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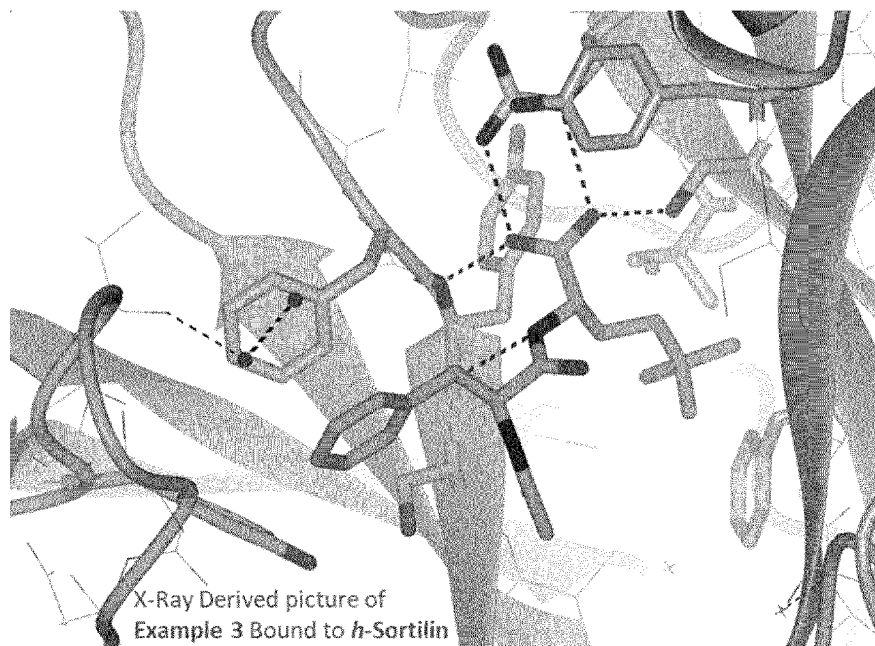
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(54) Title: MODULATORS OF SORTILIN ACTIVITY

Figure 1



(57) Abstract: The present invention relates to compounds of formula (I), which are modulators of sortilin activity. The invention also relates to pharmaceutical compositions comprising these compounds and to the use of these compounds in the treatment or prevention of medical conditions where modulation of sortilin activity is beneficial.



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MODULATORS OF SORTILIN ACTIVITY

FIELD OF THE INVENTION

The present invention relates to compounds of formula (I), which are modulators of sortilin activity. The invention also relates to pharmaceutical compositions comprising these compounds and to the use of these compounds in the treatment or prevention of medical conditions where modulation of sortilin activity is beneficial. Such medical conditions include a neurodegenerative disorder, an inflammatory disorder, a cancer, pain, diabetes mellitus, diabetic retinopathy, glaucoma, uveitis, cardiovascular disease, hereditary eye conditions or hearing loss.

BACKGROUND

Sortilin (encoded by *SORT1*) is a type 1 membrane receptor in the vacuolar protein sorting 10 protein (VPS10P) family of sorting receptors, and is abundantly expressed in the central nervous system, the inner ear, and in some peripheral tissues involved in metabolic control^{1,2,3,4}. Sortilin has an amino acid sequence according to SEQ ID NO: 1 and comprises a signal peptide, a propeptide, the Vps10p domain, a 10cc domain (10CCa + 10CCb), a transmembrane domain and a large cytoplasmic tail. The luminal domain of sortilin has 6 potential *N*-linked glycosylation sites, whilst the cytoplasmic tail enables for the recruitment of various adapter proteins.

Sortilin binds to a vast number of ligands and membrane receptors and as a result engages in functions known to be important in cellular signalling and sorting. For example, sortilin is involved in signalling by proneurotrophins: the proforms of nerve growth factor (proNGF), brain derived neurotrophic factor (proBDNF), and neurotrophin-3 (proNT3), respectively. In complex with the protein p75NTR (p75 neurotrophin receptor), sortilin has been reported to form the receptor for proneurotrophin-mediated apoptotic effects leading to degeneration and cell death in cellular and animal models^{5,6,7}.

Previous work has suggested a role for sortilin in cellular sorting and signalling associated with diseases such as diabetes and obesity (Huang *et al* 2013 Mol Biol Cell Oct;24(19):3115-22)⁸. Sortilin facilitates translocation of GLUT4 to the plasma

membrane and rescues it from degradation in the lysosomes (Pan *et al* Mol Biol Cell. 2017 Jun 15;28(12):1667-1675)⁹. Sortilin levels have been shown to be modulated by the level of inflammation associated with these diseases. The pro-inflammatory cytokine, TNF α , reduces both mRNA levels and protein levels of
5 sortilin in cultured mouse and human adipocytes, as well as *in vivo* when injected into mice (Kaddai *et al*. Diabetologia 52: 932-40, 2009)¹⁰. Sortilin can also influence cytokine secretion: targeting sortilin in immune cells has been proposed to attenuate inflammation and reduce atherosclerosis disease progression (Mortensen *et al*. J Clin Invest 124(12):5317-22, 2014)¹¹. Additionally, US
10 2016/0331746 describes various scaffolds of small molecules capable of binding to the active site of sortilin. Sortilin is involved in the regulation of glucose uptake (Shi & Kandror. Developmental Cell 9:99-108, 2005)¹² and the development of lipid disorder diseases (Gao *et al*. DNA and Cell Biology 36(12):1050-61, 2017)¹³.

Further, plasma sortilin levels have been reported to be a potential biomarker for
15 identifying patients with either coronary heart disease or diabetes mellitus (Oh *et al*. Cardiovascular Diabetology 16:92, 2017)¹⁴. Patients that showed increased sortilin levels within their plasma, and therefore identifiable as suffering from the above conditions, also displayed enhanced glucose levels suggesting sortilin as a therapeutic target for treating these conditions.

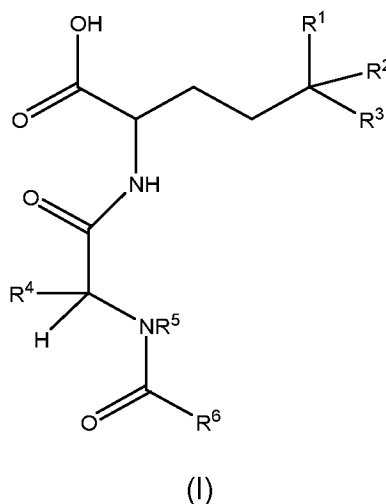
20 In view of the above, there is an unmet need for new compounds that may be used in the treatment and prevention of medical conditions in which modulation of sortilin is beneficial, such as a neurodegenerative disorder, an inflammatory disorder, a cancer, pain, diabetes mellitus, diabetic retinopathy, glaucoma, uveitis, cardiovascular disease, hereditary eye conditions or hearing loss. The
25 neurodegenerative disorder may be selected from frontotemporal dementia, Alzheimer's disease, Parkinson's disease and spinal cord injury; the inflammatory disorder may be selected from inflammatory diseases and neuroinflammation; the cancer may be selected from breast cancer, lung cancer, ovarian cancer, prostate cancer, thyroid cancer, pancreatic cancer, glioblastoma and colorectal cancer;
30 and the hearing loss may be selected from noise-induced hearing loss, ototoxicity induced hearing loss, age-induced hearing loss, idiopathic hearing loss, tinnitus and sudden hearing loss.

DESCRIPTION OF THE FIGURES

Figure 1 is an X-Ray Derived picture of Example 3 bound to *h*-Sortilin.

DISCLOSURE OF THE INVENTION

In a first aspect, the present invention provides a compound of formula (I)



or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, optical isomer, N-oxide, and/or prodrug thereof; wherein

R¹, R² and R³ are each independently selected from the group consisting of halo,
10 H, (C₁-C₄)alkyl, halo-(C₁-C₄)alkyl, (C₂-C₄)alkenyl, and halo-(C₂-C₄)alkenyl;

R⁴ is selected from the group consisting of H, (C₁-C₁₀)alkyl, halo-(C₁-C₁₀)alkyl, (C₂-C₁₀)alkenyl, halo-(C₂-C₁₀)alkenyl, (C₃-C₈)aryl, halo-(C₃-C₈)aryl, (C₃-C₈)heteroaryl, halo-(C₃-C₈)heteroaryl, (C₁-C₆)-alkylene-(C₃-C₂₀)-aryl, (C₁-C₆)-alkylene-(C₃-C₂₀)-heteroaryl, (C₁-C₆)-alkylene-(3- to 10- membered-heterocyclic ring);

15 wherein the aryl group in (C₁-C₆)-alkylene-(C₃-C₂₀)-aryl, the heteroaryl group in (C₁-C₆)-alkylene-(C₃-C₂₀)-heteroaryl or the heterocyclic ring in (C₁-C₆)-alkylene-(3- to 8- membered heterocyclic ring) is optionally substituted with one or more substituents independently selected from halo, H, -OH, (C₁-C₄)alkyl, halo-(C₁-C₄)alkyl, (C₁-C₄)alkoxy and halo-(C₁-C₄)alkoxy;

20 R⁵ is selected from the group consisting of H, (C₁-C₄)alkyl, halo-(C₁-C₄)alkyl, (C₂-C₄)alkenyl and halo-(C₁-C₄)alkenyl; and

R⁶ is selected from the group consisting of (C₁-C₆)alkyl, halo-(C₁-C₆)alkyl, (C₂-C₆)alkenyl, halo-(C₂-C₆)alkenyl, (C₃-C₈)aryl, halo-(C₃-C₈)aryl, (C₃-C₈)heteroaryl and halo-(C₃-C₈)heteroaryl.

It has been surprisingly found that compounds of formula (I) inhibit or antagonise
5 sortilin and therefore may be useful in conditions where sortilin inhibition is
beneficial. Such conditions include a neurodegenerative disorder, an
inflammatory disorder, a cancer, pain, diabetes mellitus, diabetic retinopathy,
glaucoma, uveitis, cardiovascular disease, hereditary eye conditions or hearing
loss. The neurodegenerative disorder may be selected from frontotemporal
10 dementia, Alzheimer's disease, Parkinson's disease and spinal cord injury; the
inflammatory disorder may be selected from inflammatory diseases and
neuroinflammation; the cancer may be selected from breast cancer, lung cancer,
ovarian cancer, prostate cancer, thyroid cancer, pancreatic cancer, glioblastoma
and colorectal cancer; and the hearing loss may be selected from noise-induced
15 hearing loss, ototoxicity induced hearing loss, age-induced hearing loss, idiopathic
hearing loss, tinnitus and sudden hearing loss..

As used herein, the term "sortilin" may refer to full length sortilin (also referred to
as immature sortilin), comprising a signal peptide, a propeptide, a Vps10p domain,
a 10CC domain, a transmembrane domain and a large cytoplasmic tail, having an
20 amino acid sequence according to SEQ ID NO: 1 or SEQ ID NO: 2, or it may refer
to mature sortilin, comprising a Vps10p domain, a 10CC domain, a
transmembrane domain and a large cytoplasmic tail, having an amino acid
sequence according to SEQ ID NO: 3, or a naturally occurring fragment,
homologue or variant thereof. The term "sortilin" or "sortilin molecule" are used
25 interchangeably herein. It is understood that the sortilin is capable of interacting
with a pro-neurotrophin molecule to form a sortilin/pro-neurotrophin complex. This
sortilin/pro-neurotrophin complex may or may not be capable of interacting with a
p75NTR molecule to form a trimeric complex comprising sortilin, pro-neurotrophin
and p75NTR. It is understood that this trimeric complex may be responsible for
30 adverse biological responses, such as stimulating apoptosis in retinal and
ganglion cells, and controlling growth cone retraction of projecting axons.

As used herein, the term “pro-neurotrophin” refers to the larger precursors of neurotrophins, which undergo proteolytic cleavage to yield the mature form of the neurotrophin. Neurotrophins are a family of proteins that induce the survival, development and function of neurons, and are commonly referred to as growth factors. Pro-neurotrophins are biologically active and have distinct roles compared to their neurotrophin counterparts, such as induction of apoptosis. Examples of pro-neurotrophins include proNGF, proBDNF, proNT3 and proNT4. Pro-neurotrophins may also control synaptic plasticity. Whereas mature neurotrophins induce synaptic strength, in their proforms they may weaken synapses,

The compounds of the invention may be sortilin inhibitors or antagonists. As used herein, the term “sortilin antagonist” refers to a substance that interferes with, blocks, or otherwise attenuates the effect of, a sortilin protein binding to a pro-neurotrophin (e.g., proNGF, proNT3, proBDNF) and preventing the formation of the trimeric complex between sortilin, p75NTR and the pro-neurotrophin. The term “sortilin antagonist” also includes a substance or agent that interferes with the formation of a high affinity trimeric complex. In the latter scenario, it is recognised that a trimeric complex may be formed in that sortilin can bind to p75NTR (but not proNGF) and p75NTR can simultaneously bind the NGF domain of proNGF. However, the resulting trimeric complex may be of lower affinity for its receptor and as a result have significantly reduced capacity to stimulate apoptosis via the mechanism described above. Skeldal et al (J. Biol. Chem. 2012 Dec 21;287(52):43798-809)¹⁵ demonstrated that the apoptotic function of the trimeric complex is abolished when sortilin is devoid in its intracellular domain. The term “sortilin antagonist” also includes a substance or agent that interferes with, blocks, or otherwise attenuates the effect of, a sortilin protein interacting with p75NTR. This interaction may be completely prevented, in which case the trimeric complex is prevented from forming, or only partially prevented, in which case the trimeric complex may be formed but may have reduced biological potency. Skeldal et al showed that complex formation between sortilin and p75NTR relies on contact points in the extracellular domains of the receptors and that the interaction critically depends on an extracellular juxtamembrane 23-amino acid sequence of p75NTR. Thus, the sortilin antagonist may interfere with this 23-amino acid sequence or proximal sequences in the molecules.

In the first aspect of the invention, it is preferred that R^1 , R^2 and R^3 are each independently selected from the group consisting of halo, (C₁-C₂)alkyl and halo-(C₁-C₂)alkyl.

In a preferred aspect of the invention, R^1 , R^2 and R^3 are each independently selected from F, CH₃ and CF₃. Most preferably, R^1 , R^2 and R^3 are the same. For example, in an exemplary compound of the invention, R^1 , R^2 and R^3 may each be F, CH₃ or CF₃.

In another preferred aspect of the invention, R^4 is selected from the group consisting of H, (C₁-C₆)alkyl, halo-(C₁-C₆)alkyl, (C₃-C₁₀)aryl, (C₁-C₃)-alkylene-(C₃-C₁₀)-aryl, (C₁-C₃)-alkylene-(C₃-C₂₀)-heteroaryl and (C₁-C₃)-alkylene-(3- to 10-membered-heterocyclic ring). The aryl group in (C₁-C₃)-alkylene-(C₃-C₁₀)-aryl, the heteroaryl group in (C₁-C₃)-alkylene-(C₃-C₁₀)-heteroaryl or the heterocyclic ring in (C₁-C₃)-alkylene-(3- to 8- membered heterocyclic ring) is optionally substituted with one or more substituents independently selected from halo, -OH, (C₁-C₄)alkyl, halo-(C₁-C₄)alkyl, (C₁-C₄)alkoxy and halo-(C₁-C₄)alkoxy.

The alkyl, haloalkyl, alkenyl, haloalkenyl groups and the alkyl, haloalkyl, alkoxy and haloalkoxy substituents may be linear or branched.

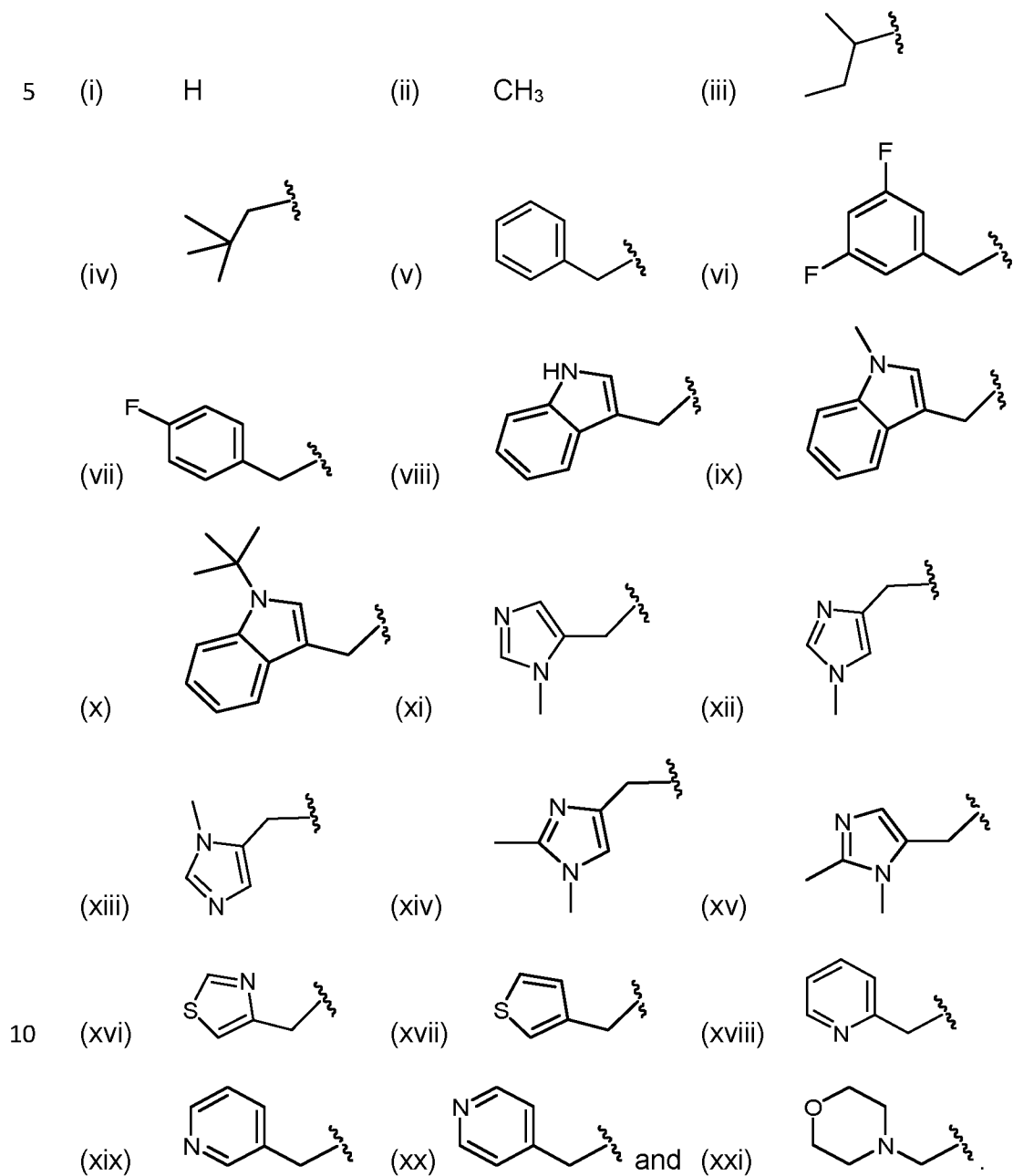
The substituent may be attached at any position of the aryl, heteroaryl or heterocyclic ring. The one or more substituents may be attached to a carbon atom, heteroatom or combinations thereof. Preferably, there are no substituents or between one to three substituents.

The heteroaryl or heterocyclic ring may comprise one, two or more heteroatoms. Preferably, the heteroaryl or heterocyclic ring comprises one or two heteroatoms. The heteroatom may be selected from N, S or O. In groups with more than one heteroatom present, the heteroatoms may be the same or they may be different.

The heterocyclic ring is may be aliphatic. It may be monocyclic, bicyclic or tricyclic. Preferably, the heterocyclic ring is monocyclic or bicyclic. Preferably, the heterocyclic ring has between 5-7 members.

The aryl and heteroaryl groups may also be monocyclic, bicyclic or tricyclic. Preferably, monocyclic or bicyclic. Preferably, the aryl and heteroaryl groups have a ring size of between 5-9 members.

In some preferred embodiments, R^4 is selected from the group consisting of:



The chirality of the carbon of formula (I) attached to R⁴ is either (R) or (S). Preferably, when groups (i)-(xii) and (ix)-(xx) above are included in formula (I), the chirality of this carbon is (S), and group (xiii) may be (S) or (R).

In another preferred aspect of the invention, R⁵ is selected from the group consisting of H, (C₁-C₃)-alkyl and (C₁-C₃)haloalkyl.

Preferably, R⁵ is selected from the group consisting of H and CH₃.

In another preferred aspect of the invention, R⁶ is selected from the group consisting of (C₁-C₄)alkyl, halo-(C₁-C₄)alkyl, (C₃-C₈)heteroaryl, and halo-(C₃-C₈)heteroaryl.

Most preferably, R⁶ is selected from the group consisting of CH₃, pyrazine and morpholine.

Particular compounds of the invention are those listed below.

