic)-I-(Pen)+IPE-OH	Method D
216	(Nva)-IC+SRS-PPIC+IPD-NH ₂	Method A
217	aIC+SRS-((Oic)-I-(Pen)+IPD-NH ₂	Method D
218	(4-Pal)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
219	(3-(Aminomethyl)benzoic acid)-IC+SRS-((tBu)A)-PP-(Pen)+IP-NH ₂	Method D
220	AIC+SRS-PPIC+(Cbz)-PD-NH ₂	Method A
221	(Tranexamic)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-(PEG1(10 atoms))-NH ₂	Method A
222	AIC+SRS-PP-(Cbz)-C+IPD-OH	Method B
223	WIC+SRS-((tBu)A)-PPI-((N-Me)C)+I-NH ₂	Method A
224	AIC+SRS-((Oic)-I-(Pen)+IPD-NH ₂	Method A
225	(Om)-IC+SRS-PP-(Cbz)-C+IPD-OH	Method B
226	(2-(3-Pyridyl)acetic acid)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
227	aIC+SRS-((Oic)-I-(Pen)+IPD-OH	Method D
228	((N-Me)G)-IC+SRS-PPIC+IPD-NH ₂ (HCl salt)	Method A, F
229	(2-(Cyclohexylamino)acetyl)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method K
230	(Abu)-IC+SRS-PPIC+IPD-NH ₂	Method A
231	AIC+SRS-((tBu)A)-P-(Oic)-I-((N-Me)C)+IPD-OH	Method B

232	(N-Me)G)-IC+SRSLPPI-(N-Me)C)+IPD-OH	Method B, C
233	aIC+SRs-(S)-(trifluoromethyl)-L-cysteine)-PPI-(Pen)+IP-NH ₂	Method A
234	AIC+SRs-(tBu)A)-P-(Oic)-I-C+IPD-NH ₂	Method A
235	AIC+SRs-(2,5-Difluoro)F)-PPI-(N-Me)C)+IPD-NH ₂	Method A
236	(Om)-IC+SRSLPPIC+I-NH ₂	Method A
237	AIC+SRSL-(L-dehydroproline)-(Oic)-I-(Pen)+IPD-OH	Method C
238	AIC+SRs-(2-Chloro)F)-PPI-(N-Me)C)+IPD-NH ₂	Method A
239	(4-(Aminomethyl)benzoic acid)-IC+SRs-(tBu)A)-PPI-(N-Me)C)+IPD-NH ₂	Method A
240	(Om)-IC+SRs-(S)-(trifluoromethyl)-L-cysteine)-PPI-(Pen)+IP-NH ₂	Method A
241	AIC+SRSL-((4aR,6aR,9S,11aS)-11-Oxo-2,3,4,4a,6a,7,8,9,11,11a-decahydro-1H-pyrido[3,2-e]pyrrolo[1,2-a]azepine-9-carboxylic acid)-IC+IPD-NH ₂	Method A
242	(trans-2-(3-(Amino)cyclohexyl)acetic acid)-IC+SRs-((tBu)A)-PPI-(Pen)+IP-NH ₂ (diastereomer #2)	Method A
243	((N,N-diMe)A)-IC+SRs-(tBu)A)-PPIC+IPD-NH ₂	Method A
244	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+I-NH ₂	Method A, D
245	AIC+SRSLP-((6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid)-IC+IPD-NH ₂	Method A
246	((N-Me)G)-IC+SRs-(tBu)A)-PPIC+IPD-OH	Method B
247	AIC+SRSLPP-(Chg)-C+I-NH ₂	Method A
248	AIC+SRs-(4-Fluoro)L)-PPIC+IPD-NH ₂	Method A
249	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-NH ₂ (Acetate)	Method FA
250	((N-Me)P)-IC+SRs-(tBu)A)-PPIC+IPD-NH ₂	Method A
251	(trans-2-(3-(Amino)cyclohexyl)acetic acid)-IC+SRs-((tBu)A)-PPI-(Pen)+IP-NH ₂ (diastereomer #1)	Method A
252	AIC+SRSNPPI-(Pen)+IPD-NH ₂	Method D

253	(N-Me)A)-IC+SRS-((tBu)A)-PPIC+IPD-NH ₂	Method A
254	AIC+SRSLPP-(Chg)-C+IPD-NH ₂	Method A
255	(Ahx)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
256	IC+SRS-((tBu)A)-PP-I-(N-Me)C)+IPD-NH ₂	Method A
257	kIC+SRS-((tBu)A)-PPI-(Pen)+I-NH ₂	Method D
258	IC+SRS-((tBu)A)-PPI-(Pen)+IP-OH	Method D
259	(Piperidin-4-ylacetic acid)-IC+SRS-((tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method K
260	A-(N-Me)I)-C+SRSLPPIC+IPD-NH ₂	Method A
261	G-(N-Me)I)-C+SRSLPPIC+IPD-NH ₂	Method A
262	AIC+SRSLPP-(Cpg)-C+IPD-NH ₂	Method A
263	(Gamma-Abu)-IC+SRS-((tBu)A)-PPI-(N-Me)C)+IPD-NH ₂	Method D
264	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-NH ₂ (Tartrate)	Method FA
265	IC+SRSLP-(Oic)-I-(Pen)+IP-OH	Method D
266	((N-Me)G)-((N-Me)G)-((N-Me)G)-((N-Me)G)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-NH ₂	Method A
267	((N-Me)G)-IC+SRS-((2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid)-P-(Oic)-I-(Pen)+IP-NH ₂	Method A
268	AIC+SRS-((tBu)A)-P-(Oic)-I-(N-Me)C)+IPD-NH ₂	Method A
269	(Tranexamic)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
270	AIC+SRSLP-(Oic)-(Chg)-C+IPD-NH ₂	Method A
271	((N-Me)G)-IC+SRSLPPIC+IPD-NH ₂	Method A
272	((2-Thiomorpholine)acetyl)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method K
273	(Cyclopropylacetic acid)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
274	(Cyclopropylacetic acid)-IC+SRS-((tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A

275	G-((N-Me)I)-C+SRSLPPIC+IPD-OH	Method B
276	G-((N-Me)I)-C+SRSLPPIC+IPd-OH	Method B
277	((N-Me)G)-IC+SRSLPPIC+(allo-I)-PD-NH ₂	Method A
278	AIC+SR-(allo-T)-LPPIC+IPD-NH ₂	Method A
279	AIC+SRS-(3-(Trimethylsilyl)-L-alanine)-P-(Oic)-I-((N-Me)C)+IPD-NH ₂	Method A
280	Ac-IC+SRS-((tBu)A)-PPI-((N-Me)C)+I-NH ₂	Method D
281	((N-Me)G)-IC+SRSLPPIC+IPD-OH	Method B, C
282	((N-Me)G)-IC+SRSLPP-(Chg)-C+IPD-NH ₂	Method A
283	(2-(Morpholine)acetyl)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method K
284	(Cyclobutylacetic)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
285	(Tranexamic)-IC+SRS-((tBu)A)-PPI-((N-Me)C)+I-NH ₂	Method A
286	GIC+SRSLPPIC+I-(Aib)-D-OH	Method B
287	((N-Me)G)-IC+SRSLPPIC-I-((trans-4-Fluoro)P)-D-OH	Method B
288	((N-Me)G)-IC+TRSLPPIC+IPD-OH	Method B
289	((N-Me)G)-IC+SRSLPP-IC+I-((2-Me)P)-D-OH	Method B
290	Ac-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
291	FIC+SRSLP-(Oic)-I-(Pen)+IP-NH ₂	Method C
292	AIC+SRSLP-(Oic)-IC+I-OH	Method E
293	IC+SRSLP-(Oic)-I-(Pen)+IPD-NH ₂	Method A, D
294	(Benzoic)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
295	(Phenylacetic acid)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
296	(Tetrahydro-2H-pyran-3-ylacetic acid)-IC+SRSLP-(Oic)-I-(Pen)+IP-NH ₂	Method A

297	(N-Me)A)-IC+SRSLPPIC+IPD-NH ₂	Method A
298	(N-Ph)G)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
299	(Om)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+(2-[(1S,2S)-1-(Amino)-2-methylbutyl]-1,3-oxazole-4-carboxylic acid)-D-NH ₂	Method A
300	(2-Hydroxyacetyl)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method K
301	(Cyclobutanecarboxylic)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
302	AIC+SRSLPP-(3-Chloro-Ph)G)-C+IPD-NH ₂	Method A
303	IC+SRS-(tBu)A)-PPI-(Pen)+IPP-NH ₂	Method D
304	(Tetrahydro-2H-pyran-3-ylacetic acid)-IC+SRSLP-(Oic)-I-(Pen)+IP-NH ₂	Method A
305	(Tetrahydropyran-4-acetic acid)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
306	(beta-A)-IC+SRSLPPIC+IPD-OH	Method B
307	(2-(N-Methyl-N-cyclopropylamino)acetyl)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method K
308	(Cyclobutanecarboxylic)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
309	(Isovaleric)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
310	(N-Me)G)-I-(Pen)+SRS-(tBu)A)-PPI-C+IP-NH ₂	Method D
311	(N-Ph)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
312	(N-Me)G)-IC+SRSLPPIC+IPd-OH	Method B
313	(N-Me)G)-IC+SRSLPPIC+I-(Abu)-D-OH	Method B
314	AIC+SRS-(AAD)-PP-I-(N-Me)C)+IPD-NH ₂	Method A
315	(Cyclopropanecarboxylic)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
316	IC+SRS-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
317	(N-Me)G)-IC+SRSLPPIC+ID-NH ₂	Method A

318	(N-Me)G)-IC+SRSLPPIC+IPD-OH (HCl- Salt)	Method B, F
319	(Tranexamic)-IC+SRS-((tBu)A)-PPI-((N-Me)C)+IPD-NH ₂	Method A
320	(PEG1(10 atoms))-AIC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
321	(N-Me)G)-IC+SRS-(Cnba)-PPI-(Pen)+IPD-NH ₂	Method D
322	((tBu)A)-GIC+SRSLP-(Oic)-I-(Pen)+IP-NH ₂	Method D
323	GIC+SRSLP-(Oic)-IC+IPD-OH	Method B
324	(N-Me)G)-IC+SRSLP-((1R,3S,5R)-2-Azabicyclo[3.1.0]hexane-3-carboxylic acid)-IC+IPD-OH	Method B
325	(N-Me)G)-IC+SRSLPPIC+(Phg)-PD-OH	Method B
326	AIC+SRSLP-(3,3-dimethyl-1,3-azasilolidine-5-carboxylic acid)-IC+IPD-NH ₂	Method A
327	(3-Methoxypropionic acid)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
328	((1S,2S,4S)-Bicyclo[2.2.1]hept-5-en-2-ylacetic acid)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂ (unknown hydroxylation by-product)	Method D
329	(N-Me)G)-IC+SRSLPPIC+I-(N-Me)G)-D-OH	Method B
330	((3-Bromo)F)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
331	A-(allo-I)C+SRSLPPIC+IPD-NH ₂	Method A
332	(Ahx)-IC+SRS-((tBu)A)-PPI-((N-Me)C)+IPD-NH ₂	Method A
333	(tert-Butylacetic acid)-IC+SRS-((tBu)A)-PPI-((N-Me)C)+I-NH ₂	Method A
334	(Cyclopentanecarboxylic acid)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH ₂	Method A
335	((tBu)A)-IC+SRSLP-(Oic)-I-(Pen)+IP-OH	Method D
336	IC+SRSLP-(Oic)-I-(Pen)+IP-NH ₂	Method D
337	AIC+SRS-((2S)-2-Amino-4,4,4-trifluorobutanoic acid)-PPI-((N-Me)C)+IPD-NH ₂	Method A
338	((2S,3S)-2-[(3R)-3-Amino-2-oxopyrrolidin-1-yl]-3-methylpentanoic acid)-C+SRSLPPIC+IAD-OH	Method B

339	(N-Me)G)-IC+SRSLPPIC+IPE-OH	Method B
340	(N-Me)G)-(N-Me)I)-C+SRSLPPIC+IPD-NH ₂	Method A
341	IC+SRSLP-(Oic)-I-(Pen)+I-NH ₂	Method D
342	(Tetrahydropyran-4-yl)-4-acetic acid)-IC+SRS-(tBu)A)-PPIC+IPD-NH ₂	Method D
343	(Cyclopentylacetic)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method A
344	AIC+SRS-(2-Amino-7-(tert-butoxy)-7-oxoheptanoic acid)-PPI-(Pen)+IPD-NH ₂	Method A
345	IC+SRS-(tBu)A)-PPI-(Pen)+I-NH ₂	Method D
346	AIC+SRSLPP-(allo-I)-C+IPD-NH ₂	Method A
347	IC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method B, D
348	GIC+SRSLPPIC+I-(Hyp)-D-OH	Method B
349	(N-Me)G)-IC+SRSLP-(Oic)-IC+IPD-OH	Method B
350	(N,N-diMe)G)-IC+SRSLPPIC+IPD-NH ₂	Method A
351	(Cyclobutylacetic)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
352	(D-(-)Biotin)-(Ahx)-AIC+SRS-(tBu)A)-PPI-(Pen)+IPD-OH	Method D
353	(Cyclopropanecarboxylic)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
354	(N-Me)G)-IC+SRSL-(trans-4-Fluoro)P)-PIC+IPD-OH	Method B
355	AIC+SRSLP-(3S-OH)P)-IC+IPD-NH ₂	Method A
356	((S)-3-Methylpentanoic acid)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method A
357	GIC+SRSL-(Morpholine-3-carboxylic)-PIC+IPD-OH	Method B
358	(Isovaleric)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method D
359	(tert-Butylacetic acid)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
360	(4-(3,5-Dimethyl-1,2-oxazol-4-yl)-L-phenylalanine)-IC+SRSLP-(Oic)-I-(Pen)+IP-NH ₂	Method C

361	(N-Me)G)-PIC+SRS-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
362	(4-(Aminomethyl)benzoic acid)-IC+SRS LPPIC-(N-Me)C)+IPD-NH ₂	Method A
363	(D-(+)-Biotin)-(Ahx)-AIC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
364	(Tranexamic)-IC+SRS LPPIC-(N-Me)C)+IPD-NH ₂	Method A
365	(4-Methylvaleric)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
366	(5-Chlorothiophene-2-carboxylic acid)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
367	(N-Me)G)-IC+SRS LPPIC+I-(Oic)-D-OH	Method B
368	IC+SRS LPPIC-(Oic)-I-(Pen)+I-OH	Method D
369	IC+SRS LPPIC-(Oic)-I-(Pen)+IPE-OH	Method D
370	fIC+SRS LPPIC-(Oic)-I-(Pen)+IP-NH ₂	Method C
371	PPIC+SRS-(tBu)A)-PPI-(Pen)+I-NH ₂	Method D
372	IC+SRS LPPIC-(Oic)-I-(Pen)+IPE-NH ₂	Method D
373	(Aib)-IC+SRS LPPIC+IPD-NH ₂	Method A
374	AIC+SRS LPPIC+(2-Methyl-D-alloisoleucine)-PD-NH ₂	Method A
375	(4-Methylvaleric)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-OH	Method D
376	(N-Me)G)-IC+SRS LPPIC-(Oic)-I-(Pen)+IP-NH ₂ (Citrate)	Method FA
377	IC+SRS LPPIC-(Oic)-I-(Pen)+IPQ-NH ₂	Method D
378	PPIC+SRS-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
379	((N-Me)I)-C+SRS-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
380	GIC+SRS LPPIC-(Hyp)-PIC+IPD-OH	Method B
381	((N-Me)G)-(Om)-(Om)-(Om)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method C
382	(Cyclobutylacetic)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A

383	(Ahx)-IC+SRSLP-(Oic)-IC+I-OH	Method E
384	GIC+SRS-(Trifluoro)l/L)-PPIC+IPD-OH	Method B
385	(Hydrocinnamic)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
386	(N-Me)G)-IC+SRSLPPIC+IpD-OH	Method B
387	(Lactic)-IC+SRS-(tBu)A)-PPIC+IPD-NH ₂	Method A
388	IC+SRSLP-(Oic)-I-(Pen)+IPQ-OH	Method D
389	AIC+SRSLPP-(Cyclobutylglycine)-C+IPD-NH ₂	Method A
390	(N-Me)G)-IC+SRSLP-(trans-4-Fluoro)P)-IC+IPD-OH	Method B
391	(Cyclohexylcarboxylic)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+IPD-NH ₂	Method A
392	(2-Hydroxyisobutyric)-IC+SRS-(tBu)A)-PPIC+IPD-NH ₂	Method A
393	((2S,3S)-2-[(3S)-2-oxopiperazin-1-yl]-3-methylpentanoic acid)-C+SRSLPPIC+IAD-OH	Method B
394	(N-Me)G)-IC+SRSLPPIC+IPN-OH	Method B
395	(Adipic acid)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
396	(tert-Butylacetic acid)-IC+SRS-(tBu)A)-PPI-(Pen)+I-NH ₂	Method D
397	((2S,3S)-2-[2-Oxopiperazin-1-yl]-3-methylpentanoic acid)-C+SRSLPPIC+IPD-OH	Method B
398	(Pivalic)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
399	(Oim)-AIC+SRS-(tBu)A)-PPI-(N-Me)C)+IPD-NH ₂	Method A
400	(Fumaric acid)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
401	GIC+SRSL-(Pip)-PIC+ID-OH	Method B
402	(N-Me)G)-I-(Pen)+SRSLPPIC+IPD-OH	Method B
403	AIC+S-(Me)R)-S-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method A
404	(Isobutyric acid)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+IPD-NH ₂	Method A

405	(N-Me)G)-(Om)-(Om)-IC+SRSLP-(Oic)-I-(Pen)+IP-NH ₂	Method C
406	(Hydrocinnamic)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-OH	Method D
407	(Suberic acid)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method D
408	(Cyclopentanecarboxylic acid)-IC+SRS-(tBu)A)-PPI-(N-Me)C)+I-NH ₂	Method A
409	(PEG1(10 atoms))-GIC+SRSLPPIC+IPD-OH	Method B
410	GIC+SRSLP-(Hyp)-IC+IPD-OH	Method B
411	AIC+SRS-(tBu)G)-PPI-C+IPD-NH ₂	Method A
412	(N-Me)G)-IC+KRS-(tBu)A)-PPI-(Pen)+IP-NH ₂	Method D
413	(N-Me)G)-IC+SR-(Dap)-LPPIC+IPD-OH	Method B
414	GIC+SRSLP-((cis-Fluoro)P)-IC+IPD-OH	Method B
415	(Cyclohexylacetic)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-NH ₂	Method A
416	GIC+SRS-((4-Bromo)F)-PPIC+IPD-OH	Method B
417	AIC+SRSLPPIC+((2S,3S)-2-((Amino)methyl)-3-methylpentanoic acid)-PD-NH ₂	Method A
418	AIC+SRSLP-(Oic)-I-(Pen)+IPN-OH	Method C
419	(N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+I-((5-Azaspino[2.4]heptane-1-carboxylic acid)-OH	Method B
420	AIC+SRSLP-(Oic)-I-(Pen)+IPD-NH ₂ (Acetate)	Method FA
421	(Tranexamic)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG2(13 atoms))-NH ₂	Method A
422	(Tranexamic)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG4-CH ₂ CO ₂ H (15 atoms))-NH ₂	Method A
423	(Tranexamic)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG3(16 atoms))-NH ₂	Method A
424	(Tranexamic)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG5-CH ₂ CO ₂ H (18 atoms))-NH ₂	Method A
425	(Tranexamic)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG5(22 atoms))-NH ₂	Method A
426	(Tranexamic)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG7(25 atoms))-NH ₂	Method A

427	(Tranexamic)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG8(28 atoms))-NH ₂	Method A
428	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IPD-(PEG9(31 atoms))-NH ₂	Method A
429	(Tranexamic)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(TTDS)-NH ₂	Method A
430	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(Om)-(Om)-NH ₂	Method A
431	AIC+SRS-IP-(Oic)-I-(Pen)+IPD-OH (sodium salt)	Method B, L
432	AIC+SRS-IP-(Oic)-I-(Pen)+IPD-OH (choline-salt)	Method B, L
433	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IPD-((N-Me)G)-((N-Me)G)-NH ₂	Method A
434	(ODD)-AIC+SRS-IP-(Oic)-I-(Pen)+IPD-OH	Method B
435	(Tranexamic)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG4(19 atoms))-NH ₂	Method A
436	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IPP-(2S)-3-(Triazol-1-yl)-2-(amino)propanoic acid)-NH ₂	Method A
437	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-(2S)-3-(Triazol-1-yl)-2-(amino)propanoic acid)-NH ₂	Method A
438	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-(5-Azasp[2.4]heptane-1-carboxylic acid)-OH	Method B
439	((N-Me)G)-IC+SRS-(tBu)A)-P-(6S)-5-Azasp[2.4]heptane-6-carboxylic acid)-I-(Pen)+IP-NH ₂	Method A
440	((N-Me)G)-IC+SRS-(2S)-3-(2,3-difluorophenyl)-2-aminopropanoic acid)-PPI-(Pen)+IP-NH ₂	Method A
441	((N-Me)G)-IC+SRS-(2S)-3-(2,3-difluorophenyl)-2-aminopropanoic acid)-P-(Oic)-I-(Pen)+IP-NH ₂	Method A
442	((N-Me)G)-IC+SRS-(tBu)A)-P-(1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid)-I-(Pen)+IPD-OH	Method C
443	((N-Me)A)-IC+SRS-(tBu)A)-P-(3R,6R)-1,1-Difluoro-5-azasp[2.4]heptane-6-carboxylic acid (enantiomer 1))-I-(Pen)+IP-NH ₂	Method A
444	((3-Me)H)-IC+SRS-IP-(Oic)-I-(Pen)+IP-NH ₂	Method A
445	((N-Me)A)-IC+SRS-(tBu)A)-P-(3R,6R)-1,1-Difluoro-5-azasp[2.4]heptane-6-carboxylic acid (enantiomer 2))-I-(Pen)+IP-NH ₂	Method A
446	((N-Me)G)-IC+SRS-IP-(Oic)-I-(Pen)+IP-(Ida)-OH	Method C

447	((N-Me)G)-IC+SRSLP-(tBu)A)-PPI-(Pen)+IP-((1-Bn)H)-OH	Method C
448	((N-Me)G)-IC+RRS-(tBu)A)-PP-(Nva)-(Pen)+IP-NH ₂	Method D
449	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-((1R,3S)-3-(Amino)cyclopentanecarboxylic acid)-OH	Method B
450	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-((R)-4-Amino-6-methylheptanoic acid)-OH	Method B
451	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-((S)-(1-Piperidin-3-yl)-acetic acid)-OH	Method B
452	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-((1S,3R)-3-(Amino)cyclopentanecarboxylic acid)-OH	Method B
453	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-((S)-3-(1-Pyrrolidine-2-yl)-propionic acid)-OH	Method B
454	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPN-OH	Method B, C
455	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(beta-homo-P)-OH	Method B
456	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-((S)-Pyrrolidine-3-carboxylic acid)-OH	Method B
457	((2,4-dioximidazolidin-1-yl)acetic acid)-IC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method B
458	AIC+SRSLP-(Oic)-I-(Pen)+IP-(*Dap)-OH	Method C
459	((R)-Piperidine-3-carboxylic acid)-IC+SRSLP-(Oic)-I-(Pen)+I-OH	Method C
460	((S)-Piperidine-3-carboxylic acid)-IC+SRSLP-(Oic)-I-(Pen)+I-OH	Method C
461	(8-aminocubane-1-carboxylic acid)-IC+SRSLP-(tBu)A)-PPI-(Pen)+IPD-OH	Method B
462	((N-Me)A)-IC+SRSLP-(Oic)-I-(Pen)+IP-((N-Benzyl)D)-NH ₂	Method I
463	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPDK-OH	Method C
464	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPGD-OH	Method C
465	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(Ahx)-OH	Method C
466	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPDD-OH	Method C
467	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(*Dap)-OH	Method J
468	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(PEG2(13 atoms))-OH	Method C

469	(Om)-IC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method C
470	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(PEG4-CH ₂ CO ₂ H (15 atoms))-OH	Method C
471	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG4-CH ₂ CO ₂ H (15 atoms))-OH	Method C
472	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(PEG5(22 atoms))-OH	Method C
473	AIC+SRSLP-(Oic)-I-(Pen)+IPD-OH (Acetate)	Method FA
474	((N-Me))-GIC+SRSLP-(Oic)-I-(Pen)+I-((S)-4-Piperazine-2-carboxylic acid)-OH	Method B
475	(Piperidin-4-ylacetic acid)-IC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method C
476	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(4-Aminomethylphenylacetic acid)-OH	Method C
477	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(3-Aminomethylphenylacetic acid)-OH	Method C
478	aIC+SRSLP-(Oic)-I-(Pen)-+IP-(3-Aminomethylphenylacetic acid)-OH	Method C
479	(Om)-IC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method C
480	(beta-P)-IC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method C
481	(beta-p)-IC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method C
482	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(3-Carboxy)F)-OH	Method C
483	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(3-Carboxy)F)-OH	Method C
484	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG2(13 atoms))-OH	Method C
485	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(PEG3(16 atoms))-OH	Method C
486	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(Om)-D-OH	Method C
487	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPR-OH	Method C
488	((R)-Piperidine-3-Carboxylic Acid)-IC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method C
489	((S)-Piperidine-3-Carboxylic Acid)-IC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method C
490	(4-Carboxybenzoic acid)-GIC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method C

