Enzyme Inhibitors

This invention relates to compounds which inhibit members of the histone deacetylase family of enzymes and to their use in the treatment of cell proliferative diseases, including cancers, polyglutamine diseases, for example Huntingdon disease, neurodegenerative diseases for example Alzheimer disease, autoimmune disease for example rheumatoid arthritis and organ transplant rejection, diabetes, haematological disorders, inflammatory disease, cardiovascular disease, atherosclerosis, and the inflammatory sequelae of infection.

Background to the Invention

In eukaryotic cells DNA is packaged with histones, to form chromatin. Approximately 150 base pairs of DNA are wrapped twice around an octamer of histones (two each of histones 2A, 2B, 3 and 4) to form a nucleosome, the basic unit of chromatin. The ordered structure of chromatin needs to be modified in order to allow transcription of the associated genes. Transcriptional regulation is key to differentiation, proliferation and apoptosis, and is, therefore, tightly controlled. Control of the changes in chromatin structure (and hence of transcription) is mediated by covalent modifications to histones, most notably of the N-terminal tails. Covalent modifications (for example methylation, acetylation, phosphorylation and ubiquitination) of the side chains of amino acids are enzymatically mediated (A review of the covalent modifications of histones and their role in transcriptional regulation can be found in Berger SL 2001 Oncogene 20, 3007-3013; See Grunstein, M 1997 Nature 389, 349-352; Wolffe AP 1996 Science 272, 371-372; and Wade PA et al 1997 Trends Biochem Sci 22, 128-132 for reviews of histone acetylation and transcription).

Acetylation of histones is associated with areas of chromatin that are transcriptionally active, whereas nucleosomes with low acetylation levels are, typically, transcriptionally silent. The acetylation status of histones is controlled by two enzyme classes of opposing activities; histone acetyltransferases (HATs) and histone deacetylases (HDACs). In transformed cells it is believed that inappropriate expression of HDACs results in silencing of tumour suppressor genes (For a review of the potential roles of HDACs in tumorigenesis see Gray SG and Teh BT 2001 Curr Mol Med 1, 401-429). Inhibitors of HDAC enzymes have been described in the literature and shown to induce transcriptional reactivation of certain genes resulting in the inhibition of cancer cell proliferation, induction of apoptosis and inhibition of tumour growth in animals (For

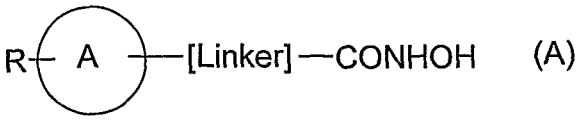
review see Kelly, WK et al 2002 Expert Opin Investig Drugs 11, 1695-1713). Such findings suggest that HDAC inhibitors have therapeutic potential in the treatment of proliferative diseases such as cancer (Kramer, OH et al 2001 Trends Endocrinol 12, 294-300, Vigushin DM and Coombes RC 2002 Anticancer Drugs 13, 1-13).

In addition, others have proposed that aberrant HDAC activity or histone acetylation is implicated in the following diseases and disorders; polyglutamine disease, for example Huntingdon disease (Hughes RE 2002 Curr Biol 12, R141-R143; McCampbell A et al 2001 Proc Soc Natl Acad Sci 98, 15179-15184; Hockly E et al 2003 Proc Soc Natl Acad Sci 100, 2041-2046), other neurodegenerative diseases, for example Alzheimer disease (Hempen B and Brion JP 1996, J Neuropathol Exp Neurol 55, 964-972), autoimmune disease and organ transplant rejection (Skov S et al 2003 Blood 101, 14 30-1438; Mishra N et al 2003 J Clin Invest 111, 539-552), diabetes (Mosley AL and Ozcan S 2003 J Biol Chem 278, 19660 - 19666) and diabetic complications, infection (including protozoal infection (Darkin-Rattray, SJ et al 1996 Proc Soc Natl Acad Sci 93, 13143-13147)) and haematological disorders including thalassemia (Witt O et al 2003 Blood 101, 2001-2007). The observations contained in these manuscripts suggest that HDAC inhibition should have therapeutic benefit in these, and other related, diseases

Many types of HDAC inhibitor compounds have been suggested, and several such compounds are currently being evaluated clinically, for the treatment of cancers. For example, the following patent publications disclose such compounds:

US 5,369,108 and WO 01/18171	WO 03/076395	WO 04/110989
	WO 03/076400	WO 04/092115
US 4,254,220	WO 03/076401	WO 04/0224991
WO 01/70675	WO 03/076421	WO 05/014588
WO 01/38322	WO 03/076430	WO 05/018578
WO 02/30879	WO 03/076422	WO 05/019174
WO 02/26703	WO 03/082288	WO 05/004861
WO 02/069947	WO 03/087057	WO 05/007091
WO 02/26696	WO 03/092686	WO 05/030704
WO 03/082288	WO 03/066579	WO 05/013958
WO 02/22577	WO 03/011851	WO 05/028447
WO 03/075929	WO 04/013130	WO 05/026907

Many of the HDAC inhibitors known in the art have a structural template, which may be represented as in formula (A):



(A)

wherein ring A is a carbocyclic or heterocyclic ring system with optional substituents R, and [Linker] is a linker radical of various types. The hydroxamate group functions as a metal binding group, interacting with the metal ion at the active site of the HDAC enzyme, which lies at the base of a pocket in the folded enzyme structure. The ring or ring system A lies within or at the entrance to the pocket containing the metal ion, with the –{Linker]- radical extending deeper into that pocket linking A to the metal binding hydroxamic acid group. In the art, and occasionally herein, the ring or ring system A is sometimes informally referred to as the "head group" of the inhibitor.

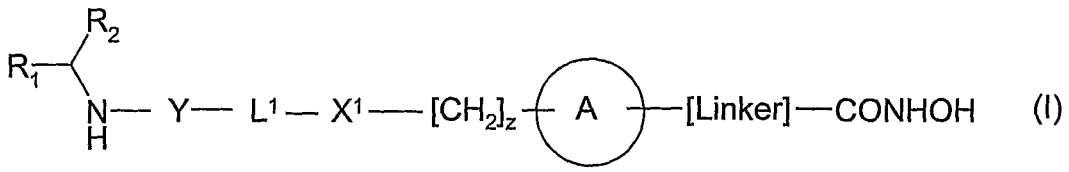
The use of prodrugs to enhance the delivery to target organs and tissues, or to overcome poor pharmacokinetic properties of the parent drug, is a well known medicinal chemistry approach. Administration of ester prodrugs, for example, which are hydrolysed by serum carboxylesterases *in vivo* to the active parent acids, can result in higher serum levels of the parent acid than administration of the acid itself.

Brief Description of the Invention

This invention is based on the finding that the introduction of an alpha amino acid ester grouping into the HDAC inhibitor molecular template (A) above facilitates penetration of the agent through the cell membrane, and thereby allows intracellular carboxylesterase activity to hydrolyse the ester to release the parent acid. Being charged, the acid is not readily transported out of the cell, where it therefore accumulates to increase the intracellular concentration of active HDAC inhibitor. This leads to increases in potency and duration of action. The invention therefore makes available a class of compounds whose structures are characterised by having an alpha amino acid ester moiety which is a substrate for intracellular carboxylesterase (also referred to herein as an "esterase motif") covalently linked to an HDAC inhibitor molecular template, and to the corresponding de-esterified parent acids, such compounds having pharmaceutical utility in the treatment of diseases such as cancers which benefit from intracellular inhibition of HDAC.

Detailed Description of the Invention

According to the present invention there is provided a compound of formula (I) or a salt, N-oxide, hydrate or solvate thereof:



(I)

wherein

R_1 is a carboxylic acid group (-COOH), or an ester group which is hydrolysable by one or more intracellular carboxylesterase enzymes to a carboxylic acid group;

R_2 is the side chain of a natural or non-natural alpha amino acid;

Y is a bond, –C(=O)-, -S(=O)$_2$-, -C(=O)O-, -C(=O)NR$_3$-, -C(=S)-NR$_3$, -C(=NH)NR$_3$ or -S(=O)$_2$NR$_3$- wherein R_3 is hydrogen or optionally substituted C_1-C_6 alkyl;

L^1 is a divalent radical of formula –(Alk^1)$_m$(Q)$_n$(Alk^2)$_p$- wherein

m, **n** and **p** are independently 0 or 1,

Q is (i) an optionally substituted divalent mono- or bicyclic carbocyclic or heterocyclic radical having 5 - 13 ring members, or (ii), in the case where both m and p are 0, a divalent radical of formula –X^2-Q^1- or –Q^1-X^2- wherein X^2 is –O-, S- or NR^A- wherein R^A is hydrogen or optionally substituted C_1-C_3 alkyl, and Q^1 is an optionally substituted divalent mono- or bicyclic carbocyclic or heterocyclic radical having 5 - 13 ring members,

Alk^1 and **Alk^2** independently represent optionally substituted divalent C_3-C_7 cycloalkyl radicals, or optionally substituted straight or branched, C_1-C_6 alkylene, C_2-C_6 alkenylene ,or C_2-C_6 alkynylene radicals which may optionally contain or terminate in an ether (–O-), thioether (-S-) or amino (–NR^A-) link wherein R^A is hydrogen or optionally substituted C_1-C_3 alkyl;

X^1 represents a bond; -C(=O); or $-S(=O)_2-$; $-NR_4C(=O)-$, $-C(=O)NR_4-$,$-NR_4C(=O)NR_5-$, $-NR_4S(=O)_2-$, or $-S(=O)_2NR_4-$ wherein R_4 and R_5 are independently hydrogen or optionally substituted C_1-C_6 alkyl;

z is 0 or 1;

A represents an optionally substituted mono-, bi- or tri-cyclic carbocyclic or heterocyclic ring system wherein the radicals R_1R_2NH-Y-L^1-X^1-$[CH_2]_z$- and HONHCO-[LINKER]- are attached different ring atoms; and

-[Linker]- represents a divalent linker radical linking a ring atom in A with the hydroxamic acid group -CONHOH, the length of the linker radical, from the terminal atom linked to the ring atom of A to the terminal atom linked to the hydroxamic acid group, is equivalent to that of an unbranched saturated hydrocarbon chain of from 3-10 carbon atoms.

Although the above definition potentially includes molecules of high molecular weight, it is preferable, in line with general principles of medicinal chemistry practice, that the compounds with which this invention is concerned should have molecular weights of no more than 600.

In another broad aspect the invention provides the use of a compound of formula (I) as defined above, or an N-oxide, salt, hydrate or solvate thereof in the preparation of a composition for inhibiting the activity of an HDAC enzyme.

The compounds with which the invention is concerned may be used for the inhibition of HDAC activity, particularly HDAC1 activity, *ex vivo* or *in vivo*.

In one aspect of the invention, the compounds of the invention may be used in the preparation of a composition for the treatment of cell-proliferation disease, for example cancer cell proliferation, polyglutamine diseases for example Huntingdon disease, neurogdeenerative diseases for example Alzheimer disease, autoimmune disease for example rheumatoid arthritis, and organ transplant rejection, diabetes, haematological disorders, infection (including but not limited to protozoal and fungal), inflammatory disease, and cardiovascular disease, including atherosclerosis.

In another aspect, the invention provides a method for the treatment of the foregoing disease types, which comprises administering to a subject suffering such disease an effective amount of a compound of formula (I) as defined above.

The term "ester" or "esterified carboxyl group" means a group $R_9O(C=O)$- in which R_9 is the group characterising the ester, notionally derived from the alcohol R_9OH.

As used herein, the term "(C_a-C_b)alkyl" wherein a and b are integers refers to a straight or branched chain alkyl radical having from a to b carbon atoms. Thus when a is 1 and b is 6, for example, the term includes methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl and n-hexyl.

As used herein the term "divalent (C_a-C_b)alkylene radical" wherein a and b are integers refers to a saturated hydrocarbon chain having from a to b carbon atoms and two unsatisfied valences.

As used herein the term "(C_a-C_b)alkenyl" wherein a and b are integers refers to a straight or branched chain alkenyl moiety having from a to b carbon atoms having at least one double bond of either E or Z stereochemistry where applicable. The term includes, for example, vinyl, allyl, 1- and 2-butenyl and 2-methyl-2-propenyl.

As used herein the term "divalent (C_a-C_b)alkenylene radical" means a hydrocarbon chain having from a to b carbon atoms, at least one double bond, and two unsatisfied valences.

As used herein the term "C_a-C_b alkynyl" wherein a and b are integers refers to straight chain or branched chain hydrocarbon groups having from two to six carbon atoms and having in addition one triple bond. This term would include for example, ethynyl, 1-propynyl, 1- and 2-butynyl, 2-methyl-2-propynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl and 5-hexynyl.

As used herein the term "divalent (C_a-C_b)alkynylene radical" wherein a and b are integers refers to a divalent hydrocarbon chain having from 2 to 6 carbon atoms, and at least one triple bond.

As used herein the term "carbocyclic" refers to a mono-, bi- or tricyclic radical having up to 16 ring atoms, all of which are carbon, and includes aryl and cycloalkyl.

As used herein the term "cycloalkyl" refers to a monocyclic saturated carbocyclic radical having from 3-8 carbon atoms and includes, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

As used herein the unqualified term "aryl" refers to a mono-, bi- or tri-cyclic carbocyclic aromatic radical, and includes radicals having two monocyclic carbocyclic aromatic rings which are directly linked by a covalent bond. Illustrative of such radicals are phenyl, biphenyl and napthyl.

As used herein the unqualified term "heteroaryl" refers to a mono-, bi- or tri-cyclic aromatic radical containing one or more heteroatoms selected from S, N and O, and includes radicals having two such monocyclic rings, or one such monocyclic ring and one monocyclic aryl ring, which are directly linked by a covalent bond. Illustrative of such radicals are thienyl, benzthienyl, furyl, benzfuryl, pyrrolyl, imidazolyl, benzimidazolyl, thiazolyl, benzthiazolyl, isothiazolyl, benzisothiazolyl, pyrazolyl, oxazolyl, benzoxazolyl, isoxazolyl, benzisoxazolyl, isothiazolyl, triazolyl, benztriazolyl, thiadiazolyl, oxadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, indolyl and indazolyl.

As used herein the unqualified term "heterocyclyl" or "heterocyclic" includes "heteroaryl" as defined above, and in its non-aromatic meaning relates to a mono-, bi- or tri-cyclic non-aromatic radical containing one or more heteroatoms selected from S, N and O, and to groups consisting of a monocyclic non-aromatic radical containing one or more such heteroatoms which is covalently linked to another such radical or to a monocyclic carbocyclic radical. Illustrative of such radicals are pyrrolyl, furanyl, thienyl, piperidinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thiadiazolyl, pyrazolyl, pyridinyl, pyrrolidinyl, pyrimidinyl, morpholinyl, piperazinyl, indolyl, morpholinyl, benzfuranyl, pyranyl, isoxazolyl, benzimidazolyl, methylenedioxyphenyl, ethylenedioxyphenyl, maleimido and succinimido groups.

Unless otherwise specified in the context in which it occurs, the term "substituted" as applied to any moiety herein means substituted with up to four compatible substituents, each of which independently may be, for example, (C_1-C_6)alkyl, (C_1-

C_6)alkoxy, hydroxy, hydroxy(C_1-C_6)alkyl, mercapto, mercapto(C_1-C_6)alkyl, (C_1-C_6)alkylthio, phenyl, halo (including fluoro, bromo and chloro), trifluoromethyl, trifluoromethoxy, nitro, nitrile (-CN), oxo, -COOH, -$COOR^A$, -COR^A, -SO_2R^A, -$CONH_2$, -SO_2NH_2, -$CONHR^A$, -SO_2NHR^A, -$CONR^AR^B$, -$SO_2NR^AR^B$, -NH_2, -NHR^A, -NR^AR^B, -$OCONH_2$, -$OCONHR^A$, -$OCONR^AR^B$, -$NHCOR^A$, -$NHCOOR^A$, -NR^BCOOR^A, -$NHSO_2OR^A$, -NR^BSO_2OH, -$NR^BSO_2OR^A$,-$NHCONH_2$, -NR^ACONH_2, -$NHCONHR^B$, -NR^ACONHR^B, -$NHCONR^AR^B$, or -$NR^ACONR^AR^B$ wherein R^A and R^B are independently a (C_1-C_6)alkyl, (C_3-C_6) cycloalkyl , phenyl or monocyclic heteroaryl having 5 or 6 ring atoms. An "optional substituent" may be one of the foregoing substituent groups.

The term "side chain of a natural or non-natural alpha-amino acid" refers to the group R^1 in a natural or non-natural amino acid of formula NH_2-CH(R^1)-COOH.

Examples of side chains of natural alpha amino acids include those of alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, histidine, 5-hydroxylysine, 4-hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, a-aminoadipic acid, α-amino-n-butyric acid, 3,4-dihydroxyphenylalanine, homoserine, α–methylserine, ornithine, pipecolic acid, and thyroxine.

Natural alpha-amino acids which contain functional substituents, for example amino, carboxyl, hydroxy, mercapto, guanidyl, imidazolyl, or indolyl groups in their characteristic side chains include arginine, lysine, glutamic acid, aspartic acid, tryptophan, histidine, serine, threonine, tyrosine, and cysteine. When R_2 in the compounds of the invention is one of those side chains, the functional substituent may optionally be protected.

The term "protected" when used in relation to a functional substituent in a side chain of a natural alpha-amino acid means a derivative of such a substituent which is substantially non-functional. For example, carboxyl groups may be esterified (for example as a C_1-C_6 alkyl ester), amino groups may be converted to amides (for example as a $NHCOC_1$-C_6 alkyl amide) or carbamates (for example as an $NHC(=O)OC_1$-C_6 alkyl or $NHC(=O)OCH_2Ph$ carbamate), hydroxyl groups may be converted to ethers (for example an OC_1-C_6 alkyl or a $O(C_1$-C_6 alkyl)phenyl ether) or esters (for example a $OC(=O)C_1$-C_6 alkyl ester) and thiol groups may be converted to

thioethers (for example a tert-butyl or benzyl thioether) or thioesters (for example a $SC(=O)C_1$-C_6 alkyl thioester).

Examples of side chains of non-natural alpha amino acids include those referred to below in the discussion of suitable R_2 groups for use in compounds of the present invention.

As used herein the term "salt" includes base addition, acid addition and quaternary salts. Compounds of the invention which are acidic can form salts, including pharmaceutically acceptable salts, with bases such as alkali metal hydroxides, e.g. sodium and potassium hydroxides; alkaline earth metal hydroxides e.g. calcium, barium and magnesium hydroxides; with organic bases e.g. N-methyl-D-glucamine, choline tris(hydroxymethyl)amino-methane, L-arginine, L-lysine, N-ethyl piperidine, dibenzylamine and the like. Those compounds (I) which are basic can form salts, including pharmaceutically acceptable salts with inorganic acids, e.g. with hydrohalic acids such as hydrochloric or hydrobromic acids, sulphuric acid, nitric acid or phosphoric acid and the like, and with organic acids e.g. with acetic, tartaric, succinic, fumaric, maleic, malic, salicylic, citric, methanesulphonic, p-toluenesulphonic, benzoic, benzenesunfonic, glutamic, lactic, and mandelic acids and the like.

Compounds of the invention which contain one or more actual or potential chiral centres, because of the presence of asymmetric carbon atoms, can exist as a number of diastereoisomers with R or S stereochemistry at each chiral centre. The invention includes all such diastereoisomers and mixtures thereof.

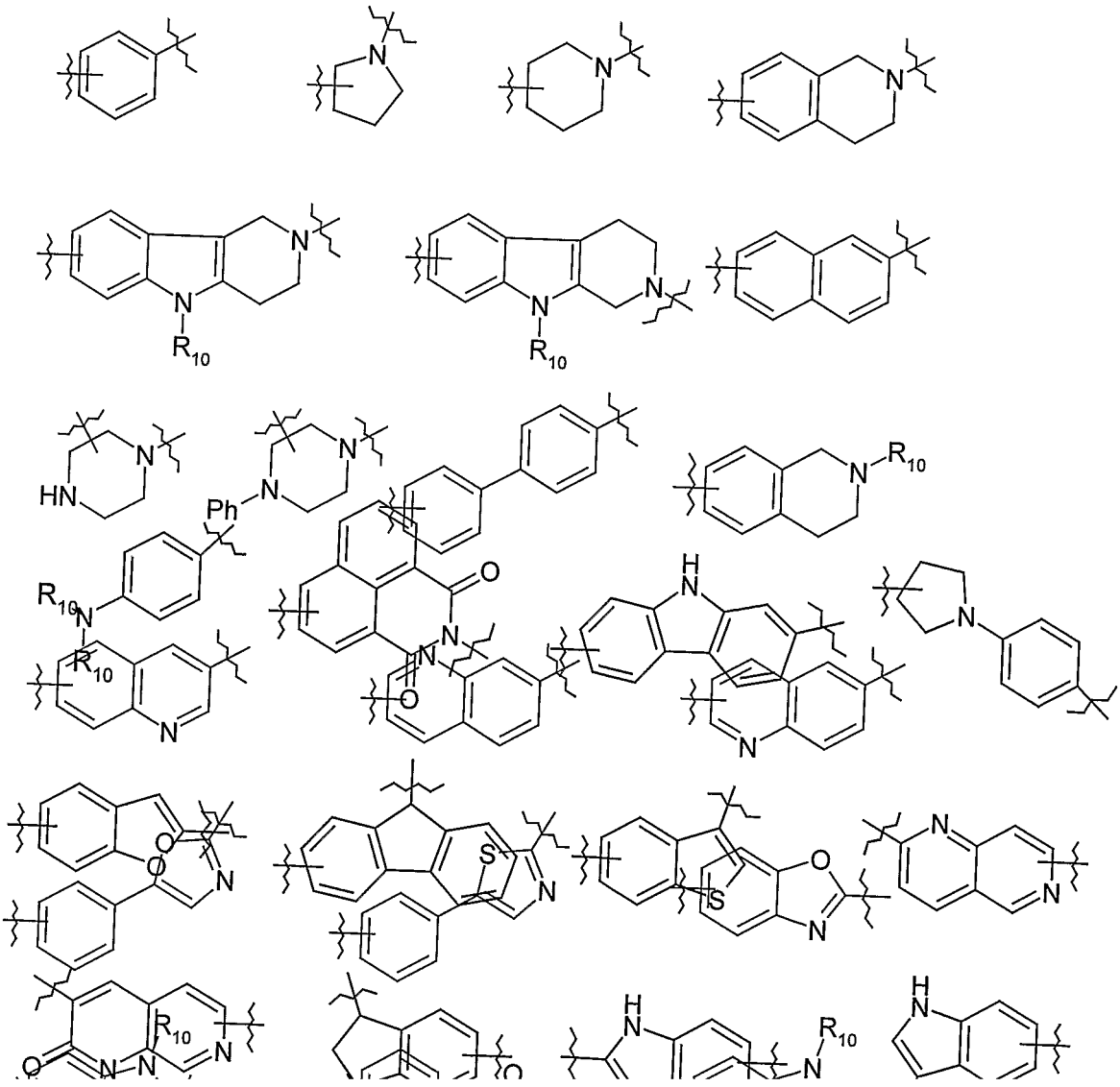
As stated above, the esters of the invention are primarily prodrugs of the corresponding carboxylic acids to which they are converted by intracellular carboxylesterases. However, for so long as they remain unhydrolised, the esters may have HDAC inhibitory activity in their own right. The compounds of the invention include not only the ester, but also the corresponding carboxylic acid hydrolysis products.