(S)-2-(2-acetamidoacetamido)-5,5-dimethylhexanoic acid;

(S)-5,5-dimethyl-2-(2-(pyrazine-2-carboxamido)acetamido)hexanoic acid;

(S)-2-((S)-2-acetamido-3-phenylpropanamido)-5,5-dimethylhexanoic acid;

(2S)-5,5-dimethyl-2-{2-[(pyrazin-2-yl)formamido]acetamido}hexanoic acid;

(2S)-2-[(2S,3S)-2-acetamido-3-methylpentanamido]-5,5-dimethylhexanoic acid;

(2S)-5,5-dimethyl-2-[(2S,3S)-3-methyl-2-[(pyrazin-2-yl)formamido]pentanamido]hexanoic acid;

(2S)-5,5,5-trifluoro-2-[(2S,3S)-3-methyl-2-[(pyrazin-2-yl)formamido]pentanamido]pentanoic acid;

(2S)-2-[(2S)-2-acetamido-3-phenylpropanamido]-5,5-dimethylhexanoic acid;

(2S)-2-[(2S)-2-acetamido-3-(4-fluorophenyl)propanamido]-5,5-dimethylhexanoic acid;

(2S)-2-[(2S)-3-(3,5-difluorophenyl)-2-acetamidopropanamido]-5,5-dimethylhexanoic acid;

(2S)-2-[(2S)-2-acetamido-3-(1H-indol-3-yl)propanamido]-5,5-dimethylhexanoic acid;

(2S)-2-[(2S)-2-acetamido-3-(1-methyl-1H-imidazol-5-yl)propanamido]-5,5-dimethylhexanoic acid;

- 5 (S)-5,5-dimethyl-2-((S)-3-phenyl-2-(pyrazine-2-carboxamido)propanamido)hexanoic acid;

(2S)-2-[(2S)-2-acetamido-3-(1-methyl-1H-imidazol-4-yl)propanamido]-5,5-dimethylhexanoic acid;

- 10 (2S)-5,5-dimethyl-2-[(2S,3S)-3-methyl-2-[(3S)-morpholin-3-yl]formamido]pentanamido]hexanoic acid;

(2S)-5,5-dimethyl-2-[(2S,3S)-3-methyl-2-[(3R)-morpholin-3-yl]formamido]pentanamido]hexanoic acid;

(2S)-5,5-dimethyl-2-[(2S,3S)-3-methyl-2-[(2S)-morpholin-2-yl]formamido]pentanamido]hexanoic acid;

- 15 (2S)-5,5-dimethyl-2-[(2S,3S)-3-methyl-2-[(2R)-morpholin-2-yl]formamido]pentanamido]hexanoic acid;

(2S)-5,5-dimethyl-2-[(2S)-3-(1-methyl-1H-imidazol-4-yl)-2-(N-methylacetamido)propanamido]hexanoic acid;

- 20 (2S)-5,5-dimethyl-2-[(2S)-3-(1-methyl-1H-imidazol-5-yl)-2-(N-methylacetamido)propanamido]hexanoic acid;

(2S)-5,5-dimethyl-2-[(2S)-3-(1-methyl-1H-imidazol-4-yl)-2-(2-oxopyrrolidin-1-yl)propanamido]hexanoic acid;

(2S) - 2 - [(2S) - 3 - (1,2 - dimethyl - 1H - imidazol - 5 - yl) - 2 - (N - methylacetamido)propanamido] - 5,5 - dimethylhexanoic acid;

- 25 (2S) - 2 - [(2S) - 3 - (1,2 - dimethyl - 1H - imidazol - 4 - yl) - 2 - (N - methylacetamido)propanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2R) - 2 - acetamido - 3 - (1H - indol - 3 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (1,3 - thiazol - 4 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

5 (2S) - 2 - [(2S) - 2 - acetamidopropanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (thiophen - 3 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (pyridin - 2 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

10 (2S) - 2 - [(2S) - 2 - acetamido - 3 - (pyridin - 3 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (pyridin - 4 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (morpholin - 4 - yl)propanamido] - 5,5 -

15 dimethylhexanoic acid; and

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (1 - methyl - 1H - indol - 3 - yl)propanamido] - 5,5 - dimethylhexanoic acid.

The compounds of formula (I) are intended for use in the treatment or prevention of a neurodegenerative disorder, an inflammatory disorder, a cancer, pain, 20 diabetes mellitus, diabetic retinopathy, glaucoma, uveitis, cardiovascular disease, hereditary eye conditions or hearing loss.

Preferably the neurodegenerative disorder is selected from frontotemporal dementia, Alzheimer's disease, Parkinson's disease and spinal cord injury.

Preferably the inflammatory disorder may be selected from inflammatory diseases and neuroinflammation;

Preferably the cancer is selected from breast cancer, lung cancer, ovarian cancer, prostate cancer, thyroid cancer, pancreatic cancer, glioblastoma and colorectal
5 cancer.

Preferably the hearing loss is selected from noise-induced hearing loss, ototoxicity induced hearing loss, age-induced hearing loss, idiopathic hearing loss, tinnitus and sudden hearing loss.

Thus, in an embodiment, the compounds for use according to the invention may
10 disrupt interaction between a sortilin molecule and a pro-neurotrophin molecule, or disrupt the interaction between a sortilin molecule and a p75NTR molecule. Said sortilin molecule may be mature sortilin.

Preferably, the compounds of the present invention are sortilin inhibitors. As used herein, the term "sortilin inhibitor" refers to a compound that binds to a sortilin
15 protein, thereby preventing it from binding to a pro-neurotrophin or a p75NTR molecule and preventing the formation of the aforementioned trimeric complex, or resulting in the formation of a trimeric complex that is less active or inactive.

Preferably, the compounds of the present invention prevent the protein-protein interaction between a sortilin molecule and a pro-neurotrophin or a p75NTR
20 molecule, further preventing the formation of the apoptotic trimeric complex usually formed between sortilin, pro-neurotrophin and the p75NTR receptor, or resulting in the formation of a low affinity trimeric complex, which is biologically less active or inactive or has minimal activity.

Thus, the compound may bind to sortilin, a pro-neurotrophin or a p75NTR molecule.
25 The antagonistic action may be due to direct blocking of protein-protein interaction or it could be by steric hindrance when bound at a site of one of these proteins apart from the binding site.

According to a second aspect of the invention, there is provided a pharmaceutical composition comprising a compound according to the first aspect of the invention
30 and one or more pharmaceutically acceptable carriers, excipients, and/or diluents.

In a third aspect of the invention, there is provided a compound according to the first aspect of the invention, or a pharmaceutical composition according to the second aspect of the invention for use in therapy.

According to a fourth aspect of the invention, there is provided a compound
5 according to the first aspect of the invention, or a pharmaceutical composition according to the second aspect of the invention for use in the treatment or prevention of a neurodegenerative disorder, an inflammatory disorder, a cancer, pain, diabetes mellitus, diabetic retinopathy, glaucoma, uveitis, cardiovascular disease, hereditary eye conditions or hearing loss.

10 Preferably, the neurodegenerative disorder is selected from frontotemporal dementia, Alzheimer's disease, Parkinson's disease and spinal cord injury.

Preferably, the hearing loss is selected from noise-induced hearing loss, ototoxicity induced hearing loss, age-induced hearing loss, idiopathic hearing loss, tinnitus and sudden hearing loss.

15 Preferably, the cancer is selected from breast cancer, lung cancer, ovarian cancer, prostate cancer, thyroid cancer, pancreatic cancer, glioblastoma, and colorectal cancer.

Preferably the cardiovascular disease is selected from atherosclerosis, cardiomyopathy, heart attack, arrhythmias, and coronary artery disease.

20 According to a fifth aspect of the invention, there is provided the use of the compound according to the first aspect of the invention for the manufacture of a medicament for the treatment or prevention of a neurodegenerative disorder, an inflammatory disorder, a cancer, pain, diabetes mellitus, diabetic retinopathy, glaucoma, uveitis, cardiovascular disease, hereditary eye conditions or hearing
25 loss.

According to a sixth aspect of the invention, there is provided a method for the treatment or prevention of a disease or condition responsive to sortilin modulation comprising administering a therapeutically effective amount of the compound according to the first aspect of the invention or the pharmaceutical composition
30 according to the second aspect of the invention.

The compounds of the invention may include isotopically-labelled and/or isotopically-enriched forms of the compounds. The compounds of the invention herein may contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. Examples of isotopes that can be incorporated into the disclosed compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, chlorine, such as ^2H , ^3H , ^{11}C , ^{13}C , ^{14}C , ^{13}N , ^{15}O , ^{17}O , ^{32}P , ^{35}S , ^{18}F , ^{36}Cl .

The compounds of the invention may be used as such or, where appropriate, as pharmacologically acceptable salts (acid or base addition salts) thereof. The pharmacologically acceptable addition salts mentioned below are meant to comprise the therapeutically active non-toxic acid and base addition salt forms that the compounds are able to form. Compounds that have basic properties can be converted to their pharmaceutically acceptable acid addition salts by treating the base form with an appropriate acid. Exemplary acids include inorganic acids, such as hydrogen chloride, hydrogen bromide, hydrogen iodide, sulphuric acid, phosphoric acid; and organic acids such as formic acid, acetic acid, propanoic acid, hydroxyacetic acid, lactic acid, pyruvic acid, glycolic acid, maleic acid, malonic acid, oxalic acid, benzenesulphonic acid, toluenesulphonic acid, methanesulphonic acid, trifluoroacetic acid, fumaric acid, succinic acid, malic acid, tartaric acid, citric acid, salicylic acid, p-aminosalicylic acid, pantoic acid, benzoic acid, ascorbic acid and the like. Compounds that have acidic properties can be converted to their pharmaceutically acceptable basic addition salts by treating the acid form with an appropriate base. Exemplary base addition salt forms are the sodium, potassium, calcium salts, and salts with pharmaceutically acceptable amines such as, for example, ammonia, alkylamines, benzathine, and amino acids, such as, e.g. arginine and lysine. The term addition salt as used herein also comprises solvates which the compounds and salts thereof are able to form, such as, for example, hydrates, alcoholates and the like.

Throughout the present disclosure, a given chemical formula or name shall also encompass all pharmaceutically acceptable salts, solvates, hydrates, N-oxides, and/or prodrug forms thereof. It is to be understood that the compounds of the invention include any and all hydrates and/or solvates of the compound formulas.

It is appreciated that certain functional groups, such as the hydroxy, amino, and like groups form complexes and/or coordination compounds with water and/or various solvents, in the various physical forms of the compounds. Accordingly, the above formulas are to be understood to include and represent those various hydrates and/or solvates.

Compounds of the invention also include tautomeric forms. Tautomeric forms result from the swapping of a single bond with an adjacent double bond together with the concomitant migration of a proton. Tautomeric forms include prototropic tautomers which are isomeric protonation states having the same empirical formula and total charge. Example prototropic tautomers include ketone - enol pairs, amide - imidic acid pairs, lactam - lactim pairs, amide - imidic acid pairs, enamine - imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, for example, 1H- and 3H-imidazole, 1H, 2H- and 4H- 1,2,4-triazole, 1H- and 2H- isoindole, and 1H- and 2H-pyrazole. Tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution.

The compounds described herein can be asymmetric (e.g. having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless otherwise indicated. Compounds of the present invention that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. Cis- and trans-geometric isomers of the compounds of the present invention are described and may be isolated as a mixture of isomers or as separated isomeric forms.

In the case of the compounds which contain an asymmetric carbon atom, the invention relates to the D form, the L form, and D,L mixtures and also, where more than one asymmetric carbon atom is present, to the diastereomeric forms. Those compounds of the invention which contain asymmetric carbon atoms, and which

as a rule accrue as racemates, can be separated into the optically active isomers in a known manner, for example using an optically active acid. However, it is also possible to use an optically active starting substance from the outset, with a corresponding optically active or diastereomeric compound then being obtained
5 as the end product.

The term "prodrugs" refers to compounds that may be converted under physiological conditions or by solvolysis to a biologically active compound of the invention. A prodrug may be inactive when administered to a subject in need thereof, but is converted in vivo to an active compound of the invention. Prodrugs
10 are typically rapidly transformed in vivo to yield the parent compound of the invention, e.g. by hydrolysis in the blood. The prodrug compound usually offers advantages of solubility, tissue compatibility or delayed release in a mammalian organism (see Silverman, R. B., The Organic Chemistry of Drug Design and Drug Action, 2nd Ed., Elsevier Academic Press (2004), page 498 to 549). Prodrugs of
15 a compound of the invention may be prepared by modifying functional groups, such as a hydroxy, amino or mercapto groups, present in a compound of the invention in such a way that the modifications are cleaved, either in routine manipulation or in vivo, to the parent compound of the invention. Examples of prodrugs include, but are not limited to, acetate, formate and succinate derivatives
20 of hydroxy functional groups or phenyl carbamate derivatives of amino functional groups.

The term "treatment" as used herein may include prophylaxis of the named disorder or condition, or amelioration or elimination of the disorder once it has been established. The term "prevention" refers to prophylaxis of the named
25 disorder or condition.

Methods delineated herein include those wherein the subject is identified as in need of a particular stated treatment. Identifying a subject in need of such treatment can be in the judgment of a subject or a health care professional and can be subjective (e.g. opinion) or objective (e.g. measurable by a test or
30 diagnostic method).

In other aspects, the methods herein include those further comprising monitoring subject response to the treatment administrations. Such monitoring may include periodic imaging or sampling of subject tissue, fluids, specimens, cells, proteins, chemical markers, genetic materials, Etc. as markers or indicators of the treatment regimen. In other methods, the subject is pre-screened or identified as in need of such treatment by assessment for a relevant marker or indicator of suitability for such treatment.

The invention provides a method of monitoring treatment progress. The method includes the step of determining a level of diagnostic marker (Marker) (e.g. any target or cell type delineated herein modulated by a compound herein) or diagnostic measurement (e.g., screen, assay) in a subject suffering from or susceptible to a disorder or symptoms thereof delineated herein, in which the subject has been administered a therapeutic amount of a compound herein sufficient to treat the disease or symptoms thereof. The level of Marker determined in the method can be compared to known levels of Marker in either healthy normal controls or in other afflicted patients to establish the subject's disease status. In preferred embodiments, a second level of Marker in the subject is determined at a time point later than the determination of the first level, and the two levels are compared to monitor the course of disease or the efficacy of the therapy. In certain preferred embodiments, a pre-treatment level of Marker in the subject is determined prior to beginning treatment according to this invention; this pretreatment level of Marker can then be compared to the level of Marker in the subject after the treatment commences, to determine the efficacy of the treatment.

A level of Marker or Marker activity in a subject may be determined at least once. Comparison of Marker levels, e.g., to another measurement of Marker level obtained previously or subsequently from the same patient, another patient, or a normal subject, may be useful in determining whether therapy according to the invention is having the desired effect, and thereby permitting adjustment of dosage levels as appropriate. Determination of Marker levels may be performed using any suitable sampling/expression assay method known in the art or described herein. Preferably, a tissue or fluid sample is first removed from a subject. Examples of suitable samples include blood, urine, tissue, mouth or cheek cells, and hair

samples containing roots. Other suitable samples would be known to the person skilled in the art. Determination of protein levels and/or mRNA levels (e.g., Marker levels) in the sample can be performed using any suitable technique known in the art, including, but not limited to, enzyme immunoassay, ELISA, radiolabeling/assay techniques, blotting/chemiluminescence methods, real-time PCR, and the like.

For clinical use, the compounds disclosed herein are formulated into pharmaceutical compositions (or formulations) for various modes of administration. It will be appreciated that compounds of the invention may be administered together with a physiologically acceptable carrier, excipient, and/or diluent (i.e. one, two, or all three of these). The pharmaceutical compositions disclosed herein may be administered by any suitable route, preferably by oral, rectal, nasal, topical (including ophthalmic, buccal and sublingual), sublingual, transdermal, intrathecal, transmucosal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. Other formulations may conveniently be presented in unit dosage form, e.g., tablets and sustained release capsules, and in liposomes, and may be prepared by any methods well known in the art of pharmacy. Pharmaceutical formulations are usually prepared by mixing the active substance, or a pharmaceutically acceptable salt thereof, with conventional pharmaceutically acceptable carriers, diluents or excipients. Examples of excipients are water, gelatin, gum arabicum, lactose, microcrystalline cellulose, starch, sodium starch glycolate, calcium hydrogen phosphate, magnesium stearate, talcum, colloidal silicon dioxide, and the like. Such formulations may also contain other pharmacologically active agents, and conventional additives, such as stabilizers, wetting agents, emulsifiers, flavouring agents, buffers, and the like. Usually, the amount of active compounds is between 0.1-95% by weight of the preparation, preferably between 0.2-20% by weight in preparations for parenteral use and more preferably between 1-50% by weight in preparations for oral administration. The formulations can be further prepared by known methods such as granulation, compression, microencapsulation, spray coating, Etc. The formulations may be prepared by conventional methods in the dosage form of tablets, capsules, granules, powders, syrups, suspensions, suppositories or injections. Liquid formulations may be prepared by dissolving or

suspending the active substance in water or other suitable vehicles. Tablets and granules may be coated in a conventional manner. To maintain therapeutically effective plasma concentrations for extended periods of time, compounds disclosed herein may be incorporated into slow release formulations.

- 5 The dose level and frequency of dosage of the specific compound will vary depending on a variety of factors including the potency of the specific compound employed, the metabolic stability and length of action of that compound, the patient's age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the condition to be treated, and the patient undergoing therapy. The daily dosage may, for example, range from about 0.001 mg to about 100 mg per kilo of body weight, administered singly or multiply in doses, e.g. from about 0.01 mg to about 25 mg each. Normally, such a dosage is given orally but parenteral administration may also be chosen.

15 DEFINITIONS

"Optional" or "optionally" means that the subsequently described event or circumstance may, but need not, occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not.

- 20 The term "heteroatom" means O, N, or S.

The term "(C₁-C_n)alkyl" denotes a straight, branched or cyclic or partially cyclic alkyl group having from 1 to n carbon atoms, i.e. 1, 2, 3... or n carbon atoms. For the "(C₁-C_n)alkyl" group to comprise a cyclic portion it should be formed of at least three carbon atoms. For parts of the range "(C₁-C_n)alkyl" all subgroups thereof are contemplated. For example, in the range (C₁-C₆)alkyl, all subgroups such as (C₁-C₅)alkyl, (C₁-C₄)alkyl, (C₁-C₃)alkyl, (C₁-C₂)alkyl, (C₁)alkyl, (C₂-C₆)alkyl, (C₂-C₅)alkyl, (C₂-C₄)alkyl, (C₂-C₃)alkyl, (C₂)alkyl, (C₃-C₆)alkyl, (C₃-C₅)alkyl, (C₃-C₄)alkyl, (C₃)alkyl, (C₄-C₆)alkyl, (C₄-C₅)alkyl, (C₄)alkyl, (C₅-C₆)alkyl, (C₆)alkyl. Examples of "C₁-C₆ alkyl" include methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, cyclobutyl, cyclopropylmethyl, branched or cyclic or partially cyclic pentyl and hexyl Etc.

The term “halo-(C₁-C_n)alkyl” denotes a C₁-C_n alkyl as described above substituted with at least one halogen atom, which is preferably, F, Cl, Br and I, more preferably F and Cl, and most preferably F.

When a term denotes a range, for instance “1 to 6 carbon atoms” in the definition
5 of (C₁-C₆)alkyl, each integer is considered to be disclosed, i.e. 1, 2, 3, 4, 5 and 6.

The term “(C₂-C_n)alkenyl” denotes a straight, branched or cyclic or partially cyclic alkyl group having at least one carbon-carbon double bond, and having from 2 to 6 carbon atoms. The alkenyl group may comprise a ring formed of 3 to 6 carbon atoms. For parts of the range “(C₂-C_n)alkenyl” all subgroups thereof are
10 contemplated. For example, the range “(C₂-C₄)alkenyl” covers (C₂-C₄)alkenyl, (C₂-C₃)alkenyl, (C₂)alkenyl. Examples of “(C₂-C₄)alkenyl” include 2-propenyl, 2-butenyl, 3-butenyl, 2-methyl-2-propenyl Etc.

The term “(C₁-C₄)alkoxy” denotes -O-((C₁-C₄)alkyl) in which a (C₁-C₄)alkyl group is as defined above and is attached to the remainder of the compound through an
15 oxygen atom. Examples of “(C₁-C₄)alkoxy” include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy and t-butoxy.

The term “halo(C₁-C₄)alkoxy” denotes a (C₁-C₄)alkoxy as described above substituted with a halogen atom, which is preferably, F, Cl, Br and I, more preferably F and Cl, and most preferably F.

20 The term “halo” means a halogen atom, and is preferably, F, Cl, Br and I, more preferably F and Cl, and most preferably F.

The term “3- to 10-membered heterocyclic ring” denotes a non-aromatic ring system having 3 to 10 ring atoms, in which at least one ring atoms is a heteroatom.

“An effective amount” refers to an amount of a compound of the invention that
25 confers a therapeutic effect on the treated subject. The therapeutic effect may be objective (i.e. measurable by some test or marker) or subjective (i.e. subject gives an indication of or feels an effect).