491	(3-Carboxybenzoic acid)-GIC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method C
492	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPT-OH	Method C
493	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPY-OH	Method C
494	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPDA-OH	Method C
495	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPGd-OH	Method C
496	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(PEG5-CH ₂ CO ₂ H(18 atoms))-OH	Method C
497	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(N-Me)G-(N-Me)G-(N-Me)G-OH	Method C
498	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(PEG1(10 atoms))-OH	Method C
499	AIC+S-(Arg(13C6,15N4))-SLP-(Oic)-I-(Pen)+IPD-OH (HCl Salt)	Method B, F
500	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG1(10 atoms))-OH	Method C
501	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG3(16 atoms))-OH	Method C
502	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG3(16 atoms))-OH	Method C
503	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG9(31 atoms))-OH	Method C
504	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG9(31 atoms))-OH	Method C
505	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG8(28 atoms))-OH	Method C
506	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(PEG4-CH ₂ CO ₂ H(15 atoms))-OH	Method C
507	AIC+SRSLP-(Oic)-I-(Pen)+IPD-(hexane-2-carboxylic acid)-PI-(Pen)+IPD-OH	Method B
508	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(hexane-2-carboxylic acid)-PI-(Pen)+IPD-OH	Method B
509	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IPD-(hexane-2-carboxylic acid)-PI-(Pen)+IPD-OH	Method B
510	((2S)-2-(morpholin-4-yl)propanoic acid)-IC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method B
511	AIC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method C
512	((ODD)-AIC+SRSLP-(Oic)-I-(Pen)+IPD-(Orn)-OH	Method C

513	(ODD)-(Om)-IC+SRSLP-(Oic)-I-(Pen)+IPD-OH	Method C
514	((N-Me)G)-IC+SRS-(tBu)A)-PPI-(Pen)+IP-(PEG2(13 atoms))-OH	Method C
515	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(AAD)-OH	Method C
516	AIC+SRSLP-((4-CF3)P)-IC+IPD-OH	Method C
517	AIC+SRS-(tBu)A)-P-((4-CF3)P)-I-(Pen)+IPD-OH	Method C
518	((N-Me)G)-FC+TRK-(tBu)A)-(Pen)+YPD-OH	Method C
519	((N-Me)G)-IC+SRS-((2,5-Difluoro)F)-PPI-(Pen)+IPD-OH	Method C
520	((N-Me)G)-IC+SRS-((2,5-Difluoro)F)-PPI-(Pen)+IPe-OH	Method C
521	((N-Me)G)-IC+SRS-((2,5-Difluoro)F)-PPI-(Pen)+IPDd-OH	Method C
522	aIC+SRS-(tBu)A)-P-((6S)-5-Azapiro[2.4]heptane-6-carboxylic acid)-I-(Pen)+IPD-OH	Method C
523	aIC+SRS-(tBu)A)-P-((6S)-5-Azapiro[2.4]heptane-6-carboxylic acid)-I-(Pen)+IPe-OH	Method C
524	AIC+SRS-(tBu)A)-P-((4-CF3)P)-IC+IPD-OH	Method C
525	((N-Me)G)-IC+SRS-((2S)-2-amino-3-(1-methylcyclopropyl)propanoic acid)-P-(Oic)-I-(Pen)+IPD-OH	Method B
526	((N-Me)G)-IC+SRS-(tBu)A)-P-((1S,2S,5R)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid)-I-(Pen)+IPD-OH	Method C
527	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(Freidinger's Lactam)-OH	Method C
528	pIC+SRSLP-(Oic)-I-(Pen)+IP-(Ida)-OH (HCl salt)	Method C
529	(Om)-IC+SRS-((2R)-2-amino-3-(trifluoromethylsulfanyl)propanoic acid)-PPI-(Pen)+IPD-OH	Method C
530	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(R)-Pyrrolidine-3-acetic acid)-OH	Method C
531	((N-Me)G)-IC+SRS-(tBu)A)-P-((3S)-2-azaspiro[4.4]nonane-3-carboxylic acid)-I-(Pen)+IPD-OH	Method C
532	((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-(R)-Pyrrolidine-3-carboxylic acid)-OH	Method C

Table 15: Analytical data for the reference peptides

Reference No	Retention time (min)	LC-MS Method 1	Purity (%)	Exact Mass Calcd (g/mol)	Exact Mass Found (g/mol)	Ionization	LC-MS Purity (%) Method W2	LC-MS Purity (%) Method W3
1	8,68	Method 1	> 99	1467,7214	1467,7344	[M+2H] ²⁺		
2	8,75	Method 1	> 99	1467,7200	1467,7400	[M+2H] ²⁺		
3	4,37	Method 2	94,79	1466,7374	1466,7408	[M+2H] ²⁺		
A	4,66	Method 6	> 99	1481,7371	1481,7600	[M+2H] ²⁺		
B	8,59	Method 1	> 99	1480,7500	1480,7600	[M+2H] ²⁺		
C	8,88	Method 1	95,00	1480,7530	1480,7632	[M+2H] ²⁺		

Table 16: Analytical data for the peptides prepared according to the invention

Example No	Retention time (min)	LC-MS Method 1	Purity (%)	Exact Mass Calcd (g/mol)	Exact Mass Found (g/mol)	Ionization	LC-MS Purity (%) Method W2	LC-MS Purity (%) Method W3
4	7,98	Method 4	93,11	1721,9208	1721,9344	[M+2H] ²⁺		
5	8,61	Method 4	96,38	1563,8153	1563,8286	[M+2H] ²⁺		
6	9,69	Method 1	> 99	1324,7359	1325,7380	[M+H] ⁺	98,49	98,16
7	7,92	Method 4	96,55	1650,9201	1650,9384	[M+2H] ²⁺		
8	6,90	Method 4	100	1433,7887	1433,807	[M+2H] ²⁺		

9	7,78	Method 4	97,50	1548,8156	1548,8322	[M+2H] ²⁺	93,70	95,10
10	9,22	Method 1	82,28	1536,8156	1536,8320	[M+2H] ²⁺	91,50	90,10
11	9,26	Method 1	93,15	1522,8000	1522,8258	[M+2H] ²⁺		
12	9,41	Method 1	> 99	1523,7840	1523,8060	[M+2H] ²⁺		
13	8,52	Method 4	> 99	1563,8153	1563,8346	[M+2H] ²⁺	98,68	97,99
14	9,97	Method 1	99,07	1577,8310	1577,8558	[M+2H] ²⁺		
15	9,33	Method 1	92,48	1562,8313	1562,8476	[M+2H] ²⁺		
16	7,67	Method 4	95,58	1654,915	1654,9296	[M+2H] ²⁺		
17	9,21	Method 1	95,00	1534,8000	1534,8246	[M+2H] ²⁺		
18	8,8	Method 1	> 99	1566,8262	1566,8502	[M+2H] ²⁺		
19	8,94	Method 1	90,00	1593,8483	1593,8616	[M+2H] ²⁺		
20	9,71	Method 1	95,90	1478,7374	1478,7458	[M+2H] ²⁺		
21	9,3	Method 1	95,59	1537,7997	1536,8342	[M+2H] ²⁺	97,00	95,80
22	9,14	Method 1	94,83	1521,8160	1521,8338	[M+2H] ²⁺	96,30	95,60
23	8,09	Method 4	> 99	1475,8356	1475,8488	[M+2H] ²⁺	88,40	89,20
24	9,21	Method 1	92,48	1536,8156	1536,8304	[M+2H] ²⁺	97,40	93,20
25	9,48	Method 1	98,21	1296,7046	1297,7152	[M+H] ⁺		
26	9,72	Method 1	> 99	1562,8313	1562,8562	[M+2H] ²⁺		
27	4,00	Method 3	> 99	1562,8313	1562,8518	[M+2H] ²⁺		
28	9,87	Method 1	> 99	1338,7516	1338,7530	[M+H] ⁺	98,77	97,66
29	8,56	Method 4	> 99	1563,8153	1563,82	[M+2H] ²⁺		
30	4,71	Method 2	> 99	1508,7843	1508,7874	[M+2H] ²⁺		

31	9,64	Method 1	> 99	1549,7997	1549,8095	[M+H] ⁺		
32	9,2	Method 1	86,00	1548,8156	1548,8232	[M+2H] ²⁺	92,70	90,10
33	7,28	Method 4	100	1459,8043	1459,8218	[M+2H] ²⁺		
34	8,08	Method 4	100	1786,9937	1788,024	[M+2H] ²⁺		
35	8,87	Method 1	95,00	1565,8422	1565,8538	[M+2H] ²⁺		
36	9,29	Method 1	95,00	1535,7840	1535,8108	[M+2H] ²⁺		
37	9,14	Method 1	> 99	1282,6890	1282,7012	[M+2H] ²⁺		
38	8,9	Method 1	> 99	1381,7686	1381,7800	[M+H] ⁺		
39	7,70	Method 4	97,08	1566,8626	1566,8794	[M+2H] ²⁺		
40	9,01	Method 1	83,81	1608,8480	1608,8640	[M+2H] ²⁺	96,30	92,40
41	9,64	Method 1	95,00	1534,8000	1534,9346	[M+2H] ²⁺		
42	7,23	Method 4	> 99	1546,8225	1546,8388	[M+2H] ²⁺	96,40	96,10
43	7,9	Method 4	> 99	1378,7829	1378,8	[M+2H] ²⁺	96,27	97,01
44	8,10	Method 4	96,58	1875,0461	1876,06	[M+2H] ²⁺		
45	9,49	Method 1	88,00	1548,8156	1548,8380	[M+2H] ²⁺		
46	9,59	Method 1	91,55	1548,8156	1548,8286	[M+2H] ²⁺		
47	9,24	Method 1	80,00	1550,8313	1550,8442	[M+2H] ²⁺	96,50	93,60
48	9,57	Method 1	> 99	1508,7843	1508,7916	[M+2H] ²⁺		
49	8,67	Method 1	> 99	1565,8422	1565,8560	[M+2H] ²⁺		
50	9,08	Method 1	> 99	1605,8735	1605,8984	[M+2H] ²⁺		
51	9,16	Method 1	71,28	1522,8000	1522,8128	[M+2H] ²⁺	95,10	94,50
52	9,28	Method 1	78/21	1534,8000	1534,8128	[M+2H] ²⁺	95,76	92,92

53	9,41	Method 1	> 99	1326,7152	1326,7209	[M+H] ⁺	98,66	97,12
54	9,45	Method 1	91,46	1550,8313	1550,8464	[M+2H] ²⁺	94,20	94,70
55	7,83	Method 4	94,68	1684,9256	1685,9558	[M+2H] ²⁺		
56	4,60	Method 4	90,76	1865,0379	1866,0744	[M+4H] ⁴⁺		
57	7,15	Method 4	98,44	1620,8844	1620,8984	[M+2H] ²⁺		
58	8,21	Method 4	100	1598,8313	1598,8554	[M+2H] ²⁺		
59	9,07	Method 1	> 99	1523,7476	1523,7686	[M+2H] ²⁺		
60	9,63	Method 1	94,50	1564,8469	1564,8598	[M+2H] ²⁺	93,30	92,20
61	7,50	Method 4	> 99	1350,7516	1350,7642	[M+2H] ²⁺	96,00	97,10
62	8,23	Method 4	100	1765,947	1765,9622	[M+2H] ²⁺		
63	8,59	Method 4	100	1839,9838	1841,0094	[M+2H] ²⁺		
64	7,12	Method 4	100	1405,7574	1405,7800	[M+2H] ²⁺		
65	9,75	Method 1	> 99	1338,7516	1338,7650	[M+H] ⁺		
66	9,11	Method 1	95,21	1508,7843	1508,8020	[M+2H] ²⁺		
67	7,72	Method 4	> 99	1447,8043	1447,8184	[M+2H] ²⁺		
68	9,62	Method 1	84,01	1564,8469	1564,8638	[M+2H] ²⁺	95,30	95,80
69	9,5	Method 1	85,69	1550,8313	1550,8490	[M+2H] ²⁺	93,00	93,90
70	7,58	Method 4	> 99	1609,832	1609,8478	[M+2H] ²⁺		
71	5,06	Method 4	99,17	1447,8043	1447,7710	[M+2H] ²⁺	98,10	96,30
72	7,34	Method 4	97,29	1610,8888	1610,9054	[M+2H] ²⁺		
73	7,5	Method 4	94,66	1640,8994	1640,913	[M+2H] ²⁺		
74	7,94	Method 4	95,69	1831,0199	1831,04	[M+2H] ²⁺		

75	8,19	Method 4	> 99	1562,8313	1562,842	[M+2H] ²⁺		
76	7,78/7,80	Method 4	100/10 0	1606,8939	1606,9108/160 6,9078	[M+2H] ²⁺		
77	8,96	Method 1	> 99	1551,8265	1551,8418	[M+2H] ²⁺		
78	8,92	Method 1	> 99	1551,8265	1551,8382	[M+2H] ²⁺		
79	8,79	Method 1	> 99	1353,7625	1353,7738	[M+2H] ²⁺		
80	9,41	Method 1	98,76	1564,8469	1564,8612	[M+2H] ²⁺		
81	9,9	Method 1	87,34	1637,8422	1637,8578	[M+2H] ²⁺	94,30	94,50
82	9,25	Method 1	87,40	1534,8000	1534,8166	[M+2H] ²⁺	94,40	93,10
83	8,22	Method 4	100	1724,9569	1724,9722	[M+2H] ²⁺		
84	8,37	Method 4	100	1853,9995	1854,0168	[M+2H] ²⁺		
85	7,64	Method 4	100	1709,9572	1709,98	[M+2H] ²⁺		
86	9,56	Method 1	> 99	1509,7684	1509,7790	[M+2H] ²⁺		
87	9,71	Method 1	88,65	1548,8156	1548,8324	[M+2H] ²⁺		
88	8,56	Method 4	> 99	1563,8153	1563,8338	[M+2H] ²⁺	99,49	98,88
89	7,71	Method 4	94,93	1742,9674	1742,9808	[M+2H] ²⁺		
90	7,25	Method 4	100	1534,8	1534,8136	[M+2H] ²⁺		
91	8,93	Method 1	92,60	1339,7468	1339,7460	[M+2H] ²⁺		
92	9,59	Method 1	98,22	1322,7203	1322,7270	[M+2H] ²⁺		
93	8,92	Method 1	79,37	1599,8265	1599,8390	[M+2H] ²⁺	97,20	94,40
94	9,69	Method 1	91,51	1604,7877	1604,7942	[M+2H] ²⁺	92,80	92,70
95	9,71	Method 1	85,46	1564,8469	1564,8626	[M+2H] ²⁺	93,20	91,60

96	7,53	Method 4	> 99	1351,7356	1351,7356	[M+2H] ²⁺	95,30	96,20
97	8,19	Method 4	96,25	1738,9725	1739,98	[M+2H] ²⁺		
98	9,19	Method 1	97,50	1522,8000	1522,8170	[M+2H] ²⁺		
99	9,75	Method 1	> 99	1336,7359	1337,7410	[M+H] ⁺		
100	6,83	Method 4	> 99	1324,7359	1324,7552	[M+2H] ²⁺	99,60	99,50
101	8,25	Method 4	99,42	1809,9733	1810,9926	[M+2H] ²⁺		
102	9,59	Method 1	92,37	1560,7404	1560,7566	[M+2H] ²⁺		
103	9,31	Method 1	> 99	1312,6995	1312,7116	[M+2H] ²⁺		
104	9,44	Method 1	> 99	1620,6792	1622,6874	[M+2H] ²⁺		
105	7,82	Method 4	96,96	1576,8469	1576,8664	[M+2H] ²⁺	96,10	97,20
106	8,12	Method 4	96,29	1576,8469	1576,8624	[M+2H] ²⁺	91,60	93,40
107	8,74	Method 4	93,03	1942,0519	1943,0778	[M+2H] ²⁺		
108	9,87	Method 1	98,00	1576,8469	1576,8632	[M+2H] ²⁺		
109	8,05	Method 4	> 99	1447,8043	1447,8228	[M+2H] ²⁺	99,50	99,30
110	8,57	Method 4	> 99	1462,8040	1462,8150	[M+2H] ²⁺	97,30	96,70
111	8,5	Method 4	> 99	1448,7884	1448,8082	[M+2H] ²⁺	99,00	99,20
112	8,34	Method 4	> 99	1577,8310	1577,8388	[M+2H] ²⁺	97,20	95,20
113	8,22	Method 4	91,92	1680,9307	1680,9486	[M+2H] ²⁺		
114	8,76	Method 1	97,00	1550,7949	1550,8186	[M+2H] ²⁺		
115	9,43	Method 1	85,65	1591,7851	1591,8010	[M+2H] ²⁺	95,50	93,70
116	9,13	Method 1	85,75	1536,8156	1536,8316	[M+2H] ²⁺	95,90	92,90
117	8,12	Method 4	> 99	1351,7356	1351,7492	[M+2H] ²⁺	99,10	98,90

118	5,19	Method 4	> 99	1576,8469	1576,8658	[M+2H] ²⁺	98,00	98,00
119	8,32	Method 4	100	1865,0155	1866,0304	[M+2H] ²⁺		
120	9,9	Method 1	97,00	1576,8469	1576,8738	[M+2H] ²⁺		
121	8,95	Method 1	> 99	1398,6999	1398,7090	[M+H] ⁺		
122	7,77	Method 4	> 99	1576,8469	1576,8580	[M+2H] ²⁺	96,70	96,70
123	10	Method 1	> 99	1433,7887	1433,7986	[M+2H] ²⁺		
124	8,84	Method 1	88,44	1588,8218	1588,8398	[M+2H] ²⁺	96,90	94,40
125	5,83	Method 4	> 99	1450,8152	1450,8322	[M+2H] ²⁺	99,60	98,60
126	7,35	Method 4	> 99	1421,7887	1421,8074	[M+2H] ²⁺	99,30	98,24
127	8,04	Method 4	94,16	1694,9463	1694,9594	[M+2H] ²⁺		
128	9,87	Method 1	> 99	1576,7717	1576,7832	[M+2H] ²⁺		
129	9,79	Method 1	> 99	1336,7359	1336,7440	[M+H] ⁺		
130	7,76	Method 4	> 99	1576,8469	1576,8588	[M+2H] ²⁺		
131	7,98	Method 4	> 99	1590,8626	1590,8774	[M+2H] ²⁺	98,50	98,20
132	7,63	Method 4	98,57	1575,8620	1575,8764	[M+2H] ²⁺	92,10	98,00
133	8,41	Method 4	100	1795,9576	1796,9876	[M+2H] ²⁺		
134	8,24	Method 4	> 99	1365,7512	1365,7660	[M+2H] ²⁺	99,60	98,90
135	8,00	Method 4	> 99	1461,8200	1461,8346	[M+2H] ²⁺	95,70	95,31
136	8,36	Method 4	100	1749,9885	1751,0162	[M+2H] ²⁺		
137	7,68	Method 4	> 99	1564,8469	1564,8598	[M+2H] ²⁺		
138	8,39	Method 4	> 99	1577,8310	1577,9858	[M+2H] ²⁺	98,90	98,80
139	7,91	Method 4	> 99	1589,8786	1589,8942	[M+2H] ²⁺	96,50	98,40

140	7,91	Method 4	94,59	1782,9987	1783,02	[M+2H] ²⁺		
141	8,78	Method 4	100	1986,0781	1987,1064	[M+2H] ²⁺		
142	2,95	Method 14	90,10	1565,7997	1565,8520	[M+2H] ²⁺		
143	7,42	Method 4	94,92	1552,8106	1552,8338	[M+2H] ²⁺	95,10	93,50
144	6,45	Method 4	> 99	1451,7993	1451,8158	[M+2H] ²⁺	96,00	96,00
145	7,92	Method 4	93,08	1698,9412	1699,9666	[M+2H] ²⁺		
146	8,54	Method 4	98,92	1898,0257	1899,0438	[M+2H] ²⁺		
147	9,03	Method 1	82,90	1494,7687	1494,7820	[M+2H] ²⁺	96,98	95,66
148	7,95	Method 4	> 99	1553,7946	1553,8008	[M+2H] ²⁺	95,10	93,50
149	7,15	Method 4	> 99	1421,7887	1421,8082	[M+2H] ²⁺	98,50	98,90
150	8,30	Method 4	96,95	1827,025	1827,04	[M+2H] ²⁺		
151	8,75	Method 4	90,85	1915,0774	1916,1114	[M+2H] ²⁺		
152	7,63	Method 4	> 99	1364,7672	1364,7830	[M+2H] ²⁺	99,30	98,10
153	9,6	Method 1	> 99	1535,7840	1535,7904	[M+2H] ²⁺		
154	8,94	Method 1	> 99	1362,7264	1362,7502	[M+2H] ²⁺		
155	10,26	Method 1	> 99	1563,8153	1563,8318	[M+2H] ²⁺		
156	7,06	Method 4	> 99	1407,7730	1407,7902	[M+2H] ²⁺	99,30	98,40
157	7,85	Method 4	> 99	1504,8258	1504,8416	[M+2H] ²⁺	98,20	99,30
158	9,49	Method 1	96,07	1562,7561	1562,7722	[M+2H] ²⁺		
159	9,93	Method 1	> 99	1336,7359	1336,7485	[M+H] ⁺		
160	7,63	Method 4	> 99	1350,7516	1350,7670	[M+2H] ²⁺	95,20	96,10
161	8,27	Method 4	> 99	1591,8466	1591,8658	[M+2H] ²⁺	98,20	98,50

162	8,52	Method 4	> 99	1577,8310	1577,8426	[M+2H] ²⁺	96,50	97,00
163	8,98	Method 1	> 99	1436,7996	1436,8210	[M+2H] ²⁺		
164	7,99	Method 4	98,33	1474,804	1476,84	[M+2H] ²⁺		
165	7,93	Method 4	96,48	1562,8313	1562,8510	[M+2H] ²⁺	96,70	96,70
166	7,37	Method 4	79,60	1550,8313	1550,8446	[M+2H] ²⁺	94,20	92,20
167	8,5	Method 4	> 99	1448,7884	1448,8034	[M+2H] ²⁺	96,60	98,20
168	8,45	Method 4	100	1871,0512	1871,06	[M+2H] ²⁺		
169	9,22	Method 1	> 99	1520,7843	1520,7968	[M+2H] ²⁺		
170	8,98	Method 1	91,66	1508,7843	1508,7936	[M+2H] ²⁺		
171	7,51	Method 4	> 99	1546,8728	1546,8896	[M+2H] ²⁺	97,10	96,20
172	5,14	Method 4	> 99	1447,8043	1447,8228	[M+2H] ²⁺	97,00	97,90
173	7,77	Method 4	> 99	1576,8469	1576,8678	[M+2H] ²⁺	98,00	97,30
174	7,54	Method 4	95,17	1654,9150	1654,9334	[M+2H] ²⁺	98,00	98,00
175	9,4	Method 1	93,13	1522,8000	1522,8092	[M+2H] ²⁺		
176	10,05	Method 1	> 99	1548,8156	1548,8388	[M+2H] ²⁺		
177	10,55	Method 1	> 99	1351,7356	1351,7490	[M+H] ⁺		
178	9,44	Method 1	87,65	1598,8313	1598,8368	[M+2H] ²⁺	92,00	95,40
179	8,11	Method 4	> 99	1590,8626	1590,8774	[M+2H] ²⁺	97,70	98,00
180	10	Method 1	> 99	1549,7997	1549,8112	[M+2H] ²⁺		
181	9,86	Method 1	99,00	1372,7359	1373,7382	[M+H] ⁺		
182	9,57	Method 1	> 99	1388,7308	1388,7400	[M+H] ⁺		
183	8,95	Method 1	> 99	1550,7949	1551,8000	[M+H] ⁺		