The hydroxamate group –C(=O)NHOH

In the compounds of the invention, the hydroxamate group functions as a metal binding group, interacting with the metal ion at the active site of the HDAC enzyme, which lies at the base of a pocket in the folded enzyme structure.

The ring or ring system A

Ring or ring system A is a mono- bi- or tri-cyclic carbocyclic or heterocyclic ring system, optionally substituted. In the compounds of the invention, when bound to the HDAC enzyme's active site, ring or ring system A lies within or at the entrance to the pocket containing the metal ion, with the –{Linker]- radical extending deeper into that pocket linking A to the metal binding hydroxamic acid group. In the art, the ring or ring system A is sometimes informally referred to as the "head group" of the inhibitor. Examples of ring systems A include the following:



wherein R_{10} is hydrogen or optionally substituted C_1-C_6 alkyl, the bond intersected by the wavy line connects to the Linker radical in the compounds (I), and wherein the grouping $R_1R_2CHNHYL_1X_1[CH_2]_z$ in the compounds (I) is linked to any convenient ring atom of the ring system shown.

The –[Linker]- radical

-[Linker]- represents a divalent linker radical linking a ring atom in A with the hydroxamic acid group CONHOH, the length of the linker radical, from the terminal atom linked to the ring atom of A to the terminal atom linked to the hydroxamic acid group, being equivalent to that of an unbranched saturated hydrocarbon chain of from 3-10 carbon atoms. An unbranched saturated hydrocarbon chain of 3 carbon atoms has a length of about 2.5 angstroms, and one of 10 carbon atoms has a length of about 11.3 angstroms. The length of ang given –[Linker]- radical can be determined from data on atom radii and bond lengths in the literature, or can be determined using chemical structure modelling software such as DS ViewerPro (Accelrys, Inc) . The defined length of the –[Linker]-radical reflects the fact that the head group A may lie at the entrance to, or within, the metal ion-containing pocket at the active site of the enzyme, and is therefore loosely related to the depth of that pocket. In many cases, the length of the linker will be equivalent to that of an unbranched saturated hydrocarbon chain of from 4 to 9 carbon atoms, for example 5, 6 or 7 carbon atoms. Specific general types of –[Linker]- radical are those discussed below as "Type 1", "Type 2", and "Type 3" linkers.

Type 1 linkers

In this type, –[Linker]- represents a divalent radical of formula $–(CH_2)_x$-Z-L^2- wherein

x is 0 or 1;

Z is a bond, $-NR_3-$, $–NR_3C(=O)-$, $-C(=O)NR_3-$, $–NR_4C(=O)-NR_3-$, $-C(=S)-NR_3$, $-C(=N)-NR_3$ $–NR_3S(=O)_2-$, or $-S(=O)_2NR_3-$ wherein R_3 is hydrogen or C_1-C_6 alkyl; -C(=O); or $-S(=O)_2-$; and

L^2 represents an optionally substituted, straight or branched, C_4-C_7 alkylene, C_4-C_6 alkenylene or C_4-C_6 alkynylene radicals which may optionally contain or terminate in an ether (–O-), thioether (-S-) or amino ($–NR^A-$) link wherein R^A is hydrogen or optionally substituted C_1-C_3 alkyl.

In one sub-class of this type of linker, in any compatible combination, x is 0; Z is –NH-, –C(=O)-, -NHC(=O)- or –C(=O)NH- and L^2 is $–(CH_2)_5–$,$–(CH_2)_6–$, or $–(CH_2)_7–$.

Type 2 linkers

In this type, –[Linker]- represents a divalent radical of formula $–(CH_2)_x– L^3-Ar^1-L^4-$ wherein

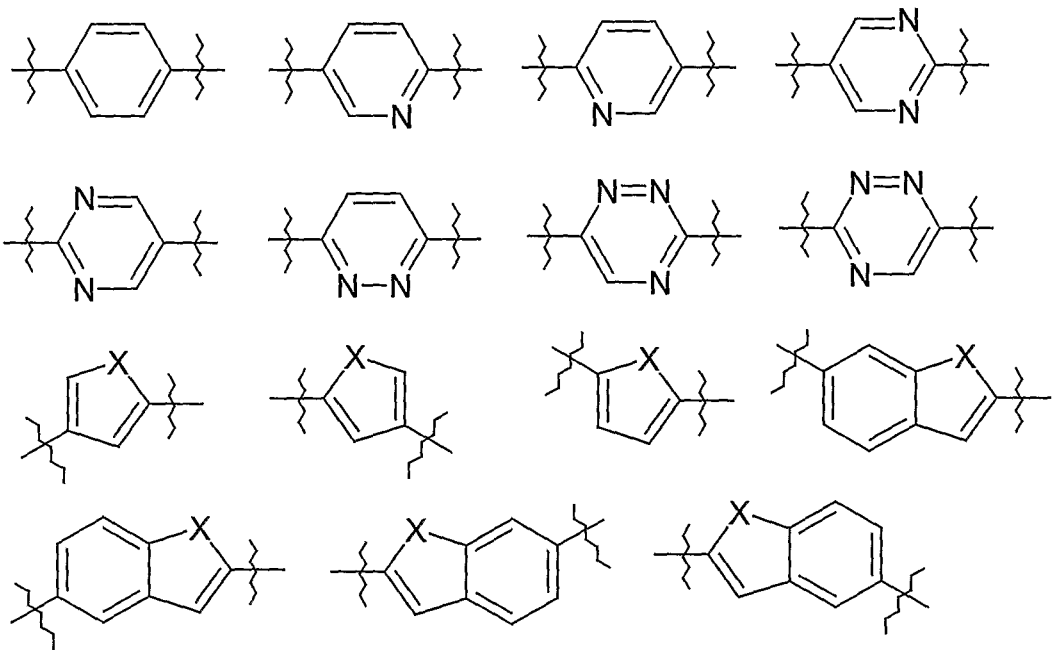
x is 0 or 1;

L^3 is Z or L^2 or Z-L^2 wherein Z is as defined in relation to Type 1 linkers and and L^2 is a bond or an optionally substituted divalent C_1-C_3 alkylene radical;

Ar^1 is a divalent phenyl radical or a divalent mono-, or bi-cyclic heteroaryl radical having 5 to 13 ring members, and

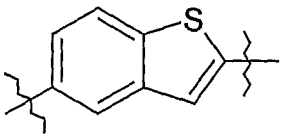
L^4 is a bond or optionally substituted $–CH_2–$ or –CH=CH-.

In one sub-class of this type of linker, in any compatible combination, x is 0 or 1; L^3 is Z or Z-L^2, wherein Z is –NH-, $-NHS(=O)_2–$, $–S(=O)_2NH-$ or $–S(=O)_2–$; L^2 is $–CH_2–$ L^4 is a bond or$–CH_2–$; and Ar^1 is divalent radical selected from the following:



wherein X is O, S or NH.

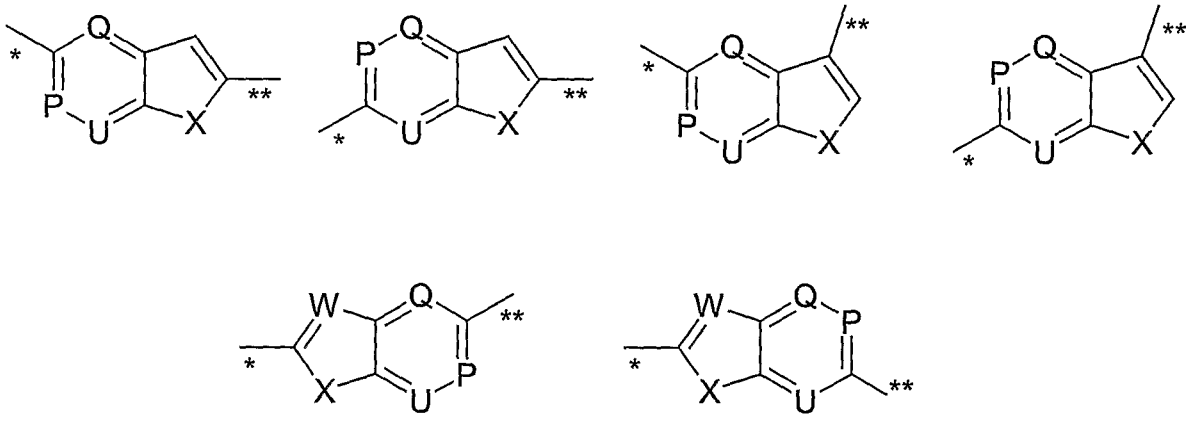
Of the above Ar^1 radicals, the benzo[b]thiophen-6-yl radical



is a particular example

In another sub-class of this type of linker, in any compatible combination, x is 0; L^3 is L^2, wherein L^2 is an straight chain C_3-C_5 alkylene radical which may optionally contain an ether (–O-), thioether (-S-) or amino (–NR^A-) link wherein R^A is hydrogen or optionally substituted C_1-C_3 alkyl, for example hydroxyethyl; and Ar^1 is divalent radical selected from those listed in the preceding paragraph.

In yet another subclass of this type, x is 0, L^3 and L^4 are bonds, and Ar^1 is a divalent phenyl radical or a divalent bicyclic heteroaryl radical having 9 to13 ring members, for example selected from the following:

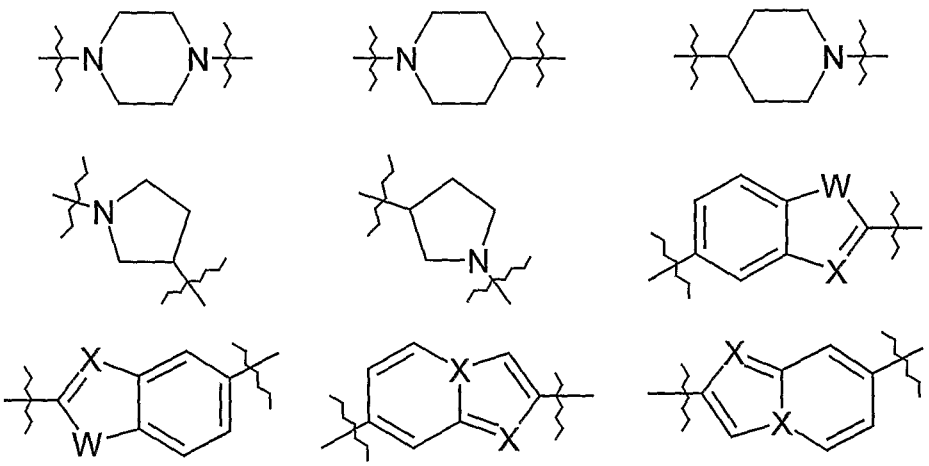


wherein **X** is selected from O, S and NH and **P**, **Q**, and **U** are independently selected from N and CH; and the bond marked ** is linked to the CONHOH group; and the bond marked * is linked to the ring or ring system A.

Type 3 linkers

In this type, –[Linker]- represents a divalent radical of formula $-(CH_2)_x-L^3-B-Ar^1-L^4-$ wherein x, Ar^1, L^3 and L^4 are as discussed with reference to Type 2 linkers above; and B is a mono- or bi-cyclic heterocyclic ring system.

In one subclass of this type of linker B is one of the following:



wherein X is N and W is NH, O or S.

The ester group R_1

The ester group R_1 must be one which in the compound of the invention is hydrolysable by one or more intracellular carboxylesterase enzymes to a carboxylic acid group. Intracellular carboxylesterase enzymes capable of hydrolysing the ester group of a compound of the invention to the corresponding acid include the three known human enzyme isotypes hCE-1, hCE-2 and hCE-3. Although these are considered to be the main enzymes, other enzymes such as biphenylhydrolase (BPH) may also have a role in hydrolysing the ester. In general, if the carboxylesterase hydrolyses the free amino acid ester to the parent acid it will, subject to the N-carbonyl dependence of hCE-2 and hCE-3 discussed below, also hydrolyse the ester motif when covalently conjugated to the HDAC inhibitor. Hence, the broken cell assay described herein provide a straightforward, quick and simple first screen for esters which have the required hydrolysis profile. Ester motifs selected in that way may then be re-assayed in the same carboxylesterase assay when conjugated to the modulator

via the chosen conjugation chemistry, to confirm that it is still a carboxylesterase substrate in that background.

Subject to the requirement that they be hydroysable by intracellular carboxylesterase enzymes, examples of particular ester groups R_1 include those of formula -(C=O)OR_9 wherein R_9 is (i) R_7R_8CH- wherein R_7 is optionally substituted (C_1-C_3)alkyl-(Z^1)$_a$-(C_1-C_3)alkyl- or (C_2-C_3)alkenyl-(Z^1)$_a$-(C_1-C_3)alkyl- wherein a is 0 or 1 and Z^1 is –O-, -S-, or –NH-, and R_8 is hydrogen or (C_1-C_3)alkyl- or R_7 and R_8 taken together with the carbon to which they are attached form an optionally substituted C_3-C_7 cycloalkyl ring or an optionally substituted heterocyclic ring of 5- or 6-ring atoms; or (ii) optionally substituted phenyl or monocyclic heterocyclic having 5 or 6 ring atoms. Within these classes, R_9 may be, for example, methyl, ethyl, n- or iso-propyl, n- or sec-butyl, cyclohexyl, allyl, phenyl, benzyl, 2-, 3- or 4-pyridylmethyl, N-methylpiperidin-4-yl, tetrahydrofuran-3-yl or methoxyethyl. Currently preferred is where R_9 is cyclopentyl.

Macrophages are known to play a key role in inflammatory disorders through the release of cytokines in particular TNFα and IL-1 (van Roon et al Arthritis and Rheumatism , 2003, 1229-1238). In rheumatoid arthritis they are major contributors to the maintenance of joint inflammation and joint destruction. Macrophages are also involved in tumour growth and development (Naldini and Carraro Curr Drug Targets Inflamm Allergy ,2005, 3-8). Hence agents that selectively target macrophage cell proliferation could be of value in the treatment of cancer and autoimmune disease. Targeting specific cell types would be expected to lead to reduced side-effects. The inventors have discovered a method of targeting HDAC inhibitors to macrophages which is based on the observation that the way in which the esterase motif is linked to the HDAC inhibitor determines whether it is hydrolysed, and hence whether or not it accumulates in different cell types. Specifically it has been found that macrophages contain the human carboxylesterase hCE-1 whereas other cell types do not. In the general formula (I) when the nitrogen of the esterase motif R_1R_2CHNH- is not directly linked to a carbonyl (-C(=O)-), ie when Y is not a –C(=O), -C(=O)O- or -C(=O)NR_3- radical, the ester will only be hydrolysed by hCE-1 and hence the HDAC inhibitors will only accumulate in macrophages. Herein, unless "monocyte" or "monocytes" is specified, the term macrophage or macrophages will be used to denote macrophages (including tumour associated macrophages) and/or monocytes.

The amino acid side chain R_2

Subject to the requirement that the ester group R_1 be hydrolysable by intracellular carboxylesterase enzymes, the identity of the side chain group R_2 is not critical.

Examples of amino acid side chains include

C_1-C_6 alkyl, phenyl, 2,- 3-, or 4-hydroxyphenyl, 2,- 3-, or 4-methoxyphenyl, 2,- 3-, or 4-pyridylmethyl, benzyl, phenylethyl, 2-, 3-, or 4-hydroxybenzyl, 2,- 3-, or 4-benzyloxybenzyl, 2,- 3-, or 4- C_1-C_6 alkoxybenzyl, and benzyloxy(C_1-C_6alkyl)-groups;

the characterising group of a natural α amino acid, in which any functional group may be protected;

groups -[Alk]$_n$$R_6$ where Alk is a (C_1-C_6)alkyl or (C_2-C_6)alkenyl group optionally interrupted by one or more -O-, or -S- atoms or -N(R_7)- groups [where R_7 is a hydrogen atom or a (C_1-C_6)alkyl group], n is 0 or 1, and R_6 is an optionally substituted cycloalkyl or cycloalkenyl group;

a benzyl group substituted in the phenyl ring by a group of formula -OCH_2COR_8 where R_8 is hydroxyl, amino, (C_1-C_6)alkoxy, phenyl(C_1-C_6)alkoxy, (C_1-C_6)alkylamino, di((C_1-C_6)alkyl)amino, phenyl(C_1-C_6)alkylamino, the residue of an amino acid or acid halide, ester or amide derivative thereof, said residue being linked via an amide bond, said amino acid being selected from glycine, α or β alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, serine, threonine, cysteine, methionine, asparagine, glutamine, lysine, histidine, arginine, glutamic acid, and aspartic acid;

a heterocyclic(C_1-C_6)alkyl group, either being unsubstituted or mono- or di-substituted in the heterocyclic ring with halo, nitro, carboxy, (C_1-C_6)alkoxy, cyano, (C_1-C_6)alkanoyl, trifluoromethyl (C_1-C_6)alkyl, hydroxy, formyl, amino, (C_1-C_6)alkylamino, di-(C_1-C_6)alkylamino, mercapto, (C_1-C_6)alkylthio, hydroxy(C_1-C_6)alkyl, mercapto(C_1-C_6)alkyl or (C_1-C_6)alkylphenylmethyl; and

a group -$CR_aR_bR_c$ in which:

each of R_a, R_b and R_c is independently hydrogen, $(C_1$-$C_6)$alkyl, $(C_2$-$C_6)$alkenyl, $(C_2$-$C_6)$alkynyl, phenyl$(C_1$-$C_6)$alkyl, $(C_3$-$C_8)$cycloalkyl; or

R_c is hydrogen and R_a and R_b are independently phenyl or heteroaryl such as pyridyl; or

R_c is hydrogen, $(C_1$-$C_6)$alkyl, $(C_2$-$C_6)$alkenyl, $(C_2$-$C_6)$alkynyl, phenyl$(C_1$-$C_6)$alkyl, or $(C_3$-$C_8)$cycloalkyl, and R_a and R_b together with the carbon atom to which they are attached form a 3 to 8 membered cycloalkyl or a 5- to 6-membered heterocyclic ring; or

R_a, R_b and R_c together with the carbon atom to which they are attached form a tricyclic ring (for example adamantyl); or

R_a and R_b are each independently $(C_1$-$C_6)$alkyl, $(C_2$-$C_6)$alkenyl, $(C_2$-$C_6)$alkynyl, phenyl$(C_1$-$C_6)$alkyl, or a group as defined for R_c below other than hydrogen, or R_a and R_b together with the carbon atom to which they are attached form a cycloalkyl or heterocyclic ring, and R_c is hydrogen, -OH, -SH, halogen, -CN, -CO_2H, $(C_1$-$C_4)$perfluoroalkyl, -CH_2OH, -$CO_2(C_1$-$C_6)$alkyl, -O$(C_1$-$C_6)$alkyl, -O$(C_2$-$C_6)$alkenyl, -S$(C_1$-$C_6)$alkyl, -SO$(C_1$-$C_6)$alkyl, -$SO_2(C_1$-$C_6)$ alkyl, -S$(C_2$-$C_6)$alkenyl, -SO$(C_2$-$C_6)$alkenyl, -$SO_2(C_2$-$C_6)$alkenyl or a group -Q-W wherein Q represents a bond or -O-, -S-, -SO- or -SO_2- and W represents a phenyl, phenylalkyl, $(C_3$-$C_8)$cycloalkyl, $(C_3$-$C_8)$cycloalkylalkyl, $(C_4$-$C_8)$cycloalkenyl, $(C_4$-$C_8)$cycloalkenylalkyl, heteroaryl or heteroarylalkyl group, which group W may optionally be substituted by one or more substituents independently selected from, hydroxyl, halogen, -CN, -CO_2H, -$CO_2(C_1$-$C_6)$alkyl, -$CONH_2$, -CONH$(C_1$-$C_6)$alkyl, -CONH$(C_1$-C_6alkyl$)_2$, -CHO, -CH_2OH, $(C_1$-$C_4)$perfluoroalkyl, -O$(C_1$-$C_6)$alkyl, -S$(C_1$-$C_6)$alkyl, -SO$(C_1$-$C_6)$alkyl, -$SO_2(C_1$-$C_6)$alkyl, -NO_2, -NH_2, -NH$(C_1$-$C_6)$alkyl, -N$((C_1$-$C_6)$alkyl$)_2$, -NHCO$(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkyl, $(C_2$-$C_6)$alkenyl, $(C_2$-$C_6)$alkynyl, $(C_3$-$C_8)$cycloalkyl, $(C_4$-$C_8)$cycloalkenyl, phenyl or benzyl.