As used herein, the terms “administration” or “administering” mean a route of administration for a compound disclosed herein. Exemplary routes of
30 administration include, but are not limited to, oral, intraocular, intravenous,

intraperitoneal, intraarterial, and intramuscular. The preferred route of administration can vary depending on various factors, e.g. the components of the pharmaceutical composition comprising a compound disclosed herein, site of the potential or actual disease and severity of disease.

- 5 The terms "subject" and "patient" are used herein interchangeably. They refer to a human or another mammal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate) that can be afflicted with or is susceptible to a disease or disorder but may or may not have the disease or disorder. It is preferred that the subject is human.
- 10 Compounds of the invention may be disclosed by the name or chemical structure. If a discrepancy exists between the name of a compound and its associated chemical structure, then the chemical structure prevails.

The invention will now be further illustrated by the following non-limiting examples. The specific examples below are to be construed as merely illustrative, and not
15 limitative of the remainder of the disclosure in any way whatsoever. Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilise the present invention to its fullest extent. All references and publications cited herein are hereby incorporated by reference in their entirety.

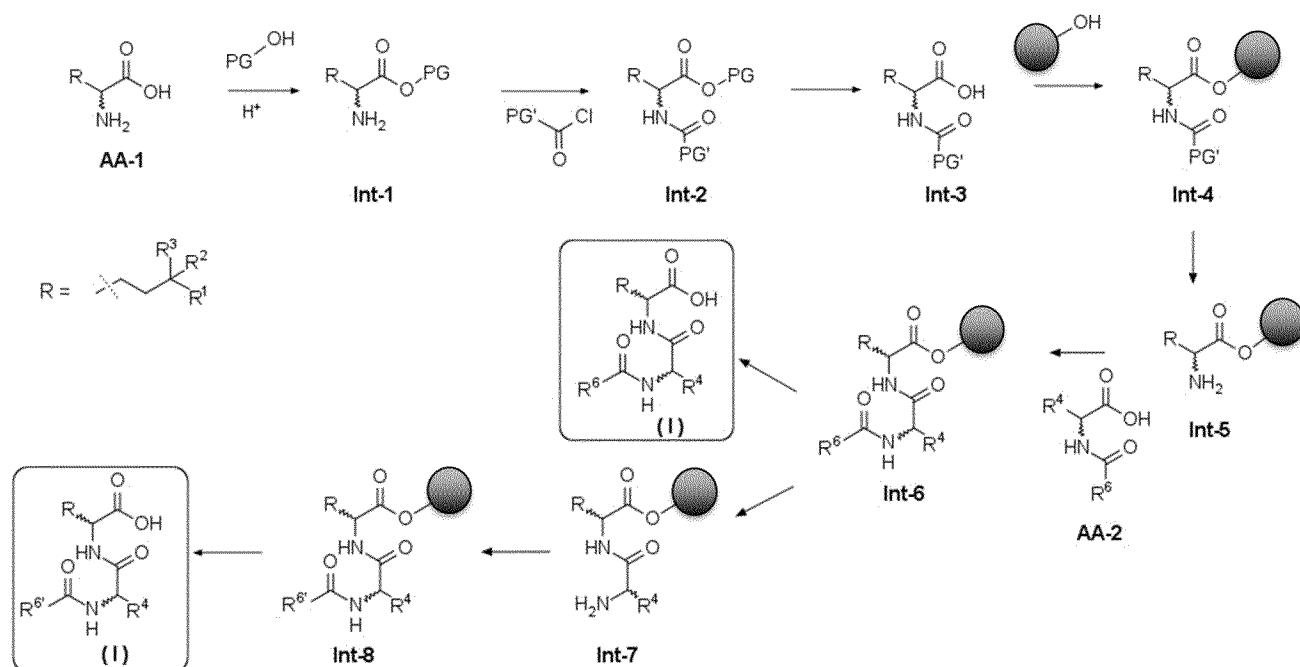
Preparation of Compounds of the Invention

- 20 The compounds of the invention can be prepared according to the following General Synthetic Schemes by methods well known and appreciated in the art. Suitable reaction conditions, for the steps of these schemes, are well known in the art and appropriate substitutions of solvents and co-reagents are within the common general knowledge of the person skilled in the art. Likewise, it will be
25 appreciated by those skilled in the art that synthetic intermediates may be isolated and/or purified by various well-known techniques as needed or desired, and that frequently, it will be possible to use various intermediates directly in subsequent synthetic steps with little or no purification. Furthermore, the skilled person will appreciate that in some circumstance, the orders in which moieties are introduced
30 is not critical. The particular order of steps required to produce the compounds of formula (I) is dependent upon the particular compound being synthesized, the

starting compound, and the relative liability of the substituted moieties as is well appreciated by those of ordinary skill in the art. All substituents, unless otherwise indicated, are as previously defined, and all reagents are well known and appreciated in the art.

- 5 The compounds of general formula (I) may be prepared by a variety of procedures, some of which are described below. One of the methods particularly suitable for the preparation of small to medium-sized peptides, as is well known and appreciated by those of ordinary skill in the art, is solid phase peptide synthesis (SPPS).
- 10 Suitable starting materials and protected amino acids of general formula AA-1, AA-2, Int-1, Int-2 or Int-3 are either commercially available or may be prepared by a variety of methods. For example, as illustrated in Scheme 1, the carboxylic acid functionality of appropriately substituted amino acids of general formula AA-1, can be chemoselectively protected as a suitable derivative, for example as a methyl
- 15 ester, using well established procedures and reagents like a mixture of methanol and thionyl chloride to yield a compound of general formula Int-1. In a subsequent step, protection of the free amine functionality in Int-1 as, for example, an amide or a carbamate, like Fmoc, affords an intermediate of general structure Int-2. The intermediate of general formula Int-3 can be obtained by selective
- 20 deprotection of the masked acid functionality present in Int-2, using for example an acidic hydrolysis. When using SPPS, Int-3 can be attached to a suitably functionalised resin, for example Wang resin, using ester formation reagents like, for exemplification, a mixture of diisopropylmethanediimine, 4-methylmorpholine and N,N-dimethylpyridin-4-amine in dichloromethane, to afford solid supported
- 25 intermediate of general formula Int-4.

GENERAL SYNTHETIC PROCEDURE 1



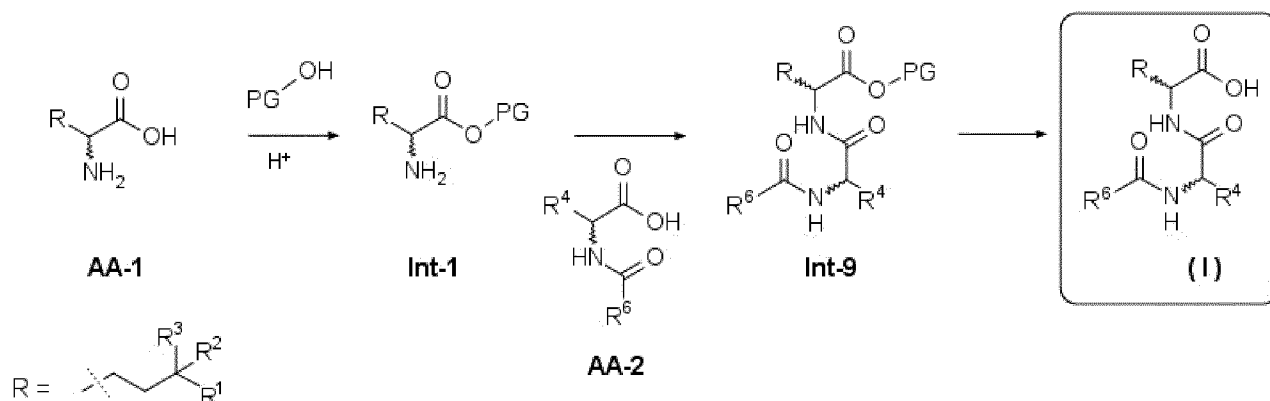
Scheme 1

- 5 When required the resin bound intermediates Int-4 to Int-7 can be analysed after cleavage from resin. Alternatively, the corresponding steps on solid support can be performed without analysis. The protecting group of the amine in Int-4 can be removed by treatment with a base like piperidine, when, for example, the Fmoc protecting group is used, to yield Int-5. The free acid functionality present in the
- 10 second N-protected-amino-acid, represented here by the compound of general formula AA-2, can be coupled with the free amine present in intermediate of general formula Int-5 to afford Int-6, using a classical amide coupling procedure, for example a mixture of HATU and a base, like N-methyl morpholine in a suitable solvent system, like a mixture of DCM-DMF. When, advantageously, the N-
- 15 protecting group in AA-2 and Int-6 has the desired substitution, R^6 , cleavage, for example, by acidic hydrolysis using a reagent like trifluoroacetic acid, of the ester bond between the solid support and the compound of general structure represented in Int-6 yields the desired compound of general formula (I).

Alternatively, AA-2 can be introduced in Int-6 with a suitable protecting group on the amine moiety that can be removed in the subsequent step, to give Int-7, using a base like piperidine, when, for example, the Fmoc protecting group is used. The free basic amine functionality present in Int-7 can be readily functionalized as an acyl derivative using a classical amide coupling procedure, for example a mixture of HATU and a base, such as *N*-methyl morpholine in a suitable solvent system, like a mixture of DCM-DMF, to yield intermediate of general formula Int-8. The final compound of general formula (I) is obtained, by cleavage, for example, using acidic hydrolysis with a reagent like trifluoroacetic acid, of the ester bond between the resin solid support and the compound of general structure shown in Int-8.

Compounds of general formula (I) can be obtained, also, as shown in general Scheme 2 below. The skilled artisan will appreciate that the amine functionality in compounds of general formula Int-1 can be coupled with the free carboxylic acid functionality present in compounds of general formula AA-2, using, as described above and for the purpose of exemplification, a mixture of a coupling reagent and a base, such as HATU and *N*-methyl morpholine in a suitable solvent system, like DCM, DMF or a mixture thereof, to yield intermediate of general formula Int-9. The chemoselective cleavage of the ester-type protecting group present in compound of general formula Int-9 can then be done to afford the desired compounds of general formula (I), for example by basic hydrolysis in an aqueous media, such as LiOH in a mixture of water and acetonitrile.

GENERAL SYNTHETIC PROCEDURE 2



Scheme 2

- 5 The products of each step can then be recovered by conventional methods including extraction, evaporation, precipitation, chromatography, filtration, trituration, crystallisation and the like.

Resin bound intermediates were analysed after cleavage from resin. All the analysis of intermediate are of free acids.

- 10 The skilled artisan will also appreciate that not all of the substituents in the compounds of formula (I) will tolerate certain reaction conditions employed to synthesise the compounds. These moieties may be introduced at a convenient point in the synthesis, or may be protected and then deprotected as necessary or desired, as is well known in the art. The skilled artisan will appreciate that the
- 15 protecting groups may be removed at any convenient point in the synthesis of the compounds of the present invention. Methods for introducing or removing protecting groups used in this invention are well known in the art; see, for example, Greene and Wuts, Protective Groups in Organic Synthesis, 4th Ed., John Wiley and Sons, New York (2006)¹⁶.

EXAMPLES

Abbreviations

approx: approximately; aq: aqueous; br: broad; *ca.*: *circa*; CDI: 1,1'-Carbonyldiimidazole; d: doublet; DCM: dichloromethane; DIC: *N,N'*-Diisopropylcarbodiimide; dioxane: 1,4-dioxane; DIPEA: diisopropylethylamine; DMF: dimethylformamide; eq.: equivalent; Et₃N: triethylamine; EtOAc: ethyl acetate; EtOH: ethanol; Fmoc: fluorenylmethoxycarbonyl; Boc: *tert*-butoxycarbonyl; h: hours; min: minutes; HATU: 2-(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl)-1,1,3,3-tetramethyl isouronium hexafluorophosphate(V); HPLC: high performance liquid chromatography; IPA, isopropanol; LC: liquid chromatography; m: multiplet; M: molar, molecular ion; MeCN: acetonitrile; MeOH: methanol; MS: mass spectrometry; NMR: nuclear magnetic resonance; PDA: photodiode array; q: quartet; rt: room temperature (*ca.* 20 °C); R_T: retention time; s: singlet, solid; SPPS: solid phase peptide synthesis. t: triplet; TBAF: tetrabutylammonium fluoride; TBME: *tert*-butyl methyl ether; TFA: trifluoroacetic acid; THF: tetrahydrofuran; UPLC: ultra-performance liquid chromatography; UV: ultraviolet.

Other abbreviations are intended to convey their generally accepted meaning.

General Experimental Conditions

All starting materials and solvents were obtained either from commercial sources or prepared according to the literature citation. Reaction mixtures were magnetically stirred and reactions performed at room temperature (*ca.* 20 °C) unless otherwise indicated.

Column chromatography was performed on an automated flash chromatography system, such as a CombiFlash Rf system, using pre-packed silica (40 µm) cartridges, unless otherwise indicated.

¹H NMR spectra were recorded, at 400 MHz or at 500 MHz, on a Bruker Avance AV-I-400 instrument, a Bruker Avance AV-II-400 instrument or a Bruker Avance III-500 HD spectrometer, equipped with a Bruker 5 mm SmartProbe™. Chemical shifts are expressed in parts per million (ppm) using either the central peaks of the residual protic solvent as references or relative to tetramethylsilane, as internal standard. The spectra were recorded at 298 K unless otherwise indicated. The

following abbreviations or their combinations are used for multiplicity of NMR signals: br = broad, d = doublet, m = multiplet, q = quartet, quint = quintet, s = singlet and t = triplet.

Analytical Methods 1 to 3:

- 5 Analytical UPLC-MS experiments to determine retention times and associated mass ions were performed using a Waters ACQUITY UPLC® H-Class system, equipped with ACQUITY PDA Detector and ACQUITY QDa Mass Detector, running one of the analytical methods described below.

- 10 Analytical LC-MS experiments to determine retention times and associated mass ions were performed using an Agilent 1200 series HPLC system coupled to an Agilent 1956, 6100 or 6120 series single quadrupole mass spectrometer running one of the analytical methods described below.

- 15 Preparative HPLC purifications were performed either using a Waters X-Select CSH C18, 5 µm, 19x50 mm column using a gradient of Acetonitrile and water, both modified with 0.1% v/v formic acid, or on a Waters X-Bridge BEH C18, 5 µm, 19x50 mm column using a gradient of Acetonitrile and 10 mM ammonium bicarbonate (aq). Fractions were collected following detection by UV at a single wavelength measured by a variable wavelength detector.

Method 1: Acidic 3 min method

- 20 Column: Waters ACQUITY UPLC® CSH C18, 1.7 µm, 2.1x30 mm at 40 °C

Detection: UV at 254 nm unless otherwise indicated, MS by electrospray ionisation

Eluent A: 0.1% v/v Formic acid in water, Eluent B: 0.1% v/v Formic acid in Acetonitrile

Gradient:

Time	%A	%B	Flow rate (ml/min)
0.00	95	5	0.77
0.11	95	5	0.77
2.15	5	95	0.77
2.56	5	95	0.77
2.83	95	5	0.77
3.00	95	5	0.77

Method 2: Basic 3 min method

Column: Waters ACQUITY UPLC® BEH C18, 1.7 µm, 2.1x30 mm at 40 °C

- 5 Eluent A: 10 mM ammonium bicarbonate (aq), Eluent B: Acetonitrile

(other parameters the same as Method 1)

Method 3: Basic 4 min method

Column: Waters X-Bridge BEH C18, 2.5 µm, 4.6x30 mm at 40 °C

Detection: UV at 254 nm unless otherwise indicated, MS by electrospray ionisation

- 10 Eluent A: 10 mM ammonium bicarbonate (aq), Eluent B: MeCN

Time	%A	%B	Flow rate (ml/min)
0.0	95.0	5.0	2.5
3.0	5.0	95.0	2.5
3.01	5.0	95.0	4.5
3.6	5.0	95.0	4.5
3.7	95.0	5.0	2.5
4.0	95.0	5.0	2.5

Analytical Methods 4 to 8:

5 Method 4: LCMS_SC_BASE

Apparatus: Agilent 1260 Bin. Pump: G1312B, degasser; autosampler, ColCom, DAD: Agilent G1315C, 210, 220 and 220-320 nm, PDA 210-320 nm, MSD: Agilent LC/MSD G6130B ESI, pos/neg 100-1000;

Column: Waters XSelect™ CSH C18, 30x2.1mm, 3.5µm, Temp: 25 °C, Flow: 1
10 mL/min, Gradient: t₀ = 5% B, t_{1.6min} = 98% B, t_{3min} = 98% B, Post time: 1.4 min

Eluent A: 10mM ammonium bicarbonate in water (pH=9.0), Eluent B: acetonitrile

Method 5: SC_ACID

Apparatus: Agilent 1260 Bin. Pump, degasser; autosampler, ColCom, DAD: Agilent G1315D, 210, 220 and 220-320 nm, PDA: 210-320 nm, MSD: Agilent
15 LC/MSD G6130B ESI, pos/neg 100-1000, ELSD Alltech 3300 gas flow 1.5 ml/min, gas temp: 40 °C

Column: Waters XSelect™ C18, 30x2.1mm, 3.5µm, Temp: 40 °C

Flow: 1 mL/min, Gradient: t₀ = 5% B, t_{1.6min} = 98% B, t_{3min} = 98% B, Post time: 1.3 min

Eluent A: 0.1% formic acid in water, Eluent B: 0.1% formic acid in acetonitrile

Method 6: UPLC_AN_BASE

Apparatus: Waters IClass; Bin. Pump: UPIBSM, SM: UPISMFTN with SO;
UPCMA, PDA: UPPDATC, 210-320 nm, SQD: ACQ-SQD2 ESI; ELSD: gas
5 pressure 40 psi, drift tube temp: 50 °C

Column: Waters XSelect CSH C18, 50x2.1mm, 2.5µm, Temp: 25 °C, Flow: 0.6
mL/min, Gradient: t₀ = 5% B, t_{2.0min} = 98% B, t_{2.7min} = 98% B, Post time: 0.3
min

Eluent A: 10mM ammonium bicarbonate in water (pH=9.5), Eluent B: acetonitrile

10 **Method 7: UPLC_AN_ACID**

Apparatus: Waters IClass; Bin. Pump: UPIBSM, SM: UPISMFTN with SO;
UPCMA, PDA: UPPDATC, 210-320 nm, SQD: ACQ-SQD2 ESI; ELSD: gas
pressure 40 psi, drift tube temp: 50 °C

Column: Waters XSelect CSH C18, 50x2.1mm, 2.5µm, Temp: 40 °C, Flow: 0.6
15 mL/min, Gradient: t₀ = 5% B, t_{2.0min} = 98% B, t_{2.7min} = 98% B, Post time: 0.3
min

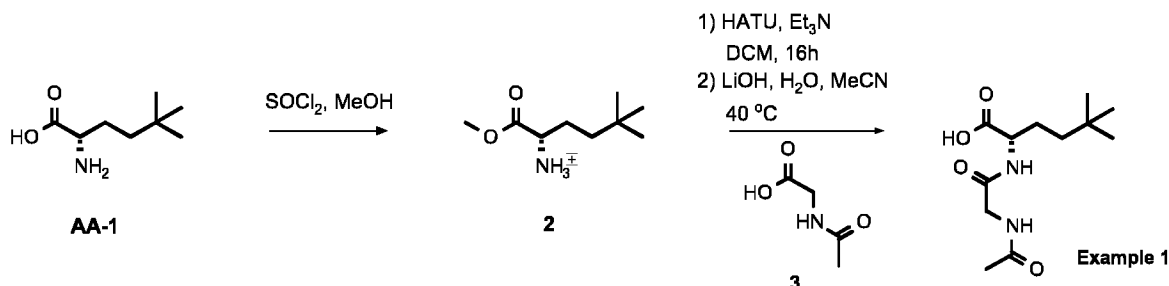
Eluent A: 0.1% formic acid in water, Eluent B: 0.1% formic acid in acetonitrile

Method 8: PREP_ACID-AS4A

Apparatus: Agilent Technologies G6130B Quadrupole; HPLC instrument type:
20 Agilent Technologies 1290 preparative LC; Column: Waters XSelect CSH (C18,
100x30mm, 10µ); Flow: 55 ml/min

Column temp: RT

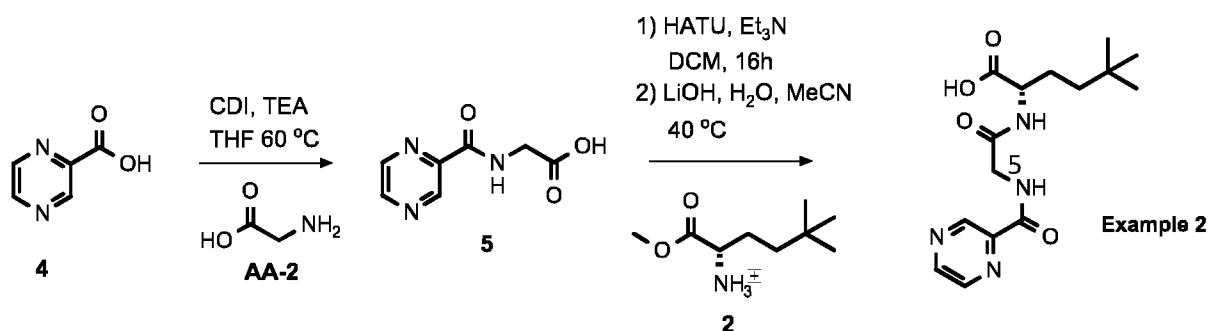
Eluent A: 0.1% formic acid in water; Eluent B: 100% acetonitrile lin. gradient: t=0
min 20% B, t=2 min 20% B, t=8.5 min 60% B, t=10 min 100% B, t=13 min 100%
25 B; Detection: DAD (220-320 nm); Detection: MSD (ESI pos/neg) mass range: 100
– 1000; fraction collection based on MS and DAD.