184	9,89	Method 1	> 99	1393,7462	1393,7700	[M+2H] ²⁺		
185	9,83	Method 1	> 99	1548,8156	1548,8244	[M+2H] ²⁺		
186	8,76	Method 1	> 99	1325,7312	1325,7359	[M+H] ⁺		
187	9,2	Method 1	85,96	1584,8156	1584,8334	[M+2H] ²⁺	97,10	95,00
188	7,97	Method 4	> 99	1351,7356	1351,7524	[M+2H] ²⁺	99,30	99,80
189	7,97	Method 4	> 99	1576,8469	1576,8658	[M+2H] ²⁺	97,60	98,50
190	8,42	Method 4	> 99	1448,7884	1448,8150	[M+2H] ²⁺	98,00	98,60
191	7,78	Method 4	> 99	1576,8469	1576,8650	[M+2H] ²⁺	98,40	96,60
192	8,07	Method 4	88,36	1576,8469	1576,8638	[M+2H] ²⁺	99,20	98,20
193	8,58	Method 4	95,98	1638,8626	1638,88	[M+2H] ²⁺		
194	4,08	Method 3	100	1474,804	1474,8212	[M+2H] ²⁺		
195	6,52	Method 4	> 99	1325,7312	1325,7488	[M+2H] ²⁺	99,10	95,10
196	8,11	Method 4	> 99	1577,8310	1577,8456	[M+2H] ²⁺	95,60	95,20
197	7,06	Method 4	100	1405,7574	1405,78	[M+2H] ²⁺		
198	10,17	Method 1	> 99	1350,7516	1350,7656	[M+H] ⁺		
199	9,16	Method 1	81,04	1508,7843	1508,7950	[M+2H] ²⁺	94,25	92,02
200	7,55	Method 4	> 99	1575,8629	1575,8758	[M+2H] ²⁺	91,60	95,90
201	8,14	Method 4	> 99	1577,8310	1577,8466	[M+2H] ²⁺	98,80	99,10
202	7,62	Method 4	> 99	1575,8629	1575,9792	[M+2H] ²⁺	98,60	99,10
203	7,95	Method 4	> 99	1576,8469	1576,8588	[M+2H] ²⁺	98,80	97,50
204	7,57	Method 4	> 99	1469,7887	1469,8062	[M+2H] ²⁺	95,90	96,20
205	8,81	Method 1	95,10	1480,7530	1480,7638	[M+2H] ²⁺		

206	7,55	Method 4	84,01	1534,8000	1534,8172	[M+2H] ²⁺	96,70	95,80
207	7,18	Method 4	97,74	1566,8626	1566,8881	[M+2H] ²⁺	98,30	98,10
208	7,16	Method 4	> 99	1336,7359	1336,7542	[M+2H] ²⁺	98,50	97,50
209	9,17	Method 1	94,43	1494,7687	1494,7826	[M+2H] ²⁺		
210	8,78	Method 1	> 99	1513,7269	1513,7372	[M+2H] ²⁺		
211	9,79	Method 1	86,55	1598,8313	1598,8430	[M+2H] ²⁺	96,39	97,13
212	10,07	Method 1	> 99	1548,8156	1548,8266	[M+2H] ²⁺		
213	9,14	Method 1	84,10	1579,8215	1579,8394	[M+2H] ²⁺	97,50	94,80
214	6,82	Method 4	94,98	1451,6699	1451,6876	[M+2H] ²⁺		
215	8,17	Method 4	> 99	1577,8310	1577,8580	[M+2H] ²⁺	98,70	98,20
216	9,02	Method 1	95,47	1508,7843	1508,8030	[M+2H] ²⁺		
217	8,00	Method 4	94,67	1562,8313	1562,8420	[M+2H] ²⁺	90,20	94,10
218	7,38	Method 4	83,21	1599,8265	1599,8452	[M+2H] ²⁺	95,50	95,40
219	7,29	Method 4	98,29	1469,7887	1469,8074	[M+2H] ²⁺	91,60	92,60
220	8,75	Method 1	91,37	1478,7374	1478,8566	[M+2H] ²⁺		
221	7,37	Method 4	98,49	1634,9252	1634,9396	[M+2H] ²⁺		
222	9,36	Method 1	> 99	1507,7527	1507,7598	[M+2H] ²⁺		
223	10,02	Method 1	> 99	1411,7500	1411,7640	[M+2H] ²⁺		
224	9,49	Method 1	> 99	1534,8000	1534,8034	[M+2H] ²⁺		
225	8,92	Method 1	98,52	1550,7949	1550,8112	[M+2H] ²⁺		
226	8,52	Method 4	> 99	1455,7730	1455,7904	[M+2H] ²⁺	98,90	98,80
227	8,32	Method 4	98,31	1563,8153	1563,8276	[M+2H] ²⁺	93,90	92,60

228	8,86	Method 1	94,17	1480,7530	1480,7624	[M+2H] ²⁺		
229	8,26	Method 4	97,70	1590,8626	1590,8782	[M+2H] ²⁺		
230	4,54	Method 2	94,53	1494,7687	1494,7734	[M+2H] ²⁺		
231	10,19	Method 1	> 99	1563,8153	1563,8254	[M+2H] ²⁺		
232	9,27	Method 1	> 99	1495,7527	1495,7612	[M+2H] ²⁺		
233	7,05	Method 4	100	1451,6699	1451,69	[M+2H] ²⁺		
234	9,66	Method 1	> 99	1548,8156	1548,8260	[M+2H] ²⁺		
235	9,52	Method 1	92,00	1564,7342	1564,7572	[M+2H] ²⁺		
236	8,62	Method 1	> 99	1311,7155	1311,7100	[M+H] ⁺		
237	8,30	Method 4	> 99	1561,7997	1561,8138	[M+2H] ²⁺	97,11	96,94
238	9,52	Method 1	99,00	1562,7141	1562,7204	[M+2H] ²⁺		
239	9,37	Method 1	96,86	1570,8190	1570,8000	[M+2H] ²⁺		
240	5,43	Method 4	98,27	1494,7121	1494,7317	[M+3H] ³⁺		
241	9,15	Method 1	> 99	1518,7687	1518,7814	[M+2H] ²⁺		
242	7,41	Method 4	100	1475,8356	1475,8544	[M+2H] ²⁺		
243	9,08	Method 1	> 99	1522,8000	1522,8114	[M+2H] ²⁺		
244	7,58	Method 4	> 99	1350,7516	1350,7668	[M+2H] ²⁺	96,10	96,70
245	9,28	Method 1	> 99	1506,7687	1506,7754	[M+2H] ²⁺		
246	9,14	Method 1	> 99	1495,7527	1495,7622	[M+2H] ²⁺		
247	9,33	Method 1	> 99	1294,6890	1294,7038	[M+2H] ²⁺		
248	8,77	Method 1	97,35	1498,7436	1498,7550	[M+2H] ²⁺		
249	7,81	Method 1	> 99	1447,8043	1447,8232	[M+2H] ²⁺		

250	9,16	Method 1	96,08	1534,8000	1534,8168	[M+2H] ²⁺		
251	7,55	Method 4	87,23	1475,8356	1475,849	[M+2H] ²⁺		
252	8,3	Method 1	85,87	1509,7432	1509,7608	[M+2H] ²⁺	96,30	96,70
253	9,04	Method 1	95,92	1508,7843	1508,7908	[M+2H] ²⁺		
254	9,18	Method 1	95,70	1506,7687	1506,7768	[M+2H] ²⁺		
255	7,26	Method 4	> 99	1449,8200	1449,8404	[M+2H] ²⁺	97,70	96,50
256	9,45	Method 1	94,64	1437,7472	1437,7622	[M+2H] ²⁺		
257	5,48	Method 4	> 99	1367,7781	1367,7956	[M+2H] ²⁺	95,40	97,30
258	7,51	Method 4	> 99	1337,7199	1337,7416	[M+2H] ²⁺	98,79	99,40
259	9,36	Method 1	> 99	1350,7516	1350,7566	[M+H] ⁺		
260	8,85	Method 1	> 99	1494,7687	1494,7762	[M+2H] ²⁺		
261	8,69	Method 1	> 99	1480,7530	1480,7618	[M+2H] ²⁺		
262	8,97	Method 1	94,47	1492,7530	1492,7622	[M+2H] ²⁺		
263	7,63	Method 4	86,77	1522,8000	1522,8100	[M+2H] ²⁺	97,20	93,30
264	8,07	Method 1	> 99	1447,8043	1447,8224	[M+2H] ²⁺		
265	8,26	Method 4	> 99	1377,7512	1377,7666	[M+2H] ²⁺	97,60	96,50
266	7,17	Method 4	100	1691,9215	1691,9382	[M+2H] ²⁺		
267	7,84	Method 4	100	1459,8043	1459,8176	[M+2H] ²⁺		
268	10,3	Method 1	> 99	1562,8313	1562,8400	[M+2H] ²⁺		
269	7,32	Method 4	> 99	1475,8356	1475,8540	[M+2H] ²⁺	98,50	98,50
270	9,77	Method 1	> 99	1560,8156	1560,8218	[M+2H] ²⁺		
271	8,7	Method 1	97,00	1480,7530	1480,7600	[M+2H] ²⁺		

272	9,47	Method 1	97,47	1594,8034	1594,8198	[M+2H] ²⁺		
273	10,12	Method 1	84,96	1533,8047	1533,8212	[M+2H] ²⁺	93,10	91,60
274	10,75	Method 1	> 99	1307,7094	1307,7134	[M+H] ⁺		
275	4,62	Method 6	92,32	1481,7371	1481,74	[M+H] ⁺		
276	4,43	Method 2	> 99	1481,7371	1481,7500	[M+2H] ²⁺		
277	8,83	Method 1	96,32	1480,7530	1480,7588	[M+2H] ²⁺		
278	4,56	Method 2	93,63	1494,7687	1494,7814	[M+2H] ²⁺		
279	10,29	Method 1	98,00	1578,8082	1578,8230	[M+2H] ²⁺		
280	10,19	Method 1	98,00	1267,6781	1267,6785	[M+H] ⁺	99,10	95,80
281	4,39	Method 2	> 99	1481,7371	1481,7378	[M+2H] ²⁺		
282	9,11	Method 1	93,00	1506,7687	1506,7738	[M+2H] ²⁺		
283	9,28	Method 1	> 99	1578,8262	1578,8412	[M+2H] ²⁺		
284	10,26	Method 4	> 99	1432,7934	1432,8096	[M+2H] ²⁺	99,40	99,10
285	9,45	Method 1	> 99	1364,7672	1364,7725	[M+H] ⁺		
286	4,43	Method 2	95,85	1455,7214	1455,7370	[M+2H] ²⁺		
287	8,81	Method 1	> 99	1499,7276	1499,7358	[M+2H] ²⁺		
288	8,94	Method 1	> 99	1495,7527	1495,7644	[M+2H] ²⁺		
289	9,01	Method 1	> 99	1495,7527	1495,7592	[M+2H] ²⁺		
290	9,66	Method 1	84,19	1493,7734	1493,7802	[M+H] ⁺	93,60	90,80
291	8,86	Method 4	93,41	1523,8356	1523,85	[M+2H] ²⁺		
292	4,85	Method 2	> 99	1323,7043	1323,7204	[M+2H] ²⁺		
293	9,8	Method 1	95,00	1491,7942	1491,8200	[M+2H] ²⁺		

294	10,33	Method 1	78,00	1555,7891	1555,8006	[M+2H] ²⁺	95,19	92,10
295	10,4	Method 1	86,71	1569,8047	1569,8000	[M+H] ⁺	93,00	90,90
296	10,43	Method 4	100	1502,8353	1502,8522	[M+2H] ²⁺		
297	8,91	Method 1	95,00	1494,7687	1494,7766	[M+2H] ²⁺		
298	11,01	Method 1	> 99	1358,7203	1358,7314	[M+2H] ²⁺		
299	9,01	Method 1	> 99	1521,7796	1521,7836	[M+2H] ²⁺		
300	9,51	Method 1	> 99	1509,7684	1509,7814	[M+2H] ²⁺		
301	10,82	Method 1	> 99	1307,7094	1307,7118	[M+H] ⁺		
302	9,26	Method 1	> 99	1534,6828	1534,6974	[M+2H] ²⁺		
303	7,31	Method 4	> 99	1433,7887	1433,8066	[M+2H] ²⁺	96,50	96,30
304	10,30	Method 4	98,25	1502,8353	1502,8516	[M+2H] ²⁺		
305	10,41	Method 1	> 99	1351,7400	1351,7415	[M+H] ⁺		
306	8,8	Method 1	> 99	1481,7371	1481,7496	[M+2H] ²⁺		
307	7,79	Method 4	> 99	1562,8313	1562,8488	[M+2H] ²⁺		
308	10,14	Method 1	87,08	1533,8047	1533,8218	[M+2H] ²⁺	96,70	95,10
309	10,3	Method 1	86,87	1535,8204	1535,8376	[M+2H] ²⁺	96,90	90,20
310	7,09	Method 4	> 99	1407,7730	1407,7906	[M+2H] ²⁺	99,20	99,20
311	10,27	Method 4	> 99	1469,7887	1469,8040	[M+2H] ²⁺	99,00	99,40
312	4,46	Method 2	> 99	1481,7371	1481,7504	[M+2H] ²⁺		
313	8,7	Method 1	> 99	1469,7371	1469,7474	[M+2H] ²⁺		
314	8,75	Method 1	91,20	1524,7429	1524,7560	[M+2H] ²⁺		
315	9,91	Method 1	83,68	1519,7891	1519,8056	[M+2H] ²⁺	95,10	93,60

316	7,06	Method 4	> 99	1336,7359	1336,7536	[M+2H] ²⁺	99,60	99,90
317	8,66	Method 1	93,96	1383,7003	1383,7078	[M+2H] ²⁺		
318	8,96	Method 1	> 99	1481,7371	1481,7454	[M+2H] ²⁺		
319	9,35	Method 1	89,58	1576,8469	1576,8584	[M+2H] ²⁺		
320	9,31	Method 1	89,56	1610,8524	1681,9082	[M+2H] ²⁺	97,20	95,40
321	6,48	Method 4	92,95	1505,7483	1505,7668	[M+2H] ²⁺	92,90	88,80
322	9,00	Method 4	98,26	1503,8669	1503,8840	[M+2H] ²⁺	96,60	95,10
323	9,35	Method 1	> 99	1521,7684	1521,7868	[M+2H] ²⁺		
324	9,09	Method 1	> 99	1493,7371	1493,7424	[M+2H] ²⁺		
325	8,9	Method 1	97,47	1501,7058	1501,7144	[M+2H] ²⁺		
326	4,71	Method 2	> 99	1524,7613	1524,7768	[M+2H] ²⁺		
327	9,77	Method 1	87,14	1537,7997	1537,8162	[M+2H] ²⁺	96,70	92,20
328	8,99	Method 4	94,93	1603,8466	1603,8682	[M+2H] ²⁺	98,50	97,80
329	8,74	Method 1	99,70	1455,7214	1455,7486	[M+2H] ²⁺		
330	9,05	Method 4	85,36	1676,7418	1676,7586	[M+2H] ²⁺	91,30	91,40
331	9,01	Method 1	82,76	1480,7530	1480,7602	[M+2H] ²⁺		
332	9,35	Method 1	92,34	1550,8313	1550,8400	[M+2H] ²⁺		
333	11,32	Method 1	> 99	1323,7407	1323,7457	[M+H] ⁺		
334	10,37	Method 1	85,11	1547,8204	1547,8366	[M+2H] ²⁺	95,80	93,90
335	10,04	Method 4	> 99	1504,8510	1504,8710	[M+2H] ²⁺	95,80	96,80
336	7,75	Method 4	> 99	1376,7672	1376,7836	[M+2H] ²⁺	98,00	98,50
337	9,06	Method 1	96,83	1520,7091	1520,7232	[M+2H] ²⁺		

338	8,42	Method 1	99,32	1467,7214	1467,7260	[M+2H] ²⁺		
339	8,81	Method 1	> 99	1495,7527	1495,7684	[M+2H] ²⁺		
340	8,87	Method 1	> 99	1494,7687	1494,7724	[M+2H] ²⁺		
341	7,26	Method 4	> 99	1279,7145	1279,7288	[M+2H] ²⁺	96,50	96,60
342	8,71	Method 4	89,03	1549,7997	1549,8172	[M+2H] ²⁺	90,40	89,40
343	10,63	Method 1	92,89	1561,8360	1561,8516	[M+2H] ²⁺		
344	8,6	Method 1	94,15	1552,7742	1552,7908	[M+2H] ²⁺		
345	6,62	Method 4	94,45	1239,6832	1239,6998	[M+2H] ²⁺	97,10	95,30
346	8,95	Method 1	> 99	1480,7530	1480,7568	[M+2H] ²⁺		
347	8,59	Method 4	> 99	1492,7782	1492,7972	[M+2H] ²⁺	98,80	98,90
348	8,48	Method 1	97,31	1483,7163	1483,7342	[M+2H] ²⁺		
349	9,6	Method 1	> 99	1535,7840	1535,7942	[M+2H] ²⁺		
350	8,88	Method 1	95,86	1494,7687	1494,7754	[M+2H] ²⁺		
351	10,41	Method 1	84,00	1547,8204	1547,8358	[M+2H] ²⁺	96,10	94,10
352	9,51	Method 4	> 99	1862,9457	1862,9551	[M+2H] ²⁺	97,70	95,00
353	10,54	Method 1	> 99	1293,6937	1293,6958	[M+H] ⁺		
354	8,97	Method 1	> 99	1499,7276	1499,7348	[M+2H] ²⁺		
355	4,52	Method 2	90,00	1496,7480	1496,7520	[M+2H] ²⁺		
356	10,58	Method 1	93,20	1549,8360	1549,8502	[M+2H] ²⁺		
357	8,82	Method 1	> 99	1483,7163	1483,7282	[M+2H] ²⁺		
358	11,05	Method 1	> 99	1309,7250	1309,6000	[M+2H] ²⁺	98,30	97,50
359	10,49	Method 4	> 99	1434,8091	1434,8240	[M+2H] ²⁺	98,60	98,90

360	9,22	Method 4	96,98	1618,8728	1618,8882	[M+2H] ²⁺	96,18	97,30
361	7,43	Method 4	95,37	1504,8258	1504,8430	[M+2H] ²⁺	98,20	97,20
362	9,2	Method 1	94,52	1556,7843	1556,8050	[M+2H] ²⁺		
363	9,35	Method 4	85,07	1861,9617	1861,9685	[M+H] ⁺	97,70	95,00
364	9,21	Method 1	97,74	1562,8313	1562,8390	[M+2H] ²⁺		
365	10,56	Method 1	80,41	1549,8360	1549,8518	[M+2H] ²⁺	94,70	92,90
366	10,82	Method 1	> 99	1595,7065	1595,7192	[M+2H] ²⁺	97,30	94,40
367	9,64	Method 1	99,58	1535,7840	1535,8040	[M+2H] ²⁺		
368	7,84	Method 4	> 99	1280,6985	1280,7178	[M+2H] ²⁺	99,60	99,70
369	8,24	Method 4	> 99	1506,7938	1506,8094	[M+2H] ²⁺	95,40	95,80
370	8,98	Method 4	> 99	1523,8356	1523,8492	[M+2H] ²⁺	97,12	97,08
371	7,10	Method 4	> 99	1433,7887	1433,8050	[M+2H] ²⁺	93,50	91,70
372	8,07	Method 4	> 99	1505,8098	1505,9486	[M+2H] ²⁺	99,40	98,80
373	4,37	Method 2	> 99	1494,7687	1494,7726	[M+2H] ²⁺		
374	9,06	Method 1	92,43	1494,7700	1494,7854	[M+2H] ²⁺		
375	10,32	Method 4	95,26	1550,8201	1550,8392	[M+2H] ²⁺		
376	7,60	Method 1	> 99	1447,8043	1447,8248	[M+2H] ²⁺		
377	7,50	Method 4	> 99	1504,8258	1504,8388	[M+2H] ²⁺	93,50	96,90
378	7,51	Method 4	> 99	1530,8415	1530,8574	[M+2H] ²⁺	99,10	99,70
379	7,20	Method 4	> 99	1350,7516	1350,7680	[M+2H] ²⁺	97,30	99,00
380	8,26/ 8,34	Method 1	70/29	1483,7163	1483,7374	[M+2H] ²⁺		
381	3,9	Method 4	> 99	1750,011	1750,0326	[M+3H] ³⁺	99,37	98,66

382	11,06	Method 1	> 99	1321,7250	1321,7286	[M+H] ⁺		
383	9,91	Method 1	> 99	1365,7500	1365,7630	[M+2H] ²⁺		
384	9,15	Method 1	87,43	1521,6931	1521,6976	[M+2H] ²⁺		
385	10,68	Method 1	83,28	1583,8204	1583,8368	[M+2H] ²⁺	96,30	91,20
386	4,55	Method 2	> 99	1481,7371	1481,7494	[M+2H] ²⁺		
387	9,46	Method 1	93,47	1495,7527	1495,7636	[M+2H] ²⁺		
388	8,07	Method 4	> 99	1505,8098	1505,8246	[M+2H] ²⁺	97,80	97,10
389	8,75	Method 1	93,22	1478,7374	1478,7462	[M+2H] ²⁺		
390	8,89	Method 1	> 99	1499,7276	1499,7360	[M+2H] ²⁺		
391	10,75	Method 1	91,00	1547,8204	1547,8280	[M+2H] ²⁺		
392	9,55	Method 1	88,42	1509,7684	1509,7812	[M+2H] ²⁺		
393	8,73	Method 1	> 99	1481,7371	1481,7432	[M+2H] ²⁺		
394	4,39	Method 2	95,41	1480,7530	1480,7642	[M+2H] ²⁺		
395	9,64	Method 1	84,41	1579,8102	1579,8260	[M+2H] ²⁺	93,40	90,50
396	10,54	Method 4	> 99	1337,7563	1337,7660	[M+H] ⁺	99,30	98,20
397	8,79	Method 1	> 99	1493,7371	1493,7510	[M+2H] ²⁺		
398	11,13	Method 1	> 99	1309,7250	1309,7295	[M+H] ⁺		
399	9,16	Method 1	88,00	1622,8637	1622,8866	[M+2H] ²⁺		
400	9,66	Method 1	85,00	1549,7633	1549,7778	[M+2H] ²⁺	95,50	93,80
401	4,54	Method 2	94,46	1384,6843	1384,7002	[M+2H] ²⁺		
402	8,86	Method 1	> 99	1509,7684	1509,7802	[M+2H] ²⁺		
403	9,29	Method 1	93,30	1536,8156	1536,8306	[M+2H] ²⁺		

404	10,21	Method 1	> 99	1507,7891	1507,7944	[M+2H] ²⁺		
405	4,24	Method 4	> 99	1790,0423	1791,0678	[M+3H] ³⁺	99,08	98,69
406	10,45	Method 4	98,71	1584,8044	1584,8186	[M+2H] ²⁺		
407	9,21	Method 4	83,34	1607,8415	1607,8578	[M+2H] ²⁺	96,00	94,40
408	11,12	Method 1	> 99	1321,7250	1321,7258	[M+H] ⁺		
409	8,77	Method 1	97,00	1626,8109	1626,8200	[M+2H] ²⁺		
410	8,59/ 8,66	Method 1	59/40	1483,7163	1483,7370	[M+2H] ²⁺		
411	8,95	Method 1	94,75	1480,7530	1480,7630	[M+2H] ²⁺		
412	5,62	Method 4	97,66	1448,836	1448,8554	[M+3H] ³⁺	96,30	97,40
413	4,36	Method 2	> 99	1480,7530	1480,7612	[M+2H] ²⁺		
414	8,7	Method 1	> 99	1485,7120	1485,7238	[M+2H] ²⁺		
415	10,87	Method 1	91,80	1575,8517	1575,8652	[M+2H] ²⁺		
416	9,36	Method 1	> 99	1579,6163	1579,6200	[M+2H] ²⁺		
417	9,15	Method 1	> 99	1494,7687	1494,7736	[M+2H] ²⁺		
418	8,39	Method 4	> 99	1562,8313	1562,9000	[M+2H] ²⁺	97,31	97,42
419	8,44	Method 4	> 99	1474,8040	1476,8178	[M+2H] ²⁺		
420	4,01	Method 3	> 99	1562,8313	1562,8422	[M+2H] ²⁺		
421	7,57	Method 4	98,63	1678,9514	1680,9710	[M+2H] ²⁺		
422	7,67	Method 4	95,55	1708,9620	1710,9742	[M+2H] ²⁺		
423	7,72	Method 4	> 99	1722,9776	1724,9942	[M+2H] ²⁺		
424	7,85	Method 4	94,30	1752,9882	1755,0024	[M+2H] ²⁺		
425	7,80	Method 4	97,93	1811,0300	1814,0484	[M+2H] ²⁺		