Examples of particular R_2 groups include hydrogen (the glycine "side chain"), benzyl, phenyl, cyclohexylmethyl, cyclohexyl, pyridin-3-ylmethyl, tert-butoxymethyl, iso-butyl, sec-butyl, tert-butyl, 1-benzylthio-1-methylethyl, 1-methylthio-1-methylethyl, 1-

mercapto-1-methylethyl, and phenylethyl. Presently preferred R_2 groups include phenyl, benzyl, and iso-butyl.

For compounds of the invention which are to be administered systemically, esters with a slow rate of carboxylesterase cleavage are preferred, since they are less susceptible to pre-systemic metabolism. Their ability to reach their target tissue intact is therefore increased, and the ester can be converted inside the cells of the target tissue into the acid product. However, for local administration, where the ester is either directly applied to the target tissue or directed there by, for example, inhalation, it will often be desirable that the ester has a rapid rate of esterase cleavage, to minimise systemic exposure and consequent unwanted side effects. In the compounds of this invention, if the carbon adjacent to the alpha carbon of the alpha amino acid ester ester is monosubstituted, ie R_2 is CH_2R^z (R^z being the mono-substituent) then the esters tend to be cleaved more rapidly than if that carbon is di- or tri-substituted, as in the case where R_2 is, for example, phenyl or cyclohexyl.

The radical $-Y-L^1-X^1-[CH_2]_z-$

This radical (or bond) arises from the particular chemistry strategy chosen to link the amino acid ester motif $R_1CH(R_2)NH-$ to the head group A of the inhibitor. Clearly the chemistry strategy for that coupling may vary widely, and thus many combinations of the variables Y, L^1, $X^1$and z are possible. However, as mentioned above, when the inhibitor is bound to the HDAC enzyme at its active site, the head group A is located at the top of, or within, the metal-ion-containing pocket of the enzyme, so by linking the amino acid ester motif to the head group it generally extends in a direction away from that pocket, and thus minimises or avoids interference with the binding mode of the inhibitor template A-[Linker]-CONHOH. Hence the precise combination of variable making up the linking chemistry between the amino acid ester motif and the head group A will often be irrelevant to the primary binding mode of the compound as a whole. On the other hand, that linkage chemistry may in some cases pick up additional binding interactions with the enzyme at the top of, or adjacent to, the metal ion-containing pocket, thereby enhancing binding.

It should also be noted that the benefits of the amino acid ester motif described above (facile entry into the cell, carboxylesterase hydrolysis within the cell, and accumulation within the cell of active carboxylic acid hydrolysis product) are best achieved when the

linkage between the amino acid ester motif and the head group is not a substrate for peptidase activity within the cell, which might result in cleavage of the amino acid from the molecule. Of course, stability to intracellular peptidases is easily tested by incubating the compound with disrupted cell contents, and analysing for any such cleavage.

With the foregoing general observations in mind, taking the variables making up the radical $-Y-L^1-X^1-[CH_2]_z-$ in turn:

z may be 0 or 1, so that a methylene radical linked to the head group A is optional;

specific preferred examples of Y when macrophage selectivity is not required include–(C=O)-, -(C=O)NH-, and -(C=O)O-; Where macrophage selectivity is required any of the other options for Y, including the case where Y is a bond, are appropriate.

In the radical L^1, examples of Alk^1 and Alk^2 radicals, when present, include $-CH_2-$, $-CH_2CH_2-$ $-CH_2CH_2CH_2-$, $-CH_2CH_2CH_2CH_2-$, $-CH=CH-$, $-CH=CHCH_2-$, $-CH_2CH=CH-$, $CH_2CH=CHCH_2$—$C\equiv C-$, $-C\equiv CCH_2-$, $CH_2C\equiv C-$, and $CH_2C\equiv CCH_2$. Additional examples of Alk^1 and Alk^2 include $-CH_2W-$, $-CH_2CH_2W-$ $-CH_2CH_2WCH_2-$, $-CH_2CH_2WCH(CH_3)-$, $-CH_2WCH_2CH_2-$, $-CH_2WCH_2CH_2WCH_2-$, and $-WCH_2CH_2-$ where W is –O-, -S-, -NH-, $-N(CH_3)-$, or $-CH_2CH_2N(CH_2CH_2OH)CH_2-$. Further examples of Alk^1 and Alk^2 include divalent cyclopropyl, cyclopentyl and cyclohexyl radicals.

In L^1, when n is 0, the radical is a hydrocarbon chain (optionally substituted and perhaps having an ether, thioether or amino linkage). Presently it is preferred that there be no optional substituents in L^1. When both m and p are 0, L^1 is a divalent mono- or bicyclic carbocyclic or heterocyclic radical with 5 - 13 ring atoms (optionally substituted). When n is 1 and at least one of m and p is 1, $L^1$is a divalent radical including a hydrocarbon chain or chains and a mono- or bicyclic carbocyclic or heterocyclic radical with 5 - 13 ring atoms (optionally substituted). When present, Q may be, for example, a divalent phenyl, naphthyl, cyclopropyl, cyclopentyl, or cyclohexyl radical, or a mono-, or bi-cyclic heterocyclicl radical having 5 to13 ring members, such as piperidinyl,

piperazinyl, indolyl, pyridyl, thienyl, or pyrrolyl radical, but 1,4-phenylene is presently preferred.

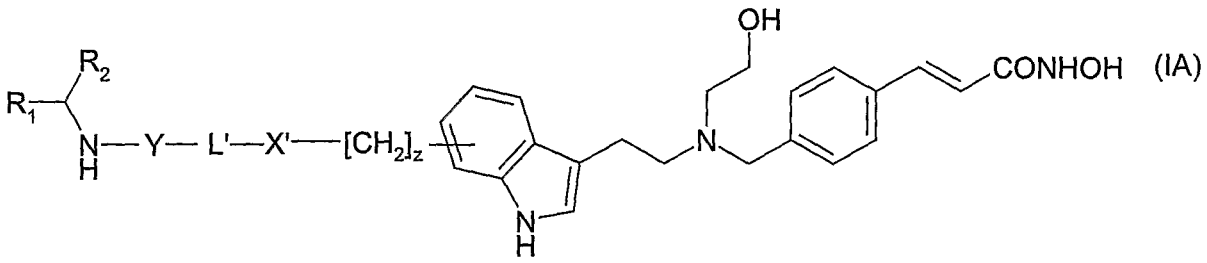
Specifically, in some embodiments of the invention, L^1, m and p may be 0 with n being 1. In other embodiments, n and p may be 0 with m being 1. In further embodiments, m, n and p may be all 0. In still further embodiments m may be 0, n may be 1 with Q being a monocyclic heterocyclic radical, and p may be 0 or 1. Alk^1 and Alk^2, when present, may be selected from $-CH_2-$, $-CH_2CH_2-$, and $-CH_2CH_2CH_2-$ and Q may be 1,4-phenylene.

Specific examples of the radical $-Y-L^1-X^1-[CH_2]_z-$ include –C(=O)- and –C(=O)NH- as well as $-(CH_2)_v-$, $-(CH_2)_vO-$, $-C(=O)-(CH_2)_v-$, $-C(=O)-(CH_2)_vO-$, $-C(=O)-NH-(CH_2)_w-$, $-C(=O)-NH-(CH_2)_wO-$

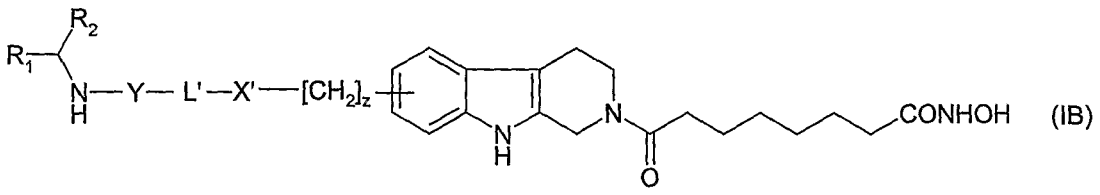


wherein v is 1, 2, 3 or 4 and w is 1, 2 or 3, such as $-CH_2-$, $-CH_2O-$, $-C(=O)-CH_2-$, $-C(=O)-CH_2O-$, $-C(=O)-NH-CH_2-$, and $-C(=O)-NH-CH_2O-$.

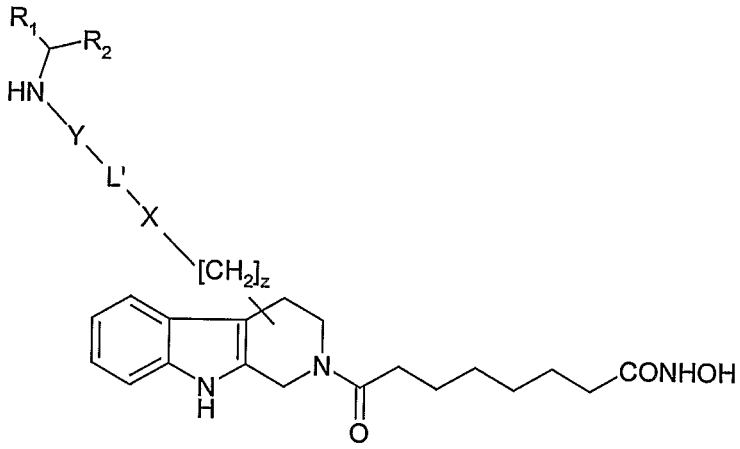
Examples of particular subsets of compounds of the invention include those of formulae (IA) to (IM):



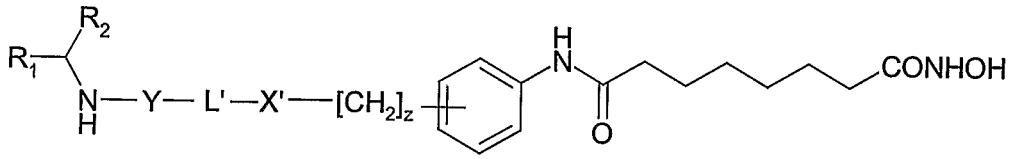
(IA)



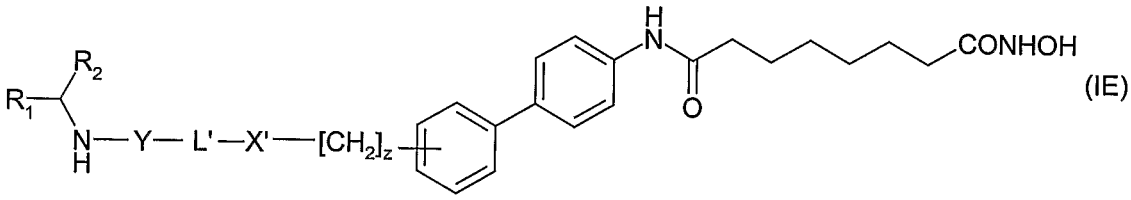
(IB)



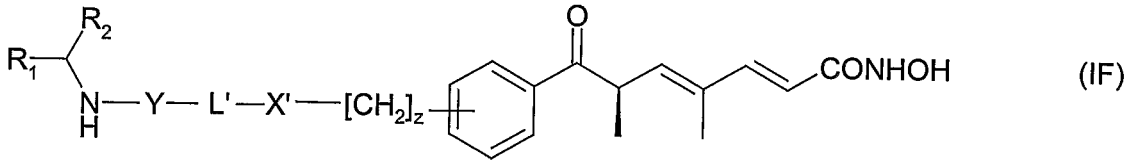
(IC)



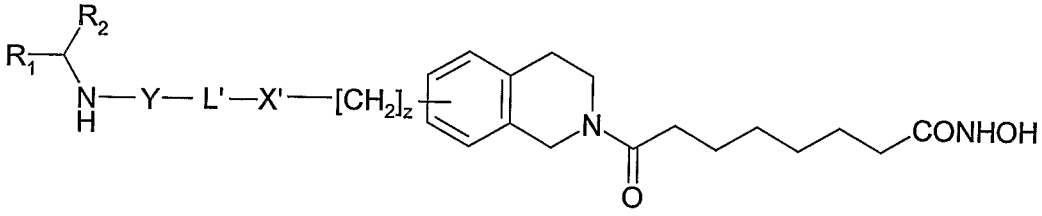
(ID)



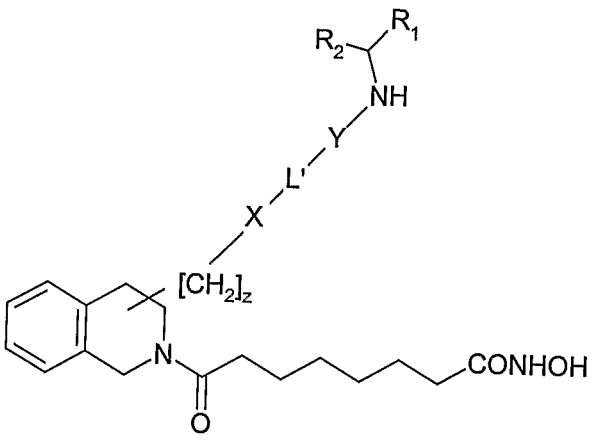
(IE)



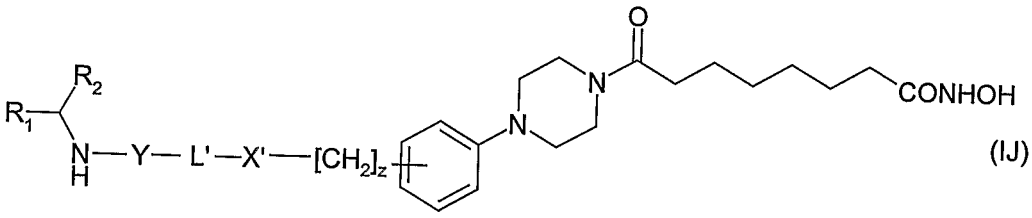
(IF)



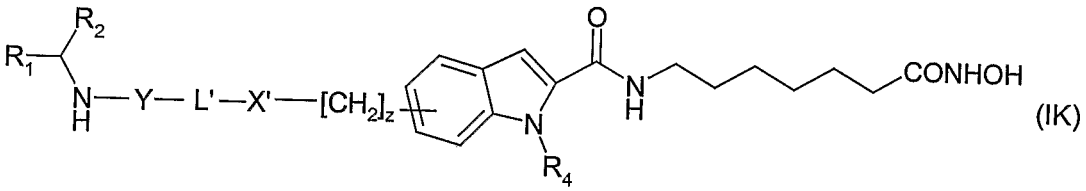
(IG)



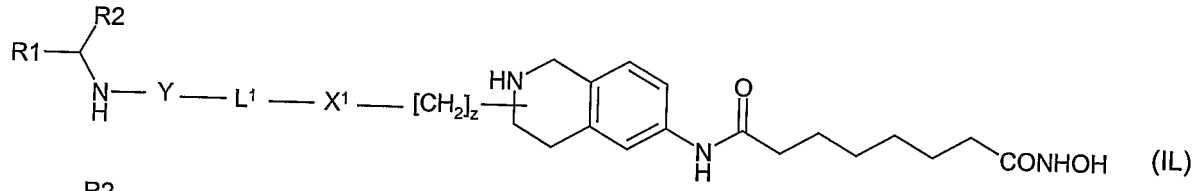
(IH)



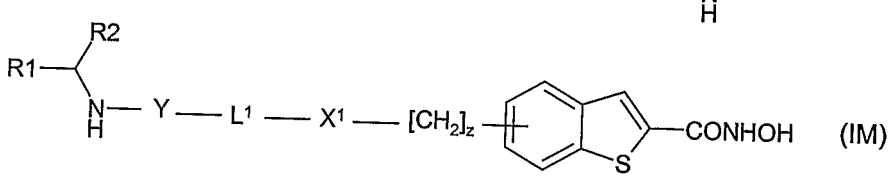
(IJ)



(IK)



(IL)



(IM)

wherein z, R_1, R_2, R_3, L^1 and $X^1$and Y are as defined in relation to formula (I), and as discussed above, including the preferences therefor.

Examples of specific compounds of the invention include the following:
(S)-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-phenyl-acetic acid cyclopentyl ester

(S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-4-phenyl-butyric acid cyclopentyl ester

(S)-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-phenyl-acetic acid cyclopentyl ester

(S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-4-methyl-pentanoic acid cyclopentyl ester

(S)-{2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenyl]-ethylamino}-phenyl-acetic acid cyclopentyl ester

(S)-2-{3-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenoxy]-propylamino}-3-phenyl-propionic acid cyclopentyl ester

(S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-3-(4-hydroxy-phenyl)-propionic acid cyclopentyl ester

(S)-3-tert-Butoxy-2-(4-{[(2-hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-propionic acid cyclopentyl ester

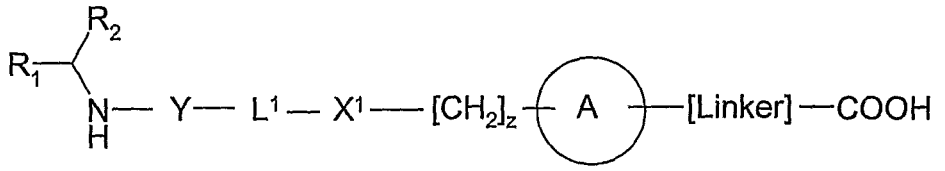
(S)-1-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzyl)-pyrrolidine-2-carboxylic acid cyclopentyl ester

(S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-propionic acid cyclopentyl ester

(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-acetic acid cyclopentyl ester

Compounds of the invention may be prepared, for example, by the methods described below and in the Examples herein.

For example, compounds of the invention may be prepared from the corresponding carboxylic acids (II)



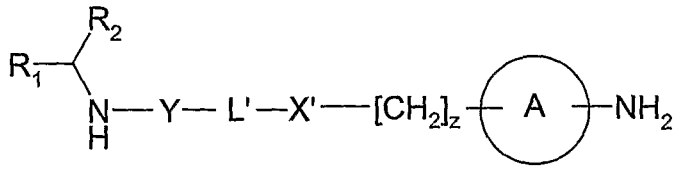
(II)

by reaction of an activated derivative thereof, such as the acid chloride, with hydroxylamine or a protected version of hydroxylamine.

Alternatively, an N- or O-protected or N,O-diprotected precursor of the desired compound (I) may be deprotected. In a useful version of this method O-protection is provided by a resin support, from which the desired hydroxamic acid (I) may be cleaved, for example by acid hydrolysis.

Carboxyl protected derivatives of compounds (II), or O-linked resin-supported derivatives of compounds (II) of the invention may be synthesised in stages by literature methods, selected according to the particular structure of the desired compound. In that connection, the patent publications listed above provide information on the synthesis of HDAC inhibitors which are structurally similar to those of the present invention.

In one approach, suitable for compounds (I) wherein Z is a sulfonamido radical – $NHSO_2$-, an amine (III)



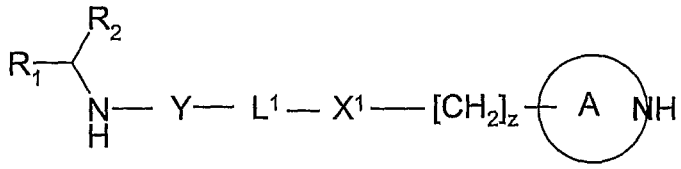
(III)

may be reacted with an activated derivative, for example the acid chloride, of a sulfonic acid $HOSO_2$-L^2-Z^2 wherein Z^2 is a protected carboxyl group, such as cleavable ester, or an O-linked resin-supported hydroxamic acid group.

In another approach, suitable for compounds (I) wherein Z is an amide radical – NHC(=O)-, an amine (III) may be reacted with a carboxylic acid HOC(=O)-L-Z^2, Z^2

being as defined in the preceding paragraph, in the presence of a carbodiimide coupling agent..

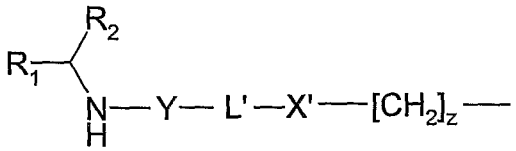
The case of compounds (I) where the ring or ring system A is linked to the –Linker-CONHOH moiety via a ring nitrogen, and Z is –(C=O)- or $-SO_2-$, the appropriate N-heterocycle (IV)



(IV)

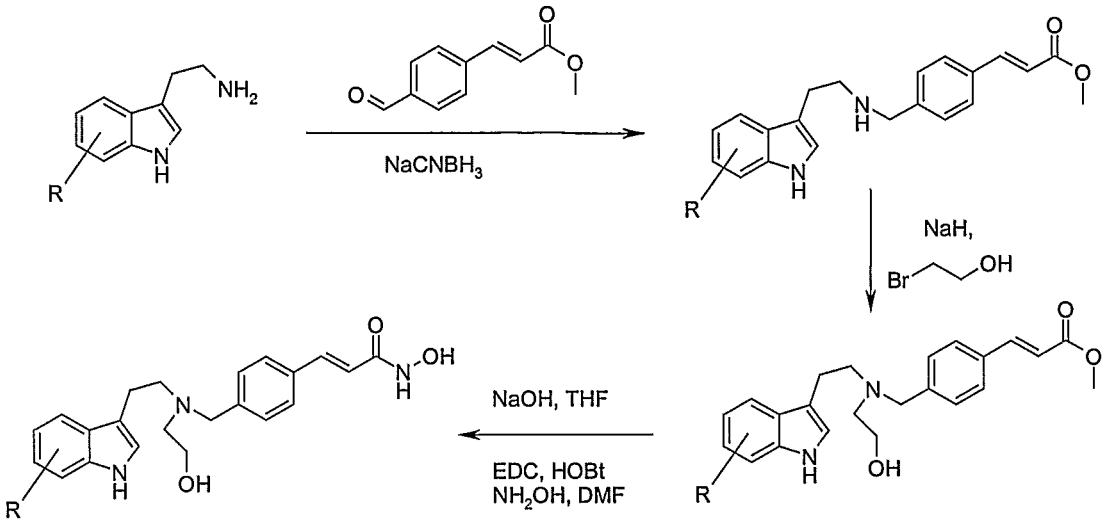
may be reacted with the corresponding carboxylic or sulfonic acid (ie $HOOC-L^2-Z^2$ or $HOSO_2-L^2-Z^2$ wherein Z^2 is as defined above), either as an activated derivative thereof such as the chloride, or in the presence of a carbodiimide coupling agent.