Example 1**(S)-2-((S)-2-acetamido-3-phenylpropanamido)-5,5-dimethylhexanoic acid.****Synthesis of methyl (S)-2-amino-5,5-dimethylhexanoate hydrochloride (2)**

- 5 Thionyl chloride (2.75 mL, 37.7 mmol) was added dropwise to a mixture of 5,5-Dimethyl-L-norleucine (**AA-1**, 2.0 g, 12.6 mmol) in methanol (10 mL). The mixture was stirred at 50 °C for 1 hour. The solvent was evaporated *in vacuo* resulting in methyl (S)-2-amino-5,5-dimethylhexanoate hydrochloride (**2**; 2.59 g, 12.4 mmol, 98 % yield) as slight yellow solid. LCMS (Method 8, 0.987 min; M+H = 174.2; calculated 174.2)

Synthesis of (S)-2-((S)-2-acetamido-3-phenylpropanamido)-5,5-dimethylhexanoic acid (Example 1)

- HATU (110 mg, 0.289 mmol) was added to a mixture of acetyl glycine **3** (33.8 mg, 0.289 mmol) and triethylamine (0.121 mL, 0.866 mmol) in dichloromethane (1 mL). The mixture was stirred for 30 minutes. Intermediate Methyl (S)-2-amino-5,5-dimethylhexanoate hydrochloride (**2**, 50 mg, 0.238 mmol) was added and the mixture was stirred overnight. The mixture was concentrated *in vacuo*. The residue was dissolved in water (1 mL) and acetonitrile (1 mL). Lithium hydroxide (69.1 mg, 2.89 mmol) was added. The mixture was stirred overnight at 40 °C. Concentrated *in vacuo* and purified by preparative HPLC (Method 8). The product containing fractions were combined and lyophilized resulting in the title compound, **Example 1** (27.6 mg, 0.107 mmol, 37 % yield, 98.5% purity). LCMS (Method 6, 0.953 min; M+H = 259.1; calculated 259.1). ¹H-NMR (400 MHz, DMSO) δ 12.68 (br, 1H), 8.12 – 8.01 (m, 2H), 4.14 (td, *J* = 8.1, 5.0 Hz, 1H), 3.73 – 3.69 (m, 2H), 1.84 (s, 3H), 1.66 (m, 1H), 1.56 (m, 1H), 1.26 – 1.10 (m, 2H), 0.85 (s, 9H).

Example 2**(2S)-5,5-dimethyl-2-{2-[(pyrazin-2-yl)formamido]acetamido}hexanoic acid****Synthesis of (pyrazine-2-carbonyl) glycine (5).**

CDI (324 mg, 2.000 mmol) was added to a suspension of pyrazine-2-carboxylic acid (**4**, 248 mg, 2 mmol) in tetrahydrofuran (dry) (3 ml). The mixture was heated at 60 °C for 1h, cooled to room temperature and triethylamine (300 µL, 2.152 mmol) and glycine (**AA-2**, 150 mg, 2 mmol) were added. The mixture was concentrated and water (10 mL) and EtOAc (25 mL) were added. The layers were separated and washed with brine (10 mL) the organic layer was dried over Na₂SO₄ and concentrated to afford a white solid (402 mg). The crude product was purified by column chromatography (12g SiO₂; 10% Methanol (7M NH₃) in DCM) to afford (pyrazine-2-carbonyl) glycine (**5**, 82 mg, 0.453 mmol, 22 % yield). LCMS (Method 4, 0.162 min; M+H = 182.0; calculated 182.1)

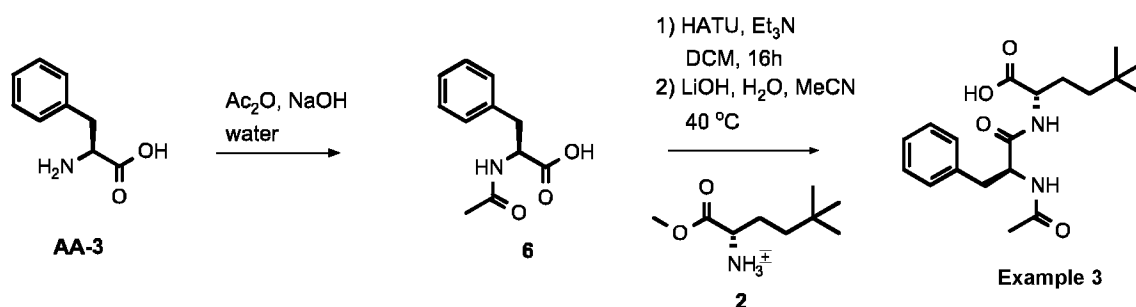
Synthesis of (2S)-5,5-dimethyl-2-{2-[(pyrazin-2-yl)formamido]acetamido}hexanoic acid (Example 2)

HATU (110 mg, 0.289 mmol) was added to a solution of (pyrazine-2-carbonyl) glycine (**5**, 52.3 mg, 0.289 mmol) and TEA (0.121 ml, 0.866 mmol) in dichloromethane (2 ml). The mixture was stirred for 30 minutes. Intermediate Methyl (S)-2-amino-5,5-dimethylhexanoate hydrochloride (**2**, 50 mg, 0.238 mmol) was added and the mixture was stirred overnight. The solvent was removed in vacuo and lithium hydroxide (69.1 mg, 2.89 mmol), acetonitrile (1 mL) and water (2 mL) were added. The mixture was stirred overnight at 40 °C. The mixture was

concentrated *in vacuo* and purified by preparative HPLC, (Method 8). The fractions were combined and freeze-dried to afford the title compound (2S)-5,5-dimethyl-2-{2-[(pyrazin-2-yl)formamido]acetamido} hexanoic acid (**Example 2**, 12.4 mg, 0.038 mmol, 16 % yield, 99.1% purity). LCMS (Method 6, 0.729 min; M+H = 323.2; calculated 323.2). ¹H-NMR (400 MHz, DMSO) δ 9.19 (d, *J* = 1.6 Hz, 1H), 9.01 (t, *J* = 5.9 Hz, 1H), 8.90 (d, *J* = 2.5 Hz, 1H), 8.82 – 8.71 (m, 1H), 7.98 (d, *J* = 7.5 Hz, 1H), 7.68 (br, 1H), 4.03 (td, *J* = 7.4, 5.1 Hz, 1H), 3.96 (d, *J* = 5.9 Hz, 2H), 1.66 (m, 1H), 1.54 (m, 1H), 1.14 (dd, *J* = 9.8, 7.4 Hz, 2H), 0.82 (s, 9H).

10 Example 3

(2S)-2-[(2S)-2-acetamido-3-phenylpropanamido]-5,5-dimethylhexanoic acid.



Synthesis of acetyl-L-phenylalanine (6)

Aqueous sodium hydroxide (1M) (1.5 ml, 1.5 mmol) was added dropwise to a stirred solution of L-phenylalanine (**AA-2**, 248 mg, 1.5 mmol) in water (3 ml) until pH 12. Acetic anhydride (0.235 ml, 2.490 mmol) was added dropwise. More aqueous sodium hydroxide (1M) was added when a precipitation was formed to keep pH > 8. The mixture was stirred for 3 hours. The pH was adjusted to pH 2 with 5M aqueous hydrochloric acid, the precipitated product was collected by filtration and dried *in vacuo* to afford acetyl-L-phenylalanine (**6**, 274 mg, 1.322 mmol, 88 % yield). LCMS (Method 5, 1.465 min; M+H = 208.1; calculated 208.1).

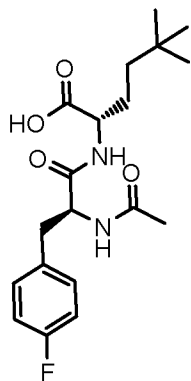
Synthesis of (S)-2-((S)-2-acetamido-3-phenylpropanamido)-5,5-dimethylhexanoic acid (Example 3)

HATU (110 mg, 0.289 mmol) was added to a mixture of acetyl-L-phenylalanine (**6**, 59.8 mg, 0.289 mmol) and triethylamine (0.121 ml, 0.866 mmol) in

dichloromethane (1 ml). The mixture was stirred for 30 minutes. Intermediate methyl (S)-2-amino-5,5-dimethylhexanoate hydrochloride (**2**, 50 mg, 0.238 mmol) was added and the mixture was stirred overnight. The solvent was removed in vacuo and lithium hydroxide (69.1 mg, 2.89 mmol), acetonitrile (1 ml) and water (1 ml) were added. The mixture was stirred overnight at 40°C. The mixture was concentrated *in vacuo* and purified by preparative HPLC, (Method 8). The fractions were combined and freeze-dried to afford the title compound (S)-2-((S)-2-acetamido-3-phenylpropanamido)-5,5-dimethylhexanoic acid (**Example 3**, 20.7 mg, 0.059 mmol, 24 % yield, 95.75% purity). LCMS (Method 7, 1.274 min; M+H = 349.2; calculated 349.2). ¹H-NMR (400 MHz, DMSO) δ 12.58 (s, 1H), 8.20 (d, *J* = 7.8 Hz, 1H), 8.08 (d, *J* = 8.6 Hz, 1H), 7.27 (s, 2H), 7.26 – 7.22 (m, 2H), 7.18 (m, 1H), 4.56 (ddd, *J* = 10.2, 8.4, 3.9 Hz, 1H), 4.14 (td, *J* = 8.1, 5.0 Hz, 1H), 3.00 (dd, *J* = 13.9, 3.9 Hz, 1H), 2.70 (dd, *J* = 13.9, 10.2 Hz, 1H), 1.73 (s, 3H), 1.72 – 1.66 (m, 1H), 1.66 – 1.51 (m, 1H), 1.30 – 1.08 (m, 2H), 0.86 (s, 9H).

15 **Example 4**

(2S)-2-[(2S)-2-acetamido-3-(4-fluorophenyl)propanamido]-5,5-dimethylhexanoic acid



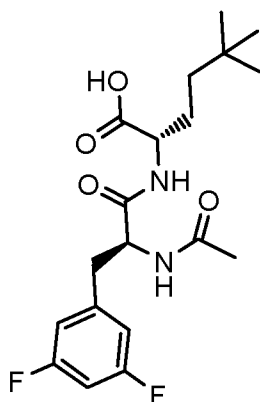
Example 4 was prepared in an analogous manner to Example 3, starting from its corresponding amino acid analogue to AA-3.

(2S)-2-[(2S)-2-acetamido-3-(4-fluorophenyl)propanamido]-5,5-dimethylhexanoic acid (**Example 4**, 28.6mg, 27% yield, 100% purity) LCMS (Method 7, 1.308 min, M+H = 367.2, calculated 367.2). ¹H-NMR (400 MHz, DMSO) δ 12.57 (s, 1H), 8.21 (d, *J* = 7.7 Hz, 1H), 8.08 (d, *J* = 8.7 Hz, 1H), 7.29 (dd, *J* = 8.6, 5.7 Hz, 2H), 7.08 (t,

J = 8.9 Hz, 2H), 4.55 (ddd, J = 10.1, 8.5, 4.1 Hz, 1H), 4.14 (td, J = 8.1, 5.0 Hz, 1H), 2.97 (dd, J = 13.9, 4.2 Hz, 1H), 2.68 (dd, J = 13.9, 10.1 Hz, 1H), 1.74 (s, 3H), 1.72 – 1.64 (m, 1H), 1.64 – 1.49 (m, 1H), 1.28 – 1.11 (m, 2H), 0.86 (s, 9H).

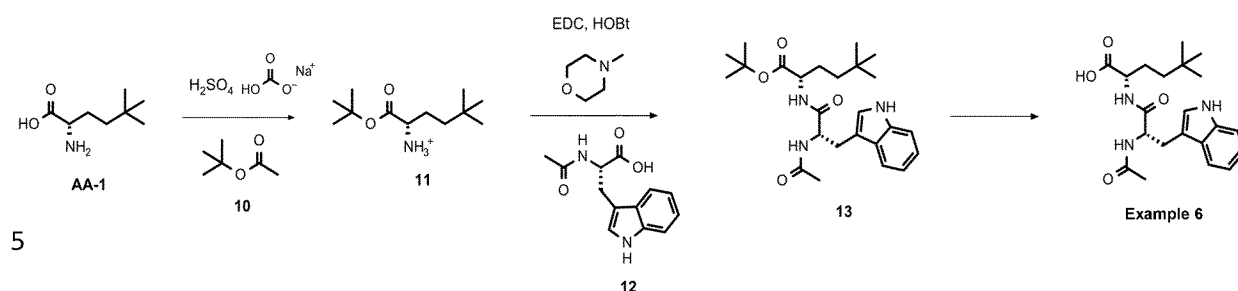
Example 5

5 **(2S)-2-[(2S)-3-(3,5-difluorophenyl)-2-acetamidopropanamido]-5,5-dimethylhexanoic acid**



Example 5 was prepared in an analogous manner to Example 3, starting from its corresponding amino acid analogue to AA-3.

- 10 (2S)-2-[(2S)-3-(3,5-difluorophenyl)-2-acetamidopropanamido]-5,5-dimethylhexanoic acid (**Example 5**, 27.8 mg, 30% yield, 98.13% purity) LCMS (Method 7, 1.308 min; M+H = 367.2; calculated 367.2). ¹H-NMR (400 MHz, DMSO) δ 12.64 (s, 1H), 8.22 (d, J = 7.8 Hz, 1H), 8.10 (d, J = 8.6 Hz, 1H), 7.05 (tt, J = 9.5, 2.4 Hz, 1H), 7.01 – 6.97 (m, 2H), 4.60 (td, J = 9.8, 4.2 Hz, 1H), 4.14 (td, J = 8.1, 4.9 Hz, 1H), 3.00 (dd, J = 13.9, 4.2 Hz, 1H), 2.74 (dd, J = 13.7, 10.0 Hz, 1H), 1.75 (s, 3H), 1.74 – 1.64 (m, 1H), 1.63 – 1.47 (m, 1H), 1.26 – 1.12 (m, 2H), 0.86 (s, 9H).
- 15

Example 6**(2S)-2-[(2S)-2-acetamido-3-(1H-indol-3-yl)propanamido]-5,5-dimethylhexanoic acid.****Synthesis of *tert*-butyl (S)-2-amino-5,5-dimethylhexanoate (11)**

H₂SO₄ (0.62 g, 0.33 mL, 2.0 Eq, 6.3 mmol) was added to a stirred suspension of (S)-2-amino-5,5-dimethylhexanoic acid (**AA-1**, 0.50 g, 1 Eq, 3.1 mmol) and 4A molecular sieves in *tert*-butyl acetate (**10**, 8.7 g, 10 mL, 24 Eq, 75 mmol) whilst maintaining the temperature below 5 °C. The reaction was then allowed to attain room temperature and stirred for 20 hours.

The reaction was added dropwise to a vigorously stirred solution of sodium bicarbonate (2.6 g, 10.0 Eq, 31 mmol) in water (20 mL) and the resulting mixture extracted with EtOAc (2 x 20 mL), dried (MgSO₄), filtered and evaporated in vacuo to afford a light brown gum. The gum was azeotroped with toluene to afford the title compound (2S)-2-[(2S)-2-acetamido-3-(1H-indol-3-yl)propanamido]-5,5-dimethylhexanoic acid (**11**, 0.54 g, 2.4 mmol, 76 %, 95%) as a cream solid. UPLC (CSH C18 Column, 130Å, 1.7 µm, 2.1 mm x 30 mm, 3 min method, 0.1% Formic acid, 2-100 % MeCN/water): m/z 216.4 (M+H)⁺ (ES+); at 0.86 min. ¹H NMR (500 MHz, DMSO-d₆) δ 7.50 (s, 2H), 3.71 (app. t, J = 5.8 Hz, 1H), 1.71 – 1.62 (m, 2H), 1.45 (s, 9H), 1.33 – 1.24 (m, 1H), 1.16 – 1.06 (m, 1H), 0.86 (s, 9H).

Synthesis of *tert*-butyl (S)-2-[(S)-2-acetamido-3-(1H-indol-3-yl)propanamido]-5,5-dimethylhexanoate (13)

HOBt (0.34 g, 1.1 Eq, 2.2 mmol) was added to a stirred solution of acetyl-L-tryptophan (**12**, 0.50 g, 1 Eq, 2.0 mmol) and *tert*-butyl (S)-2-amino-5,5-dimethylhexanoate (**11**, 0.46 g, 1.05 Eq, 2.1 mmol) in DMF (5 mL). The reaction

was cooled to 0 °C, EDC (0.41 g, 1.05 Eq, 2.1 mmol) and 4-methylmorpholine (0.41 g, 0.45 mL, 2.0 Eq, 4.1 mmol) were added, allowed to attain room temperature and stirred for 16 hours.

The reaction was quenched with water (20 mL), extracted with EtOAc (2 x 20 mL), washed with water (2 x 10 mL) and brine (5 mL), dried (MgSO₄), filtered, evaporated in vacuo and purified by chromatography on silica gel (40 g FlashPure column, 0 - 100% EtOAc/isohehexane to afford the title compound *tert*-butyl (S)-2-((S)-2-acetamido-3-(1H-indol-3-yl)propanamido)-5,5-dimethylhexanoate (**13**, 0.56 g, 1.2 mmol, 62 %, 99% Purity), 3318-07-1, as a colourless foam. LCMS (XBridge BEH C18, 130Å, 2.5 µm, 2.1 mm x 30 mm, 3 min method, 0.1% Ammonium Hydroxide, 5-100% MeCN/water): 3318-07-1, m/z 444.0 (M+H)⁺ (ES+); at 2.18 min, 99.5% purity at 210-400nm. ¹H NMR (500 MHz, DMSO-d₆) δ 10.79 (d, *J* = 2.4 Hz, 1H), 8.23 (d, *J* = 7.4 Hz, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.34 – 7.29 (m, 1H), 7.13 (d, *J* = 2.0 Hz, 1H), 7.05 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 6.97 (ddd, *J* = 8.0, 6.9, 1.1 Hz, 1H), 4.64 – 4.56 (m, 1H), 4.11 – 4.03 (m, 1H), 3.10 (dd, *J* = 14.7, 4.3 Hz, 1H), 2.87 (dd, *J* = 14.7, 9.7 Hz, 1H), 1.75 (s, 3H), 1.70 – 1.52 (m, 2H), 1.40 (s, 9H), 1.21 – 1.14 (m, 2H), 0.86 (s, 9H).