426	7,96	Method 4	98,96	1855,0563	1858,0758	[M+2H] ²⁺		
427	8,19	Method 4	> 99	1899,0825	1902,1006	[M+2H] ²⁺		
428	8,73	Method 4	94,64	2030,1043	2032,1148	[M+2H] ²⁺		
429	7,84	Method 4	> 99	1778,0198	1780,0348	[M+2H] ²⁺		
430	4,37	Method 4	> 99	1750,0110	1753,0326	[M+3H] ³⁺		
431	8,62	Method 4	> 99	1561,7997	1565,8452	[M+2H] ²⁺		
432	8,65	Method 4	> 99	1561,7997	1565,4816	[M+2H] ²⁺		
433	2,85	Method 5	85,05	1735,9113	1737,9288	[M+2H] ²⁺		
435	2,70	Method 5	94,14	1767,0038	1770,0200	[M+2H] ²⁺		
436	8,33	Method 4	98,11	1726,9011	1728,9172	[M+2H] ²⁺		
437	8,43	Method 4	> 99	1629,8483	1631,8630	[M+2H] ²⁺		
438	8,54	Method 4	97,07	1571,8568	1573,8716	[M+2H] ²⁺		
439	7,58	Method 4	> 99	1433,7887	1435,8152	[M+2H] ²⁺		
440	7,43	Method 4	> 99	1463,7229	1465,7404	[M+2H] ²⁺		
441	8,34	Method 4	> 99	1517,7699	1519,7886	[M+2H] ²⁺		
443	7,73	Method 4	> 99	1483,7855	1485,8006	[M+2H] ²⁺		
444	6,79	Method 4	> 99	1527,8418	1529,8576	[M+2H] ²⁺		
445	7,63	Method 4	> 99	1483,7855	1485,8018	[M+2H] ²⁺		
446	8,65	Method 4	91,93	1563,8153	1563,8272	[M+2H] ²⁺	95,35	98,58
447	2,65	Method 5	> 99	1635,8629	1635,8760	[M+2H] ²⁺	98,66	99,22
448	5,44	Method 4	97,04	1462,8265	1465,8501	[M+3H] ³⁺	95,79%	97,26%
449	8,33	Method 4	86,83	1559,8568	1561,8714	[M+2H] ²⁺		

450	9,24	Method 4	90,97	1589,9037	1591,9208	[M+2H] ²⁺		
451	8,61	Method 4	95,66	1573,8724	1575,8884	[M+2H] ²⁺		
452	8,24	Method 4	96,26	1559,8568	1561,8714	[M+2H] ²⁺		
453	8,53	Method 4	94,00	1573,8724	1575,8874	[M+2H] ²⁺		
454	7,96	Method 4	> 99	1562,8313	1564,8458	[M+2H] ²⁺		
455	8,47	Method 4	> 99	1559,8568	1561,8710	[M+2H] ²⁺		
456	8,28	Method 4	> 99	1545,8411	1547,8552	[M+2H] ²⁺		
457	9,44	Method 4	> 99	1632,8004	1634,8240	[M+2H] ²⁺		
458	2,61	Method 5	> 99	1534,8364	1536,8654	[M+2H] ²⁺	96,68%	96,99%
459	2,85	Method 5	> 99	1391,7669	1393,7826	[M+2H] ²⁺	89,95%	99,44%
460	2,84	Method 5	> 99	1391,7669	1393,7810	[M+2H] ²⁺	97,71%	98,46%
462	8,76	Method 4	78,21	1665,9099	1667,9238	[M+2H] ²⁺	95,17%	93,69%
463	7,12	Method 4	94,14	1691,9103	1694,9349	[M+3H] ³⁺	97,30%	90,79%
464	8,26	Method 4	> 99	1620,8368	1622,8530	[M+2H] ²⁺	97,37%	94,11%
465	8,39	Method 4	> 99	1676,8994	1678,9132	[M+2H] ²⁺	98,76%	99,20%
466	8,11	Method 4	> 99	1678,8422	1680,8566	[M+2H] ²⁺	99,18%	98,78%
467	6,96	Method 4	> 99	1534,8364	1537,8579	[M+3H] ³⁺	99,05%	99,51%
468	8,26	Method 4	94,77	1651,9041	1653,9234	[M+2H] ²⁺	96,08%	96,72%
469	6,87	Method 4	> 99	1606,8575	1609,8813	[M+3H] ³⁺	97,93%	96,22%
470	8,71	Method 4	> 99	1681,9147	1683,9302	[M+2H] ²⁺	96,68%	92,70%
471	8,40	Method 4	> 99	1796,9416	1798,9566	[M+2H] ²⁺	97,03%	95,29%
472	8,56	Method 4	> 99	1783,9828	1785,9958	[M+2H] ²⁺	97,03%	95,29%

473	8,39	Method 4	> 99	1563,8153	1563,8244	[M+2H] ²⁺		
474	6,65	Method 4	95,2	1463,7993	1463,816	[M+2H] ²⁺		
475	7,74	Method 4	> 99	1549,7997	1549,8134	[M+2H] ²⁺	97,03	95,07
476	9,69	Method 4	> 99	1595,8568	1595,8710	[M+2H] ²⁺	95,68	96,16
477	9,87	Method 4	> 99	1595,8568	1595,8712	[M+2H] ²⁺	96,85	97,09
478	9,84	Method 4	> 99	1595,8568	1595,8668	[M+2H] ²⁺	96,50	94,73
479	6,83	Method 4	> 99	1552,8106	1552,8284	[M+2H] ²⁺	96,16	94,86
480	7,51	Method 4	99,0	1549,7997	1549,8132	[M+2H] ²⁺	94,8	96,2
481	7,59	Method 4	> 99	1549,7997	1549,8206	[M+2H] ²⁺	97,55	97,23
482	8,32	Method 4	> 99	1599,8153	1599,8326	[M+2H] ²⁺	98,99	98,86
483	8,85	Method 4	94,52	1639,8466	1639,8596	[M+2H] ²⁺	98,56	98,33
484	8,50	Method 4	> 99	1766,9311	1766,9468	[M+2H] ²⁺	98,65	97,21
485	7,77	Method 4	> 99	1655,8990	1655,9152	[M+2H] ²⁺	98,60	99,16
486	2,31	Method 5	97,6	1637,8633	1637,8754	[M+2H] ²⁺	95,81	96,13
487	2,52	Method 5	> 99	1604,8895	1604,9038	[M+2H] ²⁺	99,01	98,81
488	2,95	Method 5	> 99	1603,8466	1603,8610	[M+2H] ²⁺	98,08	98,25
489	2,84	Method 5	> 99	1603,8466	1603,8684	[M+2H] ²⁺	99,49	98,23
490	3,40	Method 5	> 99	1697,8157	1697,8294	[M+2H] ²⁺	99,38	99,17
491	3,43	Method 5	> 99	1697,8157	1697,8316	[M+2H] ²⁺	96,36	96,84
492	2,86	Method 5	> 99	1549,8360	1549,8488	[M+2H] ²⁺	98,14	98,16
493	2,93	Method 5	97,60	1611,8517	1611,8664	[M+2H] ²⁺	97,58	97,80
494	2,88	Method 5	97,96	1634,8524	1634,8662	[M+2H] ²⁺	97,13	97,02

495	2,97	Method 5	> 99	1620,8368	1620,8538	[M+2H] ²⁺	98,60	98,29
496	8,11	Method 4	> 99	1685,9096	1685,9238	[M+2H] ²⁺	95,64	96,06
497	7,90	Method 4	95,72	1621,8684	1621,8810	[M+2H] ²⁺	92,88	92,81
498	7,49	Method 4	> 99	1567,8466	1567,8568	[M+2H] ²⁺	98,13	98,05
499	8,47	Method 4	> 99	1573,8030	1573,8528	[M+2H] ²⁺		
500	8,40	Method 4	> 99	1722,9048	1722,9200	[M+2H] ²⁺	98,10	99,55
501	7,86	Method 4	98,44	1770,9260	1770,9408	[M+2H] ²⁺	97,32	96,25
502	7,70	Method 4	99,09	1563,8153	1563,8320	[M+2H] ²⁺	97,46	96,62
503	8,29	Method 4	> 99	1876,0301	1876,0302	[M+2H] ²⁺	95,23	94,54
504	8,41	Method 4	> 99	1991,0571	1991,0740	[M+2H] ²⁺	96,55	96,43
505	8,23	Method 4	> 99	1832,0039	1832,0216	[M+2H] ²⁺	95,43	96,86
506	7,98	Method 4	98,67	1641,8834	1641,8986	[M+2H] ²⁺	97,81	97,21
507	7,86	Method 4	> 99	1521,7684	1521,7926	[M+2H] ²⁺		
508	7,63	Method 4	> 99	1563,7401	1563,7564	[M+2H] ²⁺		
509	7,75	Method 4	> 99	1523,7840	1523,8000	[M+2H] ²⁺		
510	8,35	Method 4	> 99	1633,8572	1633,8688	[M+2H] ²⁺		
511	2,58	Method 5	> 99	1535,6668	1535,6802	[M+2H] ²⁺	87,38	89,08
512	4,12	Method 5	> 99	1974,1298	1974,1506	[M+2H] ²⁺	95,15	95,33
513	3,96	Method 5	> 99	1903,0927	1903,1106	[M+2H] ²⁺	96,44	95,38
514	2,65	Method 5	> 99	1611,8728	1611,8852	[M+2H] ²⁺	95,68	95,22
515	2,79	Method 5	> 99	1591,8466	1591,8600	[M+2H] ²⁺	97,20	97,37
516	2,73	Method 5	> 99	1549,7244	1549,7380	[M+2H] ²⁺	93,87	95,17

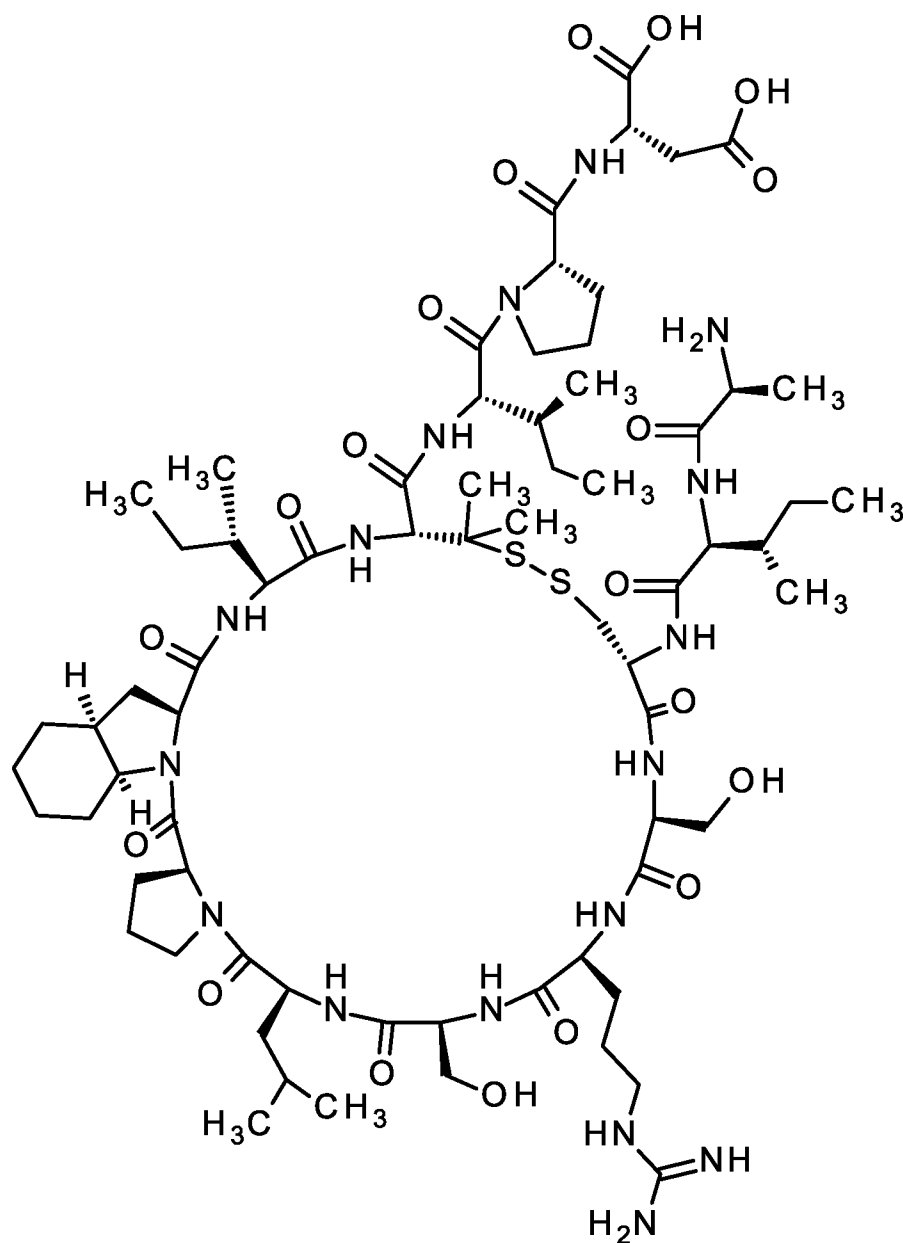
517	2,90	Method 5	> 99	1591,7714	1591,7908	[M+2H] ²⁺	91,59	93,39
518	1,75	Method 5	> 99	1355,6366	1355,6494	[M+2H] ²⁺	97,11	98,05
519	2,67	Method 5	> 99	1579,7339	1579,7548	[M+2H] ²⁺	95,69	96,40
520	2,68	Method 5	> 99	1593,7495	1595,7636	[M+2H] ²⁺	97,27	96,99
521	2,66	Method 5	> 99	1694,7608	1694,7798	[M+2H] ²⁺	95,48	95,35
522	2,79	Method 5	> 99	1549,7997	1549,8140	[M+2H] ²⁺	97,12	96,45
523	2,82	Method 5	> 99	1563,8153	1563,8312	[M+2H] ²⁺	95,87	96,34
524	2,82	Method 5	> 99	1563,7401	1573,7518	[M+2H] ²⁺	91,95	92,65
525	8,24	Method 4	84,39	1575,8153	1575,8416	[M+2H] ²⁺		
526	2,70	Method 5	> 99	1535,7840	1535,7966	[M+2H] ²⁺	96,07	95,73
527	3,11	Method 5	98,08	1644,9095	1644,9230	[M+2H] ²⁺	98,01	96,26
528	2,99	Method 5	95,62	1589,8310	1589,8490	[M+2H] ²⁺	75,28	75,57
529	6,47	Method 4	95,44	1610,7231	1610,7386	[M+2H] ²⁺	84,42	82,86
530	2,89	Method 5	> 99	1559,8568	1559,8754	[M+2H] ²⁺	97,07	96,79
531	2,94	Method 5	> 99	1577,8310	1577,8492	[M+2H] ²⁺	95,26	97,20
532	2,89	Method 5	> 99	1545,8411	1545,8590	[M+2H] ²⁺	97,87	96,49

Table 17: Maldi mass spectral data for selected peptides

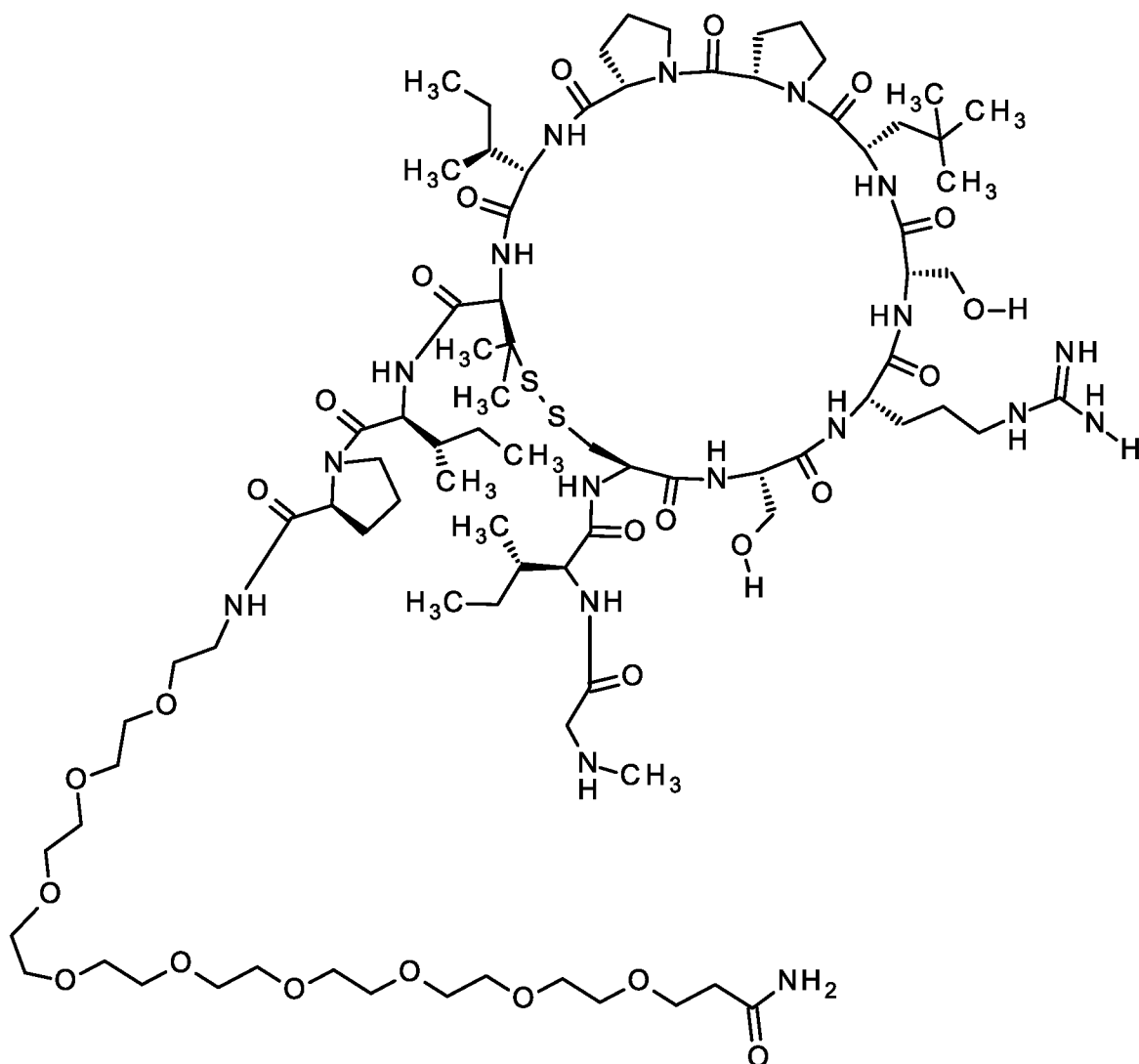
Example No	Method MALDI (Amu+1)
512	1975,223
526	1536,827
527	1645,817
529	1611,761
530	1560,875
531	1578,866
532	1546,821
142	1566,863
237	1562,785

In the following, examples 29, 44, 45, 67, 75, 109, 166, 185, 347, 443, 466 and 499 are exemplified by their chemical structure. The present invention includes pharmaceutically acceptable salts, solvates or solvates of the salts of these examples. Chemical structures are displayed as salt free forms.

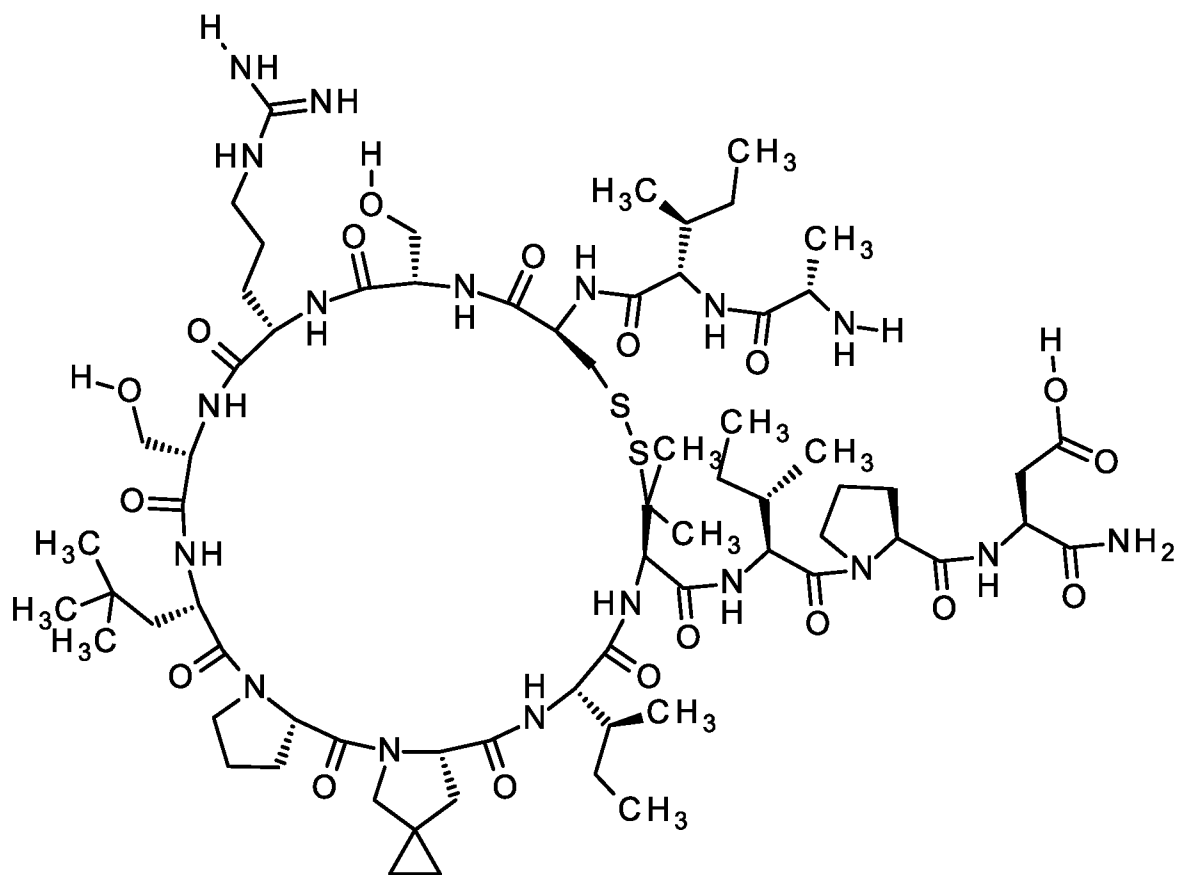
Example 29, Sequence: AIC+SRSLP-(Oic)-I-(Pen)+IPD-OH (HCl salt)



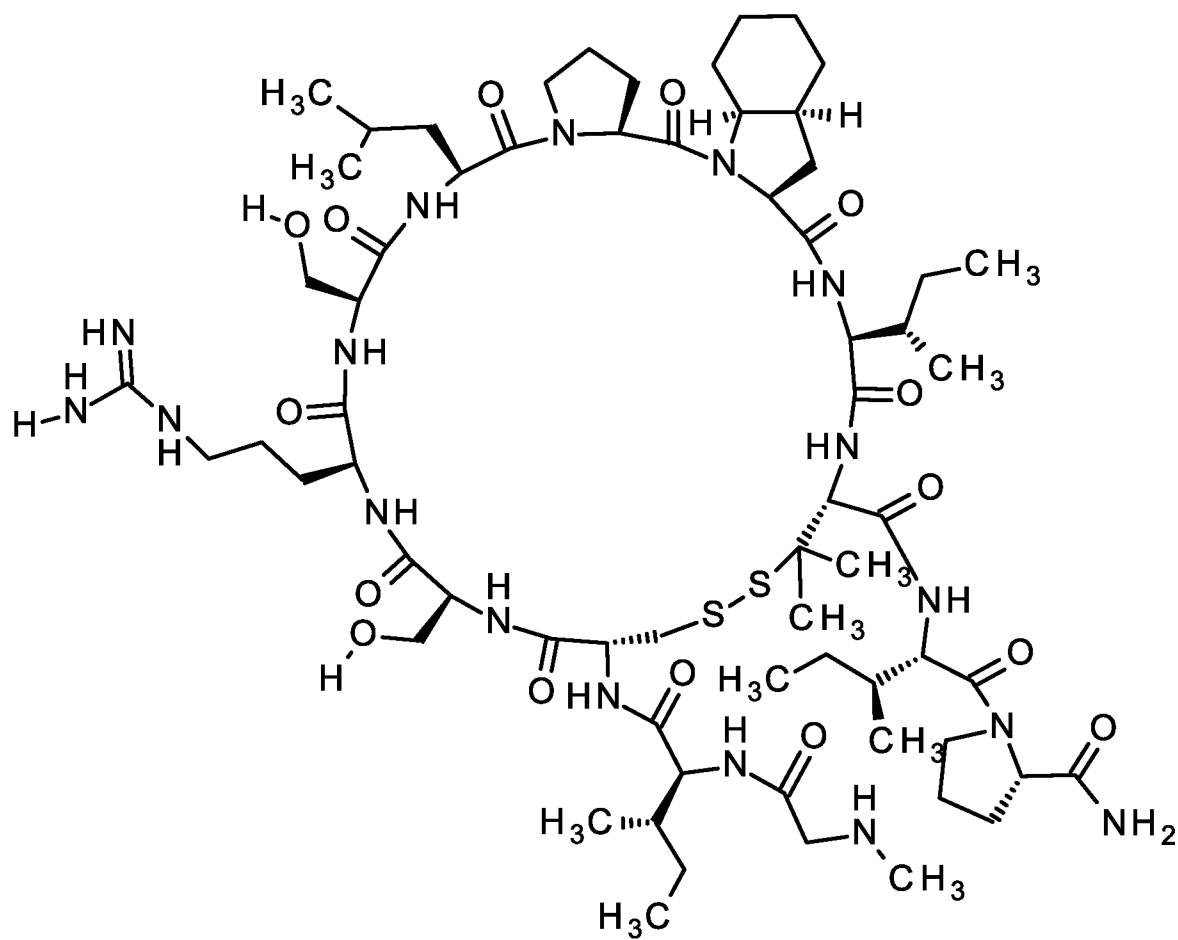
Example 44, Sequence: ((N-Me)G)-IC+SRS-((tBu)A)-PPI-(Pen)+IP-(PEG9(31 atoms))-NH₂