By way of further illustration of the use of literature methods for the synthesis of compounds within the scope of formula (I) above, the following reaction schemes 1-6 are presented. In these schemes the group R represents the radical

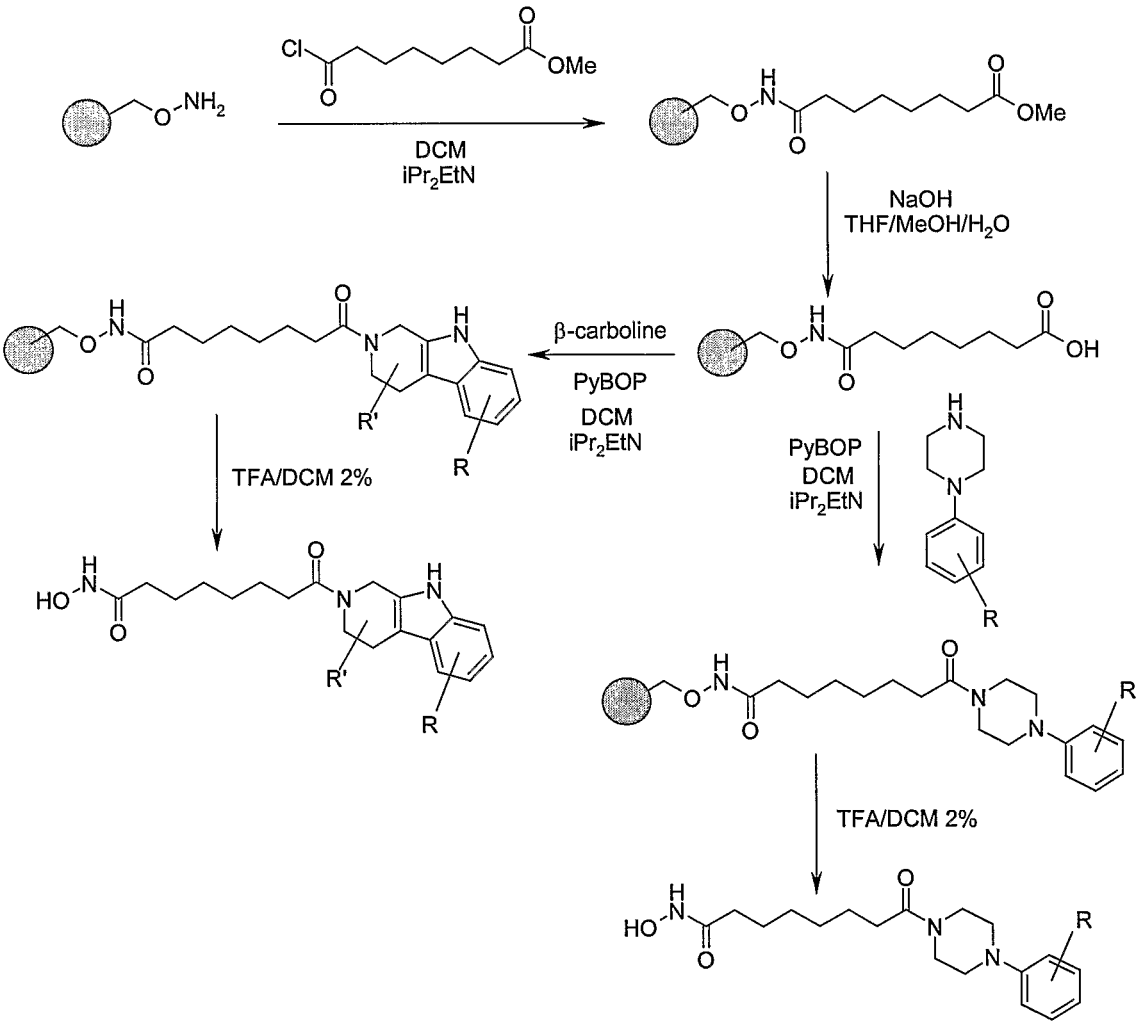


present in the compounds of the invention, or represents a functional group upon which that radical may be built up using literature methods.

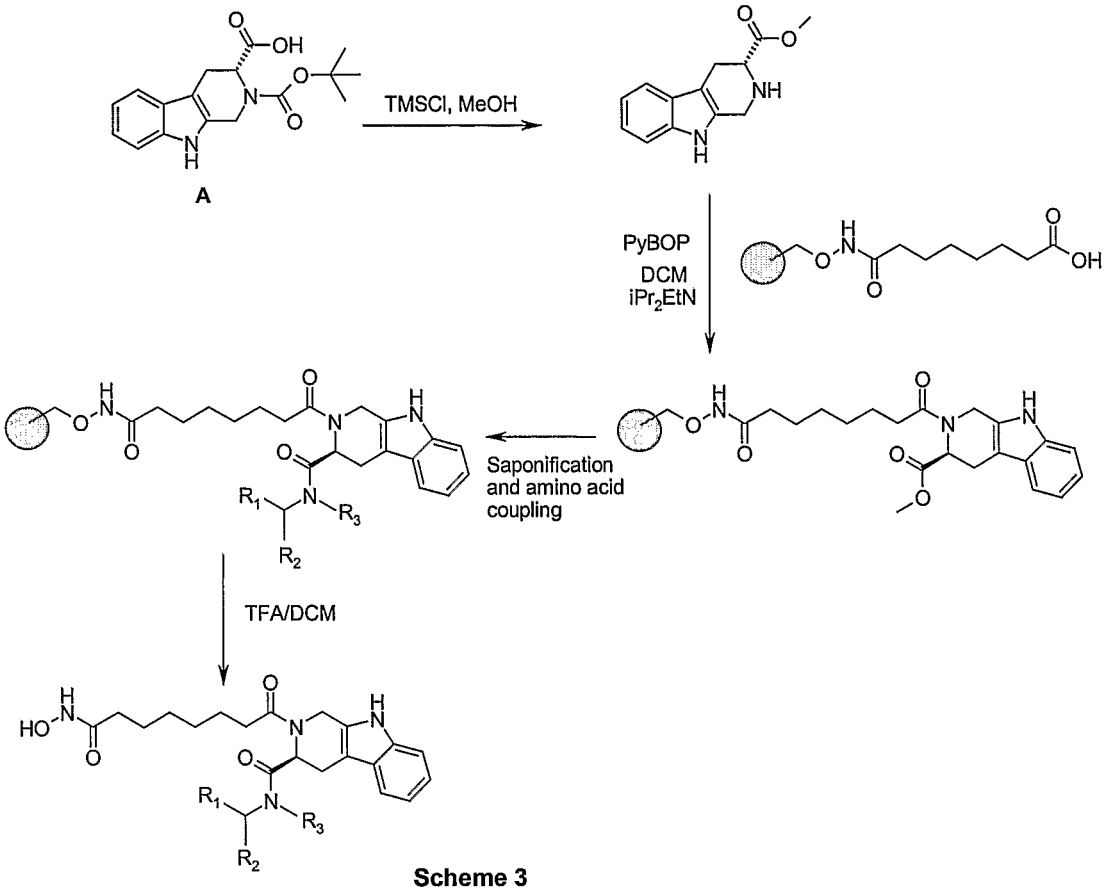
Also in the schemes, the symbol  represents a solid phase resin support.



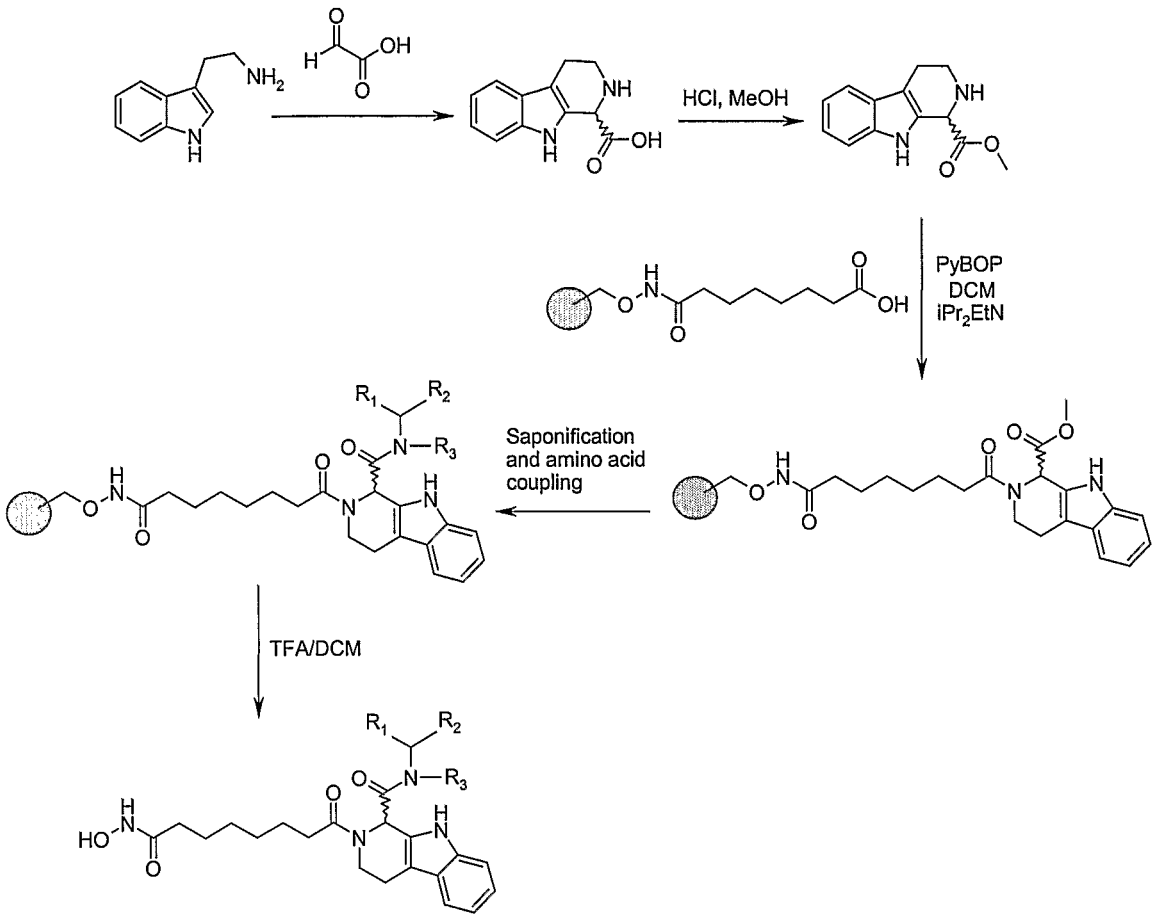
Scheme 1



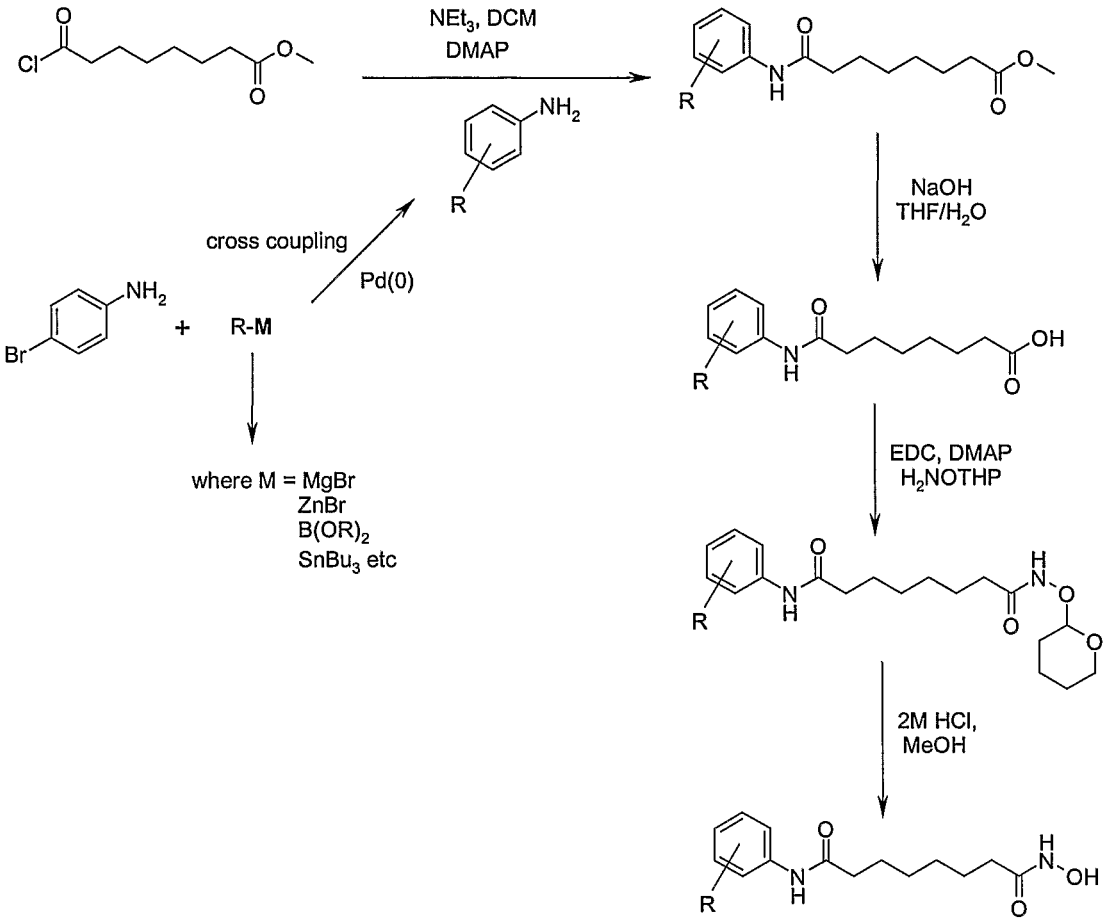
Scheme 2


TMSCl, MeOH
A
PyBOP
DCM
iPr2EtN
Saponification
and amino acid
coupling
TFA/DCM

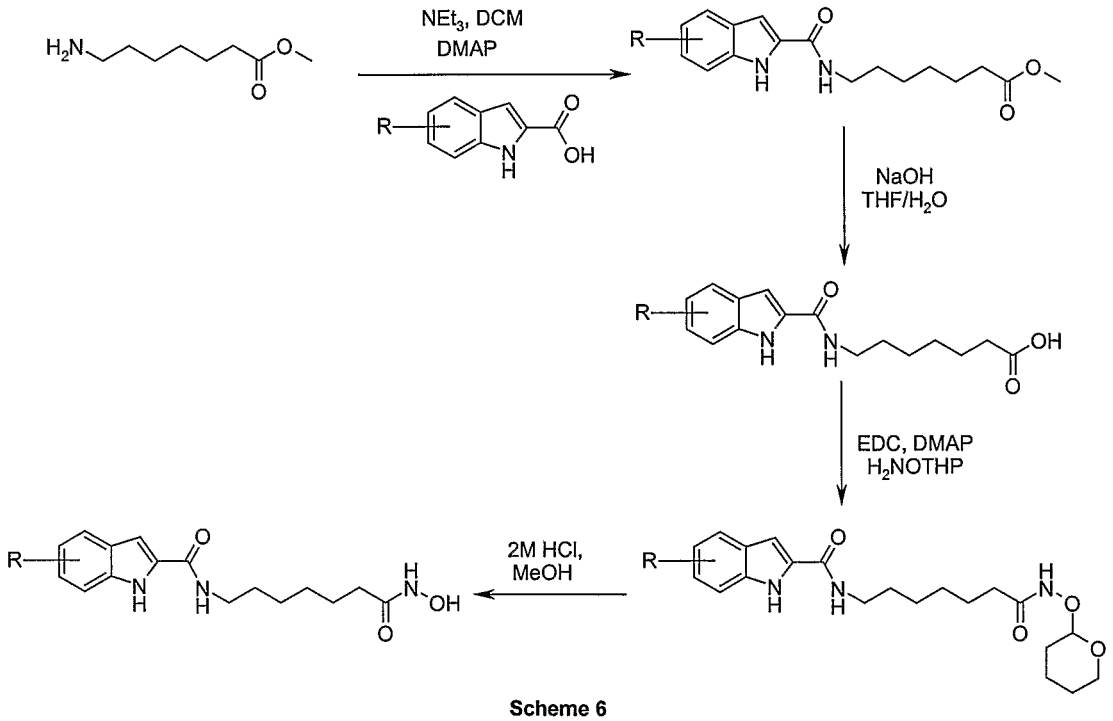
Scheme 3


HCl, MeOH
PyBOP
DCM
iPr2EtN
Saponification
and amino acid
coupling
TFA/DCM

Scheme 4



Scheme 5



Scheme 6

As mentioned above, the compounds with which the invention is concerned are HDAC inhibitors, and may therefore be of use in the treatment of cell proliferative disease, such as cancer, in humans and other mammals.

It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing treatment. Optimum dose levels and frequency of dosing will be determined by clinical trial.

The compounds with which the invention is concerned may be prepared for administration by any route consistent with their pharmacokinetic properties. The orally administrable compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

For topical application to the skin, the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations which may be used for the drug are

conventional formulations well known in the art, for example as described in standard textbooks of pharmaceutics such as the British Pharmacopoeia.

For topical application by inhalation, the drug may be formulated for aerosol delivery for example, by pressure-driven jet atomizers or ultrasonic atomizers, or preferably by propellant-driven metered aerosols or propellant-free administration of micronized powders, for example, inhalation capsules or other "dry powder" delivery systems. Excipients, such as, for example, propellants (e.g. Frigen in the case of metered aerosols), surface-active substances, emulsifiers, stabilizers, preservatives, flavorings, and fillers (e.g. lactose in the case of powder inhalers) may be present in such inhaled formulations. For the purposes of inhalation, a large number of apparata are available with which aerosols of optimum particle size can be generated and administered, using an inhalation technique which is appropriate for the patient. In addition to the use of adaptors (spacers, expanders) and pear-shaped containers (e.g. Nebulator®, Volumatic®), and automatic devices emitting a puffer spray (Autohaler®), for metered aerosols, in particular in the case of powder inhalers, a number of technical solutions are available (e.g. Diskhaler®, Rotadisk®, Turbohaler® or the inhalers for example as described in European Patent Application EP 0 505 321).

For topical application to the eye, the drug may be made up into a solution or suspension in a suitable sterile aqueous or non aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edeate; preservatives including bactericidal and fungicidal agents such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents such as hypromellose may also be included.

The active ingredient may also be administered parenterally in a sterile medium. Depending on the vehicle and concentration used, the drug can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle.

The following Examples illustrate the preparation of specific compounds of the invention, and the HDAC inhibitory properties thereof: In the Examples :

Commercially available reagents and solvents (HPLC grade) were used without further purification.

Microwave irradiation was carried out using a CEM Discover focused microwave reactor.

Solvents were removed using a GeneVac Series I without heating or a Genevac Series II with VacRamp at 30 ^{o} C or a Buchi rotary evaporator.

Purification of compounds by flash chromatography column was performed using silica gel, particle size 40–63 μm (230-400 mesh) obtained from Silicycle. Purification of compounds by preparative HPLC was performed on Gilson systems using reverse phase ThermoHypersil-Keystone Hyperprep HS C18 columns (12 μm, 100 X 21.2 mm), gradient 20-100% B (A= water/ 0.1% TFA, B= acetonitrile/ 0.1% TFA) over 9.5 min, flow = 30 ml/min, injection solvent 2:1 DMSO:acetonitrile (1.6 ml), UV detection at 215 nm.

^{1}H NMR spectra were recorded on a Bruker 400 MHz AV spectrometer in deuterated solvents. Chemical shifts (δ) are in parts per million. Thin-layer chromatography (TLC) analysis was performed with Kieselgel 60 F_{254} (Merck) plates and visualized using UV light.