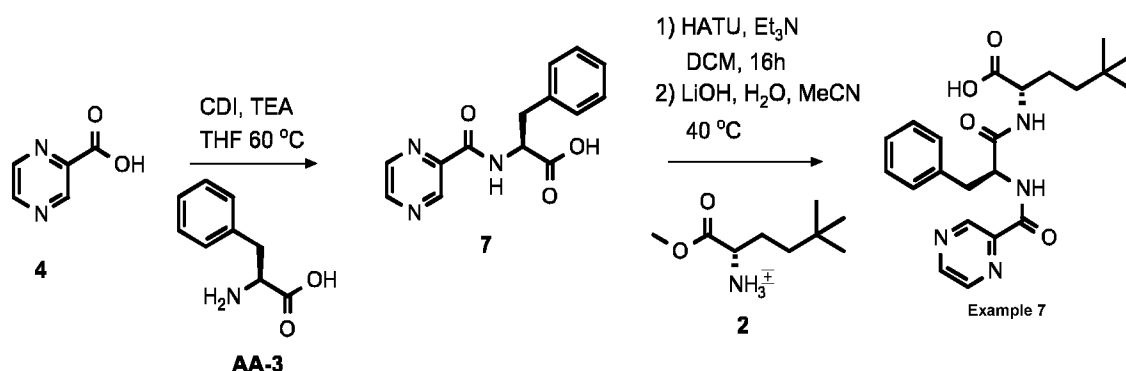
Synthesis of (2S)-2-[(2S)-2-acetamido-3-(1H-indol-3-yl)propanamido]-5,5-dimethylhexanoic acid (Example 6)

Tert-butyl (S)-2-((S)-2-acetamido-3-(1H-indol-3-yl)propanamido)-5,5-dimethylhexanoate **13** (480 mg, 1 Eq, 1.08 mmol) was dissolved in ice/cold 2,2,2-trifluoroacetic acid (50 mL), the ice bath removed and stirred for 45 min. The TFA was evaporated in vacuo at 25 °C, azeotroped with toluene (50 mL) and then dissolved in DCM (10 mL) and adsorbed onto celite. The DCM was evaporated *in vacuo* and the material purified by chromatography on RP Flash C18 (24 g cartridge, 5-50% MeCN/10 mM Ammonium Bicarbonate) to afford the title compound (2S)-2-[(2S)-2-acetamido-3-(1H-indol-3-yl)propanamido]-5,5-dimethylhexanoic acid (**Example 6**, 218 mg, 0.56 mmol, 51 %, 99%) as a white solid. UPLC (CSH C18 Column, 130Å, 1.7 µm, 2.1 mm x 30 mm, 3 min method, 0.1% Formic acid, 2-100% MeCN/water) m/z 388.9 (M+H)⁺ (ES+); at 1.27 min, 100% purity 210-400nm. LCMS (XBridge BEH C18, 130Å, 2.5 µm, 2.1 mm x 30

mm, 3 min method, 0.1% Ammonium Hydroxide, 5-100% MeCN/water): 3318-14-1, m/z 388.0 (M+H)⁺ (ES⁺); at 1.05 min, 99% purity at 260nm +/- 80nm. ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.78 (s, 1H), 8.10 (d, *J* = 8.3 Hz, 1H), 7.86 (d, *J* = 7.2 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.31 (dt, *J* = 8.1, 1.0 Hz, 1H), 7.12 (d, *J* = 2.2 Hz, 1H), 7.05 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 6.97 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 4.54 - 4.46 (m, 1H), 4.01 - 3.93 (m, 1H), 3.12 (dd, *J* = 14.8, 4.3 Hz, 1H), 2.86 (dd, *J* = 14.8, 9.6 Hz, 1H), 1.76 (s, 3H), 1.74 - 1.65 (m, 1H), 1.61 - 1.50 (m, 1H), 1.19 - 1.11 (m, 2H), 0.83 (s, 9H), CO₂H proton not observed.

Example 7

10 **(S)-5,5-dimethyl-2-((S)-3-phenyl-2-(pyrazine-2-carboxamido)propanamido)hexanoic acid.**



Synthesis of (pyrazine-2-carbonyl)-L-phenylalanine (7)

15 CDI (324 mg, 2.000 mmol) was added to a suspension of pyrazine-2-carboxylic acid (**4**, 248 mg, 2 mmol) in Tetrahydrofuran (dry) (3 ml). The mixture was heated at 60 °C for 1h, cooled to room temperature and TEA (300 μ L, 2.152 mmol) and L-phenylalanine (330 mg, 2 mmol) were added and the mixture was stirred for 2 hours. The solvent was evaporated and water and ethyl acetate were added, separated and extracted twice more with ethyl acetate. The organic layer was dried over Na₂SO₄ and concentrated *in vacuo* to afford (pyrazine-2-carbonyl)-L-phenylalanine (**7**, 80 mg, 0.295 mmol, 14.75 % yield). LCMS (Method 4, 1.379 min; (M+H)⁺ = 272.0; calculated 272.1)

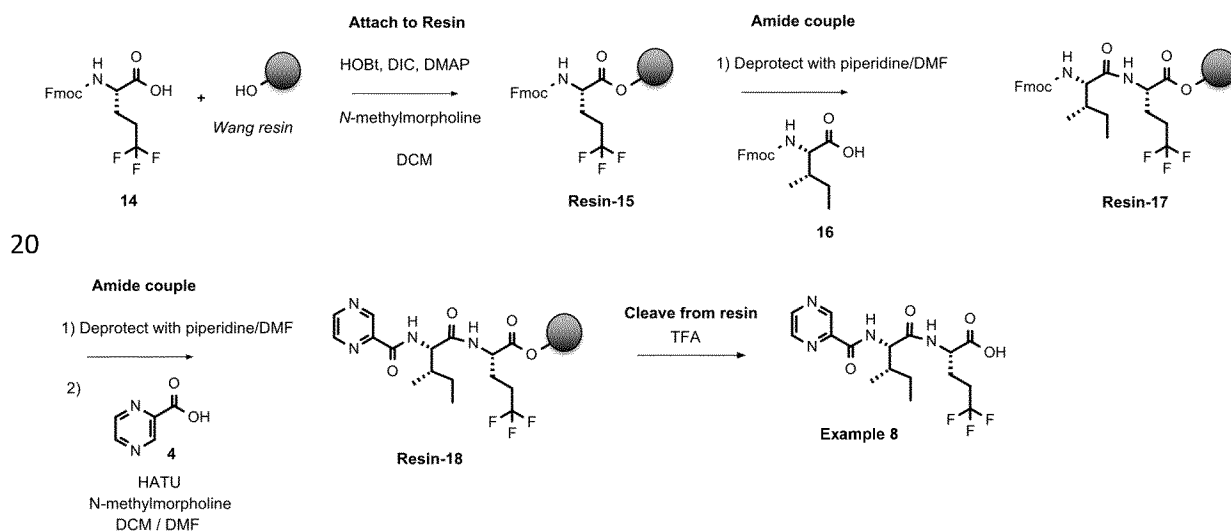
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Synthesis of (S)-5,5-dimethyl-2-((S)-3-phenyl-2-(pyrazine-2-carboxamido)propanamido)hexanoic acid (Example 7)

HATU (224 mg, 0.59 mmol) was added to a mixture of intermediate (S)-2-amino-5,5-dimethylhexanoic acid (**2**, 94 mg, 0.59 mmol), intermediate (**7**, pyrazine-2-carboxyl)-L-phenylalanine (160 mg, 0.590 mmol) and diisopropylethylamine (0.206 mL, 1.18 mmol) in *N,N*-Dimethylformamide (1 mL). The mixture was stirred at room temperature for 16 hours. The reaction mixture was purified by preparative HPLC, (Method 8). The product containing fractions were combined and lyophilised resulting in the title compound (S)-5,5-dimethyl-2-((S)-3-phenyl-2-(pyrazine-2-carboxamido)propanamido)hexanoic acid (**Example 7**, 14 mg, 0.034 mmol, 5.75 % yield). LCMS (Method 6, 0.958 min; $M+H = 413.2$; calculated 413.2). $^1\text{H-NMR}$ (400 MHz, DMSO) δ 12.77 (br, 1H), 9.12 (d, $J = 1.5$ Hz, 1H), 8.88 (d, $J = 2.5$ Hz, 1H), 8.81 – 8.71 (m, 1H), 8.69 (d, $J = 8.6$ Hz, 1H), 8.44 (d, $J = 7.8$ Hz, 1H), 7.31 – 7.10 (m, 5H), 4.90 – 4.79 (m, 1H), 4.26 – 4.10 (m, 1H), 3.17 (dd, $J = 13.9$, 4.6 Hz, 1H), 3.08 (dd, $J = 13.8$, 8.5 Hz, 1H), 1.80 – 1.66 (m, 1H), 1.66 – 1.56 (m, 1H), 1.30 – 1.12 (m, 2H), 0.84 (s, 9H).

Example 8

(2S)-5,5,5-trifluoro-2-[(2S,3S)-3-methyl-2-[(pyrazin-2-yl)formamido]pentanamido] pentanoic acid.



Synthesis of (S)-2-(((9H-Fluoren-9-yl) methoxy) carbonyl) amino)-5,5,5-trifluoropentanoic acid (14)

(S)-2-(((9H-Fluoren-9-yl) methoxy) carbonyl) amino)-5,5,5-trifluoropentanoic acid **14**, was prepared by following the procedure shown in PCT Patent Publication WO 2010/132601 A1, to prepare methyl 5,5,5-trifluoropentanoate, followed by Fmoc protection and methyl ester hydrolysis, as described in WO 2015/131100 A1.

Synthesis of (S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-5,5,5-trifluoropentanoic acid [Resin bound on C-terminal] (Resin-15)

Wang Resin (Loading: 1.0 mmol/g, 90.0 mg, 0.09 mmol) was swollen in DCM (1 mL) for 10 min and filtered, washing with DCM (3 x 5 mL). (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5,5,5-trifluoropentanoic acid (**14**, 177 mg, 0.450 mmol), 1H-benzo[d][1,2,3]triazol-1-ol hydrate (69.0 mg, 0.45 mmol), diisopropylmethanediimine (70.0 µL, 0.45 mmol), 4-methylmorpholine (49.0 µL, 0.45 mmol) and N,N-dimethylpyridin-4-amine (11.0 mg, 0.09 mmol) and DCM (5 mL) were added and the mixture was stirred at rt for 18 hours. The reaction mixture was filtered and washed with DMF (5 mL x 3), DCM (5 mL x 3), water (5 mL x 2) and DMF (5 mL x 3). DCM (5 mL), (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5,5,5-trifluoropentanoic acid (**14**, 177 mg, 0.45 mmol), 1H-benzo[d][1,2,3]triazol-1-ol hydrate (69.0 mg, 0.45 mmol), 4-methylmorpholine (49.0 µL, 0.45 mmol), diisopropylmethanediimine (69.7 µL, 0.45 mmol) and N,N-dimethylpyridin-4-amine (11.0 mg, 0.09 mmol) were added to the resin and the suspension was gently stirred for a further 12 hours at room temperature. The mixture was filtered and washed with DMF (5 mL x 3), DCM (5 mL x 3), water (5 mL x 2) and DMF (5 mL x 3) to afford the resin bound intermediate **Resin-15**. No analysis was undertaken at this stage and resin was used directly in the next step.

Synthesis of (S)-2-((2S,3S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-methylpentanamido)-5,5,5-trifluoropentanoic acid [Resin bound on C-terminal] (Resin-17)

(S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-5,5,5-trifluoropentanoic acid, resin bound (**Resin-15**, 90.0 mg, 0.09 mmol) was swollen in DMF (1 mL) for 10 minutes and filtered. 20% piperidine in DMF (5 mL) was added to the swollen resin and the suspension was agitated with nitrogen gas at room temperature for 20 minutes. The suspension was filtered, 20% piperidine in DMF (5 mL) was added and the suspension was agitated with nitrogen gas at room temperature for 20 minutes. The suspension was filtered and washed with DMF (5 mL x 3), DCM (5 mL x 3), water (5 mL x 2) and DMF (5 mL x 3). The obtained solid was used without further purification.

A mixture of DMF/DCM (1:1, 3 mL) was added followed by (((9H-fluoren-9-yl)methoxy)carbonyl)-L-isoleucine (**16**, 127 mg, 0.36 mmol), HATU (137 mg, 0.36 mmol) and 4-methylmorpholine (40.0 μ L, 0.36 mmol). The suspension was agitated with nitrogen gas at room temperature for 2 hours then filtered and washed with DMF (5 mL x 3), DCM (5 mL x 3), water (5 mL x 2) and DMF (5 mL x 3). DMF (3 mL) was added followed by (((9H-fluoren-9-yl)methoxy)carbonyl)-L-isoleucine (**16**, 127 mg, 0.36 mmol), HATU (137 mg, 0.36 mmol) and 4-methylmorpholine (40.0 μ L, 0.36 mmol). The suspension was agitated with nitrogen gas at room temperature for 15 hours then filtered and washed with DMF (5 mL x 3), DCM (5 mL x 3), water (5 mL x 2) and DMF (5 mL x 3) to afford the resin bound intermediate **Resin-17**. A small portion of resin was added to 0.5 mL of TFA and the suspension was left at room temperature for 1 hour. The suspension was concentrated *in vacuo* to afford (S)-2-((2S,3S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylpentanamido)-5,5,5-trifluoropentanoic acid (77% purity, Method 1, 1.67 min; M+H = 507.4).

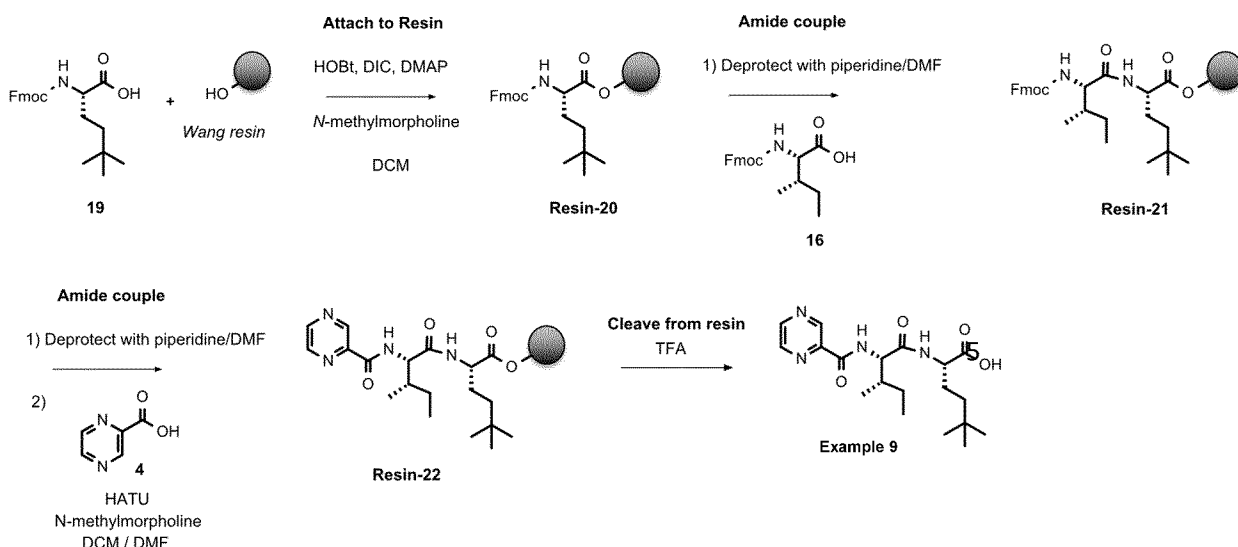
Synthesis of (S)-5,5,5-trifluoro-2-[(2S,3S)-3-methyl-2-[(pyrazin-2-yl)formamido]pentanamido]pentanoic acid (Resin-18)

(S)-2-((2S,3S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylpentanamido)-5,5,5-trifluoropentanoic acid, resin bound (**Resin-17**, 30.0 mg, 0.030 mmol) was swollen in DMF at room temperature for 10 minutes and filtered. 20% Piperidine in DMF (5 mL) was added to the swollen resin, and nitrogen gas was passed through the suspension for 20 minutes. The suspension

- was filtered and 20% piperidine in DMF (5 mL) was added, and nitrogen gas was passed through the suspension for 20 minutes. The suspension was filtered and washed with DMF (5 mL x 3), DCM (5 mL x 3), water and DMF (5 mL x 3). The obtained solid was used without further purification. DMF (5 mL) was added to the
- 5 obtained resin and pyrazine-2-carboxylic acid **4** (19 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol) and 4-methylmorpholine (15 mg, 0.15 mmol) were added. Nitrogen gas was passed through the suspension for 2 hours. The suspension was filtered and washed with DMF (5 mL x 3), DCM (5 mL x 3), water (5 mL x 2) and DMF (5 mL x 3), and DMF (10 mL) was added. pyrazine-2-carboxylic acid (**4**,
- 10 19 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol) and 4-methylmorpholine (15 mg, 0.15 mmol) were added. Nitrogen gas was passed through for 15 hours. The reaction suspension was filtered and washed with DMF (5 mL x 3), DCM (5 mL x 3), water (5 mL x 2), DMF (5 mL x 2) and DCM (5 mL x 2), to resin bound intermediate **Resin-18** (15 mg, 0.030 mmol).
- 15 A mixture of TFA/water (95:5, 1 mL) was added to the resin bound intermediate **Resin-18** (15 mg, 0.030 mmol) and stirred at room temperature for 3 hours. The reaction mixture was filtered, and resin washed with DCM (20 mL x 3). The combined filtrates were concentrated *in vacuo* and azeotroped with toluene (15 mL x 3). The crude product was purified by preparative HPLC. This resulted in
- 20 the title compound, (S)-5,5,5-trifluoro-2-((2S,3S)-3-methyl-2-(pyrazine-2-carboxamido) pentanamido)pentanoic acid (**Example 8**, 6.3 mg, 16 μ mol, 54% yield) as a white solid. 100% LCMS purity (Method 2, 0.56 min; M-H = 389.2), 100% NMR purity [^1H NMR (500 MHz, DMSO- d_6) δ 12.84 (br s, 1H), 9.20 (s, 1H), 8.91 (d, J = 2.5 Hz, 1H), 8.77 (t, J = 2.1 Hz, 1H), 8.56 (d, J = 7.7 Hz, 1H), 8.48 (d,
- 25 J = 9.0 Hz, 1H), 4.49 (dd, J = 9.1, 6.9 Hz, 1H), 4.35 – 4.26 (m, 1H), 2.39 – 2.21 (m, 2H), 2.01 – 1.77 (m, 3H), 1.57 – 1.45 (m, 1H), 1.17 – 1.06 (m, 1H), 0.93 (d, J = 6.8 Hz, 3H), 0.85 (t, J = 7.4 Hz, 3H)].

Example 9

(S)-5,5-dimethyl-2-[(2S,3S)-3-methyl-2-[(pyrazin-2-yl)formamido]pentanamido]hexanoic acid.



Synthesis of (S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-5,5-dimethylhexanoic acid [Resin bound on C-terminal] (Resin-20)

Wang resin (Loading: 1.0 mmol/g, 650 mg, 0.65 mmol) was swollen in DCM (30 mL) at room temperature for 10 minutes and filtered. DCM (30 mL) was added followed by (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5,5-dimethylhexanoic acid (**19**, 0.25 g, 0.65 mmol), DIC (0.31 mL, 2.00 mmol), 1H-benzo[d][1,2,3]triazol-1-ol hydrate (0.30 g, 2.0 mmol), 4-methylmorpholine (0.21 mL, 2.00 mmol) and DMAP (79.0 mg, 0.65 mmol) and the suspension was gently stirred at room temperature for 16 hours. The suspension was filtered and washed with DMF (20 mL x 3), DCM (20 mL x 3), water (20 mL x 2), DMF (20 mL x 2) and DCM (20 mL x 2). A mixture of DCM/DMF (1:1, 30 mL) was added followed by (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5,5-dimethylhexanoic acid (**19**, 0.25 g, 0.65 mmol), DIC (0.31 mL, 2.00 mmol), 1H-benzo[d][1,2,3]triazol-1-ol hydrate (0.30 g, 2.00 mmol), 4-methylmorpholine (0.21 mL, 2.00 mmol) and DMAP (79.0 mg, 0.65 mmol). The suspension was stirred at room temperature for 24 hours then filtered and washed with DMF (5 mL x 2), DCM (5 mL x 2), water (5 mL x 2), DMF (5 mL x 2), DMF (20 mL x 2) and DCM (20 mL x 2) to afford the

resin bound **Resin-20**. A small portion of the resin was added to TFA (0.05 mL) and the suspension was left at room temperature for 1 hour. The suspension was filtered, and concentrated *in vacuo* to afford (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5,5-dimethylhexanoic acid (100% purity), which was
5 analysed by LCMS (Method 3, 1.53 min; M+Na = 404.1).

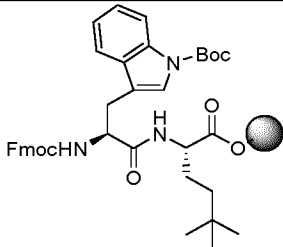
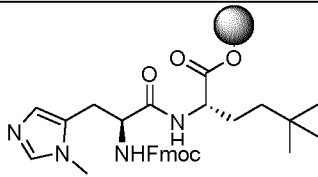
Synthesis of (S)-2-((2S,3S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-methylpentanamido)-5,5- dimethylhexanoic acid [Resin bound on C-terminal] (Resin-21)

(S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-5,5-dimethylhexanoic acid,
10 resin bound (**Resin-20**, 600 mg, 600 μ mol) was swollen in DMF (1 mL) for 10 min then filtered. 20% Piperidine in DMF (30 mL) was added to the swollen resin and the suspension was agitated with nitrogen gas at room temperature for 20 minutes. The suspension was filtered and washed with DMF (20 mL x 2), DCM (20 mL x 2), water (20 mL x 2), DCM (20 mL x 2) and DMF (20 mL x 3). The
15 suspension was filtered and 20% piperidine in DMF (30 mL) was added and nitrogen gas was passed through the suspension for 20 minutes. The suspension was filtered and washed with DMF (20 mL x 3), DCM (20 mL x 3), water (20 mL x 2) and DMF (20 mL x 3). The obtained solid was used without further purification.