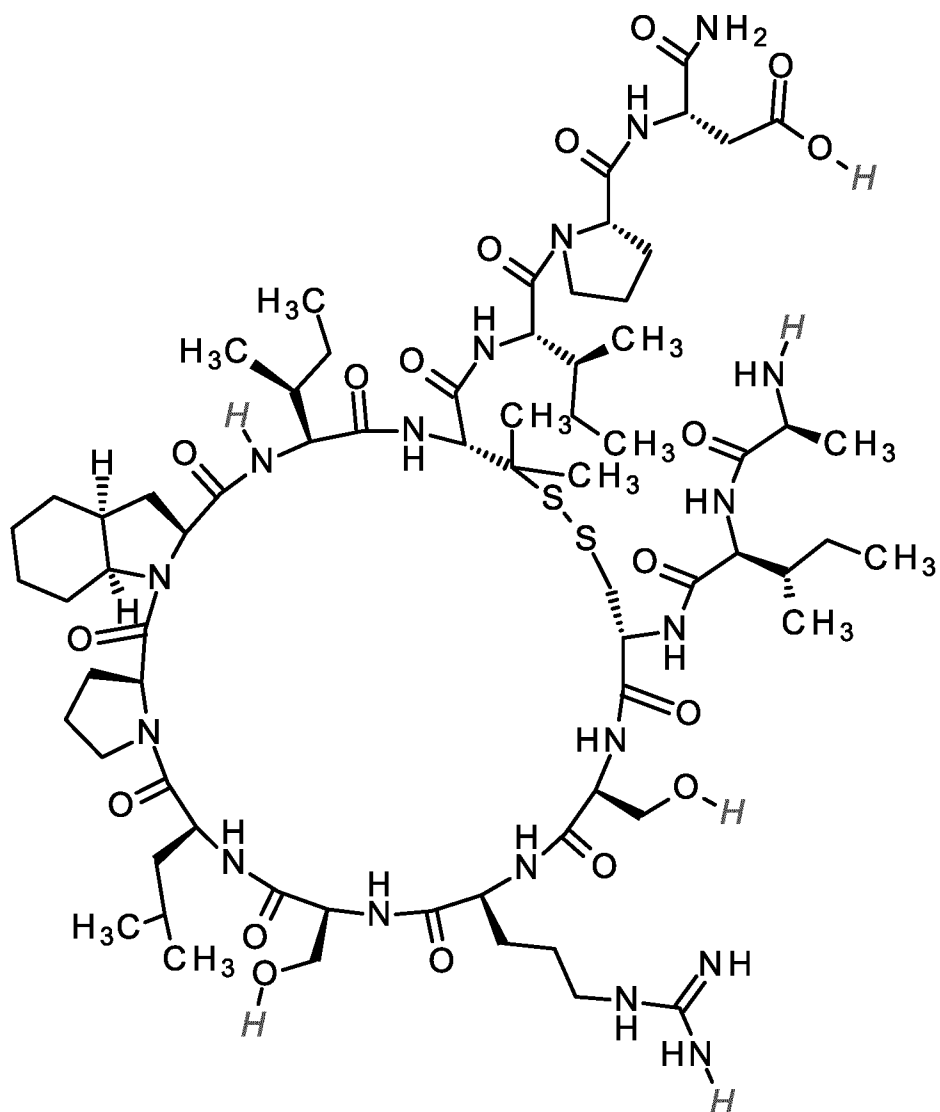
Example 45, Sequence: AI-C+SRS-((tBu)A)-P-((6S)-5-Azaspiro[2.4]heptane-6-carboxylic acid)-I-(Pen)+IPD-NH₂



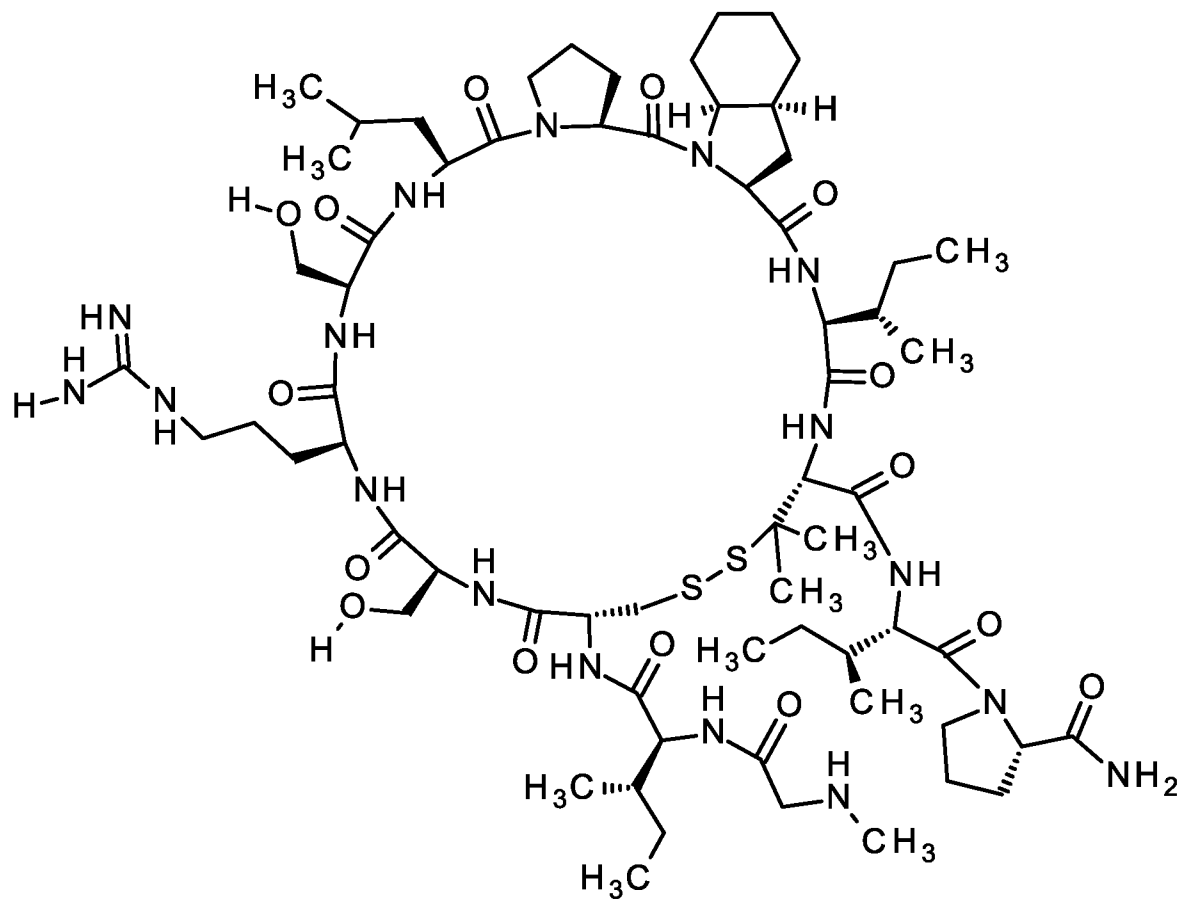
Example 67, Sequence: ((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-NH₂ (HCl salt)



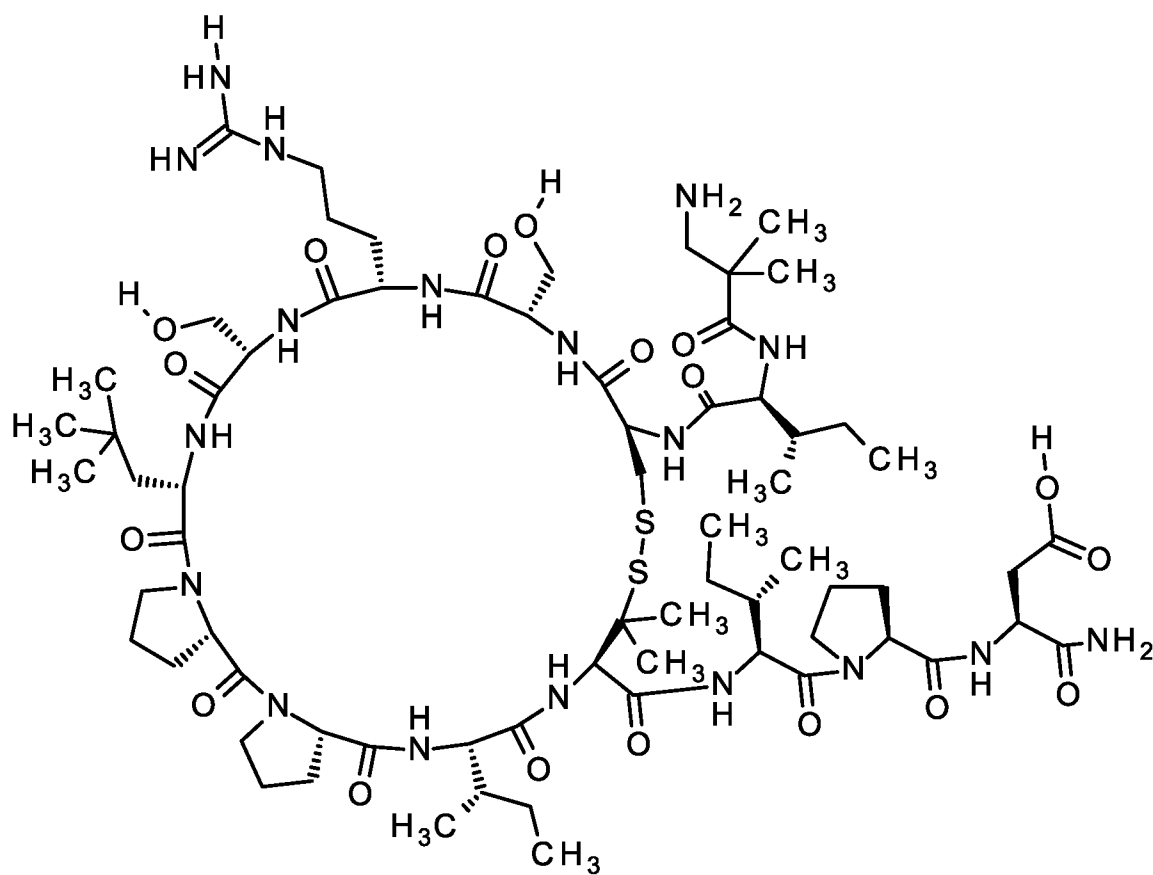
Example 75, Sequence: AIC+SRSLP-(Oic)-I-(Pen)+IPD-NH₂

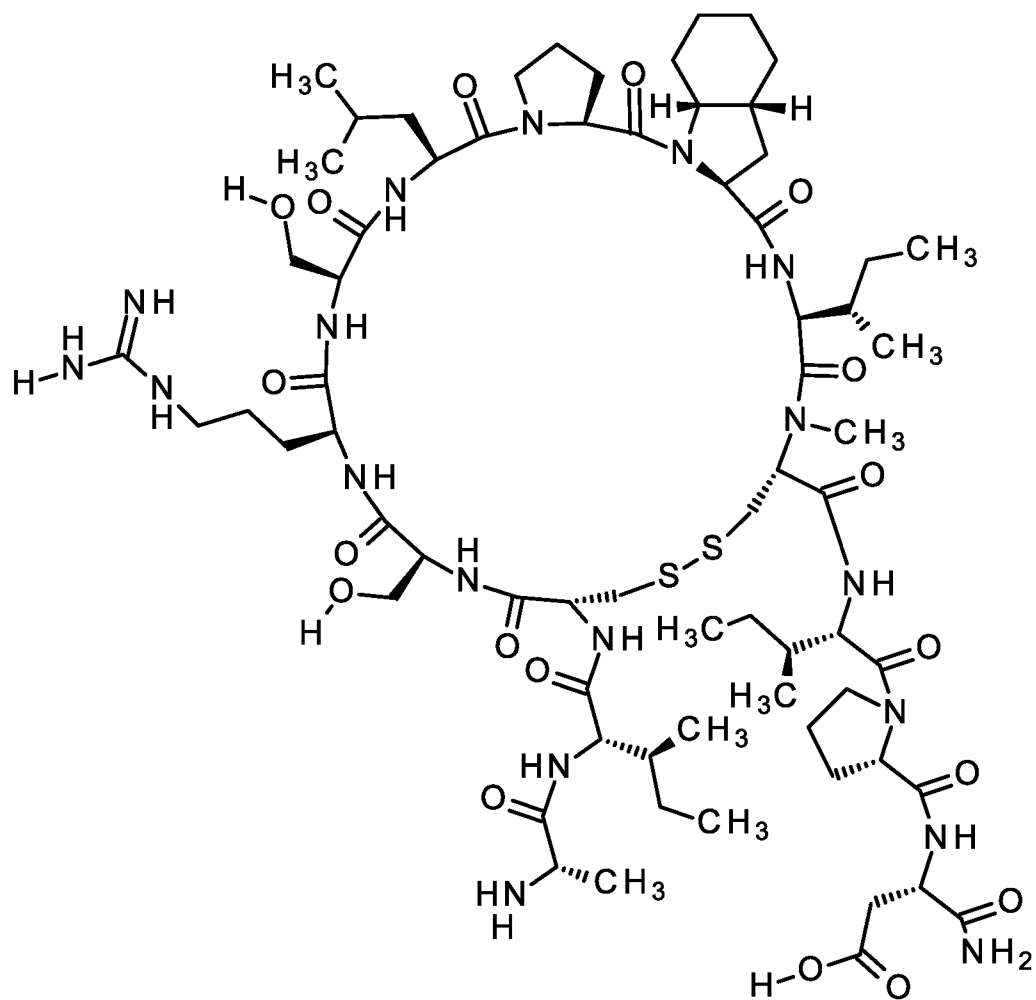


Example 109, Sequence: ((N-Me)G)-IC+SRSLP-(Oic)-I-(Pen)+IP-NH₂

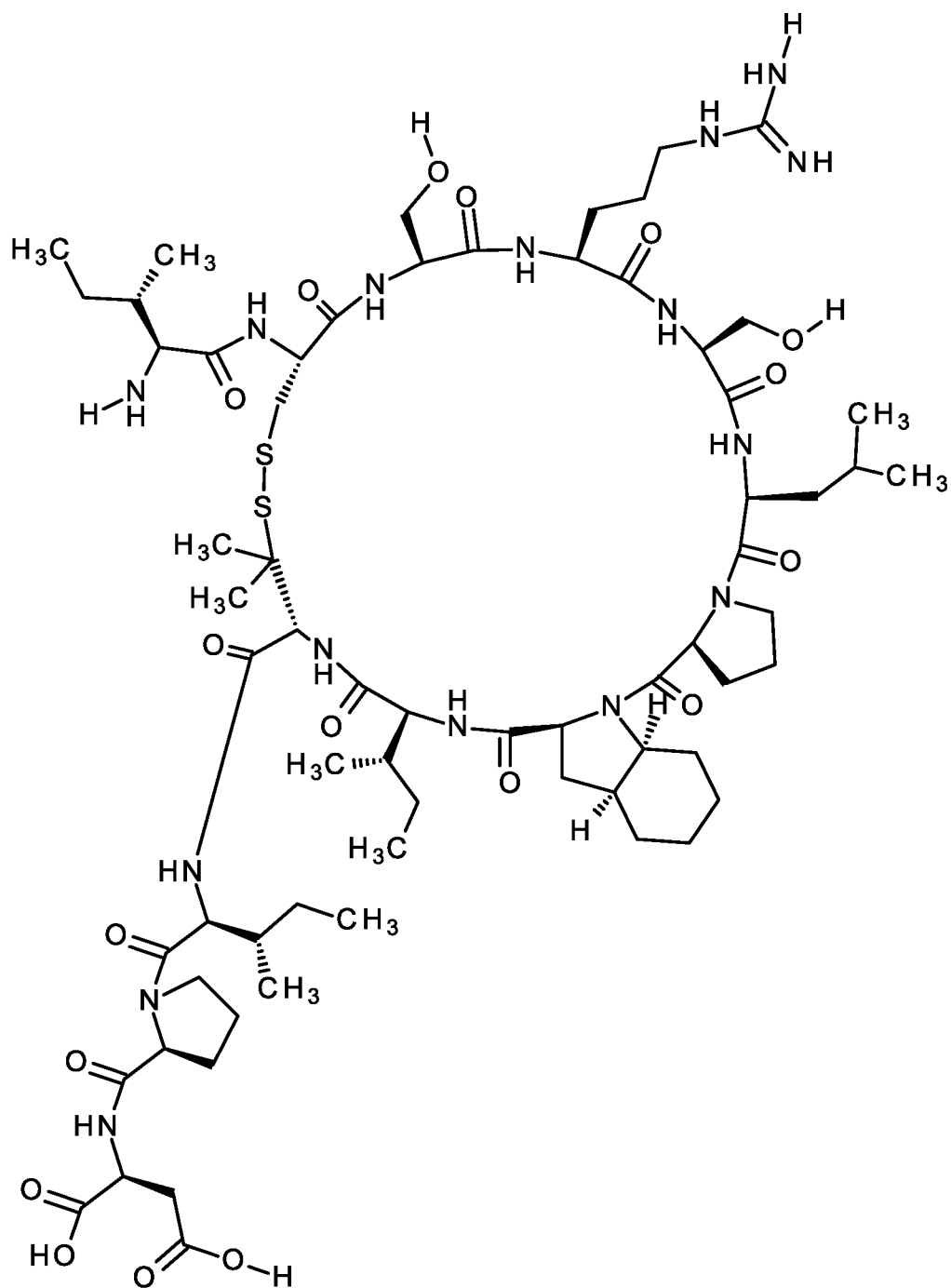


Example 166, Sequence: (3-Amino-2,2-dimethylpropionic acid)-IC+SRS-((tBu)A)-PPI-(Pen)+IPD-NH₂

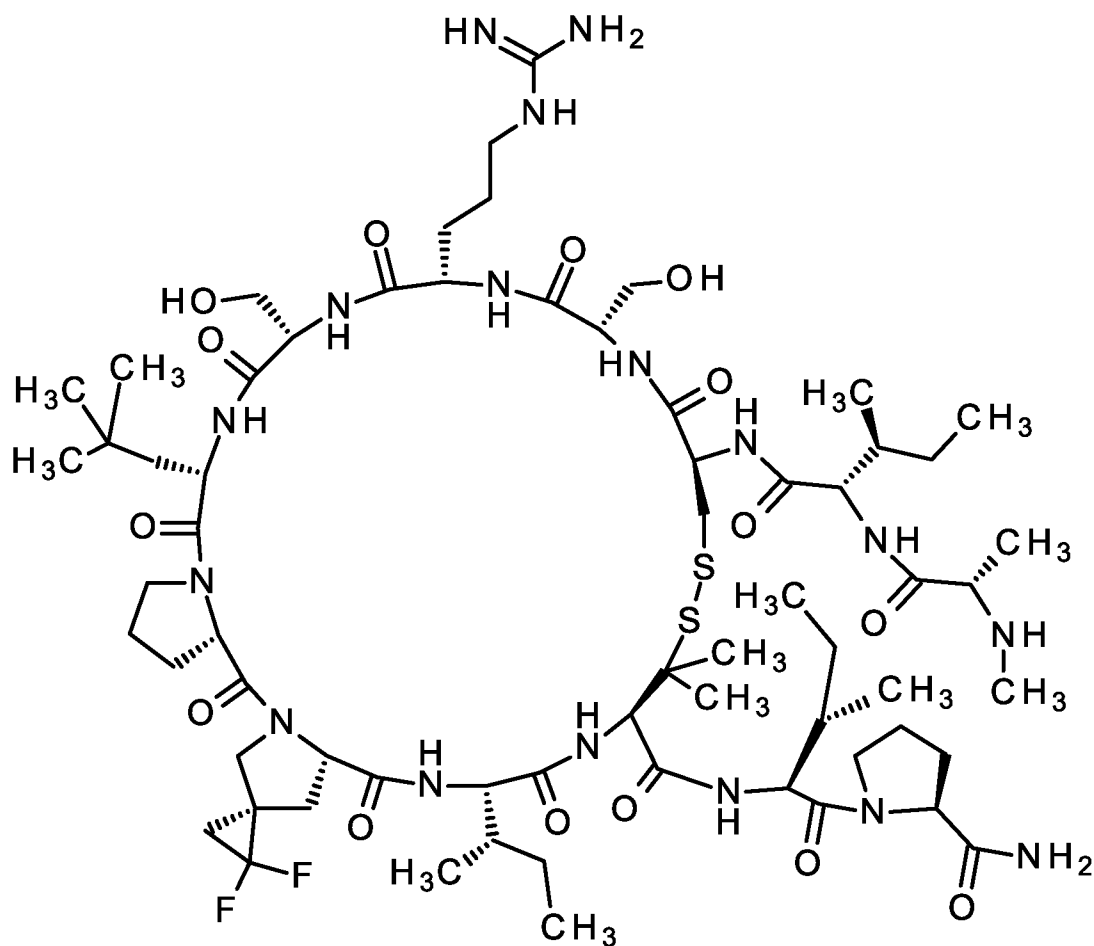


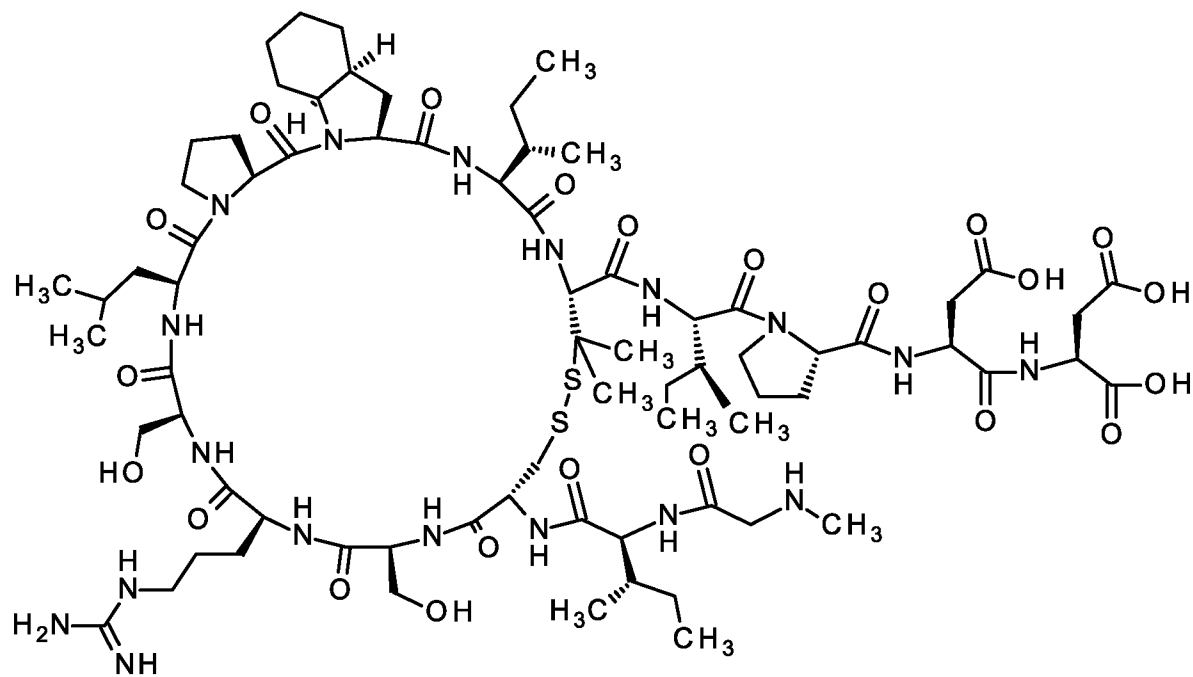


Example 347, Sequence: IC+SRLP-(Oic)-I-(Pen)+IPD-OH

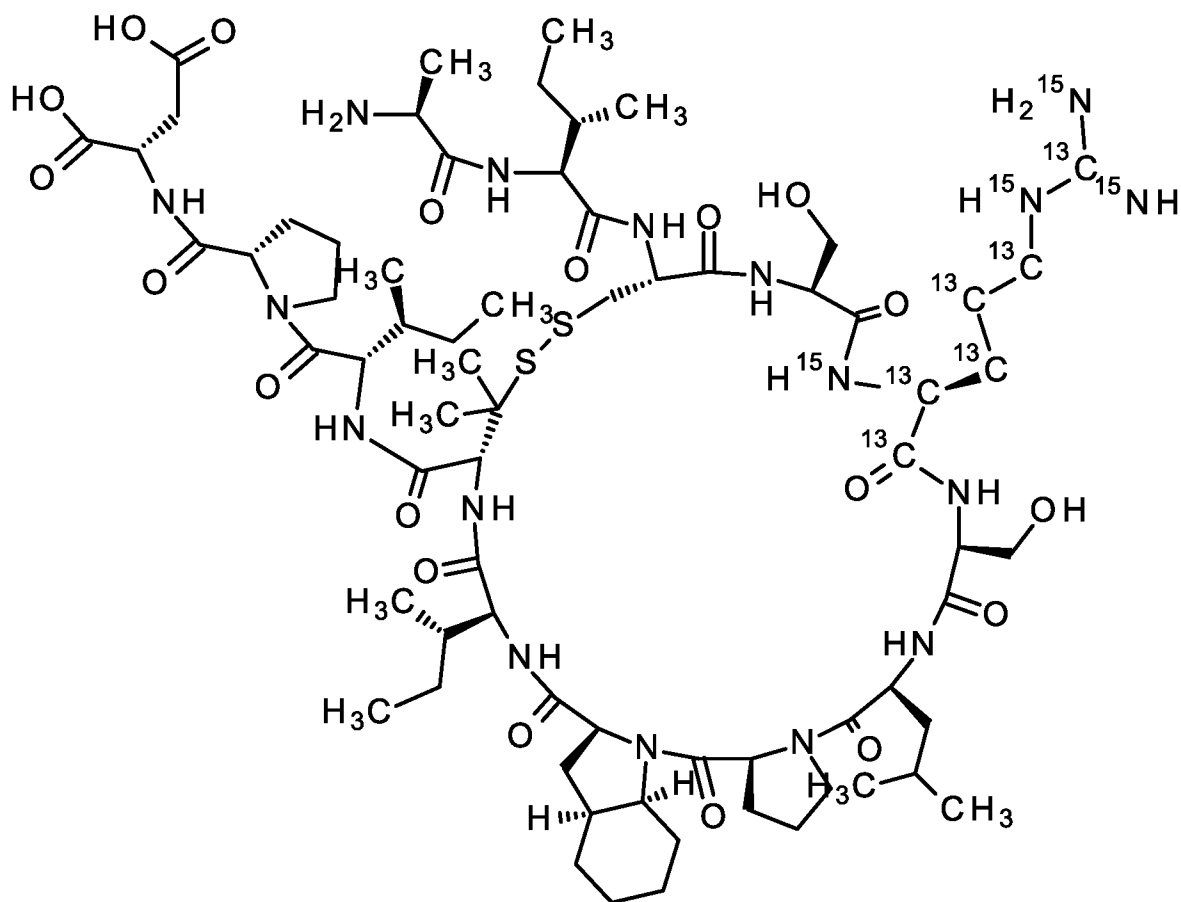


Example 443, Sequence: ((N-Me)A)-IC+SRS-((tBu)A)-P-((3R,6R)-1,1-Difluoro-5-azaspiro[2.4]heptane-6-carboxylic acid (enantiomer 1))-I-(Pen)+IP-NH₂





Example 499, Sequence: AIC+S-(Arg(13C6,15N4))-SLP-(Oic)-I-(Pen)+IPD-OH



Biological *in vitro* Testings

1. Serine protease profiling of test compounds

Test compounds were tested in a protease panel consisting of different human serine proteases including kallikrein, plasmin, FXIa, thrombin, factor Xa, tPA, and trypsin.