Analytical HPLCMS was performed on Agilent HP1100, Waters 600 or Waters 1525 LC systems using reverse phase Hypersil BDS C18 columns (5 μm, 2.1 X 50 mm), gradient 0-95% B (A= water/ 0.1% TFA, B= acetonitrile/ 0.1% TFA) over 2.10 min, flow = 1.0 ml/min. UV spectra were recorded at 215 nm using a Gilson G1315A Diode Array Detector, G1214A single wavelength UV detector, Waters 2487 dual wavelength UV detector, Waters 2488 dual wavelength UV detector, or Waters 2996 diode array UV detector. Mass spectra were obtained over the range m/z 150 to 850 at a sampling rate of 2 scans per second or 1 scan per 1.2 seconds using Micromass LCT with Z-spray interface or Micromass LCT with Z-spray or MUX interface. Data were integrated and reported using OpenLynx and OpenLynx Browser software

The following abbreviations have been used:

MeOH = methanol

EtOH = ethanol

EtOAc = ethyl acetate

Boc = tert-butoxycarbonyl

Cbz = carbobenzyloxy

DCM = dichloromethane

DCE= dichloroethane

DMF = dimethylformamide

DMSO = dimethyl sulfoxide

TFA = trifluoroacetic acid

THF = tetrahydrofuran

Na_2CO_3 = sodium carbonate

HCl = hydrochloric acid

DIPEA = diisopropylethylamine

NaH = sodium hydride

NaOH = sodium hydroxide

$NaHCO_3$ = sodium hydrogen carbonate

Pd/C = palladium on carbon

TBME = tert-butyl methyl ether

DMAP = 4-Dimethylaminopyridine

N_2 = nitrogen

PyBop = benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate

Na_2SO_4 = sodium sulphate

Et_3N = triethylamine

NH_3 = ammonia

TMSCl = trimethylchlorosilane

NH_4Cl = ammonium chloride

$LiAlH_4$ = lithium aluminium hydride

pyBrOP = Bromo-tris-pyrrolidino phosphoniumhexafluorophosphate

$MgSO_4$ = magnesium sulfate

MnO_2 = Manganese dioxide

^nBuLi = n-butyllithium

CO_2 = carbon dioxide

EDCI = *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride

Et_2O = diethyl ether

LiOH = lithium hydroxide

HOBt = 1-hydroxybenzotriazole

DIAD = Diisopropyl azodicarboxylate

HATU = O-(7-Azabenzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate

ELS = Evaporative Light Scattering

TLC = thin layer chromatography

ml = millilitre

g = gram(s)

mg = milligram(s)

mol = moles

mmol = millimole(s)

eq = mole equivalent

LCMS = high performance liquid chromatography/mass spectrometry

NMR = nuclear magnetic resonance

r.t. = room temperature

Standard wash procedure for resin chemistry

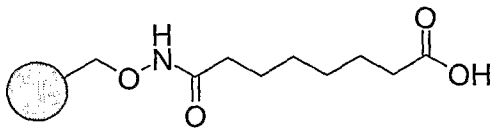
Resin was washed in the following sequence: DMF, MeOH, DMF, MeOH, DCM, MeOH, DCM, MeOH x 2, TBME x 2.

Resin test cleavage

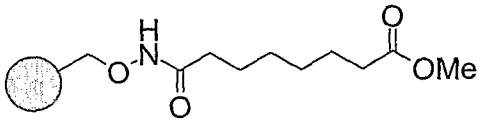
A small amount of functionalised hydroxylamine 2-chlorotrityl resin (ca 0.3ml of reaction mixture, ca 10mg resin) was treated with 2% TFA/DCM (0.5ml) for 10min at room temperature. The resin was filtered and the filtrate was concentrated by blowing with a stream of N_2 gas. LCMS of the residue was obtained.

(Note: For functionalized hydroxylamine Wang resin test cleavage was carried out using 50% TFA/ DCM).

Preparation of Suberic acid Derivatised Hydroxylamine 2-Chlorotrityl Resin

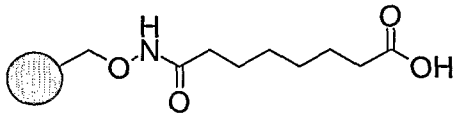


Stage 1 – Immobilisation to 2-chlorotrityl-O-NH_2 resin



To a round bottomed flask charged with 2-chlorotrityl-O-NH_2 resin (6 g, loading 1.14 mmol/g, 6.84 mmol) and DCM (60 ml) was added diisopropylethylamine (5.30, 41.0 mmol, 6 eq). Methyl 8-chloro-8-oxooctanoate (4.2 g, 20.5 mmol, 3 eq) was slowly added to the reaction mixture with orbital shaking and the reaction mixture shaken for 48 hours. The resin was filtered and washed using the standard washing procedure. The resin was dried under vacuum. LCMS purity was determined by ELS detection, 100%, m/z 204 $[M^+ + H]^+$.

Stage 2- saponification

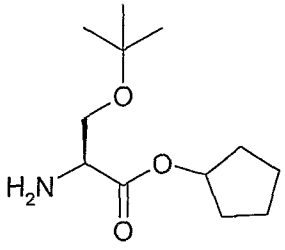


To a round bottomed flask charged with stage 1 resin (4 g, loading 1.14 mmol/g, 4.56 mmol) was added THF (16 ml) and MeOH (16 ml). To the reaction was added a solution of NaOH (0.91 g, 22.8 mmol, 5 eq) in water (16 ml). The reaction mixture was shaken for 48 hours. The resin was filtered and washed with water x 2, MeOH x 2, followed by the standard wash procedure. The resin was dried under vacuum. LCMS purity was determined by ELS detection, 100% m/z 190 $[M^+ + H]^+$.

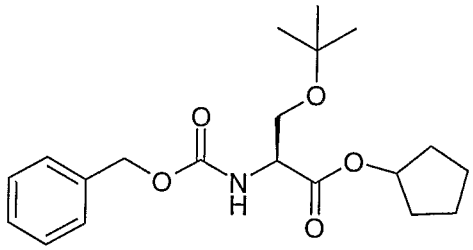
Preparation of Cyclopentyl Esters

The esters were prepared according to one of the following methods.

Method A- Synthesis of (S)-2-Amino-3-tert-butoxy-propionic acid cyclopentyl ester

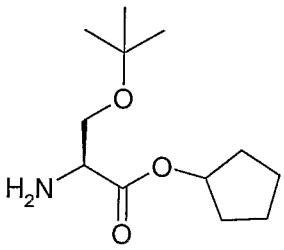


Stage 1: (S)-2-Benzyloxycarbonylamino-3-*tert*-butoxy-propionic acid cyclopentyl ester



(S)-2-Benzyloxycarbonylamino-3-*tert*-butoxy-propionic acid (4g, 0.014mol) was dissolved in DMF (40ml). Cyclopentanol (2.54ml, 0.027mol) and dimethylaminopyridine (0.165g, 0.014mol) were added. The solution was cooled to 0°C using an ice bath and to it was added N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (2.73g, 0.014mol, 1.05eq.). The mixture was stirred at 0°C for 10 minutes and then allowed to warm to r.t. and stirred for a further 18 hours. To the reaction mixture was added water (20-30ml) followed by EtOAc (40 ml). The layers were separated and the aqueous layer re-extracted with EtOAc (15 ml). The combined organic layers were washed with water (4 x 20 ml), dried ($MgSO_4$) and the solvent removed *in vacuo* to give a residue. Purification by column chromatography (1:1 EtOAc / heptane) gave the product as a colourless oil (3.82g, 78% yield). ^1H NMR (300 MHz, $CDCl_3$), δ: 1.15 (9H, s, CH_3 x 3), 1.50-1.90 (8H, m, CH_2 x 4), 3.57 (1H, dd, J = 7.4, 1.2Hz, CH), 3.85 (1H, dd, J = 7.4, 1.2Hz, CH), 4.45 (1H, m, CH), 5.15 (2H, s, CH_2), 5.25 (1H, m, CH), 5.65 (1H, d, CH, J = 7.6Hz), 7.30-7.50 (5H, m, ArH x 5).

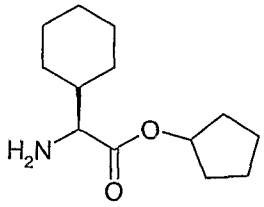
Stage 2: (S)-2-Amino-3-*tert*-butoxypropionic acid cyclopentyl ester



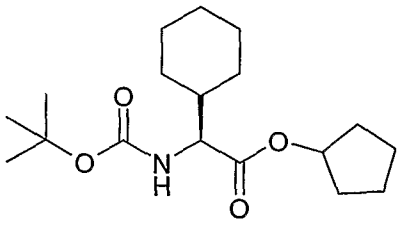
(S)-2-Benzyloxycarbonylamino-3-*tert*-butoxy-propionic acid cyclopentyl ester (3.82g, 0.011mol) was dissolved in EtOH (50ml). 20% wt. Palladium hydroxide (wet) was added cautiously to the solution. The system was evacuated and put under a hydrogen atmosphere for 4 hours. The system was evacuated and the palladium residues filtered off through Celite. The Celite was thoroughly washed with EtOH (3 x

5ml). The solvent of the filtrate was removed *in vacuo* to give the product as a colourless oil (2.41g, 100% yield). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 1.15 (9H, s, CH_3 x 3), 1.50-1.90 (10H, m, CH_2 x 4, NH_2), 3.50-3.70 (3H, m, CH_2, CH), 5.22 (1H, s, CH).

Method B- Synthesis of (S)-Amino-cyclohexyl-acetic acid cyclopentyl ester

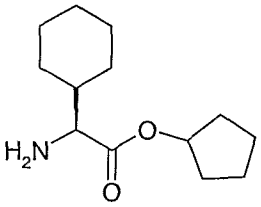


Stage 1: (S)-*tert*-Butoxycarbonylamino-cyclohexyl-acetic acid cyclopentyl ester



This was prepared in the same manner as (S)-2-benzyloxycarbonylamino-3-*tert*-butoxy-propionic acid cyclopentyl ester (Method A, Stage 1) but from (S)-*tert*-butoxycarbonylamino-cyclohexyl acetic acid. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 1.00-1.40 (10H, m, CH x 10), 1.45 (9H, s, $C(CH_3)_3$), 1.60-2.00 (8H, m, 4 x CH_2), 4.15 (1H, m, CH), 5.05 (1H, d, NH, J = 7.6Hz), 5.25 (1H, m, CH).

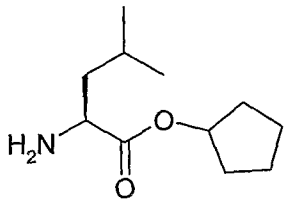
Stage 2: (S)-Amino-cyclohexyl-acetic acid cyclopentyl ester



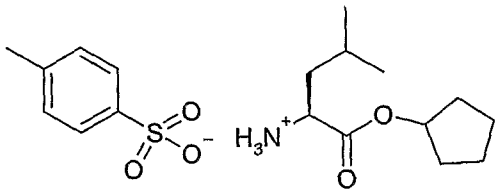
(S)-*tert*-butoxycarbonylamino-cyclohexyl-acetic acid cyclopentyl ester (1.17g, 3.60mmol) was dissolved in a TFA / DCM mixture (1:1, 10ml) at 0°C. The solution was stirred for 90 minutes, the solvent removed *in vacuo*. The residue was azetroped

with a DCM / heptane mixture (2x) to give a gum. The gum was redissolved in DCM (10ml) and washed with saturated aqueous $NaHCO_3$ solution (3 x 10ml), dried ($MgSO_4$) and filtered. The solvent of the filtrate was removed *in vacuo* to give the product as an oil (0.780g, 78% yield). ^1H NMR (300 MHz, $CDCl_3$), δ: 1.00-1.40 (10H, m, CH x 10), 1.50-2.00 (8H, m, CH x 8), 3.25 (1H, d, CH, J = 7.2Hz), 5.20 (1H, m, CH).

Method C- Synthesis of (S)-2-Amino-4-methyl-pentanoic acid cyclopentyl ester

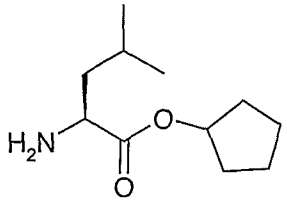


Stage 1: (S)-2-Amino-4-methyl-pentanoic acid cyclopentyl ester toluene-4-sulfonic acid



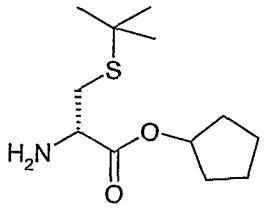
To a suspension of (S)-leucine (15g, 0.11mol) in cyclohexane (400ml) was added cyclopentanol (103.78ml, 1.14mmol) and p-toluene sulfonic acid (23.93g, 0.13mol). The suspension was heated at reflux to effect solvation. After refluxing the solution for 16 hours it was cooled to give a white suspension. Heptane (500ml) was added to the mixture and the suspension was filtered to give the product as a white solid (35g, 85% yield). ^1H NMR (300 MHz, MeOD), δ: 1.01 (6H, t, CH_3 x 2, J = 5.8Hz), 1.54-2.03 (11H, m, 11 x CH), 2.39 (3H, s, CH_3), 3.96 (1H, t, CH, J = 6.5Hz), 5.26-5.36 (1H, m, CH), 7.25 (2H, d, ArH x 2, J = 7.9Hz), 7.72 (2H, d, ArH x 2, J = 8.3Hz).

Stage 2: Synthesis of (S)-2-Amino-4-methyl-pentanoic acid cyclopentyl ester

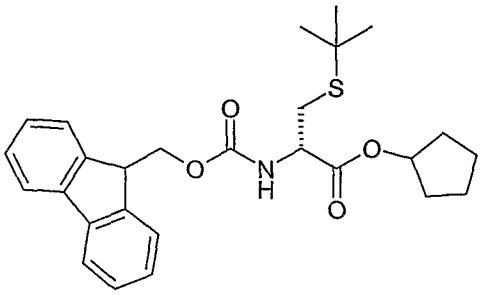


A solution of (S)-2-amino-4-methyl-pentanoic acid cyclopentyl ester toluene-4-sulfonic acid (2.57g, 0.013mol) in DCM (5ml) was washed with saturated aqueous $NaHCO_3$ solution (2 x 3ml). The combined aqueous layers were back extracted with DCM (3 x 4ml). The combined organic layers were dried ($MgSO_4$), and the solvent removed *in vacuo* to give a colourless oil (1.10g, 80% yield). ^1H NMR (300 MHz, $CDCl_3$), δ: 0.90 (6H, t, CH_3 x 2, J = 6.4Hz), 1.23-1.94 (11H, m, 5 x CH_2, CH), 3.38 (1H, dd, CH, J = 8.4, 5.9Hz), 5.11-5.22 (1H, m, CH).

Method D- Synthesis of (S)-2-Amino-3-tert-butylsulfanyl-propionic acid cyclopentyl ester

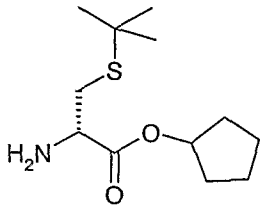


Stage 1: (S)-3-*tert*-Butylsulfanyl-2-(9H-fluoren-9-ylmethoxycarbonylamino)-propionic acid cyclopentyl ester



This was prepared in the same manner as (S)-2-Benzyloxycarbonylamino-3-*tert*-butoxy-propionic acid cyclopentyl ester (Method A, Stage 1) but from (S)-3-*tert*-Butylsulfanyl-2-(9H-fluoren-9-ylmethoxycarbonylamino)-propionic acid. ^1H NMR (300 MHz, $CDCl_3$), δ: 1.30 (9H, s, $(CH_3)_3$), 1.55-1.95 (8H, m, CH_2 x 4), 3.05 (2H, d, CH_2, J = 4.8Hz), 4.20-4.30 (1H, m, CH), 4.40 (2H, d, CH2, J = 7.5Hz), 4.65 (1H, m, CH), 5.25 (1H, m, CH), 5.70 (1H, d, NH, J = 7.8Hz), 7.30-7.50 (4H, m, ArH x 4), 7.65 (2H, d, J = 7.5Hz, ArH x 2), 7.80 (2H, d, J = 7.5Hz, ArH x 2).

Stage 2: (S)-2-Amino-3-tert-butylsulfanyl-propionic acid cyclopentyl ester



(S)-3-*tert*-Butylsulfanyl-2-(9H-fluoren-9-ylmethoxycarbonylamino)-propionic acid cyclopentyl ester (1.63g, 3.50mmol) was dissolved in a CH_3CN (25ml) at 0°C. Piperidine (21ml) was added to the solution. After stirring for 30 minutes, the solvent was removed *in vacuo* to give a residue. Purification by column chromatography (EtOAc eluent) gave the product as a colourless oil (628mg, 73% yield). ^1H NMR (300 MHz, $CDCl_3$), δ: 1.30 (9H, s, $(CH_3)_3$), 1.55-1.95 (8H, m, CH_2 x 4), 2.75 (1H, dd, CH, J = 7.2, 12.3Hz), 2.95 (1H, dd, CH, J = 4.8, 12.3Hz), 5.25 (1H, m, CH).

The following N-Cbz protected amino acids were converted to the cyclopentyl esters according to Method A (above)

(S)-2-Benzyloxycarbonylamino-succinic acid 4-tert-butyl ester

(S)-2-Benzyloxycarbonylamino-succinamic acid

(S)-2-Benzyloxycarbonylamino-4-carbamoyl-butyric acid

(S)-2-Benzyloxycarbonylamino-3-(4-tert-butoxy-phenyl)-propionic acid

(S)-2-Benzyloxycarbonylamino-3-hydroxy-butyric acid

(S)-2-Benzyloxycarbonylamino-3,3-dimethyl-butyric acid

(S)-2-Benzyloxycarbonylamino-3-(1H-indol-2-yl)-propionic acid

(S)-2-Benzyloxycarbonylamino-6-tert-butoxycarbonylamino-hexanoic acid

The following N-Boc protected amino acids were converted to the cyclopentyl esters according to Method B (above)

tert-Butoxycarbonylamino-acetic acid

(S)-2-tert-Butoxycarbonylamino-3-methyl-pentanoic acid

(S)-Pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester

(S)-2-tert-Butoxycarbonylamino-3-methyl-butyric acid

(S)-2-tert-Butoxycarbonylamino-4-methylsulfanyl-butyric acid

The following free amino acids were converted to the cyclopentyl esters according to Method C (above)

(S)-Amino-phenyl-acetic acid

(S)-2-Amino-3-phenyl-propionic acid

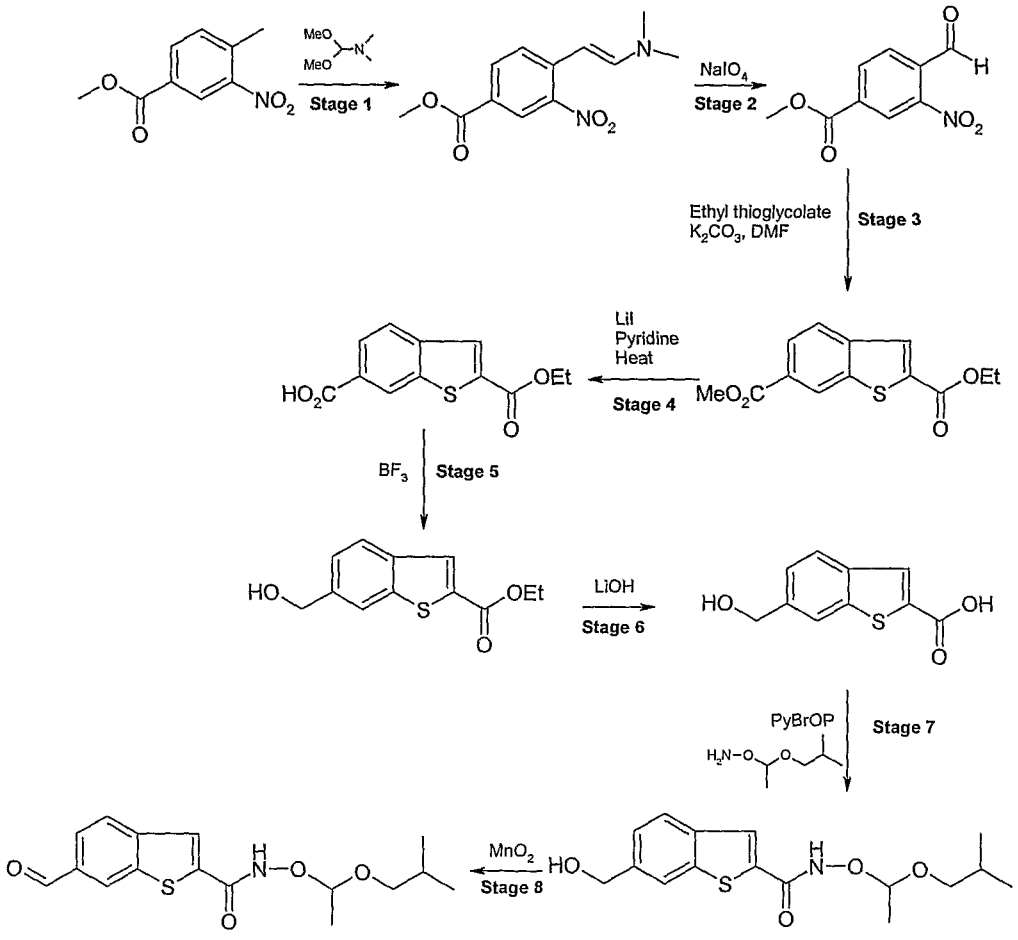
(S)-2-Amino-propionic acid

(S)-2-Amino-4-methyl-pentanoic acid

Preparation of 6-formyl-benzo[b]thiophene-2-carboxylic acid (1-isobutoxy-ethoxy) amide

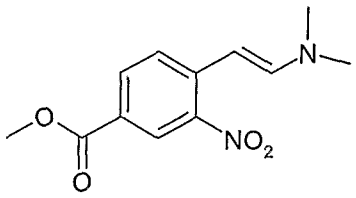
The Synthesis is outlined below in Scheme 7.

Additional literature references relating to this route can be found within *Tetrahedron Letters,* **35**, 2, 219-222 & WO 05/034880



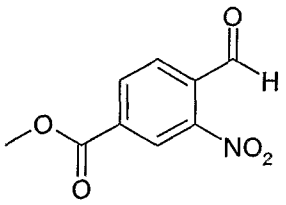
Scheme 7

Stage 1: 4-(2-dimethylamino-vinyl)-3-nitrobenzoic acid methyl ester



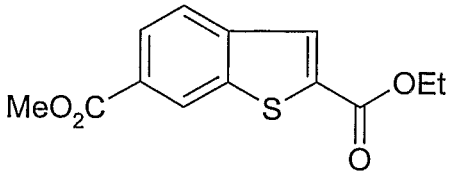
Methyl 4-methyl-3-nitrobenzoate (5 g, 25.6 mmol) was dissolved in DMF (25 mL, 5 vol) and to this was added *N,N*-dimethylformamide dimethylacetal (4.4 mL, 33.3 mmol). The mixture was allowed to stir at 140 °C for 3 h. The resulting deep red solution was allowed to cool and concentrated under vacuum. The residue was triturated with methanol and filtered. The filtrate was washed with methanol and dried on the sinter to yield 4-(2-dimethylamino-vinyl)-3-nitrobenzoic acid methyl ester (5.2g, 80%). ^{1}H NMR (300 MHz, DMSO), δ: 2.98 (6H, s, 2 x CH_3), 3.87 (3H, s, CH_3), 5.58 (1H, m, CH), 7.72 – 7.83 (3H, m, ArH), 8.32 (1H, m, CH).