DCM (2.6 mL) and DMF (0.4 mL) were added followed by additions of (((9H-fluoren-9-yl)methoxy)carbonyl)-L-isoleucine (**16**, 850 mg, 2.40 mmol), HATU (910
20 mg, 2.40 mmol) and 4-methylmorpholine (0.26 mL, 2.40 mmol). The reaction was stirred gently at room temperature for 16 hours under an atmosphere of nitrogen then filtered and washed with DMF (20 mL x 2), DCM (20 mL x 2), water (20 mL x 2), DCM (20 mL x 2) and DMF (20 mL x 3). (((9H-fluoren-9-yl)methoxy)carbonyl)-L-isoleucine (**16**, 850 mg, 2.40 mmol), HATU (910 mg, 2.40
25 mmol) and 4-methylmorpholine (0.26 mL, 2.40 mmol) were added. Nitrogen gas was passed through for 15 hours. The reaction suspension was filtered and the washed with DMF (20 mL x 3), DCM (20 mL x 3), water (20 mL x 2), DMF (20 mL x 2) and DCM (20 mL x 2), to afford resin bound intermediate **Resin-21**. A small
30 portion of the resin was added to TFA (0.05 mL) and stirred at room temperature for 30 minutes. The suspension was filtered, washing with DCM (5 mL) and the combined filtrates were concentrated *in vacuo* to afford (S)-2-((2S,3S)-2-((((9H-

Fluoren-9-yl)methoxy)carbonyl)amino)-3-methylpentanamido)-5,5-dimethylhexanoic acid (96% purity) which was analysed by LCMS (Method 2, 1.17 min; M+H = 496.56).

Table 2: Intermediates Resin-23 and Resin-24 were prepared from the corresponding starting material by a manner analogous to Intermediate Resin-21.

Intermediate	Structure
Resin-23	
Resin-24	

Synthesis of (S)-5,5-Dimethyl-2-((2S,3S)-3-methyl-2-(pyrazine-2-carboxamido)pentanamido)hexanoic acid (Example 9)

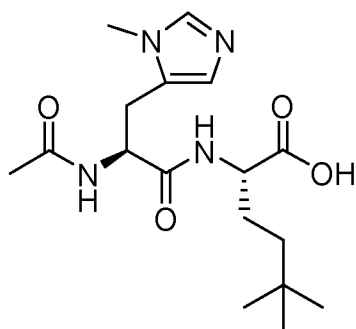
(S)-2-((2S,3S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-methylpentanamido)-5,5-dimethylhexanoic acid, resin bound (**Resin-21**, 39.0 mg, 0.08 mmol) was swollen in DMF at room temperature for 10 minutes and filtered. 20% Piperidine in DMF (5 mL) was added to the swollen resin, and nitrogen gas was passed through the suspension for 20 minutes. The suspension was filtered and 20% piperidine in DMF (5 mL) was added, and nitrogen gas was passed through the suspension for 20 minutes. The suspension was filtered and washed with DMF (5 mL x 3), DCM (5 mL x 3), water and DMF (5 mL x 3). The obtained solid was used without further purification. DMF (5 mL) was added to the obtained resin and pyrazine-2-carboxylic acid (**4**, 40.0 mg, 0.32 mmol), HATU (0.12 mg, 0.32 mmol) and 4-methylmorpholine (38.0 μ L, 0.32 mmol) were added. Nitrogen gas was passed through the suspension for 2 hours. The suspension was filtered

and washed with DMF (5 mL x 3), DCM (5 mL x 3), water (5 mL x 2) and DMF (5 mL x 3), and DMF (10 mL) was added. Pyrazine-2-carboxylic acid (**4**, 40.0 mg, 0.32 mmol), HATU (0.12 mg, 0.32 mmol) and 4-methylmorpholine (38.0 μ L, 0.32 mmol) were added. Nitrogen gas was passed through for 15 hours. The reaction suspension was filtered and washed with DMF (5 mL x 3), DCM (5 mL x 3), water (5 mL x 2), DMF (5 mL x 2) and DCM (5 mL x 2), to afford resin bound intermediate **Resin-22** (39 mg, 0.030 mmol).

A mixture of TFA/water (95:5, 1 mL) was added to the resin bound intermediate **Resin-22** (39.0 mg, 0.08 mmol) and stirred at room temperature for 3 hours. The reaction mixture was filtered, and resin washed with DCM (20 mL x 3). The combined filtrates were concentrated *in vacuo* and azeotroped with toluene (15 mL x 3). The crude product was purified by preparative HPLC. This resulted in the title compound, (S)-5,5-dimethyl-2-((2S,3S)-3-methyl-2-(pyrazine-2-carboxamido) pentanamido) hexanoic acid (**Example 9**, 13.71 mg, 33 μ mol, 41% yield, 90% Purity) as a white solid. 100% LCMS purity (Method 1, 1.39 min; M+H = 379.3); 90% NMR purity [^1H NMR (500 MHz, DMSO- d_6) δ 12.55 (br s, 1H), 9.20 (d, J = 1.5 Hz, 1H), 8.91 (d, J = 2.5 Hz, 1H), 8.79 – 8.75 (m, 1H), 8.47 (d, J = 9.3 Hz, 1H), 8.43 (d, J = 7.6 Hz, 1H), 4.54 (dd, J = 9.3, 7.0 Hz, 1H), 4.14 (td, J = 8.0, 5.0 Hz, 1H), 1.93 – 1.85 (m, 1H), 1.70 – 1.45 (m, 3H), 1.24 – 1.05 (m, 3H), 0.92 (d, J = 6.8 Hz, 3H), 0.86 – 0.80 (m, 12H), containing 0.5% TBME].

Example 10

(S)-2-((S)-2-Acetamido-3-(1-methyl-1H-imidazol-5-yl)propanamido)-5,5-dimethylhexanoic acid

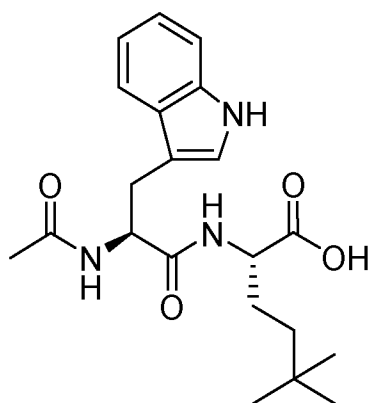


Example 10 was prepared in an analogous manner to Example 9.

(S)-2-((S)-2-Acetamido-3-(1-methyl-1H-imidazol-5-yl)propanamido)-5,5-dimethylhexanoic acid (4.4% yield, 97% LCMS purity). 97% LCMS purity (Method 1, 0.57 min; M+H = 353.3); 90 % NMR purity [¹H NMR (500 MHz, DMSO-d₆) δ 12.64 (br s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 8.6 Hz, 1H), 7.54 (s, 1H), 6.67 (s, 1H), 4.65 (dd, J = 8.2, 6.5 Hz, 1H), 4.11 (td, J = 8.2, 4.9 Hz, 1H), 3.57 (s, 3H), 2.86 (dd, J = 15.1, 6.5 Hz, 1H), 2.74 (dd, J = 15.1, 7.9 Hz, 1H), 1.81 (s, 3H), 1.65 – 1.55 (m, 1H), 1.52 – 1.43 (m, 1H), 1.09 – 1.05 (m, 2H), 0.82 (s, 9H), containing 4% IPA.].

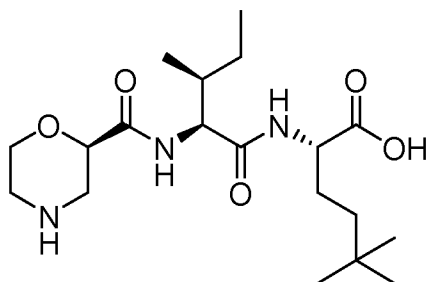
Example 11

10 Synthesis of (S)-2-((S)-2-Acetamido-3-(1H-indol-3-yl)propanamido)-5,5-dimethylhexanoic acid



Example 11, which is the same compound as Example 6, was additionally prepared in an analogous manner to Example 9.

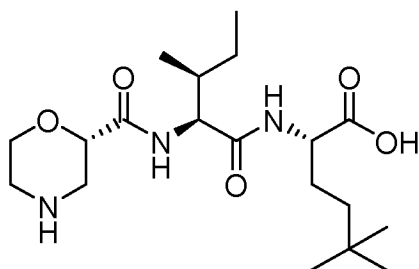
15 (S)-2-((S)-2-Acetamido-3-(1H-indol-3-yl)propanamido)-5,5-dimethylhexanoic acid (3.0% yield, 100% LCMS purity). 100% LCMS purity (Method 1, 1.18 min, M+H = 388.3). 98% NMR purity [¹H NMR (500 MHz, DMSO-d₆) δ 10.79 – 10.75 (m, 1H), 8.12 (d, J = 8.2 Hz, 1H), 7.79 (s, 1H), 7.57 (d, J = 7.9 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.13 – 7.11 (m, 1H), 7.06 – 7.02 (m, 1H), 6.99 – 6.94 (m, 1H), 4.50 – 4.42 (m, 1H), 3.92 – 3.87 (m, 1H), 3.13 (dd, J = 14.7, 4.3 Hz, 1H), 2.86 (dd, J = 14.7, 9.7 Hz, 1H), 1.76 (s, 3H), 1.74 – 1.65 (m, 1H), 1.59 – 1.50 (m, 1H), 1.16 – 1.08 (m, 2H), 0.83 (s, 9H), containing 2% DCM, CO₂H peak not observed.]

Example 12**Synthesis of (S)-5,5-Dimethyl-2-((2S,3S)-3-methyl-2-((R)-morpholine-2-carboxamido)pentanamido)hexanoic acid**

- 5 Example 12 was prepared in an analogous manner to Example 9.

(S)-5,5-Dimethyl-2-((2S,3S)-3-methyl-2-((R)-morpholine-2-carboxamido)pentanamido)hexanoic acid (3% yield, LCMS purity unconfirmed due to weak chromophore, Method 1, 0.67 min, M+H = 386.2). 97% NMR purity [¹H NMR (500 MHz, DMSO-d₆) δ 8.03 (br s, 1H), 7.28 (d, J = 9.2 Hz, 1H), 4.25 – 4.18 (m, 1H), 4.03 – 3.94 (m, 1H), 3.88 – 3.82 (m, 2H), 3.56 – 3.46 (m, 1H), 2.99 – 2.93 (m, 1H), 2.72 – 2.58 (m, 2H), 2.48 – 2.42 (m, 1H), 1.78 – 1.72 (m, 1H), 1.70 – 1.62 (m, 1H), 1.58 – 1.48 (m, 1H), 1.46 – 1.38 (m, 1H), 1.18 – 1.11 (m, 2H), 1.06 – 0.95 (m, 1H), 0.85 – 0.82 (m, 11H), 0.82 – 0.78 (m, 4H), NH and CO₂H peaks not observed.]

15 **Example 13**

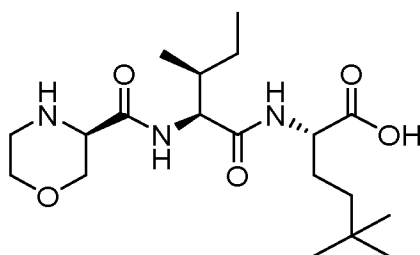
Synthesis of (S)-5,5-Dimethyl-2-((2S,3S)-3-methyl-2-((S)-morpholine-2-carboxamido)pentanamido)hexanoic acid

Example 13 was prepared in an analogous manner to Example 9.

(S)-5,5-Dimethyl-2-((2S,3S)-3-methyl-2-((S)-morpholine-2-carboxamido)pentanamido)hexanoic acid (20% yield, LCMS purity unconfirmed due to weak chromophore, Method 1, 0.66 min, M+H = 386.2). 95% NMR purity [¹H NMR (500 MHz, DMSO-d₆) δ 9.72 - 8.66 (m, 1H), 8.09 (br s, 1H), 7.27 (d, J = 9.2 Hz, 1H), 4.26 (dd, J = 9.2, 6.9 Hz, 1H), 4.04 – 3.96 (m, 1H), 3.87 – 3.79 (m, 2H), 3.50 (td, J = 10.9, 3.2 Hz, 1H), 3.01 (dd, J = 12.5, 2.8 Hz, 1H), 2.71 – 2.62 (m, 2H), 2.48 – 2.41 (m, 1H), 1.78 – 1.70 (m, 1H), 1.69 – 1.61 (m, 1H), 1.58 – 1.49 (m, 1H), 1.45 – 1.36 (m, 1H), 1.18 – 1.11 (m, 2H), 1.05 – 0.95 (m, 1H), 0.83 (d, J = 2.0 Hz, 9H), 0.83 – 0.78 (m, 6H), containing 3% Acetonitrile, no CO₂H peak observed.]

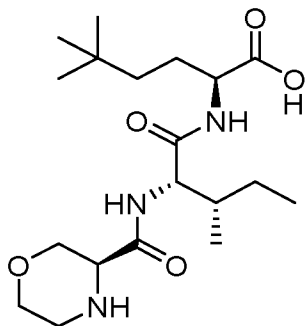
Example 14

Synthesis of (S)-5,5-Dimethyl-2-((2S,3S)-3-methyl-2-((R)-morpholine-3-carboxamido)pentanamido)hexanoic acid



Example 14 was prepared in an analogous manner to Example 9.

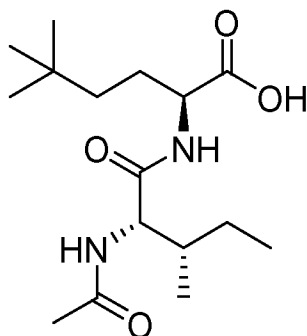
(S)-5,5-Dimethyl-2-((2S,3S)-3-methyl-2-((R)-morpholine-3-carboxamido)pentanamido)hexanoic acid (7.2% yield, LCMS purity unconfirmed due to weak chromophore, Method 1, 0.67 min, M+H = 386.2). 95% NMR purity 95% [¹H NMR (500 MHz, DMSO-d₆) δ 9.16 - 8.74 (m, 1H), 8.12 (br s, 1H), 7.25 (d, J = 9.2 Hz, 1H), 4.26 (dd, J = 9.2, 6.9 Hz, 1H), 4.04 – 3.97 (m, 1H), 3.86 – 3.78 (m, 2H), 3.50 (td, J = 10.9, 3.2 Hz, 1H), 3.01 (dd, J = 12.1, 2.8 Hz, 1H), 2.71 – 2.60 (m, 2H), 2.45 (dd, J = 12.4, 10.2 Hz, 1H), 1.77 – 1.69 (m, 1H), 1.69 – 1.61 (m, 1H), 1.60 – 1.49 (m, 1H), 1.44 – 1.36 (m, 1H), 1.18 – 1.11 (m, 2H), 1.05 – 0.95 (m, 1H), 0.83 (s, 9H), 0.83 – 0.79 (m, 6H), containing 2% Acetonitrile, CO₂H peak not observed.]

Example 15**Synthesis of (S)-5,5-Dimethyl-2-((2S,3S)-3-methyl-2-((S)-morpholine-3-carboxamido)pentanamido)hexanoic acid**

- 5 Example 15 was prepared in an analogous manner to Example 9.

(S)-5,5-Dimethyl-2-((2S,3S)-3-methyl-2-((S)-morpholine-3-carboxamido)pentanamido)hexanoic acid (15% yield, Method 1, 0.66 min, M+H = 386.2). 87% NMR purity [¹H NMR (500 MHz, DMSO-d₆) δ 8.17 – 8.07 (m, 1H), 7.78 (d, J = 9.2 Hz, 1H), 4.27 (dd, J = 9.2, 6.6 Hz, 1H), 4.08 – 4.01 (m, 1H), 3.70 (dd, J = 10.9, 3.6 Hz, 1H), 3.55 (dt, J = 11.2, 3.7 Hz, 2H), 3.46 (dd, J = 10.9, 7.7 Hz, 1H), 2.91 – 2.82 (m, 1H), 2.78 – 2.61 (m, 3H), 1.79 – 1.71 (m, 1H), 1.71 – 1.61 (m, 1H), 1.61 – 1.49 (m, 1H), 1.49 – 1.39 (m, 1H), 1.22 – 1.11 (m, 2H), 1.08 – 0.99 (m, 1H), 0.85 (s, 2H), 0.84 (s, 9H), 0.82 (t, J = 7.9 Hz, 4H), CO₂H peak not observed.]

15 **Example 16**

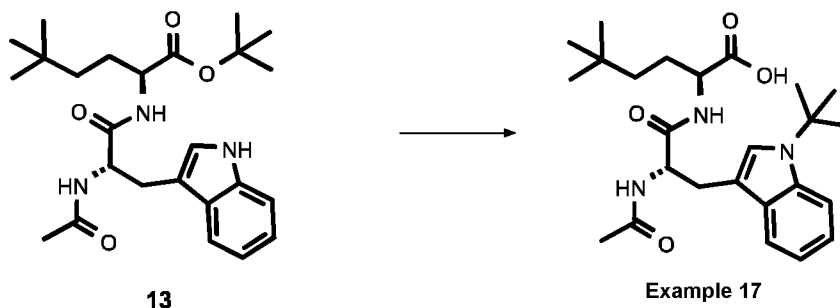
Synthesis of (S)-2-((2S,3S)-2-Acetamido-3-methylpentanamido)-5,5-dimethylhexanoic acid

Example 16 was prepared in an analogous manner to Example 9.

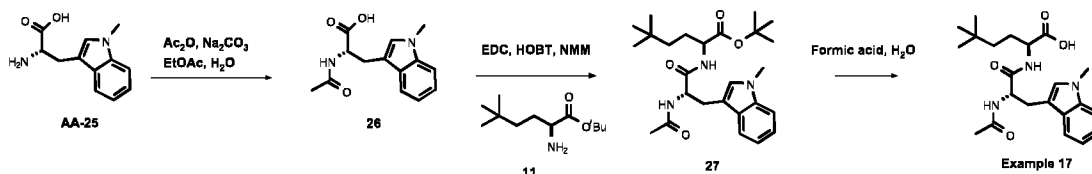
(S)-2-((2S,3S)-2-Acetamido-3-methylpentanamido)-5,5-dimethylhexanoic acid (30% yield, 100% LCMS purity =, Method 3, 1.09 min, M+H = 315.2). 98% NMR purity [¹H NMR (500 MHz, DMSO-d₆) δ 12.46 (br s, 1H), 8.06 (d, J = 7.3 Hz, 1H), 7.85 (d, J = 9.1 Hz, 1H), 4.23 (dd, J = 9.0, 7.5 Hz, 1H), 4.12 – 4.03 (m, 1H), 1.84 (s, 3H), 1.74 – 1.62 (m, 2H), 1.61 – 1.50 (m, 1H), 1.47 – 1.38 (m, 1H), 1.25 – 1.13 (m, 2H), 1.13 – 1.02 (m, 1H), 0.85 (s, 9H), 0.83 (s, 3H), 0.80 (t, J = 7.4 Hz, 3H).]

Example 17

(2S)-2-[(2S)-3-(1-tert-butyl-1H-indol-3-yl)-2-acetamidopropanamido]-5,5-dimethylhexanoic acid



Tert-butyl (S)-2-((S)-2-acetamido-3-(1H-indol-3-yl)propanamido)-5,5-dimethylhexanoate **13** (480 mg, 1.08 mmol) was dissolved in ice/cold 2,2,2-trifluoroacetic acid (50 mL), the ice bath removed and stirred for 45 minutes. The TFA was evaporated in vacuo at 25 °C, azeotroped with toluene (50 mL) and then dissolved in DCM (10 mL) and adsorbed onto celite. The DCM was evaporated in vacuo and the material purified by chromatography on RP Flash C18 (24 g cartridge, 5-50% MeCN/10 mM ammonium bicarbonate) to afford the title compound (2S)-2-[(2S)-3-(1-tert-butyl-1H-indol-3-yl)-2-acetamidopropanamido]-5,5-dimethylhexanoic acid (**Example 17**, 4 mg, 0.009 mmol, 0.8 %) as a pale brown solid. 98% purity LCMS (Method 1, 1.61 min; M+H = 444.2). ¹H NMR (500 MHz, DMSO-d₆) δ 12.61 (s, 1H), 8.15 (d, J = 7.7 Hz, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.65 - 7.57 (m, 2H), 7.28 (s, 1H), 7.07 (ddd, J = 8.4, 6.9, 1.3 Hz, 1H), 6.99 (app. t, J = 7.4 Hz, 1H), 4.63 - 4.55 (m, 1H), 4.16 – 4.09 (m, 1H), 3.08 (dd, J = 14.7, 5.1 Hz, 1H), 2.84 (dd, J = 14.8, 8.8 Hz, 1H), 1.77 (s, 3H), 1.73 – 1.65 (m, 1H), 1.64 (s, 9H), 1.61 – 1.49 (m, 1H), 1.24 – 1.11 (m, 2H), 0.85 (s, 9H).