Test description

Inhibitory potency and/or selectivity of test compounds were determined. The assays are based on the fluorescent detection of aminomethylcoumarine (AMC), released from the fluorogenic peptidic protease substrates upon protease catalyzed cleavage. The active proteases or zymogenes, typically purified from human plasma or for trypsin from human pancreas, and corresponding substrates are commercially available.

Serine protease assays comprise of the following enzymes and substrates. All enzymes and substrates are diluted in assay buffer (50 mM Tris/HCl pH7.4, 100 mM NaCl, 5 mM CaCl₂, 0.1% BSA). The final assay concentrations are given:

- Kallikrein (Kordia; 0.2 nM), H-Pro-Phe-Arg-AMC (Bachem I-1295; 5 μ M)
- Plasmin (Kordia; 0.1 μ g/mL, 1.2 nM), MeOSuc-Ala-Phe-Lys-AMC (Bachem I-1275; 50 μ M)
- Factor XIa (Kordia; 0.15 nM), Boc-Glu(OBzl)-Ala-Arg-AMC (Bachem I-1575; 5 μ M)
- Thrombin (Kordia; 0.02 nM), Boc-Asp(OBzl)-Pro-Arg-AMC (Bachem I-1560; 5 μ M)
- Factor Xa (Kordia; 1.3 nM), Boc-Ile-Glu-Gly-Arg-AMC (Bachem I-1100; 5 μ M)
- Tissue plasminogen activator (tPA, Loxo; 2 nM), CH₃SO₂-D-Phe-Gly-Arg-AMC (Pentaparm 091-06; 5 μ M)
- Trypsin (Sigma; 0.042 U/mL), substrate Boc-Ile-Glu-Gly-Arg-AMC (Bachem I-1100; 5 μ M)

For determination of test compound potency, the enzyme and corresponding substrate dilutions are used to perform protease assays:

To 384 well microtiter plates (white, Greiner), containing 1 μ L/well serial dilutions of test or reference compounds, 20 μ L assay buffer, 20 μ L enzyme dilution, and 20 μ L substrate are added. Control reactions do not contain test compound (DMSO only). After incubation for typically 30 min (linear reaction kinetics) at room temperature, fluorescence (ex 360 nm, em 465 nm) is measured in a microtiter plate fluorescence reader (e.g Tecan Safire II). IC₅₀ values are determined by plotting log test compound concentration against the percentage protease activity.

2. Biochemical Human MASP-1 and MASP-2 assay

2.1 Recombinant expression and protein production of recombinant human MASP1 and MASP2 active proteases.

A truncated cDNA sequence of human MASP1 encoding the fragment corresponding to the amino acids 297-699 with an C-terminal His Tag and N-terminal Ig-kappa secretion signal (SEQ ID No: 2) was sub-cloned into the mammalian expression vector pcDNA3.1 (Invitrogen).

A truncated cDNA sequence of human MASP1 encoding the fragment corresponding to the amino acids 297-686 with an C-terminal His Tag and N-terminal Ig-kappa secretion signal (SEQ ID No: 3) was sub-cloned into the mammalian expression vector pcDNA3.1 (Invitrogen).

The MASP1 or MASP2 expression vectors were transfected into the HEK293 (ATCC No. CRL-1573) cell line using Lipofectamine LTX® Reagent (Thermo-Fischer), as described by the manufacturer. The mature form of the recombinant human MASP1 and MASP2 proteases were secreted into the culture medium. The MASP1 and MASP2 proteins were purified from the conditioned media by affinity chromatography on Ni-NTA Superflow resin (Qiagen) as described by the manufacturer.

2.2 Biochemical human MASP1 assay

Recombinant human MASP1 enzyme produced in the HEK 293 cells was diluted in the reaction buffer (50 mM HEPES pH 8,0; 100 mM NaCl; 0,01% CHAPS; 0,5 mM Gluthathione) to the concentration of 20 nM and 25 µl was transferred into each single well of 384-well white microtiter plate (Greiner Bio One 781075). 1 µl of the inhibitor compound solution (dissolved in DMSO, at the corresponding concentration) or pure DMSO as a control was added to the same wells. The enzymatic reaction was initiated by addition of 25 µl of 20 µM solution of the FRET substrate ABZ-MYGGARRL-Lys (Dnp)-NH₂; (ABZ - 2-amino-benzoyl; DNP - 2,4-dinitrophenyl; custom synthesis by Jerini Peptide Technologies, Berlin) in the reaction buffer. The microtiter plate was incubated for 60-120 min at the temperature of 32 °C. The increase of fluorescence intensity was measured in appropriate fluorescence plate reader (e.g. TECAN Ultra) using excitation wavelength of 320 nm and emission wavelength of 420 nm. IC₅₀ values were calculated from percentage of inhibition of human MASP1 activity as a function of test compound concentration.

2.3 Biochemical human MASP2 assay

Recombinant human MASP2 enzyme produced in the HEK 293 cells was diluted in the reaction buffer (50 mM HEPES pH 8,0; 100 mM NaCl; 0,01% CHAPS; 0,5 mM Gluthathione) to the concentration of 20 nM and 25 µl was transferred into each single well of 384-well white microtiter plate (Greiner Bio One 781075). 1 µl of the inhibitor compound solution (dissolved in DMSO, at the corresponding concentration) or pure DMSO as a control was added to the same wells. The enzymatic reaction was initiated by addition of 25 µl of 60 µM solution of the FRET substrate DABCYL-KISPQGYGRR-Glu(EDANS)-NH₂; (Dabcyl - 4 - ((4 - (dimethylamino)phenyl)azo)benzoic acid; Edans - 5-[(2-Aminoethyl) amino]naphthalene-1-sulfonyl; custom synthesis by Jerini Peptide Technologies, Berlin) in the reaction buffer. The microtiter plate was incubated for 60-120 min at the temperature of 32 °C. The increase of fluorescence intensity was measured in appropriate fluorescence plate reader (e.g. TECAN Ultra) using excitation wavelength of 340 nm and emission wavelength of 490 nm. IC₅₀ values were calculated from percentage of inhibition of human MASP2 activity as a function of test compound concentration.

3. C3 Deposition assay (human, rat, mouse, dog, mini-pig)

The C3 deposition assay was conducted essentially as described (reference). Multiwell plates (Greiner-Nunc 384 Maxi Sorp #464718) were coated over night with Mannan from *Saccharomyces cerevisiae* (Sigma M7504, 10 µg/mL in 0.05 M carbonate-bicarbonate buffer, pH 9.6) at 4°C. Wells were washed three times with TBS and subsequently incubated for 2 hours with 50 µL of 1% bovine serum albumin (BSA) in Tris-buffered saline (TBS) at 37°C in order to block non-specific binding. After this step and each of the following incubation steps wells were washed three times with C3 wash buffer (TBS; 0.05% Tween 20; 5 mM CaCl₂). Wells were next incubated for 30 min at 37°C with 50 µL of a mixture of test compounds with diluted serum in Veronal buffer (Veronal Puffer (Lonza 12624E). Serum was used at

concentrations that did not show detectable C3 deposition to uncoated plates in pre-tests. Appropriate dilutions were found to be in the range of 1:100 – 1:200 for human, rat, mouse and dog serum and 1:20 – 1:100 for mini pig serum, respectively. In typical experiments compounds were tested in a range of concentrations between 1×10^{-9} and 5×10^{-5} mol/L. After washing C3 deposition was detected by incubation with a polyclonal rabbit anti-human C3 antibody (Dako (Biozol) A0062) for 1 hour followed by washing and incubation with a peroxidase conjugated Anti Rabbit IgG (Sigma A1949) for 30 min at 37°C and subsequent washing and incubation with TMB substrate solution in the dark. When appropriately developed the color reaction was stopped by addition of 25 μ L of stop solution (Sigma S5814) and quantified on a photometer by measuring absorption at a wavelength of 450 nm. Antibodies were diluted in C3 wash buffer supplemented with 0.5% BSA.

4. Biochemical Rat MASP-1 and MASP-2 assay

4.1 Recombinant expression and protein production of recombinant human and rat MASP1 and MASP2 active proteases.

A truncated cDNA sequence of rat MASP1 encoding the fragment corresponding to the amino acids 302-704 with an C-terminal His Tag and N-terminal Ig-kappa secretion signal (SEQ ID No: 4) was subcloned into the mammalian expression vector pcDNA3.1 (Invitrogen).

A truncated cDNA sequence of rat MASP2 encoding the fragment corresponding to the amino acids 296-685 with a C-terminal His Tag and N-terminal Ig-kappa secretion signal (SEQ ID No: 5) was subcloned into the mammalian expression vector pcDNA3.1 (Invitrogen).

The MASP1 or MASP2 expression vectors were transfected into the HEK293 (ATCC No. CRL-1573) cell line using Lipofectamine LTX® Reagent (Thermo-Fischer), as described by the manufacturer. The mature form of the recombinant rat MASP1 and MASP2 proteases were secreted into the culture medium. The MASP1 and MASP2 proteins were purified from the conditioned media by affinity chromatography on Ni-NTA Superflow resin (Qiagen) as described by the manufacturer.

4.2 Biochemical rat MASP1 assay.

Recombinant rat MASP1 enzyme produced in the HEK 293 cells was diluted in the reaction buffer (50 mM HEPES pH 8,0; 100 mM NaCl; 0,01% CHAPS; 0,5 mM Gluthathione) to the concentration of 4 nM and 25 μ l was transferred into each single well of 384-well white microtiter plate (Greiner Bio One 781075). 1 μ l of the inhibitor compound solution (dissolved in DMSO, at the corresponding concentration) or pure DMSO as a control was added to the same wells. The enzymatic reaction was initiated by addition of 25 μ l of 40 μ M solution of the FRET substrate Dabcyl-MYGGARRL-Glu(Edans)-NH₂; (Dabcyl - 4 - ((4 - (dimethylamino)phenyl)azo)benzoic acid; Edans - 5-[2-Aminoethyl) amino]naphthalene-1-sulfonyl;

custom synthesis by Jerini Peptide Technologies, Berlin) in the reaction buffer. The microtiter plate was incubated for 60-120 min at the temperature of 32 C. The increase of fluorescence intensity was measured in appropriate fluorescence plate reader (e.g. TECAN Ultra) using excitation wavelength of 340 nm and emission wavelength of 490 nm. IC50 values were calculated from percentage of inhibition of rat MASP2 activity as a function of test compound concentration.

4.3 Biochemical rat MASP2 assay.

Recombinant rat MASP2 enzyme produced in the HEK 293 cells was diluted in the reaction buffer (50 mM HEPES pH 8,0; 100 mM NaCl; 0,01% CHAPS; 0,5 mM Gluthathione) to the concentration of 20 nM and 25 µl was transferred into each single well of 384-well white microtiter plate (Greiner Bio One 781075). 1 µl of the inhibitor compound solution (dissolved in DMSO, at the corresponding concentration) or pure DMSO as a control was added to the same wells. The enzymatic reaction was initiated by addition of 25 µl of 30 µM solution of the FRET substrate Abz-IEGRTSED-(Lys)Dnp-NH₂; (ABZ - 2-aminobenzo-yl; DNP - 2,4-dinitrophenyl; custom synthesis by Jerini Peptide Technologies, Berlin) in the reaction buffer. The microtiter plate was incubated for 60-120 min at the temperature of 32 C. The increase of fluorescence intensity was measured in appropriate fluorescence plate reader (e.g. TECAN Ultra) using excitation wavelength of 320 nm and emission wavelength of 420 nm. IC50 values were calculated from percentage of inhibition of rat MASP2 activity as a function of test compound concentration.

Table 18: Average IC50 of the reference peptides

Reference No	human MASP1 IC50 [mol/L]	human MASP2 [IC50 [mol/L]	Human C3-DEPOSITION SERUM IC50 [mol/L]
1	1,56E-08	1.09 E-06	1,52E-07
2	1,80E-08	6,10E-07	1,85E-07
3	1,80E-08	6,10E-07	5,10E-08
A	7,45E-09	9.14 E-07	3,94 E-08
B	8,00E-09	3.90 E-07	2.82E-08
C	3,60E-09	3,00E-06	2.57E-08

Table 19: Average IC50 of the peptides of the invention

Example No	human MASP1 IC50 [mol/L]	human MASP2 [IC50 [mol/L]	Human C3-DEPOSITION SERUM IC50 [mol/L]
4	9,30E-10	6,80E-08	8,11E-09
5	9,60E-10	9,70E-08	1.03E-08
6	1,10E-09	5,00E-08	1,39E-08
7	1,10E-09	2,30E-08	8,71 E-08

8	1,10E-09		1,45E-08
9	1,14E-09	5,00E-08	2,85E-08
10	1,19E-09	3,60E-08	2,75E-08
11	1,20E-09	6,20E-07	3,86E-08
12	1,20E-09	3,70E-08	1,42E-07
13	1,85E-09	1,00E-07	6,62E-09
14	1,30E-09	6,20E-08	1,18E-07
15	1,30E-09	8,70E-08	1,56E-08
16	1,30E-09	2,80E-08	5,01E-09
17	1,33E-09	1,80E-08	3,82E-07
18	1,35E-09	4,00E-08	2,09E-07
19	1,36E-09	1,00E-07	1,24E-07
20	1,40E-09	7,20E-07	3,65E-08
21	1,40E-09	6,20E-08	1,63E-08
22	1,40E-09	5,30E-08	1,39E-08
23	1,40E-09	4,00E-08	6,25E-09
24	1,43E-09	9,80E-08	2,83E-08
25	1,50E-09	1,00E-07	1,28E-08
26	1,50E-09	9,30E-08	1,04E-08
27	1,50E-09	1,40E-07	8,50E-09
28	1,50E-09	5,60E-08	1,31E-08
29	2,45E-09	4,20 E-08	6,99E-09
30	1,53E-09	1,40E-07	3,61E-08
31	1,57E-09	1,77E-08	4,28E-08
32	1,60E-09	1,10E-07	2,93E-08
33	1,60E-09		1,02E-08
34	1,60E-09	4,50E-08	3,92 E-08
35	1,65E-09	1,20E-07	1,57E-07
36	1,65E-09	2,60E-08	4,51E-07
37	1,70E-09	1,20E-07	1,46E-08
38	1,70E-09	8,70E-08	7,26E-08
39	1,70E-09	3,10E-08	1,08E-08
40	1,70E-09	5,90E-08	1,21E-08
41	1,80E-09	4,00E-08	1,21E-08
42	1,80E-09	3,60E-08	1,41E-08
43	1,80E-09	7,60E-08	2,73E-08
44	1,80E-09	7,30E-08	6,85E-08
45	1,83E-09	2,45E-08	1,86E-08
46	1,90E-09	4,30E-07	2,57E-08
47	1,90E-09	1,20E-07	4,34E-08

48	1,95E-09	2,25E-07	1,02E-07
49	2,00E-09	1,10E-07	3,56E-07
50	2,00E-09	7,60E-08	3,19E-08
51	2,00E-09	4,40E-08	3,95E-08
52	2,00E-09	2,40E-08	2,30E-08
53	2,00E-09	8,00E-08	3,03E-08
54	2,00E-09	7,30E-08	8,81E-09
55	2,00E-09	4,20E-08	5,40E-08
56	2,00E-09	1,30E-07	6,97E-09
57	2,00E-09	4,70E-08	1,17E-08
58	2,00E-09	4,20E-08	2,13E-08
59	2,09E-09		2,38E-07
60	2,10E-09	7,20E-08	1,15E-08
61	2,10E-09	9,80E-08	3,94 E-08
62	2,10E-09	9,60E-08	5,91 E-08
63	2,10E-09	1,30E-07	7,91 E-08
64	2,10E-09	2,00E-08	2,98 E-08
65	2,20E-09	9,80E-08	9,82E-08
66	2,20E-09	1,20E-07	3,60E-07
67	2,20E-09	4,30E-08	1,21E-07
68	2,20E-09	7,00E-08	1,40E-08
69	2,20E-09	5,10E-08	1,31E-08
70	2,20E-09	6,50E-08	4,84E-08
71	2,20E-09	6,30E-08	7,11 E-08
72	2,20E-09	4,20E-08	5,98 E-08
73	2,20E-09	3,00E-08	7,04 E-08
74	2,20E-09	5,20E-08	5,20E-08
75	2,23E-09	1,50E-07	3.20 E-08
76	2,30E-09	8,70E-08	1,18E-08
77	2,40E-09	1,50E-07	5,33E-08
78	2,40E-09	1,70E-07	1,46E-08
79	2,40E-09	1,10E-07	2,59E-07
80	2,40E-09	5,70E-08	2,00E-08
81	2,40E-09	6,40E-08	1,62E-08
82	2,40E-09	6,80E-08	1,33E-08
83	2,40E-09	1,10E-07	3,48E-08
84	2,40E-09	1,00E-07	7,00 E-08
85	2,40E-09	5,80E-08	4,82E-08
86	2,45E-09	1,85E-07	1,21E-06
87	2,50E-09	1,90E-07	1,52E-08

88	2,50E-09	1,20E-07	8,08E-08
89	2,50E-09	4,80E-08	4,30E-08
90	2,50E-09	7,00E-08	1,21E-07
91	2,60E-09	1,30E-07	1,12E-08
92	2,60E-09	3,60E-07	3,77E-08
93	2,60E-09	3,00E-08	4,00E-08
94	2,60E-09	4,70E-08	2,98E-08
95	2,60E-09	3,80E-08	1,21E-08
96	2,60E-09	9,20E-08	1,89E-08
97	2,60E-09	1,30E-07	4,84E-08
98	2,60E-09	8,30E-08	1,04E-07
99	2,70E-09	4,90E-07	1,35E-08
100	2,70E-09	3,20E-07	1,09E-08
101	2.70 E-09	2.00 E-07	8.13 E-08
102	2,80E-09	2,80E-06	2,51E-07
103	2,80E-09	2,30E-07	4,30E-07
104	2,80E-09	4,80E-08	3,29E-07
105	2,80E-09	1,50E-07	5,69E-08
106	2,80E-09	1,45E-07	1,21E-08
107	2,80E-09	1,80E-07	1,10 E-07
108	2,80E-09	1,30E-07	1,07E-07
109	2,90E-09	6,96E-08	1,35E-07
110	2,90E-09	2,00E-07	8,24E-08
111	2,90E-09	1,60E-07	4,87E-08
112	2,90E-09	1,30E-07	4,50E-08
113	2,90E-09	8,90E-08	3,89E-08
114	3,00E-09	3,30E-07	1,40E-08
115	3,00E-09	3,00E-08	1,92E-08
116	3,00E-09	4,60E-08	1,54E-08
117	3,00E-09	3,60E-07	6,81E-08
118	3,00E-09	1,40E-07	5,03E-08
119	3,00E-09	2,00E-07	7,81E-08
120	3,10E-09	5,90E-07	3,65E-08
121	3,10E-09	8,30E-08	5,78E-08
122	3,10E-09	1,60E-07	8,44 E-08
123	3,20E-09	9,70E-08	5,55E-09
124	3,20E-09	7,50E-08	1,40E-08
125	3,20E-09	9,30E-08	2,83E-08
126	3,20E-09	2,40E-08	1,49E-08
127	3,20E-09	7,90E-08	3,96E-08

128	3,20E-09	1,60E-06	1,24E-07
129	3,30E-09	1,60E-07	2,76E-08
130	3,30E-09	7,10E-08	5,43E-08
131	3,30E-09	2,00E-07	4,02E-08
132	3,30E-09	1,40E-07	1,07E-07
133	3,30E-09	1,60E-07	1,17E-07
134	3,40E-09	4,10E-07	5,12E-08
135	3,40E-09	9,90E-08	5,12E-08
136	3,40E-09	8,70E-08	5,53E-08
137	3,50E-09	9,80E-08	5,34E-08
138	3,50E-09	1,40E-07	1,14E-07
139	3,50E-09	1,90E-07	6,94E-08
140	3,50E-09	9,00E-08	6,80E-08
141	3,50E-09	2,10E-07	1,83E-07
142	2,13E-09	1.26 E-07	1.24E-08
143	3,60E-09	7,45E-08	4,11E-08
144	3,60E-09	1,30E-07	5,53E-08
145	3,60E-09	6,00E-08	1,01E-07
146	3,60E-09	1,70E-07	9,88E-08
147	3,70E-09	6,80E-08	6,12E-08
148	3,70E-09	5,60E-08	5,00E-08
149	3,70E-09	5,90E-07	7,80E-08
150	3,70E-09	9,70E-08	7,41E-08
151	3,70E-09	1,00E-07	3,00E-08
152	3,75E-09	1,95E-07	4,23E-08
153	4,65E-09	3.16 E-07	2.05E-08
154	3,80E-09	1,40E-07	2,24E-07
155	3,80E-09	2,10E-07	2,24E-08
156	3,80E-09	4,40E-08	2,73E-08
157	3,80E-09	6,30E-08	1,57E-06
158	3,90E-09	9,30 E-07	1,21E-08
159	3,90E-09	1,70E-07	2,10E-08
160	3,90E-09	1,00E-07	6,39E-08
161	3,90E-09	1,70E-07	7,56E-08
162	3,90E-09	1,60E-07	8,22E-08
163	4,00E-09	6,40E-08	1,77E-08
164	4,00E-09	5,70E-08	8,16E-08
165	4,05E-09	1,50E-07	3,60E-08
166	4,20E-09	1,30E-07	3,34E-08
167	4,20E-09	2,30E-07	7,05E-08