Stage 2: 4-Formyl-3-nitrobenzoic acid methyl ester



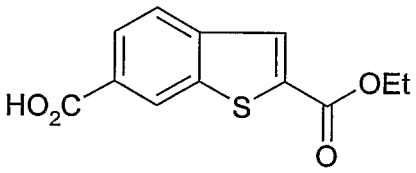
To a solution of the enamine (5 g, 20.0 mmol) in THF (50 mL, 10 vol) and water (50 mL, 10 vol) was added sodium periodate (12.8g, 60.0 mmol) and the mixture allowed to stir for 2 h. The mixture was filtered and the resulting solids washed with EtOAc (500 mL). The organic layer was isolated, washed with $NaHCO_3$ (3 x 100 mL) and dried ($MgSO_4$). Concentration under vacuum afforded 4-formyl-3-nitrobenzoic acid methyl ester (3.9g, 93%). LCMS m/z 210 $[M^+ + H]^+$, ^{1}H NMR (300 MHz, DMSO), δ: 3.96 (3H, s, OMe), 8.01 (1H, d, ArH), 8.39 (1H, d, ArH), 8.54 (1H, s, ArH), 10.31 (1H, s, CHO).

Stage 3: Benzo[b]thiophene-2,6-dicarboxylic acid 2-ethyl ester 6-methyl ester



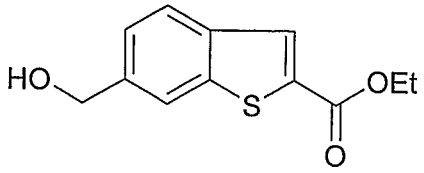
A mixture of 4-formyl-3-nitrobenzoic acid methyl ester (3.9 g, 18.7 mmol), mercapto-acetic acid ethyl ester (2.2 mL, 20.4 mmol) and K_2CO_3 (3.3 g, 24 mmol) in DMF (40 ml, 10 vol) was heated to 50 °C overnight. After cooling to r.t. the mixture was poured onto ice-cold water (250 mL) and the resulting mixture stirred for 40 min. The solid formed was isolated by filtration, washed with water (4 x 50 mL) and dried under vacuum to afford the title compound (3.9 g, 80%). LCMS m/z 265 $[M^+H]^+$, ^1H NMR (300 MHz, $CDCl_3$) δ: 1.40 (3H, t J = 6.8 Hz, CH_3), 3.95 (3H, s, OMe), 4.40 (2H, q J = 7.2 Hz, CH_2), 7.88 (1H, d J = 8.0 Hz, ArH), 7.97 – 8.09 (2H, m. ArH), 8.56 (1H, s, ArH).

Stage 4: Benzo[b]thiophene-2,6-dicarboxylic acid 2-ethyl ester



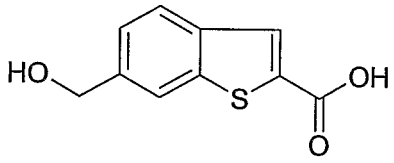
A mixture of benzo[b]thiophene-2,6-dicarboxylic acid 2-ethyl ester 6-methyl ester (3.9 g, 14.77 mmol) and Lithium iodide (10 g, 74.6 mmol) in anhydrous pyridine (30 ml, 9 vol) was stirred at reflux for 16 h. After cooling to r.t., the mixture was added (either as a melt or chipped out) to ice-cold 2N HCl (200 mL). The solid formed was isolated by filtration and washed with water (3 x 50 mL). The product was purified by recrystallisation from methanol to give the title compound (1.8 g, 49%). LCMS m/z 251 $[M^+H]^+$,^1H NMR (300 MHz, DMSO), δ: 1.35 (3H, t J=6.9 Hz, CH_3), 4.38 (2H, q J=7.1 Hz, CH_2), 7.99 (1H, d J=8.3 Hz, ArH), 8.12 (1H, d J=8.3 Hz, ArH), 8.27 (1H, s, ArH), 8.70 (1H, s, ArH).

Stage 5: 6-Hydroxymethyl-benzo[b]thiophene-2-carboxylic acid ethyl ester



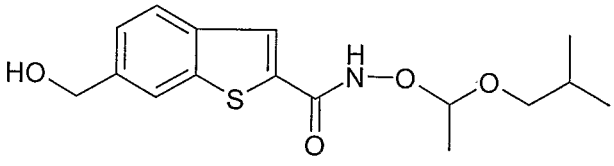
A solution of benzo[b]thiophene-2,6-dicarboxylic acid 2-ethyl ester (1.6g, 6.4 mmol) in anhydrous THF (40 mL, 25 vol) was cooled to 0 °C. To this BH_3 (1M in THF, 30 mL, 30.0 mmol) was added slowly. The reaction was allowed to warm to r.t. and stirred for 3 h. The solution was then cooled to 0 °C and quenched using 1N HCl (7.5 mL). The reaction mixture was concentrated under vacuum to remove all THF and the resulting solid isolated by filtration and dried under vacuum to give 6-hydroxymethyl-benzo[b]thiophene-2-carboxylic acid ethyl ester (1.3g, 87%). LCMS m/z 237 $[M^+ + H]^+$, ^1H NMR (300 MHz, DMSO), δ: 1.34 (3H, t J=6.9 Hz, CH_3), 4.35 (2H, q J=7.1 Hz, CH_2), 4.65 (2H, s, CH_2), 6.53 (1H, br s, OH), 7.42 (1H, d J=9.4 Hz), 7.98 (3H, m, ArH), 8.18 (1H, s, ArH).

Stage 6: 6-Hydroxymethyl-benzo[b]thiophene-2-carboxylic acid



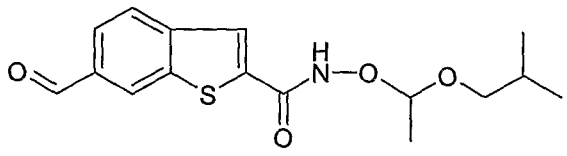
6-Hydroxymethyl-benzo[b]thiophene-2-carboxylic acid ethyl ester (2.4 g, 9.6 mmol, 1 eq) was dissolved in THF (10 mL, 4 vol) and water added (10 mL) along with LiOH (0.69 g, 28.8 mmol). The reaction mixture was stirred at 50 °C for 3 h and then concentrated to dryness and taken onto the next step without purification.

Stage 7: 6-hydroxymethyl-benzo[b]thiophene-2-carboxylic acid (1-isobutoxy-ethoxy) amide



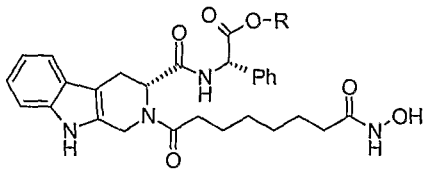
To a solution of 6-hydroxymethyl-benzo[b]thiophene-2-carboxylic acid (1.76 g, 8.4 mmol, 1 eq) in DMF was added PyBrOP (4.3 g, 9.2 mmol), O-(isobutoxy-ethyl)-hydroxylamine (11.5 mL, 84.0 mmol) (prepared *via* procedure in WO0160785) and DIPEA (2.9 mL, 16.7 mmol). The reaction mixture was allowed to stir at r.t. for 2 h then diluted with water (40 mL) and EtOAc (40 mL). The organic layer was isolated, washed with brine (50 mL) and concentrated. The residue was purified by chromatography on silica gel eluting with EtOAc / heptane (1:1) to afford the title compound (1.8g, 67% over 2 steps). LCMS m/z 322 $[M^+-H]^+$, ^1H NMR (300 MHz, MeOD), δ: 0.83 (6H, d J = 6.6Hz, 2 x CH_3), 1.32 (3H, d J= 5.9 Hz, CH_3), 1.75 (1H, m, CH), 3.38 (2H, m, CH_2), 4.63 (2H, s, CH_2), 4.95 (1H, m, CH), 7.32 (1H, d J = 8.2Hz, ArH), 7.77 (3H, m, ArH).

Stage 8: 6-formyl-benzo[b]thiophene-2-carboxylic acid (1-isobutoxy-ethoxy) amide

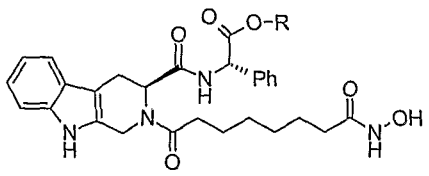


To a solution of 6-hydroxymethyl-benzo[b]thiophene-2-carboxylic acid (1-isobutoxy-ethoxy) amide (600 mg, 1.86 mmol) in DCM (3 mL) was added MnO_2 (2.1 g, 24.1 mmol). The mixture was stirred at ambient temperature for 30 min and then filtered through celite. The filtrate was concentrated to afford the title compound (435 mg, 82%). LCMS m/z 320 $[M^+-H]^+$, ^1H NMR (300 MHz, MeOD), δ: 0.94 (6H, d J = 6.7 Hz, 2 x CH_3), 1.45 (3H, d J = 5.3 Hz, CH_3), 1.87 (1H, m, CH), 3.40 (2H, m, CH2), 5.08 (1H, dd J = 5.2, 10.6 Hz, CH), 7.89-8.09 (3H, m, ArH), 8.55 (1H, s, ArH), 10.11 (1H, s, CHO).

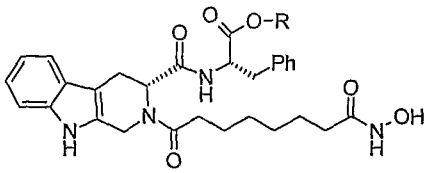
Synthesis of Compounds in Figure 1 as Exemplified by Compound (1) and Compound (2)



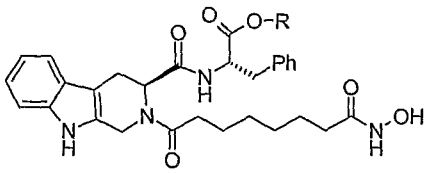
R= cyclopentyl 1
R= H 2



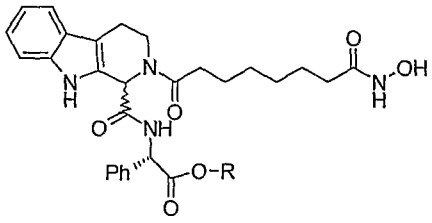
R= cyclopentyl 3
R= H 4



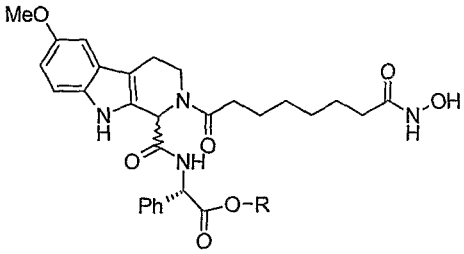
R= ethyl 5
R= H 6



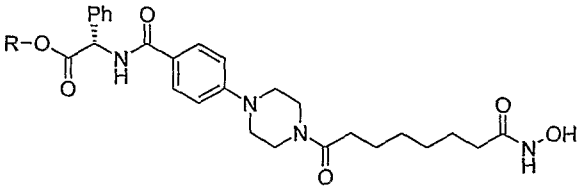
R= ethyl 7
R= H 8



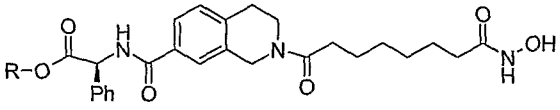
R= cyclopentyl 9
R= H 10



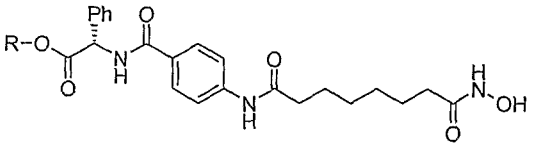
R= cyclopentyl 11
R= H 12



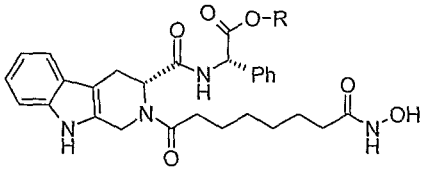
R= cyclopentyl 13
R= H 14



R= cyclopentyl 15
R= H 16



R= cyclopentyl 17
R= H 18

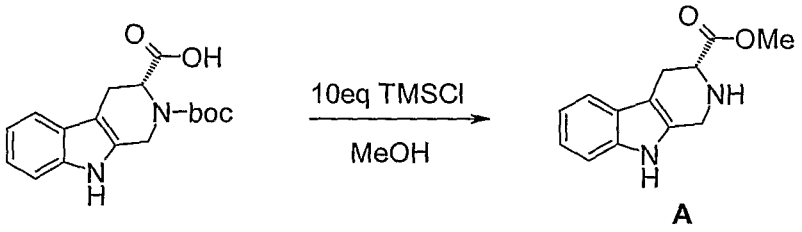


R= t-butyl 19

Figure 1

Preparation of Building Blocks A-G

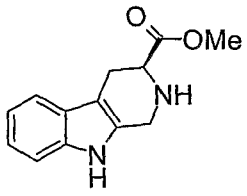
Building Blocks A and B



A

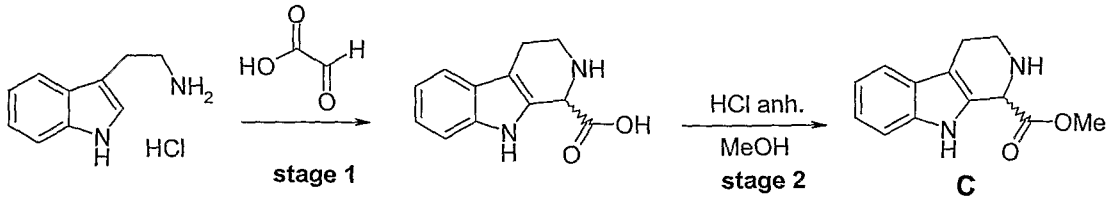
N-Boc-D-tetrahydro-beta-carboline-3-carboxylic acid (5.0g, 15.8mmol) and TMSCl (20ml, 158mmol) in MeOH (50ml) were heated under reflux for 2h. The reaction mixture was evaporated to dryness to yield (R)-2,3,4,9-Tetrahydro-1H-beta-carboline-3-carboxylic acid methyl ester (building block **A**). LCMS purity 100%. m/z 231 $[M^+H]^+$, 461 $[2M^+H]^+$. Building block **A** was used without further purification.

(S)-2,3,4,9-Tetrahydro-1H-beta-carboline-3-carboxylic acid methyl ester (building block **B**) was obtained by the same procedure as block **A** using N-boc-L-tetrahydro-beta-carboline-3-carboxylic acid.



B

Building Block C



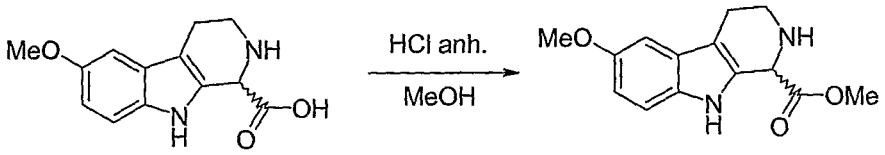
C

Stage 1: A solution of glyoxylic acid monohydrate (1.51g, 16.4mmol) in water (10ml) was added dropwise to a stirred solution of tryptamine.HCl (3.0g, 15.3mmol) in water (200ml). KOH (0.827g, 14.7mmol) in water (10ml) was added. The reaction mixture

was stirred at room temperature for 1 h after which time precipitation occurred. Following filtration under reduced pressure the tetrahydro-beta-carboline-1-carboxylic acid was collected and washed with water. Yield 1.9g (58%); m/z 217 $[M^+H]^+$.

Stage 2: A solution of tetrahydro-beta-carboline-1-carboxylic acid (7.4g) in MeOH (250ml) was saturated with HCl gas for 20min. The reaction mixture was gently stirred at room temperature for 18h. and ca. 80% conversion was observed. The reaction mixture was re-treated with HCl gas and allowed to stir for another 18h. Upon completion of the reaction the mixture was concentrated in vacuo to yield 2,3,4,9-tetrahydro-1H-beta-carboline-1-carboxylic acid methyl ester (building block **C**), LCMS purity 95%, m/z 231 $[M^+H]^+$. The product was used without further purification.

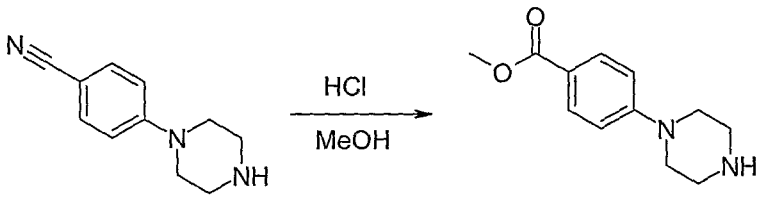
Building Block D



D

6-Methoxy-2,3,4,9-tetrahydro-1H-beta-carboline-1-carboxylic acid methyl ester (building block **D**) was obtained from esterification of 6-methoxy-tetrahydro-beta-carboline-1-carboxylic acid using the procedure as for building block **C**. Building block **D**: LCMS purity 98%, m/z 261 $[M^+H]^+$. Building block **D** was used without further purification.

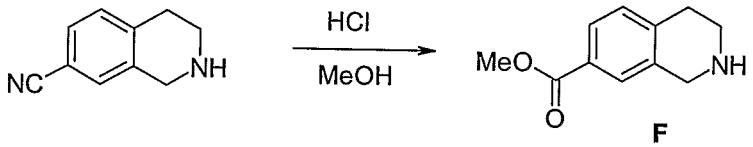
Building Block E



A solution 4-piperazin-1-yl-benzonitrile (1.5g, 8.0mmol) in MeOH (150ml) was saturated with HCl gas. Water (0.17ml) was added and the mixture was heated under reflux for 18 h. The reaction mixture was cooled to r.t. and was resaturated with HCl

gas. This was refluxed for a further 24 h. The mixture was concentrated under reduced pressure yielding 4-Piperazin-1-yl-benzoic acid methyl ester (building block **E**). LCMS purity 90%, m/z 221 $[M^+ + H]^+$. Building block E was used without further purification.

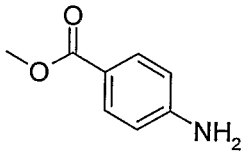
Building Block F



F

1,2,3,4-Tetrahydro-isoquinoline-7-carboxylic acid methyl ester (building block **F**) was prepared following the same procedure as for building block **E**. LCMS purity 89%. m/z 193 $[M^+ + H]^+$. This product was used without further purification.

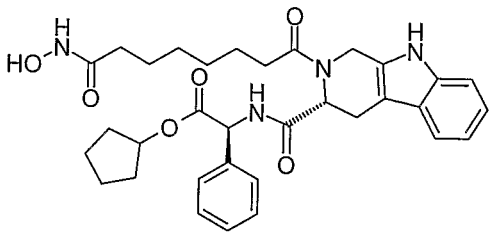
Building Block G



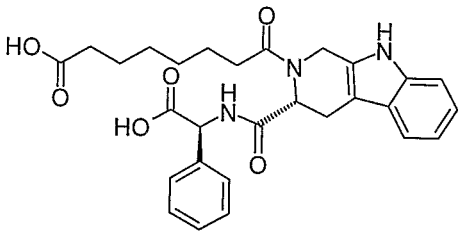
G

4-Amino-benzoic acid methyl ester (building block G) was commercially available

Synthesis of Compounds (1) and Compound (2)

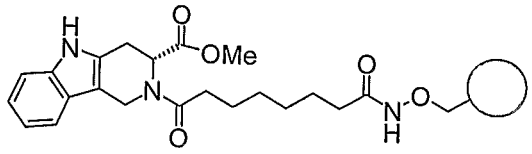


1



2

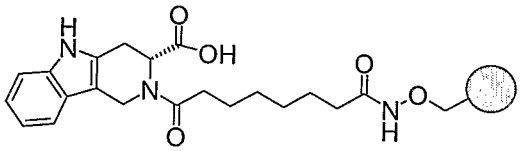
Stage 1: Coupling with building block



Hydroxylamine 2-chlorotrityl resin derivatized with suberic acid (4.6g, loading 1.14mmol, 5.24mmol) was swollen in anhydrous DCM (50ml). Building block A (4.76g, 15.72mmol) was added, followed by pyBOP (8.18g, 15.72mmol, 3eq) and DIPEA (6.77g, 52.4mmol, 10eq). The reaction was shaken for 18h, filtered and washed using the standard wash procedure. The resin was dried under vacuum.

Note: For building block G, coupling using the above condition gave ca. 10% conversion. Thus an alternative condition was used: Stage 2 resin (1.0g, loading 1.14mmol) was swollen in anhydrous DCM (100ml). 1-Chloro-N,N-2-trimethylpropenylamine (Ghosez reagent)[1] (7.53ml, 57.0mmol, 50eq) was added at 0 °C under the atmosphere of N_2. The mixture was allowed to warm to room temperature and gently shaken for 1-2h. The aniline building block G (8.6g, 57.0mmol, 50 eq) was added portionwise over 20min. Et_3N (8.0ml, 57.0mmol, 50eq) was added. The mixture was shaken for 18h. LCMS after a test cleave shows 70% conversion, m/z 323 $[M^+H]^+$, 645 $[2M^+H]^+$. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

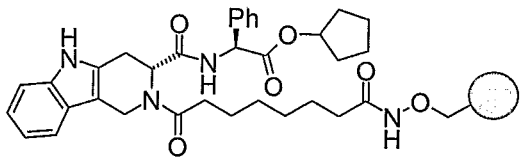
Stage 2: Saponification



Stage 1 resin (4.8g, loading 1.14mmol, 5.47mmol) was suspended in MeOH (17.5ml) and THF (17.5ml). A solution of NaOH (1.1g, 27.5 mmol, 5eq) in water (17.5ml) was added. The mixture was shaken for 18h. LCMS of the test cleave confirmed the completion of reaction, m/z 388 $[M^+H]^+$, 775 $[2M^+H]^+$. The resin was filtered and washed with water x 2, MeOH x 2, followed by the standard wash procedure. The resin was dried under vacuum.