Example 18**(2S)-2-[(2S)-2-acetamido-3-(1-methyl-1H-indol-3-yl)propanamido]-5,5-dimethylhexanoic acid****5 Synthesis of (2S)-2-acetamido-3-(1-methyl-1H-indol-3-yl)propanoic acid (26)**

Ac₂O (0.16 mL, 1.6 mmol) was added dropwise to a vigorously stirred mixture of 1-methyl-L-tryptophan (0.30 g, 1.4 mmol) and sodium carbonate (0.44 g, 4.1 mmol) in EtOAc (10 mL) and water (10 mL) and then stirred for 2 hours. The reaction was adjusted to pH 3 with 2M HCl, extracted with EtOAc (20 mL), washed with brine (10 mL), dried (MgSO₄), filtered and evaporated in vacuo to afford (2S)-2-acetamido-3-(1-methyl-1H-indol-3-yl)propanoic acid (**26**, 0.31 g, 1.1 mmol, 83 %) as an off-white solid. 97% purity LCMS (Method 1, 0.98 min; M+H = 261.1). ¹H NMR (500 MHz, DMSO-d₆) δ 12.59 (s, 1H), 8.13 (d, J = 7.8 Hz, 1H), 7.54 (d, J = 7.8, Hz, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.13 (dd, J = 8.1, 6.9 Hz, 1H), 7.10 (s, 1H), 7.02 (dd, J = 8.0, 6.9 Hz, 1H), 4.44 (td, J = 8.2, 5.2 Hz, 1H), 3.72 (s, 3H), 3.13 (dd, J = 14.6, 5.3 Hz, 1H), 2.98 (dd, J = 14.6, 8.5 Hz, 1H), 1.80 (s, 3H).

Synthesis of *tert*-butyl (2S)-2-[(2S)-2-acetamido-3-(1-methyl-1H-indol-3-yl)propanamido]-5,5-dimethylhexanoate (27)

HOBt (97 mg, 0.63 mmol) was added to a stirred solution of N α -acetyl-1-methyl-L-tryptophan (0.15 g, 0.58 mmol) and *tert*-butyl (2S)-2-amino-5,5-dimethylhexanoate (0.13 g, 0.61 mmol) in DMF (1 mL). The reaction was cooled to 0 °C, EDC (0.12 g, 0.61 mmol) and 4-methylmorpholine (0.13 mL, 1.2 mmol) added, allowed to attain room temperature and stirred for 4 h. The reaction was quenched with water (20 mL), extracted with EtOAc (2 x 20 mL), washed with water (2 x 10 mL) and brine (5mL), dried (MgSO₄), filtered, evaporated in vacuo

and purified by chromatography on silica gel (24 g FlashPure column, 0 - 100% EtOAc/isohexane) to afford *tert*-butyl (2*S*)-2-((2*S*)-2-acetamido-3-(1-methyl-1*H*-indol-3-yl)propanamido)-5,5-dimethylhexanoate (**27**, 0.17 g, 0.36 mmol, 63 %) as a colourless gum. 100% purity LCMS (Method 2, 1.83 min; *M*+*H* = 458.1). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.23 (d, *J* = 7.4 Hz, 1H), 8.02 (d, *J* = 8.3 Hz, 1H), 7.66 - 7.62 (m, 1H), 7.37 - 7.34 (m, 1H), 7.14 - 7.10 (m, 1H), 7.09 (s, 1H), 7.03 - 6.99 (m, 1H), 4.58 (td, *J* = 8.8, 4.6 Hz, 1H), 4.08 (td, *J* = 7.4, 5.7 Hz, 1H), 3.72 (s, 3H), 3.09 (dd, *J* = 14.6, 4.6 Hz, 1H), 2.87 (dd, *J* = 14.7, 9.3 Hz, 1H), 1.76 (s, 3H), 1.69 - 1.52 (m, 2H), 1.40 (s, 9H), 1.21 - 1.16 (m, 2H), 0.86 (s, 9H).

10 **Synthesis of (S)-2-((S)-2-acetamido-3-(1-methyl-1*H*-indol-3-yl)propanamido)-5,5-dimethylhexanoic acid (Example 18)**

tert-butyl (2*S*)-2-((2*S*)-2-acetamido-3-(1-methyl-1*H*-indol-3-yl)propanamido)-5,5-dimethylhexanoate (165 mg, 361 μmol) was dissolved in an ice/cold mixture of formic acid (5 mL) and water (0.5 mL). The ice bath was then removed and the reaction stirred for 20 hours. The solvent was evaporated in vacuo, azeotroped with toluene and treated with ether to afford the title compound (2*S*)-2-((2*S*)-2-acetamido-3-(1-methyl-1*H*-indol-3-yl)propanamido)-5,5-dimethylhexanoic acid (**Example 18**, 142 mg, 0.35 mmol, 96 %) as an off-white solid. 98% purity LCMS (Method 1, 1.39 min; *M*+*H* = 402.5). ¹H NMR (500 MHz, DMSO-*d*₆) δ 12.60 (s, 1H), 8.17 (d, *J* = 7.7 Hz, 1H), 8.02 (d, *J* = 8.3 Hz, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.12 (t, *J* = 7.6 Hz, 1H), 7.09 (s, 1H), 7.01 (t, *J* = 7.4 Hz, 1H), 4.57 (td, *J* = 8.6, 4.8 Hz, 1H), 4.16 (td, *J* = 8.1, 5.3 Hz, 1H), 3.71 (s, 3H), 3.09 (dd, *J* = 14.7, 4.8 Hz, 1H), 2.87 (dd, *J* = 14.7, 9.0 Hz, 1H), 1.76 (s, 3H), 1.73 - 1.65 (m, 1H), 1.63 - 1.53 (m, 1H), 1.26 - 1.11 (m, 2H), 0.86 (s, 9H).

BIOLOGICAL DATA**Neurotensin scintillation proximity assay**

The data of exemplified compounds of the invention tested in a Neurotensin (NTS) scintillation proximity assay (SPA). The IC₅₀ data is shown in the table below.

- 5 NTS, which is a 13 amino acid neuropeptide, is a sortilin ligand. The IC₅₀ is a measure of the amount of the compound required to inhibit the binding of NTS to sortilin by 50%. The skilled person will recognise that the lower the IC₅₀ value, the less of the compound needed to achieve the desired effect, and as a result, the chances of undesirable off-target effects are reduced.
- 10 Compound affinity was determined by measuring the displacement of [3H]-neurotensin or [125I]-neurotensin binding to hSortilin in SPA format. Total volume of 40 µl in 50 mM HEPES pH 7.4 assay buffer containing 100 mM NaCl, 2.0 mM CaCl₂, 0.1% BSA and 0.1% Tween-20. Compound pre-incubation for 30 minutes at room temperature with 150 nM of 6his-Sortilin before 5 nM [3H]-
- 15 Neurotensin or [125I]-neurotensin and Ni chelate imaging beads (Perkin Elmer) were added, after 6 hours plate was read on a ViewLux with 360 s exposure time. Dose-response evaluation of compounds was performed with 8 concentrations of drugs (covering 3 decades). IC₅₀ values were calculated by nonlinear regression using the sigmoid concentration-response (variable slope) using CDD Vault
- 20 software. All values reported are average of at least 2 determinations.

The data in the table below shows that the compounds disclosed herein are sortilin inhibitors.

Representative Examples	IC₅₀ [3H]Neurotensin SPA	IC₅₀ [125I]Neurotensin SPA
Example 1	1670 nM	-
Example 3	90 nM	-
Example 4	140 nM	-
Example 5	270 nM	-
Examples 6 and 11	60 nM	-

Example 8	-	2.17 μ M
Example 9	-	620 nM
Example 10	2.03 μ M	-
Example 12	150 nM	
Example 13	120 nM	-
Example 14	240 nM	-
Example 15	310 nM	-
Example 16	-	290 nM
Example 17	100 nM	-
Example 18	90 nM	-

X-RAY DATA

Materials and methods

sSortilin, the luminal domain of sortilin, was obtained as previously described (Andersen et al., Acta Cryst. D, 2017)¹⁷. Prior to crystallization 12 μ l of 4 mg/ml sSortilin in 50 mM Tris–HCl pH 8.0, 150 mM NaCl was mixed with 1.2 μ l of the two compounds INS1767 and INS1783 dissolved in DMSO at concentrations of 16.7 mM and 13.2 mM respectively.

Sitting drops were set up by adding 2 μ l of the sortilin ligand mixture to 2 μ l of reservoir solution composed of 100 mM Hepes pH 7.3, 400 mM malonate pH 7.3, 8% v/v glycerol, 22.5% w/v PEG3350. The sitting drops were left to equilibrate by vapor diffusion with 500 μ l reservoir solution.

Crystals were mounted in litho-loops without further cryoprotection and were flash cooled in liquid nitrogen.

Diffraction data were collected at beamline P13 EMBL/DESY, Hamburg, and processed using the XDS package (Kabsch, W., Acta Cryst. D, 2010)¹⁸ (Table 1)

Phases for the structure factors were obtained by molecular replacement using the known structure of sortilin (PDB entry: 3F6K) as search model and the program Phaser implemented in the Phenix software package (Afonine et al., Acta Cryst. D, 2012)¹⁹. A refined model was obtained by several cycles of model building in Coot (Emsley P. et al., Acta Cryst. D, 2010)²⁰ and maximum likelihood refinement using Phenix. The resulting X-ray derived picture of the compound of Example 3 bound to *h*-sortilin is shown in Figure 1. Refinement statistics and agreement of the models with standard geometry are shown in the table below.

Refinement statistics and agreement of the models with standard geometry

(Statistics for the highest resolution shell are shown in parentheses)

	Example 3
Wavelength	
Resolution range	47.2 - 2.6 (2.69 -2.6)
Space group	C 1 2 1
Unit cell (a,b,c) Å (α,β,γ) °	162.54 78.24 112.5 90 126.951 90
Total reflections	462840 (44942)
Unique reflections	34897 (3450)
Multiplicity	13.3 (13.0)
Completeness (%)	99.89 (99.83)
Mean I/sigma(I)	15.36 (1.17)
Wilson B-factor	86.37
R-merge	0.09716 (2.081)
R-meas	0.1012 (2.166)
R-pim	0.02794 (0.5955)
CC1/2	0.999 (0.645)
CC*	1 (0.886)
Reflections in refinement	34878 (3445)
Reflections used for R-free	1394 (138)

R-work	0.2017 (0.4356)
R-free	0.2343 (0.4964)
CC(work)	0.954 (0.761)
CC(free)	0.933 (0.747)
Number of non-hydrogen atoms macromolecules	5396
Protein residues	657
RMS(bonds) Å	0.002
RMS(angles) °	0.42
Ramachandran favored (%)	95.39
Ramachandran allowed (%)	4.30
Ramachandran outliers (%)	0.31
Rotamer outliers (%)	1.97
Clashscore	3.97
Average B-factor macromolecules	107.08
Number of TLS groups	6

REFERENCES

1. Tauris, J., et al., Proneurotrophin-3 May Induce Sortilin-Dependent Death In Inner Ear Neurons. *Eur J Neuroscience* (2020), 33(4), pp.622-31.
2. Goettsch, C., et al., Sortilin and Its Multiple Roles in Cardiovascular and Metabolic Diseases. *Atherosclerosis, Thrombosis and Vascular Biology* (2017), 38(1), pp. 19-25.
3. Willnow, T.E., et al., Sortilins: new players in lipoprotein metabolism. *Current Opinion in Lipidology* (2011), 22(2), pp. 79-85.
4. Kjolby, M., et al., Sort1, encoded by the cardiovascular risk locus 1p13.3, is a regulator of hepatic lipoprotein export. *Cell Metabolism* (2010), 12(3), pp. 213-223.
5. Jansen, P., et al., Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. *Nature Neuroscience* (2007), 10(11), pp.1449-1457.
6. Tenk, H.K., et al., ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *J Neuroscience* (2005), 10(11), pp.1449-1457.
7. Nykjaer, A., et al., Sortilin is essential for proNGF-induced neuronal cell death. *Nature* (2004), 427(6977), pp.843-848.
8. Huang, G. et al., Insulin responsiveness of glucose transporter 4 in 3T3-L1 cells depends on the presence of sortilin. *Mol Biol Cell* (2013), 24(19), pp.3115-3122.
9. Pan, X. et al., Sortilin and retromer mediate retrograde transport of Glut4 in 3T3-L1 adipocytes. *Mol Biol Cell* (2017), 28(12), pp.1667-1675.
10. Kaddai, V. et al. Involvement of TNF- α in abnormal adipocyte and muscle sortilin expression in obese mice and humans. *Diabetologia* (2009) 52, pp. 932-940.

11. Mortensen, M.B. et al., Targeting sortilin in immune cells reduces proinflammatory cytokines and atherosclerosis. *J Clin Invest* (2014), 124(12), pp. 5317-5322.
12. Shi, J. & Kandror, K. V., Sortilin Is Essential and Sufficient for the Formation
5 of Glut4 Storage Vesicles in 3T3-L1 Adipocytes. *Developmental Cell* (2005), 9,
pp. 99-108.
13. Gao, A. et al., Implications of Sortilin in Lipid Metabolism and Lipid Disorder
Diseases. *DNA and Cell Biology* (2017), 36(12), pp.1050-1061.
14. Oh, T.J. et al., Circulating sortilin level as a potential biomarker for coronary
10 atherosclerosis and diabetes mellitus. *Cardiovascular Diabetology* (2017), 16(92).
15. Skeldal, S. et al., Mapping of the Interaction Site between Sortilin and the p75
Neurotrophin Receptor Reveals a Regulatory Role for the Sortilin Intracellular
Domain in p75 Neurotrophin Receptor Shedding and Apoptosis. *J Biol Chem*
(2012), 21(287), pp. 43798-43809.
16. Wuts, P.G.M. and Greene, T.W, Greene's Protective Groups in Organic
15 Synthesis, 4th Edition, John Wiley and Sons, New York (2006).
17. Andersen, K R et al., Introducing site-specific cysteines into nanobodies for
mercury labelling allows de novo phasing of their crystal structures. *Acta
Crystallographia Section. D* (2017) 73(1), pp. 804-813.
18. Kabsch, W, XDS. *Acta Crystallographia Section. D* (2010) 66(2), pp. 125-132.
20
19. Afonine, P V et al., *Acta Crystallographia Section. D* (2012) 68(4), pp. 352-
367.
20. Emsley, P. et al., *Acta Crystallographia Section. D* (2010) 66(4), pp. 486-501.

Sequences referenced throughout the specification and forming part of the description

SEQ ID NO: 1 (full length sortilin- isoform 1)

```

5   1   MERPWGAADG LSRWPHGLGL LLLLQLLPPS TLSQDRLDAP PPPAAPLPRW
    51  SGPIGVSWGL RAAAAGGAFP RGGRWRRSAP GEDEECGRVR DFVAKLANNT
   101  HGHVFDLRLG SVLSWVGDS TGVILVLTTF HVPLVIMTFG QSKLYRSEDY
   151  GKNFKDITDL INNTFIRTEF GMAIGPENSG KVVLTAEVSG GSRGGRIFRS
   201  SDFAKNFVQT DLPFHPLTQM MYSPQNSDY LALSTENGLW VSKNFGGKWE
  10  251  EHKAVCLAK WGSNTIFFT TYANGSCKAD LGALELWRTS DLGKSFKTIG
    301  VKIYSFGLGG RFLFASVMAD KDTTRRIHVS TDQGDWTSMA QLPSVGQEQF
    351  YSILAANDDM VFMHVDEPGD TGFGTIFTSD DRGIVYSKSL DRHLYTTTGG
    401  ETDFTNVTSL RGVYITSVLS EDNSIQTMIT FDQGGRWTHL RKPENSECDA
    451  TAKNKNESCL HIHASYSISQ KLNVPMAPLS EPNAVGVIA HGSVGDAISV
  15  501  MVPDVYISDD GGYSWTKMLE GPHYTYILDS GGIIVAIHS SRPINVIKFS
    551  TDEGQCWQTY TFRDPIYFT GLASEPGARS MNISIWGFTE SFLTSQWWSY
    601  TIDFKDILER NCEEKDYTIW LAHSTDPEDEY EDGCILGYKE QFLRLRKSSM
    651  CQNGRDYVVT KQPSICLCSL EDFLCDFGYR RPENDSKCQE QPELKGHDLE
    701  FCLYGREEHL TTNGYRKIPG DKCQGGVNPV REVKDLKKKC TSNFLSPEKQ
  20  751  NKSNSVPII LAIVGLMLVT VVAGVLIVKK YVCGGRFLVH RYSLVQQHAE
    801  ANGVGDVDAL DTASHTNKSG YHDDSDEDL E

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SEQ ID NO: 2 (full length sortilin- isoform 2)

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25  1   MERPWGAADG LSRWPHGLGL LLLLQLLPPS TLSQDRLDAP PPPAAPLPRW
    51  SGPIGVSWGL RAAAAGGAFP RGGRWRRSAP GEDEECGRVR DFVAKLANNT
   101  HGHVFDLRLG SVLSWVGDS TGVILVLTTF HVPLVIMTFG QSKLYRSEDY
   151  GKNFKDITDL INNTFIRTEF GMAIGPENSG KVVLTAEVSG GSRGGRIFRS
   201  SDFAKNFVQT DLPFHPLTQM MYSPQNSDY LALSTENGLW VSKNFGGKWE
  30  251  EHKAVCLAK WGSNTIFFT TYANGSCTDL GALELWRTSD LGKSFKTIGV
    301  KIYSFGLGGR FLFASVMADK DTTTRRIHVST DQGDWTSMAQ LPSVGQEQFY
    351  SILAANDDMV FMHVDEPGDT GFGTIFTSD RGVYSKSLD RHLYTTTGG E
    401  TDFTNVTSLR GVIYITSLSE DNSIQTMITF DQGGRWTHLR KPENSECDAT
    451  AKNKNESCLH IHASYSISQK LNVPMAPLSE PNAVGVIAH GSVGDAISVM
  35  501  VPDVYISDDG GYSWTKMLEG PHYTYILDSG GIIVAIHSS RPINVIKFS
    551  DEGQCWQTYT FTRDPIYFTG LASEPGARSM NISIWGFTE FLTSQWWSYT
    601  IDFKDILERN CEEKDYTIWL AHSTDPEDEY DGCILGYKEQ FLRLRKSSVC
    651  QNGRDYVVT KQPSICLCSLE DFLCDFGYR PENDSKCQE PELKGHDLEF
    701  CLYGREEHLT TNGYRKIPG DKCQGGVNPV EVKDLKKKCT SNFLSPEKQN
  40  751  SKNSVPII AIVGLMLVT VAGVLIVKK YVCGGRFLVH RYSLVQQHAE
    801  NGVDGVDALD TASHTNKSGY HDDSDEDLLE

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SEQ ID NO: 3 (mature sortilin)

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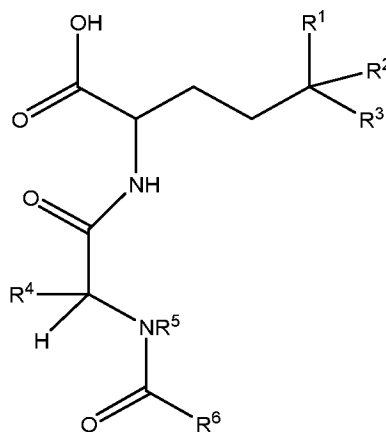
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   101  NGLWWSKNFG GKWEEIHKAV CLAKWGSNT IFFTTYANGS CTDLGALELW
   151  RTSDLGKSFK TIGVKIYSFG LGGRFLFASV MADKDTTRRI HVSTDQGDW
   201  SMAQLPSVGQ EQFYSLAAN DDMVFMHVDE PGDTGFGTIF TSDDRGIVYS
  50  251  KSLDRHLYTT TGGETDFTNV TSLRGVYITS VLSEDNSIQT MITFDQGGRW
    301  THLRKPENSE CDATAKNKNE CSLHIHASYS ISQKLNVPMA PLSENAVGI
    361  VIAHGSVGDA ISVMVPDVI SDDGGYSWTK MLEGPHYTYI LDSGGIIVAI
    401  EHSSRPINVI KFSTDEGQCW QTYTFTRDPI YFTGLASEPG ARSMNISIWG
    451  FTESFLTSQW VSYTIDFKDI LERNCEEKDY TIWLAHSTD P EDYEDGCILG
  55  501  YKEQFLRLRK SSVQCNGRDY VTKQPSICL CSLEDFLCDF GYYRPENDSK

```

551 CVEQPELKGH DLEFCLYGRE EHLTTNGYRK IPGDKCQGGV NPVREVKDLK
601 KKCTSNFLSP EKQNSKSNSV PIILAIVGLM LVTVVAGVLI VKKYVCGGRF
651 LVHRYSVLQQ HAEANGVDGV DALDTASHTN KSGYHDDSDE DLLE

CLAIMS

1. A compound of formula (I)



5

(I)

or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, optical isomer, N-oxide, and/or prodrug thereof; wherein

R¹, R² and R³ are each independently selected from the group consisting of halo, H, (C₁-C₄)alkyl, halo-(C₁-C₄)alkyl, (C₂-C₄)alkenyl, and halo-(C₂-C₄)alkenyl;

- 10 R⁴ is selected from the group consisting of H, (C₁-C₁₀)alkyl, halo-(C₁-C₁₀)alkyl, (C₂-C₁₀)alkenyl, halo-(C₂-C₁₀)alkenyl, (C₃-C₂₀)aryl, halo-(C₃-C₂₀)aryl, (C₃-C₈)heteroaryl, halo-(C₃-C₂₀)heteroaryl, (C₁-C₆)-alkylene-(C₃-C₂₀)-aryl, (C₁-C₆)-alkylene-(C₃-C₂₀)-heteroaryl, (C₁-C₆)-alkylene-(3- to 10- membered-heterocyclic ring);

- 15 wherein the aryl group in (C₁-C₆)-alkylene-(C₃-C₂₀)-aryl, the heteroaryl group in (C₁-C₆)-alkylene-(C₃-C₂₀)-heteroaryl or the heterocyclic ring in (C₁-C₆)-alkylene-(3- to 8- membered heterocyclic ring) is optionally substituted with one or more substituents independently selected from halo, H, -OH, (C₁-C₄)alkyl, halo-(C₁-C₄)alkyl, (C₁-C₄)alkoxy and halo-(C₁-C₄)alkoxy;

- 20 R⁵ is selected from the group consisting of H, (C₁-C₄)alkyl, halo-(C₁-C₄)alkyl, (C₂-C₄)alkenyl and halo-(C₁-C₄)alkenyl; and

R^6 is selected from the group consisting of (C₁-C₆)alkyl, halo-(C₁-C₆)alkyl, (C₂-C₆)alkenyl, halo-(C₂-C₆)alkenyl, (C₃-C₂₀)aryl, halo-(C₃-C₂₀)aryl, (C₃-C₂₀)heteroaryl and halo-(C₃-C₂₀)heteroaryl.