168	4,20E-09	1,20E-07	4,75E-08
169	4,25E-09	5,20E-08	8,68E-07
170	4,30E-09	6,80E-08	1,33E-08
171	4,30E-09	3,00E-08	1,95E-08
172	4,30E-09	5,90E-08	3,59E-08
173	4,30E-09	2,00E-07	1,03E-07
174	4,35E-09	6,55E-08	2,80E-07
175	4,40E-09	1,30E-07	1,31E-08
176	4,40E-09	2,40E-07	1,37E-08
177	4,40E-09	8,50E-07	2,75E-08
178	4,40E-09	6,80E-08	4,68E-08
179	4,40E-09	1,70E-07	8,65E-08
180	4,40E-09	1,80E-07	1,11E-08
181	4,50E-09	1,70E-07	1,35E-05
182	4,50E-09	6,80E-08	1,25E-07
183	4,70E-09	1,10E-06	4,07E-08
184	4,70E-09	2,00E-07	4,30E-08
185	4,80E-09	2,45E-07	6,74E-09
186	4,80E-09	1,70E-07	1,16E-08
187	4,90E-09	8,10E-09	1,65E-08
188	4,90E-09	5,50E-07	9,66E-08
189	4,90E-09	1,40E-07	9,90E-08
190	4,90E-09	2,00E-07	2,81E-07
191	5,00E-09	2,00E-07	9,12E-08
192	5,00E-09	1,50E-07	1,15E-07
193	5,00E-09	1,40E-07	4,15E-08
194	5,10E-09	1,00E-07	8,29E-08
195	5,10E-09	8,10E-08	2,27E-08
196	5,10E-09	1,80E-07	9,68E-08
197	5,10E-09	1,70E-08	6,34E-08
198	5,20E-09	2,50E-07	3,89E-08
199	5,20E-09	1,90E-08	4,36E-08
200	5,20E-09	1,90E-07	5,26E-08
201	5,30E-09	1,00E-07	1,56E-07
202	5,30E-09	1,30E-07	1,10E-07
203	5,40E-09	1,70E-07	1,53E-07
204	5,40E-09	1,60E-08	2,17E-08
205	5,50E-09	1,40E-05	2,51E-08
206	5,70E-09	4,40E-08	3,34E-08
207	5,70E-09	7,40E-08	2,89E-07

208	5,70E-09	1,50E-08	1,39E-08
209	5,80E-09	5,10E-08	1,48E-08
210	5,80E-09	6,10E-08	2,29E-08
211	5,80E-09	7,30E-08	3,55E-08
212	6,00E-09	7,40E-07	1,43E-08
213	6,10E-09	1,70E-07	5,82E-08
214	6,10E-09	2,70E-08	2,24E-08
215	6,20E-09	1,80E-07	1,27E-07
216	6,30E-09	2,20E-07	2,51E-08
217	6,30E-09	1,30E-07	6,43E-08
218	6,40E-09	6,50E-08	2,86E-08
219	6,40E-09	1,60E-08	2,89 E-08
220	6,50E-09	4,10E-07	1,48E-07
221	6,50E-09	1,30E-08	1,67E-08
222	6,50E-09	1,40E-07	7,36E-08
223	6,60E-09	1,20E-07	2,70E-07
224	6,90E-09	4,00E-07	1,55E-08
225	6,90E-09	4,90E-08	6,88E-08
226	7,00E-09	8,50E-09	3,03E-08
227	7,00E-09	1,00E-07	1,20E-07
228	7,10E-09	4,20E-06	3,10E-08
229	7,10E-09	2,00E-07	2,97E-08
230	7,20E-09	3,00E-07	4,31E-07
231	7,20E-09	1,90E-07	9,22E-09
232	7,30E-09	1,70E-06	1,25E-07
233	7,30E-09	1,90E-08	1,48E-08
234	7,40E-09	2,60E-07	9,46E-09
235	7,60E-09	6,30E-08	4,91E-08
236	7,80E-09	1,90E-07	2,72E-08
237	9,30 E-09	6,40 E-08	1,47 E-08
238	8,10E-09	1,30E-07	1,25E-07
239	8,10E-09	3,50E-08	5,19E-08
240	8,10E-09	5,60E-08	2,99E-08
241	8,20E-09	7,25E-08	2,20E-08
242	8,20E-09		1,98E-08
243	8,30E-09	1,20E-07	3,36E-08
244	8,45E-09	2,95E-07	1,74E-07
245	8,50E-09	3,30E-08	2,08E-08
246	8,80E-09	2,60E-06	2,17E-07
247	8,90E-09	9,20E-08	2,67E-08

248	8,90E-09	3,00E-07	2,71E-08
249	1,40E-09	3,70E-08	
250	9,30E-09	2,60E-07	2,53E-08
251	9,30E-09		1,66E-08
252	9,40E-09	4,80E-07	6,48E-08
253	9,50E-09	4,40E-07	7,70E-08
254	9,80E-09	7,35E-08	1,53E-08
255	9,90E-09	1,60E-08	2,82E-08
256	1,00E-08	6,20E-07	1,94E-07
257	1,00E-08	1,10E-07	2,48E-08
258	1,00E-08	4,00E-07	1,40E-08
259	1,00E-08	3,20E-08	7,83E-08
260	1,01E-08	2,70E-06	5,63E-08
261	1,10E-08	1,50E-06	1,62E-07
262	1,10E-08	1,70E-07	3,55E-08
263	1,10E-08	2,10E-07	6,34E-08
264	1,10E-09	3,50E-08	7,17E-09
265	1,10E-08	1,20E-06	3,20E-06
266	1,10E-08	3,80E-08	2,18E-08
267	1,10E-08	5,70E-08	8,29E-08
268	1,20E-08	3,70E-07	1,62E-07
269	1,20E-08	1,70E-08	2,23E-08
270	1,20E-08	2,90E-07	1,35E-07
271	1,30E-08	5,00E-07	3,48E-08
272	1,30E-08	2,90E-08	2,96E-08
273	1,30E-08	4,70E-09	1,82E-08
274	1,30E-08	5,50E-08	1,79E-07
275	1,40E-08	1,10E-06	4,71E-08
276	1,40E-08	1,30E-06	3,38E-06
277	1,40E-08	1,70E-07	2,45E-07
278	1,40E-08	3,30E-07	2,16E-08
279	1,40E-08	1,10E-06	3,44E-08
280	1,40E-08	6,10E-08	8,78E-08
281	1,50E-08	5,35E-07	4,47E-08
282	1,50E-08	8,91E-07	1,59E-07
283	1,50E-08	4,20E-08	6,98E-08
284	1,50E-08	9,30E-09	1,86E-08
285	1,50E-08	2,60E-08	2,64E-07
286	1,60E-08	1,60E-07	4,29E-06
287	1,60E-08	4,90E-07	2,70E-07

288	1,60E-08	6,50E-06	6,96E-07
289	1,60E-08	1,60E-07	4,11E-07
290	1,60E-08	2,50E-08	2,54E-08
291	1,60E-08	8,10E-08	3,29E-08
292	1,60E-08	9,30E-07	1,30E-08
293	1,70E-08	6,03E-07	3,87E-07
294	1,70E-08	4,90E-09	4,19E-08
295	1,70E-08	1,10E-08	6,37E-08
296	1,70E-08	1,20E-08	1,26E-08
297	1,80E-08	5,00E-07	1,20E-07
298	1,80E-08	4,20E-08	2,32E-08
299	1,80E-08	9,10E-07	7,36E-07
300	1,80E-08	4,50E-08	5,98E-08
301	1,80E-08	1,90E-08	1,46E-07
302	1,90E-08	1,40E-06	7,96E-08
303	1,90E-08	5,00E-07	1,67E-07
304	1,90E-08	2,70E-08	1,84E-08
305	1,90E-08	9,40E-09	7,34E-08
306	2,00E-08	4,50E-07	3,17E-07
307	2,00E-08	4,30E-08	2,65E-08
308	2,00E-08	5,00E-09	1,68E-08
309	2,00E-08	1,20E-08	2,06E-08
310	2,00E-08	3,00E-06	2,26E-07
311	2,00E-08	4,90E-08	1,94E-08
312	2,10E-08	5,30E-07	2,45E-06
313	2,10E-08	2,50E-07	3,16E-07
314	2,10E-08	2,60E-07	2,09E-07
315	2,10E-08	4,50E-09	2,27E-08
316	2,10E-08	6,60E-07	2,00E-07
317	2,20E-08	2,10E-07	3,86E-07
318	2,20E-08	6,10E-07	3,47E-07
319	2,20E-08	1,80E-08	2,13E-08
320	2,20E-08	1,90E-07	6,43E-08
321	2,20E-08	1,80E-06	2,74E-07
322	2,20E-08	1,50E-07	1,13E-07
323	6,96E-09	5,60E-07	2,32E-08
324	2,30E-08	7,20E-08	3,51E-07
325	2,30E-08	4,50E-07	1,19E-06
326	2,30E-08	7,10E-07	2,15E-08
327	2,30E-08	2,50E-08	1,94E-08

328	2,30E-08	1,30E-08	3,16E-08
329	2,40E-08	2,60E-07	6,86E-06
330	2,40E-08	1,30E-07	3,30E-08
331	2,40E-08	6,10E-07	1,81E-07
332	2,50E-08	2,30E-08	2,43E-08
333	2,50E-08	8,40E-09	1,40E-08
334	2,50E-08	5,50E-09	7,97E-09
335	2,50E-08	1,80E-07	5,66E-08
336	2,50E-08	6,90E-07	2,72E-07
337	2,50E-08	2,00E-06	2,51E-05
338	2,60E-08	3,60E-07	3,35E-07
339	2,60E-08	6,40E-07	4,73E-07
340	2,60E-08	3,00E-07	8,69E-07
341	2,60E-08	4,30E-07	2,89E-07
342	2,60E-08	1,70E-08	4,06E-08
343	2,70E-08	1,20E-08	1,60E-08
344	2,70E-08	5,10E-08	4,43E-08
345	2,70E-08	3,90E-07	1,93E-07
346	2,70E-08	1,90E-07	6,84E-08
347	2,75E-08	8,55E-07	1,51E-07
348	2,80E-08	6,90E-07	4,91E-06
349	9,60E-09	4,20E-07	7,06E-08
350	2,80E-08	3,70E-07	1,68E-07
351	2,80E-08	1,20E-08	7,74E-09
352	2,80E-08	7,10E-08	2,85E-07
353	2,80E-08	5,00E-08	4,47E-07
354	2,90E-08	5,10E-07	5,94E-07
355	2,90E-08	9,30E-07	1,62E-07
356	2,90E-08	1,60E-08	2,88E-08
357	3,10E-08	1,30E-06	1,15E-05
358	3,10E-08	1,90E-08	4,38E-08
359	3,10E-08	3,40E-08	3,26E-08
360	3,10E-08	5,50E-08	9,13E-08
361	3,20E-08	1,00E-06	2,30E-07
362	3,30E-08	6,50E-08	3,94E-08
363	3,30E-08	1,20E-07	1,30E-07
364	3,30E-08	9,80E-08	2,28E-08
365	3,40E-08	1,04E-08	4,57E-08
366	3,40E-08	9,00E-09	4,71E-09
367	3,50E-08	6,00E-07	1,31E-06

368	3,50E-08	1,20E-06	5,58E-07
369	3,50E-08	4,20E-07	6,06E-08
370	3,50E-08	9,35E-08	1,30E-08
371	3,60E-08	8,10E-07	3,15E-07
372	3,60E-08	6,60E-07	4,55E-07
373	3,70E-08	4,50E-07	5,24E-07
374	3,70E-08	1,40E-05	6,70E-05
375	3,70E-08	9,60E-09	1,13E-07
376	1,10E-09	3,50E-08	1,38E-08
377	3,70E-08	8,10E-07	2,67E-07
378	3,80E-08	1,10E-06	3,10E-07
379	3,90E-08	3,60E-07	1,92E-07
380	4,00E-08	8,10E-07	9,47E-07
381	4,00E-08	5,50E-07	5,47E-08
382	4,00E-08	4,10E-08	1,27E-07
383	4,00E-08	8,00E-07	1,53E-05
384	4,20E-08	2,60E-07	8,12E-07
385	4,25E-08	8,95E-09	5,64E-08
386	4,30E-08	7,50E-07	3,05E-07
387	4,40E-08	2,20E-06	8,76E-08
388	4,50E-08	7,50E-07	4,33E-07
389	4,60E-08	3,30E-07	6,03E-08
390	4,70E-08	1,30E-06	1,15E-06
391	4,70E-08	9,50E-09	2,57E-08
392	4,70E-08	8,00E-08	6,24E-08
393	4,90E-08	3,80E-06	5,45E-07
394	5,20E-08		1,12 E-07
395	5,20E-08	1,20E-08	4,59E-08
396	5,30E-08	2,80E-08	2,55E-08
397	5,40E-08	3,80E-06	7,20E-07
398	5,40E-08	2,20E-08	1,92E-08
399	5,50E-08	5,20E-07	3,42E-07
400	5,50E-08	9,00E-09	5,26E-08
401	5,60E-08		4,48E-07
402	5,80E-08	2,10E-06	2,55E-05
403	5,90E-08	7,40E-07	6,59E-07
404	6,20E-08	1,70E-08	3,51E-08
405	6,30E-08	9,80E-07	8,69E-08
406	6,40E-08	1,30E-08	6,13E-08
407	6,40E-08	1,20E-08	1,54E-08

408	6,90E-08	3,20E-08	2,86E-07
409	7,10E-08	5,70E-07	1,53E-05
410	7,60E-08	2,70E-06	4,32E-06
411	7,60E-08	2,50E-06	2,84E-07
412	7,70E-08	5,00E-06	1,83E-07
413	7,80E-08	2,00E-06	1,05E-05
414	8,60E-08	4,00E-06	2,38E-05
415	8,60E-08	2,00E-08	1,86E-08
416	8,70E-08	1,90E-07	4,14E-05
417	8,90E-08	1,70E-05	2,07E-07
418	1,80E-09	1,30E-07	5,39E-08
419	5,10E-09	1,00E-07	8,29E-08
421	5,00E-09	9,80E-09	5,22E-08
422	8,10E-09	1,20E-08	5,91E-08
423	5,70E-09	1,20E-08	4,77E-08
424	6,60E-09	1,10E-08	5,26E-08
425	7,70E-09	1,30E-08	4,78E-08
426	9,10E-09	1,50E-08	4,60E-08
427	1,00E-08	1,50E-08	6,39E-08
428	3,70E-09	3,20E-07	6,03E-08
429	6,90E-09	1,20E-08	4,72E-08
430	1,40E-09	9,10E-08	4,05E-08
431	2,00E-09	1,70E-07	8,64E-08
432	2,70E-09	3,70E-07	1,73E-07
433	1,70E-09	1,20E-07	8,02E-08
434	1.00 E-06	1.00 E-06	
435	9,20E-09	1,70E-08	5,82E-08
436	2,30E-09	4,40E-08	5,19E-08
437	2,20E-09	7,60E-08	5,61E-08
438	2,90E-09	8,30E-08	5,08E-08
439	3,20E-09	1,50E-08	2,84E-08
440	1,80E-08	2,60E-08	2,15E-07
441	6,50E-08	2,20E-07	2,53E-07
443	7,80E-10	7,40E-08	2,52E-08
444	2,80E-09	6,10E-08	2,95E-08
445	3,00E-09	3,00E-07	4,99E-08
446	2.70 E-09	7.20 E-08	
447	4.70 E-09	6.55 E-08	
448	4,40E-07	1,00E-06	3.45E-06
449	1,80E-09	9,30E-08	5.58E-08

450	5,80E-09	2,30E-07	7.34E-08
451	2,60E-09	6,10E-08	5.69E-08
452	2,00E-09	1,00E-07	3.78E-08
453	2,50E-09	5,60E-08	5.98E-08
454	2,00E-09	9,90E-08	6.57E-08
455	2,90E-09	8,50E-08	
456	1,90E-09	1,10E-07	5.55E-08
457	1,70E-08	4,50E-08	5.06E-08
458	5,10E-09	2,10E-07	1.07E-07
459	4,30 E-09	2,00 E-07	1.04E-07
460	8,20 E-09	1,40 E-07	6.20E-08
462	1,20 E-08	1,30 E-07	6.10E-08
463	3,80 E-09	2,60 E-07	6.39E-08
464	1,00 E-09	4,50 E-08	7.76E-08
465	4,10 E-10	1,20 E-07	9.94E-08
466	7,20 E-10	4,00 E-08	7.16E-08
467	6,60 E-09	5,50 E-08	4.15E-08
468	4,50 E-09	9,70 E-08	5.77E-08
469	1,90 E-09	1,10 E-07	4.00E-08
470	1,80 E-09	5,80 E-08	4.12E-08
471	2,60 E-09	8,20 E-08	4.49E-08
472	4,00 E-09	5,10 E-08	4.39E-08
474	2.40E-09	7.80E-08	
475	2.60E-08	1.80E-08	
476	3.80E-09	6.10E-08	
477	9.60E-09	1.72E-07	
478	3.70E-09	3.70E-08	
479	5.85E-09	1.75E-07	
480	3.00E-09	3.50E-08	
481	2.50E-09	5.50E-08	
482	2.80E-09	2.50E-08	
483	5.40E-09	6.40E-08	
484	5.50E-09	1.20E-07	
485	2.10E-09	3.50E-08	
486	3.80E-09	3.20E-08	
487	2.35E-09	4.80E-08	
488	5.20E-09	1.10E-07	
489	2.40E-09	3.20E-08	
490	1.95E-08	2.10E-08	4.64E-08
491	5.80E-08	4.30E-08	

492	3.00E-09	1.80E-07	
493	3.20E-09	1.20E-07	
494	2.60E-09	8.40E-08	
495	3.40E-09	3.70E-08	
496	2.00E-09	3.10E-08	
497	2.30E-09	4.70E-08	
498	2.30E-09	3.90E-08	
500	7.70E-09	1.50E-07	
501	4.80E-09	5.90E-08	
502	6.50E-09	1.10E-07	
503	6.30E-09	6.20E-08	
504	6.80E-09	1.20E-07	
505	7.90E-09	7.60E-08	
506	6.20E-09	7.60E-08	
507	1.70E-09	2.70E-08	
508	1.40E-08	3.30E-07	
509	6.50E-08	7.00E-08	
510	7.00E-08	1.70E-07	
511	5.26E-07	1.00E-06	
512	1.00E-06	1.00E-06	
513	7.40E-07	1.00E-06	
514	5.30E-09	4.60E-08	
515	7.00E-09	1.50E-07	
516	7.50E-09	1.00E-06	
517	3.10E-09	5.10E-07	
518	1.00E-06	1.00E-06	
519	1.80E-08	4.70E-08	
520	2.10E-08	6.40E-08	
521	1.70E-08	2.00E-08	
522	4.40E-09	2.20E-08	
523	4.40E-09	2.40E-08	
524	7.80E-09	1.00E-06	
525	2.00E-08	2.30E-07	
526	6.60E-09	1.04E-07	4.85E-08
527	2.30E-08	9.60E-08	1.54E-08
528	7.50E-09	6.50E-08	2.03E-08
529	3.10E-09	5.40E-08	3.95E-08
530	6.78 E-09	1.16 E-07	2.14 E-08
531	1.71 E-08	2.61 E-07	3.41E-08
532	2.80 E-08	1.66 E-07	2.03E-08

Table 20: Average IC50 of the reference peptides

Reference No	rat MASP1 IC50 [mol/L]	rat MASP2 IC50 [mol/L]	rat C3-DEPOSITION SERUM IC50 [mol/L]
1	6.86 E-08	6.80 E-08	1.70E-06
3			1.14E-06
A	5.02 E-08	6.84 E-08	1.41E-06
B	8.34 E-08	5.42 E-08	6.49E-07
C	2.10 E-07		5.79E-07

Table 21: Average IC50 of the peptides of the invention

Example No	rat MASP1 IC50 [mol/L]	rat MASP2 IC50 [mol/L]	rat C3-DEPOSITION SERUM IC50 [mol/L]
5	2.80 E-08	4.90 E-08	2.14E-07
7	3.00E-08	1.60E-08	
12	1.30E-08		
13	9.32 E-09	1.64 E-08	2.42E-07
14	2.30E-08		
17	3.10E-08		
18	2.60E-08		
19	2.30E-08		
20	1.30E-08		
21	6.70E-08	4.00E-08	
26	5.50E-08	5.34E-08	1.50E-07
27	9.81 E-09	9.10 E-09	1.46E-07
29	1.05 E-08	1.74 E-08	1.96E-07
30	2.80E-08		
31	2.20E-08		
32	5.60E-08	4.80E-08	
35	4.60E-08		
36	2.40E-08		
37	1.40E-07		
38	1.20E-08		
39	4.90E-08	3.70E-08	
41	2.80E-08	1.30E-08	
44	8.70E-08	3.50E-08	
45	4.00E-08		

48	5.15E-08		
49	6.00E-08		
54	1.60E-07	4.80E-08	
55	6.50E-08	2.70E-08	
59	2.50E-07		
62	5.50E-08	5.00E-08	
65	7.20E-09		
66	7.00E-08		
67	7.20E-08	3.60E-08	
72	4.30E-08	2.70E-08	
73	4.30E-08	2.80E-08	
74	7.10E-08	2.80E-08	
75	3.50E-08	3.49E-08	1.83E-07
77	6.60E-08		
78	4.80E-08		
79	8.50E-08		
83	1.20E-07	4.80E-08	
85	7.50E-08	3.70E-08	
86	1.60E-08		
88	3.60E-08	6.80E-08	
90	6.70E-08	5.50E-08	
97	1.20E-07	5.10E-08	
98	1.70E-08		
99	2.20E-07		
101	7.60E-08	5.00E-08	
102	1.40E-07		
103	1.10E-07		
104	7.70E-08		
108	1.10E-08		
109	7.60E-08	4.20E-08	
119	1.30E-07	7.00E-08	
123	5.90E-08		
126	1.10E-08	1.00E-08	
128	3.50E-08		
133	9.80E-08	6.70E-08	
140	1.00E-07	5.00E-08	
141	1.40E-07	9.50E-08	
142	1.09 E-08	1.71 E-08	4.72E-07
146	1.10E-07	6.80E-08	
150	1.20E-07	5.30E-08	

153	4.00 E-08	5.03 E-08	8.00E-07
154	7.20E-08		
156	2.10E-08	1.60E-08	
158	2.47E-07	4.20E-07	
164	7.90E-08	2.80E-08	
168	1.40E-07	5.30E-08	
169	3.70E-07		
170	5.40E-08	9.82 E-09	2.36E-07
171	4.20E-08	2.10E-08	
174	6.56E-08	3.51E-08	
175	2.80E-08		
180	4.20E-08		
181	1.30E-07		
182	1.30E-08		
185	5.70E-08	1.09 E-08	2.36E-07
186	3.80E-07		
187	7.70E-08	3.70E-09	
194	7.00 E-08	4.20 E-08	
197	1.00E-07	1.50E-08	
199	1.80E-07	4.90E-08	
205	3.00E-07		
207	4.30E-08	3.36E-08	
208	6.10E-08	8.60E-09	
209	3.30E-07		
210	1.90E-08		
211	7.50E-08	2.30E-08	
212	2.46 E-08	3.45 E-08	2.31E-07
216	2.40E-07		
222	2.40E-07		
223	1.20E-07		
224	6.04 E-08	4.24 E-08	7.83E-07
225	2.30E-07		
226	2.90E-08	2.90E-09	
228	2.60E-07		
230	3.70E-07		
231	3.80E-08		
232	6.73E-08	1.25E-07	
234	7.80E-08		
235	3.80E-07	2.90E-08	
236	8.90E-07		

237	1.00 E-06	1.00 E-06	
238	1.50E-07		
241	1.30E-07		
243	1.40E-07		
246	7.80E-08		
247	7.00E-07		
248	2.60E-07		
250	7.70E-08		
253	1.30E-07		
254	3.20E-07		
259	2.70E-08		
260	1.60E-07		
261	3.00E-07		
262	4.90E-07		
267	1.30E-07	4.20E-08	
268	1.00E-07		
269	1.20E-07	9.60E-09	
270	1.70E-07		
271	8.87 E-08	5.52 E-08	1.06E-06
274	8.90E-08		
275	1.50E-07		
276	2.00E-07		
277	1.90E-07		
278	8.30E-08		
281	2.70E-07	3.08E-07	1.41E-06
282	4.70E-07		
284	6.00E-08	9.70E-09	
286	2.30E-07		
287	2.50E-07		
289	1.80E-07		
294	9.70E-08	9.60E-09	
305	7.60E-08	5.30E-09	
313	2.20E-07		
323	5.18 E-08	6.01 E-08	1.14E-06
343	1.40E-07	1.90E-08	
347	1.00 E-06	1.00 E-06	
348	3.50E-07		
360	1.20E-07	7.30E-08	
367	1.20E-07		
370	2.35E-07	1.70E-07	

381	6.00E-07	4.70E-07	
400	4.50E-08	1.10E-08	
405	6.50E-07	5.10E-07	
419	7.00E-08	4.20E-08	
436	3.90E-08	3.10E-08	
437	4.60E-08	4.00E-08	
438	3.10E-08	2.20E-08	
439	3.60E-08	9.40E-09	
440	6.10E-07	1.40E-08	
441	4.10E-07	1.20E-07	
443	2.90E-08	4.70E-09	
444	1.30E-07	9.80E-09	
445	1.50E-07	9.40E-08	
447	2.10 E-08	3.50 E-08	
448	5.00E-06	5.00E-06	
449	4.50E-08	4.45E-08	
450	3.10E-08	5.20E-08	
451	4.60E-08	4.60E-08	
452	3.60E-08	3.70E-08	
453	3.80E-08	3.20E-08	
454	1.27E-07	7.90E-08	
455	4.10E-08	3.20E-08	
456	3.90E-08	3.00E-08	
457	2.00E-08	5.00E-09	2.34E-07
458	2.50E-07	1.00E-07	
459	1.80E-07	5.10E-08	
460	2.10E-07	6.90E-08	
462	1.60E-07	1.50E-07	
463	1.90E-07	1.00E-07	
464	9.40E-08	1.20E-07	
465	7.20E-08	1.40E-07	
466	1.76E-08	7.20E-08	
467	1.70E-07	7.00E-08	
468	1.30E-07	8.00E-08	
469	1.20E-07	3.40E-08	
470	1.00E-07	5.40E-08	
471	7.80E-08	7.10E-08	
472	1.00E-07	4.90E-08	
474	8.70E-08	2.60E-08	
475	2.50E-07	2.50E-08	

476	5.30E-08	6.10E-08	
477	1.54E-07	1.23E-07	
478	6.80E-08	4.90E-08	
479	8.40E-08	4.50E-08	
480	2.40E-08	4.10E-08	
481	2.00E-08	3.60E-08	
482	1.80E-08	3.30E-08	
483	3.40E-08	5.00E-08	
484	6.30E-08	8.80E-08	
485	3.60E-08	3.20E-08	
486	6.90E-08	6.80E-08	
487	6.70E-08	3.80E-08	
488	4.10E-08	3.60E-08	
489	4.20E-08	2.20E-08	
490	1.25E-08	1.50E-08	3.66E-07
491	2.00E-08	2.30E-08	
492	6.50E-08	8.10E-08	
493	4.40E-08	4.90E-08	
494	3.80E-08	6.20E-08	
495	4.50E-08	6.60E-08	
496	3.10E-08	3.10E-08	
497	5.80E-08	3.90E-08	
498	4.40E-08	4.20E-08	
526	4.85E-07	2.53E-07	
527	2.40E-07	4.20E-08	
528	4.20E-07	8.20E-09	
529	7.30E-07	1.00E-07	
530	2.33 E-08	1.84 E-08	2.82E-07
531	2.60 E-08	7.41 E-08	
532	2.42 E-08	1.76 E-08	

4. Kidney ischemia reperfusion injury (IRI) in rats after unilateral nephrectomy

All procedures conformed to national legislation (dt. Tierschutzgesetz) and EU directives for the use of animals for scientific purposes and were approved by the institutional animal care office of Bayer AG and by the competent regional authority (LANUV Recklinghausen). Standard laboratory diet and tap water were available ad libitum. In a typical experiment, the number of animals used was n = 6 to 12. Animals were randomly assigned to experimental groups. Kidney ischemia reperfusion injury (IRI) was performed in male unilaterally nephrectomized Wistar rats of a preferred body weight in the range of 250 to 350 g.