Note: For building block E, saponification was carried out using 10eq. of 2.7M NaOH and was shaken for 72h.

Stage 3: Coupling with L-phenylglycine cyclopentyl ester

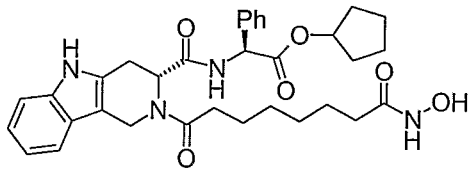


Stage 2 resin (2.4g, loading 1.14mmol, 2.7mmol) was suspended in anhydrous DCM (30ml). L-phenylglycine cyclopentylester tosyl salt (3.2g, 8.1mmol, 3eq) was added, followed by pyBOP (4.2g, 8.1mmol, 3eq) and DIPEA (3.5g, 27.0mmol, 10eq). The mixture was shaken for 18h. The LCMS of the test cleave (i.e. a small quantity of resin was washed using the standard wash procedure, dried, and cleaved in 2% TFA/DCM. Resin was filtered off and filtrate concentrated to dryness. LCMS was obtained) confirmed the completion of reaction, m/z 589 $[M^+H]^+$. The whole sample of resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

Note.

For compounds **5-8**, L-phenylalanine ethyl ester (3eq) was used.
For compound **19**, L-phenylglycine t-butyl ester (3eq) was used.

Stage 4: (S)-{[(R)-2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-3-carbonyl]-amino}-phenyl-acetic acid cyclopentyl ester (**1**)

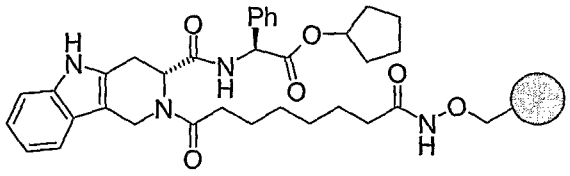


1

Stage 3 resin (1.0g, loading 1.14mmol) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was collected and evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and after 20mins. The combined filtrates were evaporated to dryness under reduced pressure at r.t, the residue (ca. 300mg) was purified by preparative HPLC to yield compound **(1)**, m/z 589 $[M^+H]^+$; ^1H NMR (400 MHz, CD_3OD) δ: 1.3-1.7(16 H, m, CH_2), 2.1-2.3 (2 H, m, CH_2), 2.5 (2 H, m, CH_2), 3.0-3.5 (2 H, m, CH_2), 4.5-4.8 (2 H, m, CH_2), 5.1 (1 H, m, $CO_2C\underline{H}$), 5.2 (1 H, dd, $CH_2C\underline{H}NCO$), 5.5-5.9 (1 H, d, $CONHC\underline{H}Ph$), 7.0-7.5 (9 H, m, Ar).

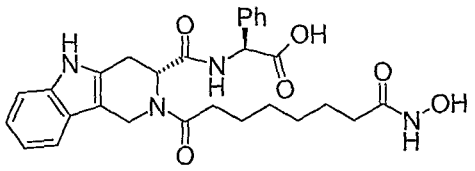
The corresponding carboxylic acid was obtained by the following procedure

Stage 5: Saponification



Stage 3 resin (1.0g, loading 1.14mmol) was suspended in MeOH (4ml) and THF (4ml). A solution of NaOH (0.23g, 5.7mmol) in water (4ml) was added. The mixture was shaken for 18h. LCMS of the test cleave confirmed the completion of reaction, m/z 521 $[M^+H]^+$. Resin was filtered and washed with water x 2, MeOH x 2, followed by the standard wash procedure. Resin was dried under vacuum.

Stage 6: (S)-{[(R)-2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-3-carbonyl]-amino}-phenyl-acetic acid (**2**)



2

Stage 5 resin (1.0g, loading 1.14mmol) was cleaved using the procedure outlined for Stage 6 yielding compound **(2)**, m/z 521 $[M^+ + H]^+$; ^1H NMR (400 MHz, CD_3OD), δ: 1.3-1.5 (4 H, 2 x CH_2), 1.6-1.8 (4 H, 2 x CH_2), 2.1-2.2 (2 H, m, CH_2), 2.4-2.7 (2 H, m, CH_2), 3.0-3.2 (1 H, m), 3.5 (1H, m), 4.55 (m), 4.9 (m), 5.1- 5.35 (2 H, m), (2 H, m, CH_2NCO), 5.75-5.8 (1 H, 2 x d, NHCHPh), 7.0-7.5 (9 H, m, Ar), 7.6 (d), 7.7 (d), 8.35 (d), 8.95 (s), 9.05 (s).

The following compounds were prepared according to the procedure described for Compound **(1)** and Compound **(2)**

(S)-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-3-carbonyl]-amino}-phenyl-acetic acid cyclopentyl ester (3)

Building block B used

LCMS purity 98%, m/z 589 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.20-1.40 (8 H, m, 4 x CH_2), 1.40-1.80 (8 H, m, 4 x CH_2), 2.10 (2 H, m, CH_2), 3.45 (2 H, m, CH_2), 5.0 (2 H, m, CH_2 overlaps with D_2O peak), 5.25-5.45 (2 H, m, 2 x CH), 5.50 (1 H, s, CONHCHPh), 7.00-7.50 (9 H, m, Ar).

(S)-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-3-carbonyl]-amino}-phenyl-acetic acid (4)

Building block B used

LCMS purity 100%, m/z 521 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ 1.30-1.50 (4 H, m, 2 x CH_2), 1.55-1.80 (4 H, m, 2 x CH_2), 2.15 (2 H, m, CH_2), 2.60 (2 H, m, CH_2), 3.00 - 3.25 (2 H, m, CH_2), 3.40-3.55 (2 H, m, CH_2), 5.20-5.30 (1 H, m, CHCON), 5.35 (1 H, s, NHCHPh), 7.05-7.50 (9 H, m, Ar).

(S)-2-{[(R)-2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-3-carbonyl]-amino}-3-phenyl-propionic acid ethyl ester (5)

Building block A used

LCMS purity 100% m/z 563 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ 1.00 (3 H, t, CH_3), 1.20-1.24 (4 H, m, 2 x CH_2), 1.50-1.70 (4 H, m, 2 x CH_2), 2.00 (2 H, m, CH_2), 2.30-2.50 (2 H, m, CH_2), 2.80-3.00 (2 H, m, CH_2), 4.05 (2 H, q, CO_2CH_2), 4.35-4.50 (1 H, m, CH), 4.80-5.05 (2 H, m, CH_2), 5.40 (1 H, s, NHPhCO), 6.80-7.30 (9 H, m, Ar).

(S)-2-{[(R)-2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-3-carbonyl]-amino}-3-phenyl-propionic acid (6)

Building block A used

LCMS purity 100%, m/z 534 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ 1.30-1.50 (4 H, m, 2 x CH_2), 1.60-1.80 (4 H, m, 2 x CH_2), 2.15 (2 H, m, CH_2), 2.50 (2 H, m, CH_2), 3.00 (2 H, m, CH_2), 3.20 (2 H, m, CH_2), 4.30-4.80 (2 H, m, CH_2), 5.15 (1 H, m, CH), 6.90-7.50 (9 H, m, Ar).

(S)-2-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-3-carbonyl]-amino}-3-phenyl-propionic acid ethyl ester (7)

Building block B used

LCMS purity 100%, m/z 563 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ 1.00 (3 H, t, CH_3), 1.30-1.50 (4 H, m, 2 x CH_2), 1.60-1.70 (4 H, m, 2 x CH_2), 2.10 (2 H, m, CH_2), 2.30-2.65 (2 H, m, CH_2), 2.95-3.20 (2 H, m, CH_2), 3.45 (1 H, m, CH), 4.05 (2 H, q, CO_2CH_2), 4.35-4.50 (1 H, m, CH), 4.80-5.05 (2 H, m, CH_2), 5.50 (1 H, s, NHPhCO), 6.90-7.50 (9 H, m, Ar).

(S)-2-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-3-carbonyl]-amino}-3-phenyl-propionic acid (8)

Building block B used

LCMS purity 100%, m/z 534 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ 1.30-1.50 (4 H, m, 2 x CH_2), 1.60-1.80 (4 H, m, 2 x CH_2), 2.15 (2 H, m, CH_2), 2.50 (2 H, m, CH_2), 2.95-3.15 (2 H, m, CH_2), 3.20-3.50 (2 H, m, CH_2), 4.30-4.50 (2 H, m, 2 x CH), 4.80-5.20 (2 H, m, CH_2), 6.90-7.50 (9 H, m, Ar).

(S)-{[2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carb oline-1-carbonyl]-amino}-phenyl-acetic acid cyclopentyl ester (9)

Building block C used

LCMS purity 100%, m/z 589 $[M^+H]^+$, ^1H NMR (400 MHz, $CDCl_3$), δ 1.30-1.80 (16 H, m, 8 x CH_2), 2.15 (2 H, m, CH_2), 2.45-2.70 (2 H, m, CH_2), 2.95 (2 H, m, CH_2), 3.55 (1 H, m, CH), 4.35 (1 H, m, CH), 5.15 (1 H, m, CO_2CH), 5.45 (1 H, m, CH), 6.20 (1 H, d, PhCHNH) 7.00-7.80 (9 H, m, Ar), 8.80-9.20 (1 H, broad m, CHNHOH).

(S)-{[2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carb oline-1-carbonyl]-amino}-phenyl-acetic acid (10)

Building block C used

LCMS purity 100%, m/z 521 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ 1.30-1.50 (4 H, m, 2 x CH_2), 1.60-1.80 (4 H, m, 2 x CH_2), 2.15 (2 H, m, CH_2), 2.50-2.65 (2 H, m, CH_2),

2.95 (2 H, m, CH_2), 3.70 (1 H, dd, CH), 4.30 (1 H, dd, CH), 5.50 (1 H, m, CH), 6.10 and 6.20 (0.5 H each, s, PhCHNH) 7.00-7.50 (9 H, m, Ar).

(S)-{[2-(7-Hydroxycarbamoyl-heptanoyl)-6-methoxy-2,3,4,9-tetrahydro-1H-beta-carboline-1-carbonyl]-amino}-phenyl-acetic acid cyclopentyl ester (11)

Building block D used

LCMS purity 100%, m/z 619 $[M^+ + H]^+$, ^1H NMR (400 MHz, $CDCl_3$), δ 1.30-1.80 (16 H, m, 8 x CH_2), 2.15 (2 H, m, CH_2), 2.50-2.65 (2 H, m, CH_2), 2.85 (2 H, m, CH_2), 3.70 (1 H, dd, CH), 3.80 (3 H, s, OMe), 4.30 (1 H, dd, CH), 5.20 (1 H, m, CO_2CH), 5.30-5.50 (1 H, m, CH), 6.15-6.20 (1 H, d, PhCHNH) 6.80-7.80 (8 H, m, Ar), 8.80-9.00 (1 H, m, CONHOH).

(S)-{[2-(7-Hydroxycarbamoyl-heptanoyl)-6-methoxy-2,3,4,9-tetrahydro-1H-beta-carboline-1-carbonyl]-amino}-phenyl-acetic acid (12)

Building block D used

LCMS purity 100%, m/z 551 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ 1.30-1.60 (8 H, m, 4 x CH_2), 2.05 (2 H, m, CH_2), 2.50-2.65 (2 H, m, CH_2), 2.80 (2 H, m, CH_2), 3.55 (1 H, dd, CH), 3.70 (3 H, s, OMe), 4.30 (1 H, dd, CH), 5.30-5.50 (1 H, m, CH), 5.90-6.10 (0.5 H each, s, PhCHNH), 6.65 (1 H, m, Ar), 6.80 (1 H, m, Ar), 7.10 (1 H, m, Ar), 7.35 (5 H, m, Ar).

(S)-{[2-(7-Hydroxycarbamoyl-heptanoyl)-6-methoxy-2,3,4,9-tetrahydro-1H -beta-carboline-1-carbonyl]-amino}-phenyl-acetic acid cyclopentyl ester (13)

Building block E used

LCMS purity 95%, m/z 579 $[M^+ + H]^+$, ^1H NMR (400 MHz, $CDCl_3$), δ 1.40-1.90 (16 H, m, 8 x CH_2), 2.15 (2 H, m, CH_2), 2.45 (2 H, m, CH_2), 3.40 (4 H, m, 2 x CH_2N), 3.60-3.80 (4 H, m, 2 x CH_2N), 5.30 (1 H, m, CO_2CH), 5.70 (1 H, d, PhCHNH), 6.90 (2 H, d, Ar), 7.30-7.50 (6 H, m, Ar), 7.80 (2 H, d, Ar).

(S)-{[2-(7-Hydroxycarbamoyl-heptanoyl)-6-methoxy-2,3,4,9-tetrahydro-1H -beta-carboline-1-carbonyl]-amino}-phenyl-acetic acid (14)

Building block E used

LCMS purity 100%, m/z 511 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ 1.30 (4 H, m, 2 x CH_2), 1.50 (4 H, m, 2 x CH_2), 2.00 (2 H, t, CH_2), 2.35 (2 H, t, CH_2), 3.30 (4 H, m, 2 x CH_2N), 3.70 (4 H, m, 2 x CH_2N), 5.55 (1 H, s, PhCHNH), 6.90 (2 H, d, Ar), 7.30 (3 H, m, Ar), 7.40 (2 H, m, Ar), 7.70 (2 H, d, Ar).

(S)-{[2-(7-Hydroxycarbamoyl-heptanoyl)-1,2,3,4-tetrahydro-isoquinoline-7-carbonyl]-amino}-phenyl-acetic acid cyclopentyl ester (15)

Building block F used

LCMS purity 100%, m/z 550 $[M^+H]^+$, ^1H NMR (400 MHz, $CDCl_3$), δ 1.30-1.80 (16 H, m, 8 x CH_2), 2.15 (2 H, m, CH_2), 2.45 (2 H, m, CH_2), 2.95 (2 H, m, 2 x CH_2), 3.70-3.90 (2 H, m, 2 x CH_2), 4.60-4.70 (2 H, m, CH_2), 5.25 (1 H, m, CO_2CH), 5.70 (1 H, m, PhCHNH), 7.20-7.70 (8 H, m, Ar).

(S)-{[2-(7-Hydroxycarbamoyl-heptanoyl)-1,2,3,4-tetrahydro-isoquinoline -7-carbonyl]-amino}-phenyl-acetic acid (16)

Building block F used

LCMS purity 87%, m/z 482 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ 1.30-1.50 (4 H, m, 2 x CH_2), 1.60-1.70 (4 H, m, 2 x CH_2), 2.15 (2 H, m, CH_2), 2.50 (2 H, m, CH_2), 2.95 (2 H, m, 2 x CH_2), 3.70 (2 H, m, CH_2), 4.80 (2 H, m, CH_2), 5.70 (1 H, s, PhCHNH), 7.20-7.80 (8 H, m, Ar).

(S)-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzoylamino]-phenyl-acetic acid cyclopentyl ester(17)

Building block G used

LCMS purity 94%, m/z 510 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ 1.30-1.80 (16 H, m, 8 x CH_2), 2.00-2.20 (4 H, m, 2 x CH_2), 5.10-5.30 (1 H, m, CO_2CH), 5.70 (1 H, m, PhCHNH), 7.30-7.80 (9 H, m, Ar).

(S)-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzoylamino]-phenyl-acetic acid (18)

Building block G used

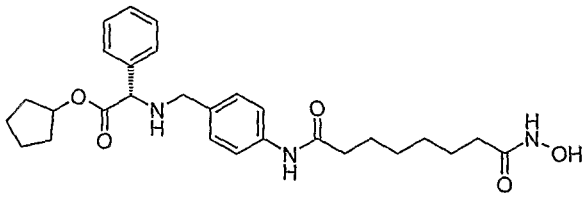
LCMS purity 100%, m/z 442 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ 1.30-1.40 (4 H, m, 2 x CH_2), 1.50-1.70 (4 H, m, 2 x CH_2), 2.20 (2 H, t, CH_2), 2.35 (2 H, t, CH_2), 5.70 (1 H, s, PhCHNH), 7.25-7.40 (3 H, m, Ar), 7.50 (2 H, d, Ar), 7.65 (2 H, d, Ar), 7.80 (2 H, d, Ar).

(S)-{[(R)-2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-3-carbonyl]-amino}-phenyl-acetic acid tert-butyl ester (19)

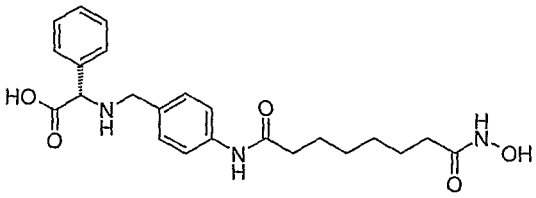
Building block A used

LCMS purity 100%, m/z 577 $[M^+H]^+$, ^1H NMR (400 MHz, $CDCl_3$), δ 1.20-1.40 (17 H, m, 4 x CH_2 and $C(CH_3)_3$), 2.10 (2 H, m, CH_2), 2.45 (2 H, m, CH_2), 3.15-3.60 (2 H, m, CH_2), 4.75 (2 H, m, CH_2), 5.35 (2 H, m, PhCHNH and CH), 6.90-7.50 (9 H, m, Ar).

Synthesis of Compound (20) and Compound (21)

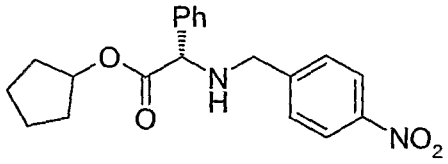


20



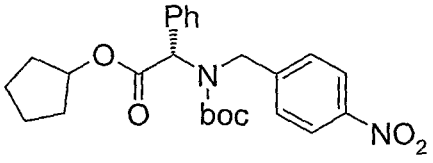
21

Stage 1: (S)-(4-Nitro-benzylamino)-phenyl-acetic acid cyclopentyl ester



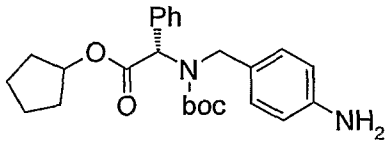
A mixture of 4-nitrobenzyl bromide (15g, 69.4mmol), L-phenylglycine cyclopentylester tosyl salt (27.1g, 60.4mmol) and potassium carbonate (19.6g, 138.8mmol) in DMF (250ml) was stirred at room temperature for 18h. The reaction mixture was diluted with EtOAc (300ml) and washed with water (3 x 200ml). The EtOAc layer was isolated, dried (Na_2SO_4), filtered and concentrated to dryness yielding an orange colour oil. A crude weight of 21g was isolated. LCMS purity 81%, m/z 355 $[M^+H]^+$, . This product was used without further purification.

Stage 2: (S)-[tert-Butoxycarbonyl-(4-nitro-benzyl)-amino]-phenyl-acetic acid cyclopentyl ester



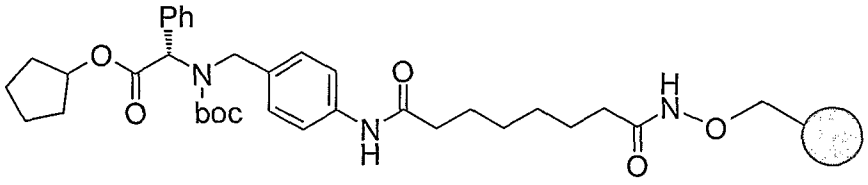
To a solution of Stage 1 product (15g, 42.37mmol) in THF (150ml) was added K_2CO_3 (6.9g, 50.8mmol), followed by di-t-butyldicarbonate (22.2g, 101.7mmol). Water (150ml) was added and the reaction stirred at room temperature for 36h. The reaction mixture was evaporated to dryness. The residue was redissolved in EtOAc (300ml), washed consecutively with 0.1 M HCl (150ml), sat. aq. $NaHCO_3$ and water (150 ml). The EtOAc layer was dried (Na_2SO_4), filtered and concentrated to dryness yielding a yellow oil. After purification by column chromatography (10% EtOAc/ hexane) the product was obtained as clear yellow oil (12g, 62% yield). LCMS purity 95%, m/z 455 $[M^+H]^+$, 496 $[M^+H+41]^+$.

Stage 3: (S)-[(4-Amino-benzyl)-tert-butoxycarbonyl-amino]-phenyl-acetic acid cylopentyl ester



A mixture of Stage 2 product (12g, 26.4mmol) and 10% Pd/C (2.0g) in EtOAc (350ml) was hydrogenated at room temperature for 18h. The Pd/C catalyst was filtered off through a pad of celite. The filtrate was concentrated under reduced pressure to yield a white solid (10.1g, 90% yield). LCMS purity 100%, m/z 425 $[M^+H]^+$, 466 $[M^+H+41]^+$.