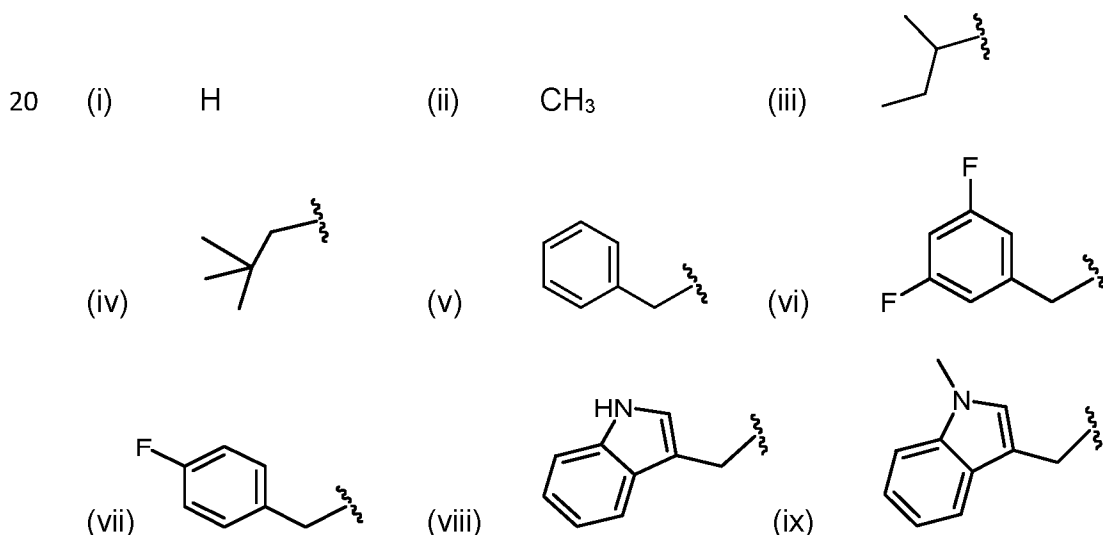
2. The compound according to claim 1, wherein R^1 , R^2 and R^3 are each independently selected from the group consisting of halo, (C₁-C₂)alkyl and halo-(C₁-C₂)alkyl.

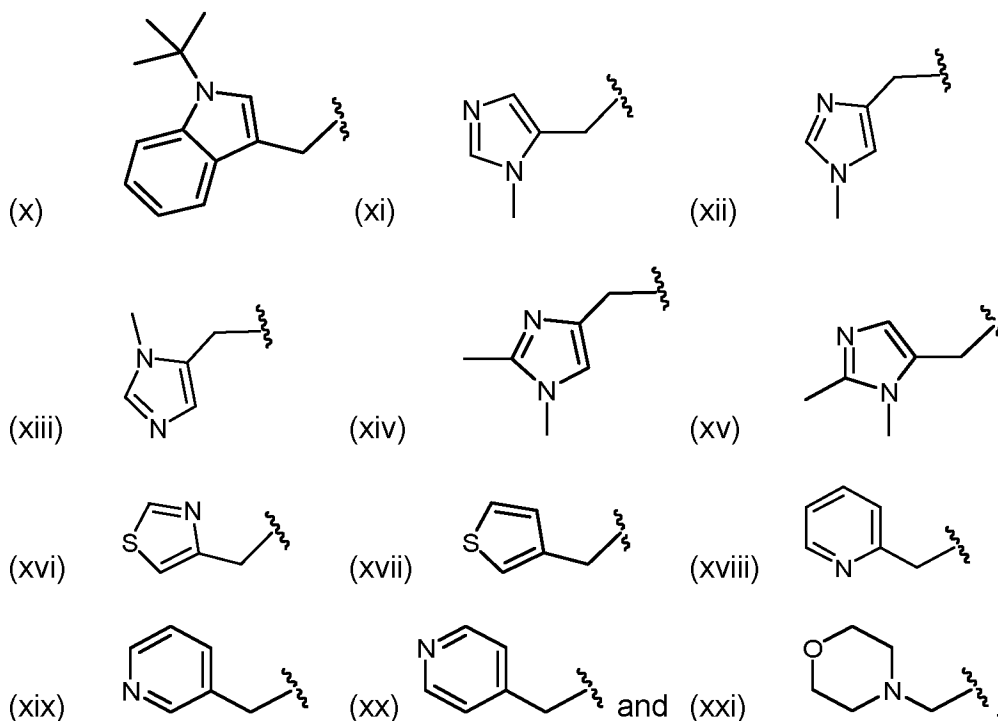
3. The compound according to claim 1 or claim 2, wherein R^1 , R^2 and R^3 are each independently selected from F, CH₃ and CF₃.

4. The compound according to any preceding claim, wherein R^4 is selected from the group consisting of H, (C₁-C₆)alkyl, halo-(C₁-C₆)alkyl, (C₃-C₁₀)aryl, (C₁-C₃)-alkylene-(C₃-C₁₀)-aryl, (C₁-C₃)-alkylene-(C₃-C₂₀)-heteroaryl and (C₁-C₃)-alkylene-(3- to 10- membered-heterocyclic ring);

wherein the aryl group in (C₁-C₃)-alkylene-(C₃-C₁₀)-aryl, the heteroaryl group in (C₁-C₃)-alkylene-(C₃-C₁₀)-heteroaryl or the heterocyclic ring in (C₁-C₃)-alkylene-(3- to 8- membered heterocyclic ring) is optionally substituted with one or more substituents independently selected from halo, -OH, (C₁-C₄)alkyl, halo-(C₁-C₄)alkyl, (C₁-C₄)alkoxy and halo-(C₁-C₄)alkoxy.

5. The compound according to any preceding claim, wherein R^4 is selected from the group consisting of:





5 6. The compound according to any preceding claim, wherein R^5 is selected from the group consisting of H, (C₁-C₃)-alkyl and (C₁-C₃)haloalkyl.

7. The compound according to any preceding claim, wherein R^5 is selected from the group consisting of H and CH₃.

8. The compound according to any preceding claim, wherein R^6 is selected
 10 from the group consisting of (C₁-C₄)alkyl, halo-(C₁-C₄)alkyl, (C₃-C₈)heteroaryl, and halo-(C₃-C₈)heteroaryl.

9. The compound according to any preceding claim, wherein R^6 is selected from the group consisting of CH₃, pyrazine and morpholine.

10. The compound according to any preceding claim, wherein the compound
 15 of Formula (I) is:

(S)-2-(2-acetamidoacetamido)-5,5-dimethylhexanoic acid;

(S)-5,5-dimethyl-2-(2-(pyrazine-2-carboxamido)acetamido)hexanoic acid;

(S)-2-((S)-2-acetamido-3-phenylpropanamido)-5,5-dimethylhexanoic acid;

(2S)-5,5-dimethyl-2-{2-[(pyrazin-2-yl)formamido]acetamido}hexanoic acid;

- (2S)-2-[(2S,3S)-2-acetamido-3-methylpentanamido]-5,5-dimethylhexanoic acid;
- (2S)-5,5-dimethyl-2-[(2S,3S)-3-methyl-2-[(pyrazin-2-yl)formamido]pentanamido]hexanoic acid;
- (2S)-5,5,5-trifluoro-2-[(2S,3S)-3-methyl-2-[(pyrazin-2-yl)formamido]pentanamido]pentanoic acid;
- 5 (2S)-2-[(2S)-2-acetamido-3-phenylpropanamido]-5,5-dimethylhexanoic acid;
- (2S)-2-[(2S)-2-acetamido-3-(4-fluorophenyl)propanamido]-5,5-dimethylhexanoic acid;
- (2S)-2-[(2S)-3-(3,5-difluorophenyl)-2-acetamidopropanamido]-5,5-
- 10 dimethylhexanoic acid;
- (2S)-2-[(2S)-2-acetamido-3-(1H-indol-3-yl)propanamido]-5,5-dimethylhexanoic acid;
- (2S)-2-[(2S)-2-acetamido-3-(1-methyl-1H-imidazol-5-yl)propanamido]-5,5-dimethylhexanoic acid;
- 15 (S)-5,5-dimethyl-2-[(S)-3-phenyl-2-(pyrazine-2-carboxamido)propanamido]hexanoic acid;
- (2S)-2-[(2S)-2-acetamido-3-(1-methyl-1H-imidazol-4-yl)propanamido]-5,5-dimethylhexanoic acid;
- (2S)-5,5-dimethyl-2-[(2S,3S)-3-methyl-2-[(3S)-morpholin-3-yl]formamido}pentanamido]hexanoic acid;
- 20 (2S)-5,5-dimethyl-2-[(2S,3S)-3-methyl-2-[(3R)-morpholin-3-yl]formamido}pentanamido]hexanoic acid;
- (2S)-5,5-dimethyl-2-[(2S,3S)-3-methyl-2-[(2S)-morpholin-2-yl]formamido}pentanamido]hexanoic acid;
- 25 (2S)-5,5-dimethyl-2-[(2S,3S)-3-methyl-2-[(2R)-morpholin-2-yl]formamido}pentanamido]hexanoic acid;

(2S)-5,5-dimethyl-2-[(2S)-3-(1-methyl-1H-imidazol-4-yl)-2-(N-methylacetamido)propanamido]hexanoic acid;

(2S)-5,5-dimethyl-2-[(2S)-3-(1-methyl-1H-imidazol-5-yl)-2-(N-methylacetamido)propanamido]hexanoic acid;

- 5 (2S)-5,5-dimethyl-2-[(2S)-3-(1-methyl-1H-imidazol-4-yl)-2-(2-oxopyrrolidin-1-yl)propanamido]hexanoic acid;

(2S) - 2 - [(2S) - 3 - (1,2 - dimethyl - 1H - imidazol - 5 - yl) - 2 - (N - methylacetamido)propanamido] - 5,5 - dimethylhexanoic acid;

- 10 (2S) - 2 - [(2S) - 3 - (1,2 - dimethyl - 1H - imidazol - 4 - yl) - 2 - (N - methylacetamido)propanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2R) - 2 - acetamido - 3 - (1H - indol - 3 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (1,3 - thiazol - 4 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

- 15 (2S) - 2 - [(2S) - 2 - acetamidopropanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (thiophen - 3 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (pyridin - 2 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

- 20 (2S) - 2 - [(2S) - 2 - acetamido - 3 - (pyridin - 3 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (pyridin - 4 - yl)propanamido] - 5,5 - dimethylhexanoic acid;

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (morpholin - 4 - yl)propanamido] - 5,5 - dimethylhexanoic acid; and

(2S) - 2 - [(2S) - 2 - acetamido - 3 - (1 - methyl - 1H - indol - 3 - yl)propanamido] - 5,5 - dimethylhexanoic acid.

5 11. A pharmaceutical composition comprising a compound according to any preceding claim and a pharmaceutically acceptable carrier, excipient, and/or diluent.

12. The compound according to any one of claims 1 to 10, or the pharmaceutical composition of claim 10, for use in therapy.

10 13. The compound according to any one of claims 1 to 9, or the pharmaceutical composition of claim 10, for use in the treatment or prevention of a neurodegenerative disorder, an inflammatory disorder, a cancer, pain, diabetes mellitus, diabetic retinopathy, glaucoma, uveitis, cardiovascular disease, hereditary eye conditions or hearing loss.

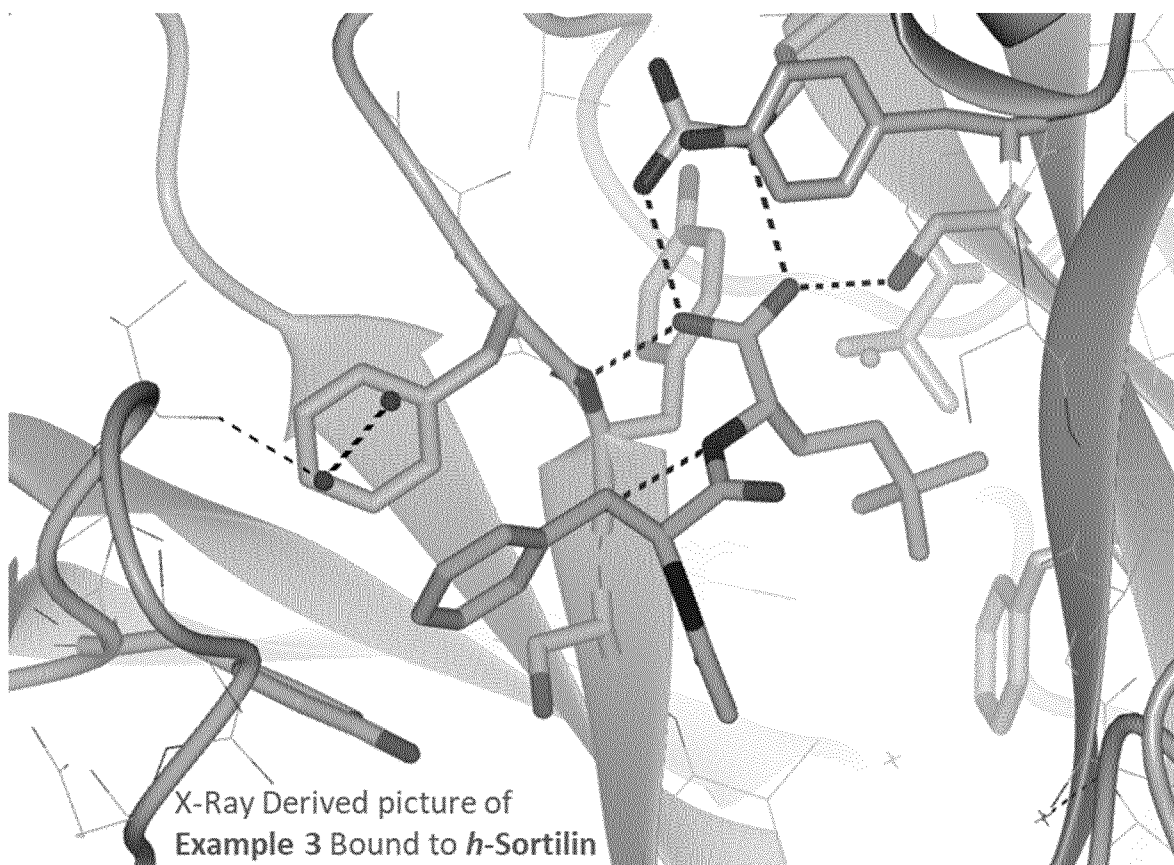
15 14. The compound or pharmaceutical composition for use according to claim 12,

wherein the neurodegenerative disorder is selected from frontotemporal dementia, Alzheimer's disease, Parkinson's disease and spinal cord injury;

wherein the inflammatory disorder may be selected from inflammatory diseases
20 and neuroinflammation;

wherein the cancer is selected from breast cancer, lung cancer, ovarian cancer, prostate cancer, thyroid cancer, pancreatic cancer, glioblastoma and colorectal cancer; and

wherein the hearing loss is selected from noise-induced hearing loss, ototoxicity
25 induced hearing loss, age-induced hearing loss, idiopathic hearing loss, tinnitus and sudden hearing loss.

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/060742

A. CLASSIFICATION OF SUBJECT MATTER INV. C07K5/097 C07K5/065 C07K5/062 C07K5/078 C07K5/06 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		
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		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2016/331746 A1 (PATEL NIKETA A [US] ET AL) 17 November 2016 (2016-11-17) cited in the application claim 1 -----	1-14
A	WO 2014/114779 A1 (LUNDBECK & CO AS H [DK]) 31 July 2014 (2014-07-31) figure 10 -----	1-14
X	US 5 185 324 A (RUEGER WOLFGANG [DE] ET AL) 9 February 1993 (1993-02-09) example 1c, starting material -----	1, 4-8
X	US 5 399 743 A (HIGUCHI NAOKI [JP] ET AL) 21 March 1995 (1995-03-21) example 2, starting material -----	1, 4, 6-9
-/--		
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search	Date of mailing of the international search report	
23 August 2022	06/09/2022	
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 Schleifenbaum, A	

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2022/060742

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CAS Caplus [Online] 1 January 1930 (1930-01-01), Abderhalden Emil ET AL: "Further studies on the nature of enzyme actions, performed with enzymes of the polypeptidase group", XP55939713, Database accession no. 1930:14771 abstract -----	1, 4-8
X	GREGORY M. LAMONTE ET AL: "Development of a Potent Inhibitor of the Plasmodium Proteasome with Reduced Mammalian Toxicity", JOURNAL OF MEDICINAL CHEMISTRY, vol. 60, no. 15, 25 July 2017 (2017-07-25) , pages 6721-6732, XP055484335, US ISSN: 0022-2623, DOI: 10.1021/acs.jmedchem.7b00671 synthetic intermediate fragment comprising P4-P3-P2 -----	1, 4-7
X	JOSE B. ROQUE ET AL: "Deconstructive fluorination of cyclic amines by carbon-carbon cleavage", SCIENCE, vol. 361, no. 6398, 13 July 2018 (2018-07-13), pages 171-174, XP055658244, US ISSN: 0036-8075, DOI: 10.1126/science.aat6365 compound 4b -----	1, 4-8
X	ANONYMOUS: REGISTRY, STN, 28 November 2016 (2016-11-28), XP009537547, the whole document -----	1-9
X	ANONYMOUS: REGISTRY, STN, 25 November 2016 (2016-11-25), XP009537548, the whole document -----	1-9
X	ANONYMOUS: REGISTRY, STN, 24 November 2016 (2016-11-24), XP009537549, the whole document ----- -/--	1-9

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2022/060742

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANONYMOUS: REGISTRY, STN, 16 November 2016 (2016-11-16), XP009537550, the whole document -----	1-9
X	ANONYMOUS: REGISTRY, STN, 15 November 2016 (2016-11-15), XP009537551, the whole document -----	1-9
X	ANONYMOUS: REGISTRY, STN, 14 November 2016 (2016-11-14), XP009537552, the whole document -----	1-9
X	ANONYMOUS: REGISTRY, STN, 28 June 2016 (2016-06-28), XP009537553, the whole document -----	1-9
X	ANONYMOUS: REGISTRY, STN, 27 June 2016 (2016-06-27), XP009537554, the whole document -----	1-9
X	ANONYMOUS: REGISTRY, STN, 18 November 2016 (2016-11-18), XP009537626, the whole document -----	1-9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2022/060742

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2022/060742

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2016331746 A1	17-11-2016	US 2016331746 A1	17-11-2016
		US 2018318296 A1	08-11-2018
WO 2014114779 A1	31-07-2014	CN 104955456 A	30-09-2015
		EP 2948147 A1	02-12-2015
		HK 1217431 A1	13-01-2017
		JP 6409004 B2	17-10-2018
		JP 2016505063 A	18-02-2016
		US 2015368231 A1	24-12-2015
		US 2017239226 A1	24-08-2017
		WO 2014114779 A1	31-07-2014
US 5185324 A	09-02-1993	AT 93863 T	15-09-1993
		AU 632519 B2	07-01-1993
		CA 2005422 A1	14-06-1990
		DE 3842067 A1	21-06-1990
		DK 629889 A	15-06-1990
		EP 0373549 A2	20-06-1990
		ES 2059688 T3	16-11-1994
		HU 205369 B	28-04-1992
		JP H02204475 A	14-08-1990
		KR 900009691 A	05-07-1990
		PT 92558 A	29-06-1990
		US 5185324 A	09-02-1993
		ZA 899507 B	29-08-1990
US 5399743 A	21-03-1995	AT 147755 T	15-02-1997
		DE 69307414 T2	26-06-1997
		EP 0578978 A1	19-01-1994
		JP H05345754 A	27-12-1993
		US 5399743 A	21-03-1995