For unilateral nephrectomy rats were kept anesthetized under inhalation of 2% isoflurane in air. Analgesia was provided as a subcutaneous injection of 400 µl/kg of a mixture of 25% Ketavet and 8% Rompun in 0.9 NaCl. Unilateral nephrectomy was performed after protruding the right kidney through a small incision in the dorsolateral abdominal wall and ligating of its peduncle. After unilateral nephrectomy abdominal incision was closed by surgical sutures in layers and animals were allowed to recover for 7 to 8 days before IRI. IRI was performed under anesthesia and analgesia as described above. The remnant left kidney was protruded through a small incision of the abdominal wall and blood vessels of the kidney peduncle were clamped with an atraumatic microvascular clamp for 45 minutes in a typical setting. During this time the kidney together with the clamp in situ was repositioned into the abdominal cavity to ensure warm ischemia. After 45 min the clamp was opened and removed, and the incision closed by sutures as described above. Test compound or vehicle was administered intravenously via a polyethylene catheter placed before surgery into the jugular vein.

Compounds were dissolved in appropriate vehicle and administered either preventive before IRI or therapeutically after completion of IRI. Typical dose range applied was 0.1 – 30 mg/kg i.v.. Vehicle without compound was administered to animals that served as controls. Sham control animals underwent the whole procedure described above without closure of the clamp for induction of ischemia.

Blood samples were taken under anesthesia at day 1 and 8 after IRI. In a typical setting, animals were sacrificed 8 days after IRI and kidneys were sampled and frozen in liquid nitrogen. In another typical setting the animals were sacrificed 1 day after IRI.

Typical laboratory parameters measured in plasma samples to assess kidney function were creatinine and urea. For determination of creatinine clearance animals were kept in metabolic cages and urine was collected for at least 16 hours. After determination of urine volume flow (V_U) and determination of urinary and plasma creatinine concentrations ($[Crea]_U$ and $[Crea]_{PI}$, respectively) creatinine clearance (Cl_{Crea}) was calculated according to the standard formula: $Cl_{Crea} = V_U * [Crea]_U / [Crea]_{PI}$

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction: Total RNA was extracted from tissue samples by the Trizol method. Integrity of obtained RNA was checked on a Bioanalyzer (Agilent). For reverse transcription, 1 µg of total RNA was first digested with RNase-free DNase I (Gibco) for 15 min at room temperature and then reversetranscribed using Promiscript (Promega) in a total reaction volume of 40 µl according to the standard protocol of the kit supplier. After inactivation of the enzyme by heating for 15 min to 65 °C, the obtained cDNA was diluted to a final volume of 150 µl with bidest. water and 4 µl were chosen per PCR reaction. Real-Time PCR including normalization of raw data to cytosolic beta-actin as a housekeeping gene was carried out as described (Ellinghaus et al., 2005). The resulting expression is given in arbitrary units. Sequences of used oligonucleotide primers and probes are given in table 1.

5. Kidney ischemia reperfusion injury (IRI) in pigs after aortic balloon occlusion

All procedures conformed to national legislation (dt. Tierschutzgesetz) and EU directives for the use of animals for scientific purposes and were approved by the institutional animal care office of Bayer AG and by the competent regional authority (LANUV Recklinghausen). Female Göttingen mini pigs (Ellegaard, Denmark) of a body weight ranging preferably from 12 to 16 kg were used for the experiments. Animals were randomly assigned to experimental groups.

Minimal invasive methods were applied with modifications as described (Simon et al., Effects of intravenous sulfide during porcine aortic occlusion-induced kidney ischemia/reperfusion injury. *Shock*. 2011;35:156-163; Matejkova et al., Carbamylated erythropoietin-FC fusion protein and recombinant human erythropoietin during porcine kidney ischemia/reperfusion injury. *Intensive Care Med*. 2011;39:497–510). In brief: Pigs were kept anesthetized by a continuous i.v.-infusion of Ketavet®, Dormicum® and Pancuronium® after premedication with an intramuscular injection of Ketavet® / Stresnil®. After intratracheal intubation animals were artificially ventilated using a pediatric respirator (Avance CS², GE Healthcare) with an oxygen air mixture at a tidal volume of 6 to 8 mL/kg at a constant positive end-expiratory pressure (PEEP) of 3 - 4 cm H₂O and a frequency of 13 to 20 min⁻¹. Ventilation was adjusted to keep arterial PaCO₂ at about 40 mmHg at baseline. A catheter was placed into the right jugular vein for drug and fluid administration. Ringer-lactate solution was infused intravenously at a constant rate of 10 mL/kg/h. Animals received 50 i.E./kg Heparin i.v.. Routinely the following cardiovascular and respiratory parameters were measured after placement of necessary probes and catheters fitted to appropriate pressure transducers and recording equipment: central venous pressure (via left jugular vein), arterial blood pressure and heart rate (BP and HR; via left carotid artery) and cardiac output (CO) and systemic vascular resistance (SVR) by use of the PiCCO® system (Pulsion, Germany) connected to a Pulsion 4F Thermodilution-catheter (PV2014L08N) placed into the right carotid artery. Catheters for measurement of CVP, BP and HR were fitted to a Ponemah recording system via Combitrans transducers (Braun, REF 5203660). A Fogarty occlusion catheter (8F/14F, Edwards Lifesciences, REF 6208014F) was inserted into the abdominal aorta via the left femoral artery so that the tip with the inflatable balloon was placed upstream of the kidney arteries. A catheter was introduced into the urinary bladder via a small abdominal incision and urine continuously collected. Arterial blood samples were collected at regular intervals in which creatinine, urea, liver enzymes, blood cells and compound concentrations were determined. Arterial pO₂, pCO₂ and pH were determined on a Stat Profile® PRIME® (Nova Biomedical) blood gas analyzer at regular intervals in arterial blood samples. Kidney perfusion was assessed at regular intervals by Doppler ultrasound determination of the resistive index using a LOGIQ e Veterinary ultrasound apparatus (General Electrics) fitted with a 2,0 to 5,0 MHz broad-spectrum convex transducer (C1-5-RS, REF 5384874). Renal resistive index (RRI) is a suitable parameter to assess severity of acute kidney injury in patients (Darmon et al., Diagnostic accuracy of Doppler renal resistive index for reversibility of acute kidney injury in critically ill patients. *Intensive Care Med*. 2011;37(1):68-76)

When cardiovascular parameters showed a stable baseline (which was normally the case 60 min after surgery) recordings were started and samples for baseline parameters were collected. HR and MABP were continuously measured and for recording averaged over 2 min intervals. At the end of experimentation pigs were sacrificed by exsanguination.

Kidney injury was induced by inflating the balloon of the Fogarty balloon catheter with saline which immediately interrupted blood flow to the kidneys and the abdominal organs and led to a sharp increase of aortic blood pressure upstream of the balloon. Stop of blood flow was further confirmed by Doppler ultrasound examination of the kidney blood vessels. In typical experiments the aorta is kept occluded for 90 to 120 min until reperfusion by deflating the balloon. After reperfusion the Ringer-lactate infusion rate is doubled to 20 mL/kg/h in order to stabilize blood pressure and to enable diuresis. All parameters were monitored for up to 6 hours after reperfusion.

Compounds were dissolved in appropriate vehicle and administered either preventive before IRI or therapeutically after completion of IRI. Typical dose range applied was 0.1 – 10 mg/kg i.v.. In a typical experiment up to three doses were tested in up to 6 animals per dose group. Vehicle without compound was administered to animals that served as controls. Sham control animals underwent the whole procedure described above without induction of ischemia.

As a measure for kidney function after reperfusion preferably but not exclusively changes in diuresis, serum creatinine, serum potassium, serum bicarbonate and in resistive index determined by Doppler ultrasound examination were used.

Table 22: Sequence Listing

SEQ ID	No	Sequence
SEQ ID	1	GIC+SRSLPPIC+IPD-OH
SEQ ID	2	METDTLLLWVLLLWVPGSTGDAGNECPQLQPPVHG- KIEPSQAKYFFKDQVLVSCDTGYKVLKDNVEMDTFQIECLKDGTWSNKIP TCKIVDCRAPGELEHGLITFSTRNNLTYYKSEIKYSCQEPYYKMLNNNT- GIYTCSAQGVWMNKVLGRSLPTCLPVCGLPKFSRKL MARIFNGRPAQKGT TPWIAMLSHLNGQPF CGGSLLGSSWIVTAAHCLHQSLDPEDPTLRDSDL- SPSDFKIILGKHWRLRSDENEQHLGVKHTTLHPQYDPNTFENDVALVELLE SPVLNAFVMPICLPEGPQQEGAMVIVSGWGKQFLQRF- PETLMEIEIPIVDHSTCQKAYA- PLKKKVTRDMICAGEKEGGKDACAGDSGGPMVTLNRERGQWYLVGTVS WGDDCGKKDRYGVYSYIHHNKDWIQRVTGVRNHHHHHH
SEQ ID	3	METDTLLLWVLLLWVPGSTGDAQPCPYPMAPPNGHVSPVQAKY- ILKDSFSIFCETGYELLQGHLPKLSFTAVCQKDGSWDRMPACSI VDCGPP DDLPSGRVEYITGPGVTTYKAVIQYSCEETFYTMKVNDGKYVCEAD- GFWTSSKGEKSLPVCEPVCGLSARTTGGRIYGGQKAKPGDFPWQVLILGG TTAAGALLYDNWVLTAAHAVYEQKHDASALDIRMGTLKRL- SPHYTQAWSEAVFI- HEGYTHDAGFDNDIALIKLNNKVVINSNITPICLPRKEAESFMRTDDIGTAS GWGLTQRGFLARNLMYVDIPIVDHQCTAAY- EKPPYPRGSVTANMLCAGLESGGKD- SCRGDSGGALVFLDSETERWFGGIVSWGSMNCGEAGQYGVYTKVINYI PWNIISDFHHHHHH
SEQ ID	4	METDTLLLWVLLLWVPGSTGDAGNECPKLQPPVYGKIEPSQAVYS- FKDQVLISCDTGYKVLKDNVEMDTFQIECLKDGAWSNKIPTCKIVDCGVP AVLKHGLVTFSTRN- NLTTYKSEIRYSCQQPYKMLHNTTGVYTCSAHGTWT- NEVLKRSLPTCLPVCGLPKFSRKHISRIFNGRPAQKGTTPWIAMLSQLNGQ PFCGGSLLGSNWVLTAAHCLHHPLDPEEPILHNSHLL- SPSDFKIIMGKHWRRRSDEDEQHL- HVKHIMLHPLYNPSTFENDLGLVELSESPRLNDFVMPVCLPEHPSTEGTM VIVSGWGKQFLQRLPENLMEIEIPIVNYHTCQEAYTPLGKKVTQDMI- CAGEKEGGKDACAGDSGGPMVTKDAERDQWYLVGVVSWGDCGKKDR YGVYSYIYPNKDWIQRVTGVRNHHHHHH

SEQ ID	5	METDTLLLWVLLLWVPGSTGDTAQPCPDPTAPPN- GHISPVQATYVLKDSFSVFCKTGFELLQGSVPLKSFTAVCQKDGSWDRPIP ECSHDCGPPDDL PNGHVDYITGPEVTTYKAVIQYSCEET- FYTMSNGKYVCEAD- GFWTSSKGEKSLPVCKPVCGLSTHTSGGRIIGGQPAKPGDFPWQVLLLGET TAAGALIHDDWVLTAAHAVYGKTEAMSSLDIRMGILKRLSLIYTQAW- PEAVFIHEGYTHGAG- FDNDIALIKLKNKVTINRNIMPICLPRKEAASLMKTD FVGT VAGWGLTQK GFLARNLMFVDIPIVDHQKCATAYTKQPYP- GAKVTVNMLCAGLDRGGKDSCRGDSSGAL- VFLDNETQRWFVGGIVSWG SINCGGSEQYGVYTKVTNYIPWIENIINNFHH HHHH
SEQ ID	6	ABZ-MYGGARRL-Lys (Dnp)-NH ₂
SEQ ID	7	DABCYL-KISPQGYGRR-Glu(EDANS)-NH ₂
SEQ ID	8	Dabcyl-MYGGARRL-Glu(Edans)-NH ₂
SEQ ID	9	Abz-IEGRTSED-(Lys)Dnp-NH ₂
SEQ ID	10	AIC+SRSLPPIC+IPD-OH

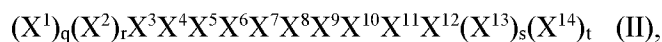
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Claims

1. A compound containing a peptide of the following formula (II),



or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein

X^1 represents a natural amino acid or an unnatural amino acid,

q represents the integer 0 or 1,

X^2 represents the natural amino acid I,

r represents the integer 0 or 1,

X^3 represents the natural amino acid C or the unnatural amino acid L-Penicillamine (Pen),

X^4 represents the natural amino acid S,

X^5 represents the natural amino acid R or the unnatural amino acid N(5)-Methyl-L-arginine ((Me)R),

X^6 represents the natural amino acid S,

X^7 represents the natural amino acid L or the unnatural amino acid L-tert-Butylalanine ((tBu)A),

X^8 represents the natural amino acid P or the unnatural amino acid L-Proline (3,4-₂H),

X^9 represents the natural amino acid P or the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic),

X^{10} represents the natural amino acid I,

X^{11} represents an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen),

X^{12} represents the natural amino acid I,

X^{13} represents the natural amino acid P,

s represents the integer 0 or 1,

X¹⁴ represents a natural amino acid selected from a list consisting of D, Q and E,

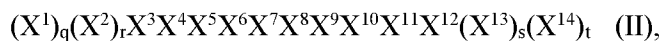
t represents the integer 0 or 1,

wherein the N-terminus of the peptide is unsubstituted, acetylated or mono- or disubstituted with C₁-C₂₀-alkyl,

wherein the C-terminus of the peptide is unsubstituted or amidated, and

wherein the peptide is cyclized via a linkage connecting X³ and X¹¹.

2. A compound containing a peptide of formula (II),



or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein

X¹ represents a natural amino acid or an unnatural amino acid,

q represents the integer 0 or 1,

X² represents the natural amino acid I,

r represents the integer 0 or 1,

X³ represents the natural amino acid C or the unnatural amino acid L-Penicillamine (Pen),

X⁴ represents the natural amino acid S,

X⁵ represents the natural amino acid R or the unnatural amino acid N(5)-Methyl-L-arginine ((Me)R),

X⁶ represents the natural amino acid S,

X⁷ represents the natural amino acid L or the unnatural amino acid L-tert-Butylalanine ((tBu)A),

X⁸ represents the natural amino acid P or the unnatural amino acid L-Proline (3,4-₂H),

X⁹ represents the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic),

X¹⁰ represents the natural amino acid I,

X¹¹ represents the natural amino acid C, or an unnatural amino acid selected from a list consisting of L-N-Methylcysteine ((N-Me)C) and L-Penicillamine (Pen),

X¹² represents the natural amino acid I,

X¹³ represents the natural amino acid P,

s represents the integer 0 or 1,

X¹⁴ represents a natural amino acid selected from a list consisting of D, Q and E,

t represents the integer 0 or 1,

wherein the N-terminus of the peptide is unsubstituted, acetylated or mono- or disubstituted with C₁-C₂₀-alkyl,

wherein the C-terminus of the peptide is unsubstituted or amidated, and

wherein the peptide is cyclized via a linkage connecting X³ and X¹¹.

3. A compound containing a peptide according to Claim 1, or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein

X⁹ represents the unnatural amino acid 2,3,3a,4,5,6,7,7a-Octahydroindole-2-carboxylic acid (Oic).

4. A compound containing a peptide according to any of Claims 1 to 3, or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein

X¹ represents a natural amino acid selected from a list consisting of A and G, or an unnatural amino acid selected from a list consisting of N-Methyl-L-Alanine (N-Me)A, N-Methyl-Glycine ((N-Me)G), L-Norleucine (Nle), L-Norvaline (Nva) and L-Ornithine (Orn).

5. A compound containing a peptide according to any of Claims 1 to 4, or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein the N-terminus and C-terminus of the peptide are unsubstituted.

6. A compound containing a peptide according to any of Claims 1 to 5 or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, wherein the peptide is cyclized via a disulfide bond connecting X³ and X¹¹.

7. A compound containing a peptide according to any of Claims 1 to 6 or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, whereas the peptide is substituted by at least one PEG group.
8. A compound containing a peptide according to any of Claims 1 to 7 or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, which acts as a MASP-1 and/or MASP-2 inhibitor and/or which inhibits C3 deposition.
9. A process for preparing a compound containing a peptide according to any of Claims 1 to 8 or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, using solid phase peptide synthesis.
10. A compound containing a peptide according to any of Claims 1 to 8 or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, for the use in the prophylaxis and/or treatment of diseases.
11. A compound containing a peptide according to any of Claims 1 to 8 or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, for the use in the prophylaxis and/or treatment of cardiovascular and cardiopulmonary disorders, shock, inflammatory disorders, cardiovascular, pulmonary, cerebral and renal sequels of sepsis, ischemia and/or reperfusion-related damage, acute kidney injury, transplant protection and delayed graft function, diseases of the blood and blood-forming organs and the immune system, sequels of diabetes mellitus, inflammatory diseases of the nervous system, diseases of the eye, diseases of the skin, diseases of the respiratory, digestive or genitourinary system and sequels of burns and injuries.
12. A pharmaceutical composition comprising at least one compound containing a peptide according to any of Claims 1 to 8 or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, in combination with one or more inert, nontoxic, pharmaceutically suitable excipients.
13. A pharmaceutical composition comprising at least one compound containing a peptide according to any of Claims 1 to 8 or a derivative, prodrug, analogue, pharmaceutically acceptable salt, solvate or solvate of the salt, in combination with one or more further active ingredients selected from the group consisting of inhibitors of phosphodiesterases, stimulators or activators of guanylate cyclase, IP receptor agonists, mineralocorticoid-receptor antagonist, diuretic, PPAR-gamma agonist, PPAR-delta agonist, corticosteroids, active ingredients which reduce damage to organs under oxidative stress, compounds which inhibit induction of cell death and apoptosis pathway, compounds which inhibit inflammatory response and T cell proliferation, antithrombotic agents, platelet aggregation

inhibitor, thrombin inhibitor, GPIIb/IIIa antagonist, factor Xa inhibitor, heparin or a low molecular weight (LMW) heparin derivative and inhibitors of coagulation factor XI.

14. A pharmaceutical composition according to Claims 12 or 13 for the prophylaxis and/or treatment of cardiovascular and cardiopulmonary disorders, shock, inflammatory disorders, cardiovascular, pulmonary, cerebral and renal sequels of sepsis, ischemia and/or reperfusion-related damage, acute kidney injury, transplant protection and delayed graft function, diseases of the blood and blood-forming organs and the immune system, sequels of diabetes mellitus, inflammatory diseases of the nervous system, diseases of the eye, diseases of the skin, diseases of the respiratory, digestive or genitourinary system and sequels of burns and injuries.
15. Method for treatment and/or prevention of cardiovascular and cardiopulmonary disorders, shock, inflammatory disorders, cardiovascular, pulmonary, cerebral and renal sequels of sepsis, ischemia and/or reperfusion-related damage, acute kidney injury, transplant protection and delayed graft function, diseases of the blood and blood-forming organs and the immune system, sequels of diabetes mellitus, inflammatory diseases of the nervous system, diseases of the eye, diseases of the skin, diseases of the respiratory, digestive or genitourinary system and sequels of burns and injuries in humans and animals by administration of an effective amount of at least one compound as defined in any of Claims 1 to 8, or of a pharmaceutical composition according to Claims 12 or 13.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/062040

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K7/08
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)
C07K A61K

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, WPI Data, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/136831 A1 (EOTVOS LORAND TUDOMANYEGYETEM [HU] ET AL.) 2 December 2010 (2010-12-02) cited in the application the whole document ----- -/--	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"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

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"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

18 June 2020

Date of mailing of the international search report

03/07/2020

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Cervigni, S

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2020/062040

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANDREA KOCSIS ET AL: "Selective Inhibition of the Lectin Pathway of Complement with Phage Display Selected Peptides against Mannose-Binding Lectin-Associated Serine Protease (MASP)-1 and -2: Significant Contribution of MASP-1 to Lectin Pathway Activation", THE JOURNAL OF IMMUNOLOGY, AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 185, 2010, pages 4169-4178, XP007915332, ISSN: 0022-1767, DOI: 10.4049/JIMMUNOL.1001819 [retrieved on 2010-09-03] cited in the application the whole document -----	1-15
X	D. HEJA ET AL: "Monospecific Inhibitors Show That Both Mannan-binding Lectin-associated Serine Protease-1 (MASP-1) and -2 Are Essential for Lectin Pathway Activation and Reveal Structural Plasticity of MASP-2", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 287, no. 24, 8 June 2012 (2012-06-08), pages 20290-20300, XP055285722, US ISSN: 0021-9258, DOI: 10.1074/jbc.M112.354332 cited in the application the whole document -----	1-15
X	TUNJUNG MAHATMANTO: "Review seed biopharmaceutical cyclic peptides: From discovery to applications : Seed Biopharmaceutical Cyclic Peptides", BIOPOLYMERS, vol. 104, no. 6, 19 September 2015 (2015-09-19), pages 804-814, XP055706394, US ISSN: 0006-3525, DOI: 10.1002/bip.22741 the whole document -----	1-15
X	WO 2013/192240 A2 (OMEROS CORP [US]; UNIV LEICESTER [GB]) 27 December 2013 (2013-12-27) the whole document -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2020/062040

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