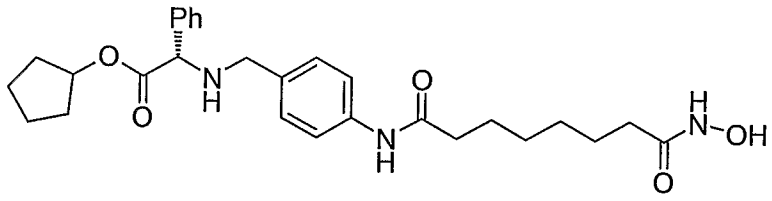
Stage 4: Coupling of stage 3 aniline



Hydroxylamine 2-chlorotrityl resin derivatized with suberic acid (1.0g, loading 0.94mmol) was swollen in anhydrous DCM (100ml). 1-Chloro-N,N-2-trimethylpropenylamine (Ghosez reagent)[1] (0.373ml, 2.82mmol, 3eq) was added at 0 °C under the atmosphere of N_2. The mixture was allowed to warm to room temperature and gently shaken for 1-2h. Stage 3 aniline (1.2g, 2.82mmol, 3eq) was

added portionwise over 20min. Et_3N (0.53ml, 3.76mmol, 4eq) was added. The mixture was shaken for 1h. LCMS after a test cleave shows 70% conversion, m/z 596 $[M^+H]^+$. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

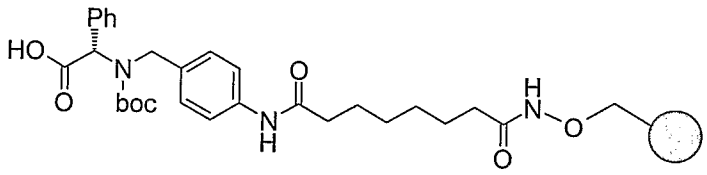
Stage 5: (S)-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-phenyl-acetic acid cyclopentyl ester **(20)**



20

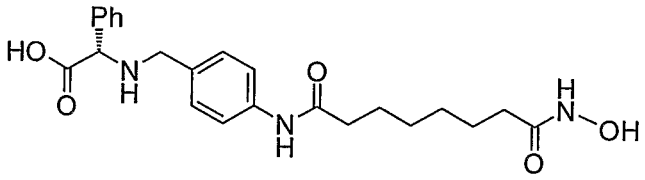
Stage 4 resin (1.5g, loading 0.94mmol) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was collected and evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and after 20mins filtered. The combined filtrates were evaporated to dryness under reduced pressure at r.t to give a residue. This residue was allowed to stand in 20% TFA/DCM for 40mins. After evaporation to dryness, also under reduced pressure at r.t, the residue was purified by preparative HPLC to yield compound **(20)** as the TFA salt, LCMS purity 95%, m/z 496 $[M^+H]^+$, ^1H NMR (400 MHz, DMSO), δ: 1.30-1.50 (6 H, m, 3 x CH_2), 1.50-1.70 (8 H, m, 4 x CH_2), 1.80 (2 H, m, CH_2), 2.10 (2 H, t, CH_2), 2.45 (2 H, t, CH_2), 4.1 (2 H, dd, $C\underline{H}_2NH$), 5.25 (1 H, m, $C\underline{H}OCO$), 5.35 (1 H, m, $OCOC\underline{H}Ph$), 7.45 (2 H, d, Ar), 7.60 (5 H, m, Ar), 7.80 (2 H, d, Ar), 10.00-10.10 (2 H, br s), 10.50 (1 H, s).

Stage 6: Saponification of cyclopentyl ester



Stage 4 resin (2.5g, loading, 0.94mmol, 2.35mmol) was suspended in MeOH (8.7ml) and THF (8.7ml). An aq. solution of 2.7 N NaOH (8.8ml, 10eq, 23.5mmol) was added. The mixture was shaken for 36h. LCMS of the test cleave confirmed the completion of reaction, m/z 528 $[M^+H]^+$. The resin was filtered and washed with water x 2, MeOH x 2, followed by the standard wash procedure. The resin was dried under vacuum.

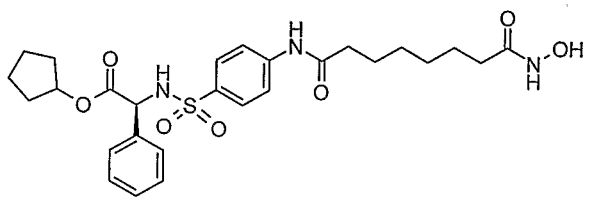
Stage 7: (S)-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-phenyl-acetic acid **(21)**



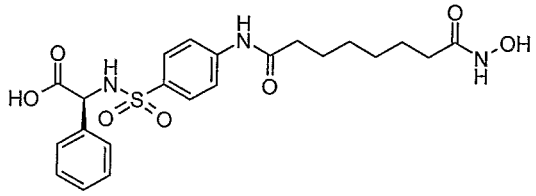
21

Stage 6 resin (2.5g, loading 0.94mmol, 2.35mmol) was cleaved and boc deprotected using the procedure outlined for Stage 5. The crude product (0.40g) was purified by preparative HPLC giving compound **(21)** as the TFA salt. LCMS purity 100%, m/z 428 $[M^+H]$, ^1H NMR (400 MHz, CD_3OD), δ: 1.30 (4 H, 2 x CH_2), 1.55 (4 H, 2 x CH_2), 2.00 (2 H, t, CH_2), 2.30 (2 H, t, CH_2), 3.90 (1 H, s, $NHCH_2$), 4.05 (2 H, dd, $NHCH_2$), 4.95 (1 H, s, CHPh), 7.35 (2 H, d, Ar), 7.40 (5 H, m, Ar), 7.55 (2 H, d, Ar).

Synthesis of Compound (22) and Compound (23)

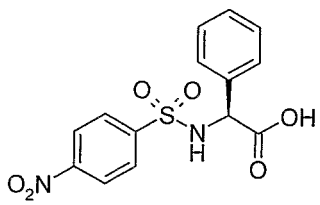


22



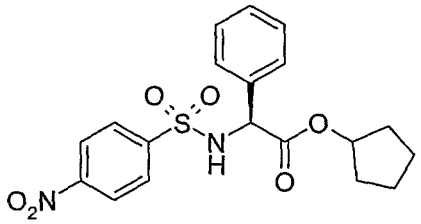
23

Stage 1: (S)-(4-Nitro-benzenesulfonylamino)-phenyl-acetic acid



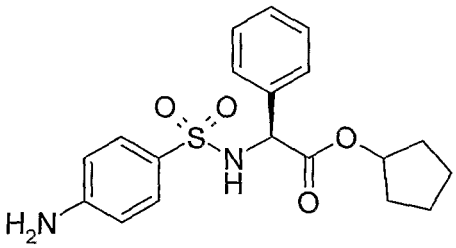
To a solution of L-phenylglycine (0.227g, 1.5mmol) in water (5 ml) and dioxane (5ml) was added triethylamine (0.42ml, 3.0mmol) followed by slow addition of 4-nitrobenzene sulphonyl chloride (0.5g, 2.3mmol) in dioxane (5ml) at 0°C. After stirring for 45 minutes the reaction mixture was evaporated to dryness, re-dissolved in EtOAc and washed with saturated $NaHCO_3$ solution (2x20ml) and water (10ml). The EtOAc layer was dried over Na_2SO_4, filtered and evaporated to dryness. LCMS purity 75%, (molecular ion not observed) yield 0.58g, (76%). This material was used without any purification.

Stage 2: (S)-(4-Nitro-benzenesulfonylamino)-phenyl-acetic acid cyclopentyl ester



To a solution of stage 1 acid (4.32g, 12.8mmol) in cyclopentanol (60ml) at 0°C was added slowly thionyl chloride (9.3ml, 128mmol). The reaction mixture was stirred and heated under reflux at 70°C for 2 hours. The excess thionyl chloride was removed by evaporation *in vacuo*, the reaction mixture was extracted into EtOAc and washed with saturated $NaHCO_3$ solution and dried over Na_2SO_4, filtered and evaporated to dryness. Flash column chromatography purification with DCM gave the required product (3.6g, 70% yield). LCMS purity of 100%, (molecular ion not observed).

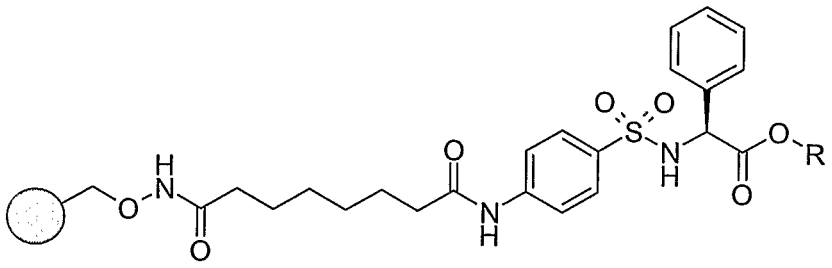
Stage 3: (S)-(4-Amino-benzenesulfonylamino)-phenyl-acetic acid cyclopentyl ester



A mixture of (S)-(4-Nitro-benzenesulfonylamino)-phenyl-acetic acid cyclopentyl ester (5.29g, 13.1mmol) and 10% Pd/C (5.0g) in EtOAc (350ml) was hydrogenated under balloon pressure at room temperature for 24h. The Pd/C catalyst was filtered off

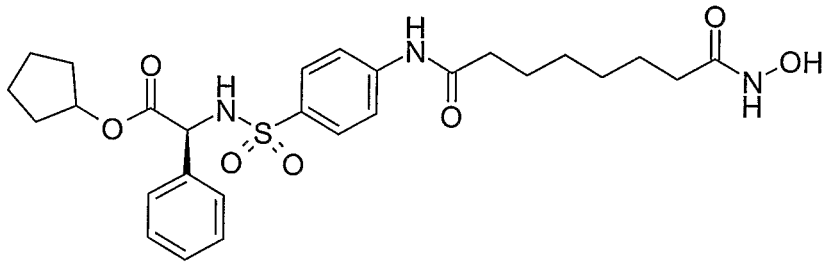
through a pad of celite. The filtrate was concentrated under reduced pressure to yield the required product (4.54g, 92% yield). LCMS purity 100%, m/z 375 $[M^+ + H]^+$.

Stage 4: Coupling of anline



Hydroxylamine 2-chlorotrityl resin derivatized with suberic acid (0.39g, loading 1.14mmol/g) was swollen in anhydrous DCM (25ml) and at 0°C under N_2 atmosphere 1-chloro-N,N, 2-trimethylpropenylamine (0.175ml, 1.33mmol) added dropwise. The reaction mixture was shaken for 1.5 hours. A solution of stage 3 aniline (0.5g, 1.33mmol) in DCM (25ml) was added followed by triethylamine (0.25ml, 1.76mmol). The reaction mixture was shaken for a further 10 minutes. LCMS after a test cleave showed 61% conversion, m/z 546 $[M^+ + H]^+$. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum and used in the next step.

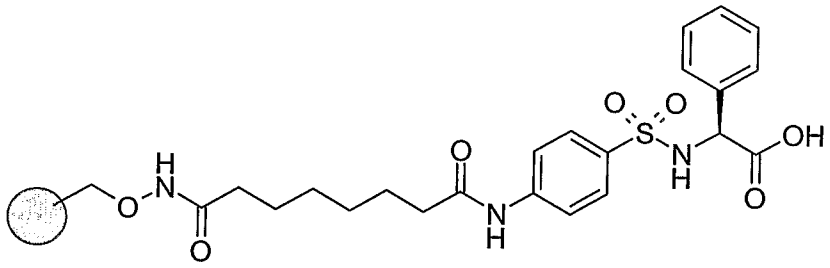
Stage 5: (S)-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzenesulfonylamino]-phenyl-acetic acid cyclopentyl ester **(22)**



Stage 4 resin (1.12g, loading 1.14mmol/g) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was collected and evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and after 20mins filtered. The combined filtrates were evaporated to dryness under reduced pressure at room temperature to give a residue. The residue was

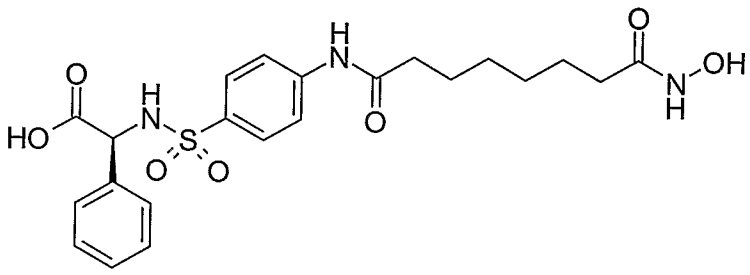
purified by preparative HPLC to yield compound **(22)**. LCMS purity 93%, m/z 546 $[M^+H]^+$, ^1H NMR (400 MHz, DMSO), δ: 1.20-1.68 (16 H, m, 8 x CH_2), 1.93 (2 H, t, CH_2), 2.33 (2H, t, CH_2), 4.80 (1 H, m, CHOCO), 4.81 (1 H, d, OCOCHPh), 7.27 (5 H, m, Ar), 7.65 (2 H, d, Ar), 7.71 (2 H, d, Ar), 8.67 (1 H, br s), 8.75 (1 H, d), 10.24 (1 H, s), 10.34 (1 H, s).

Stage 6: Saponification of cyclopentyl ester



Stage 4 resin (1.2g, loading 1.14mmol/g) was suspended in THF (8ml) and methanol (8ml) and 2.7M sodium hydroxide (5.1ml, 13.68mmol) was added. The mixture was shaken for 48h. LCMS of the test cleave confirmed the completion of reaction, m/z 478 $[M^+H]^+$. The resin was filtered and washed with water x 2, MeOH x 2, followed by the standard wash procedure. The resin was dried under vacuum.

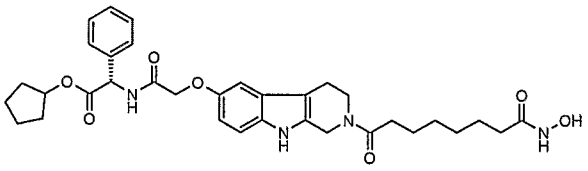
Stage 7: (S)-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzenesulfonylamino]-phenyl-acetic acid **(23)**



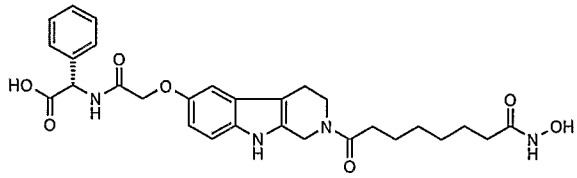
Stage 6 resin (1.2g, loading 1.14mmol/g) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was collected and evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and after 20mins filtered. The combined filtrates were evaporated to dryness under reduced pressure at room temperature to give a residue. The residue was purified by preparative HPLC to yield compound **(23)**. LCMS purity 91%, m/z 478

$[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.44 (4 H, m, 2 x CH_2), 1.62-1.74 (4 H, m, 2 x CH_2), 2.12 (2 H, t, CH_2), 2.34 (1 H, m, OCOCHPh), 2.41 (2 H, t, CH_2), 7.25 (5 H, m, Ar), 7.69 (2 H, d, Ar), 7.72 (2 H, d, Ar).

Synthesis of Compounds (24) and Compound (25)

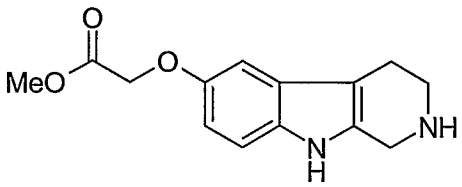


24



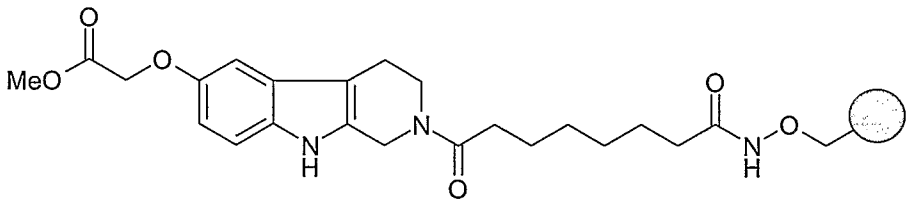
25

Stage 1: (2,3,4,9-Tetrahydro-1H-beta-carbolin-6-yloxy)-acetic acid methyl ester



A mixture of 5-carboxymethoxy tryptamine (1.24g, 4.56mmol), 36% aq formaldehyde and MeOH (25ml) was heated under reflux for 1.5 h. The reaction mixture was evaporated to dryness. MeOH (50ml) and TMSCl (1.24ml) were sequentially added. Reflux was continued for 1 h. Reaction mixture was evaporated to dryness and was used in the next stage without purification.

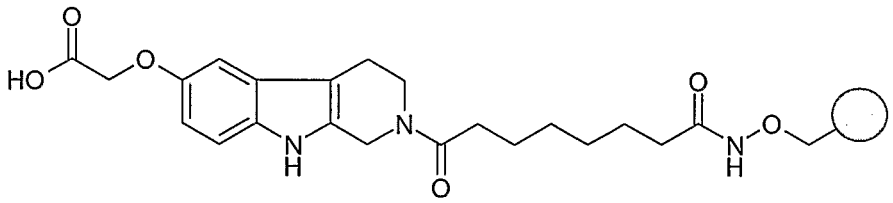
Stage 2: Amidation



Hydroxylamine 2-chlorotrityl resin derivatized with suberic acid (2.0g, loading 1.14mmol/g, 2.28mmol) was suspended in DCM (40ml). pyBOP (3.56g) was added followed by a DCM solution (40ml) of Stage 1 amine (4.56mmol) and DIPEA (3.9ml, 22.8mmol). The reaction was shaken at room temperature for 18 h. LCMS after test

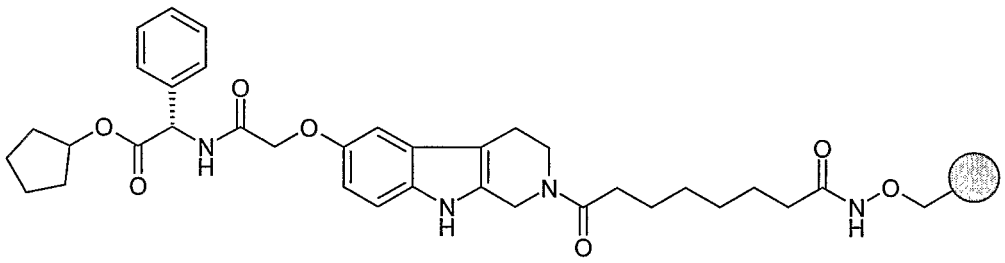
cleave confirmed the completion of reaction. The resin was filtered and washed using the standard wash procedure and was thoroughly dried.

Stage 3: Saponification of methyl ester



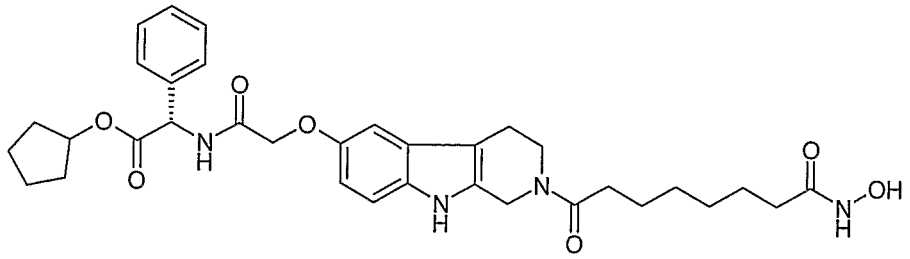
Stage 2 resin (2.0g, 1.14 mmol/g, 2.28 mmol) was suspended in a mixture of THF (10ml) and MeOH (10ml). 1.4M NaOH (10ml) was added over 5 min. The mixture was shaken for 18 h. LCMS after test cleave confirmed the completion of reaction. The resin was filtered and washed using the standard wash procedure.

Stage 4: Coupling with L-phenylglycine cyclopentyl ester



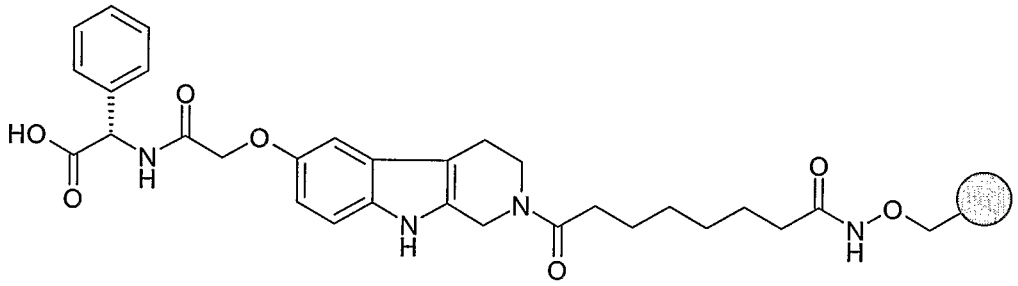
Stage 3 resin (2.0g, loading 1.14mmol/g, 2.28mmol) was suspended in DCM (30ml). pyBOP (3.56g, 6.84mmol) was added, followed by L-phenylglycine cyclopentyl ester (2.59g, 6.84mmol) and DIPEA (3.9ml, 22.8mmol). The mixture was shaken for 18 h. LCMS after test cleave confirmed completion of reaction. The resin was filtered, washed using standard wash procedure and dried under vacuum.

Stage 5: (S)-{2-[2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carbolin-6-yloxy]-acetylamino}-phenyl-acetic acid cyclopentyl ester **(24)**



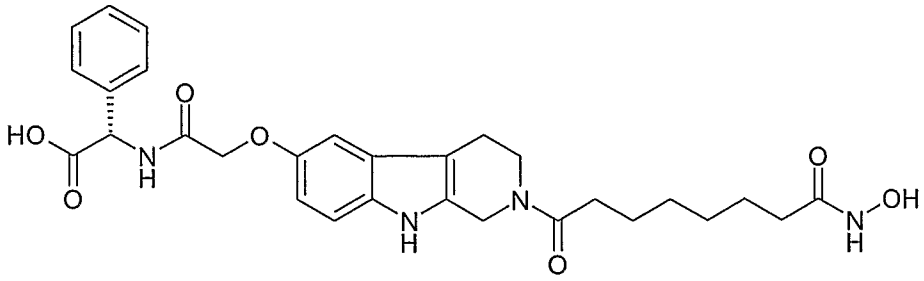
Stage 4 resin (0.8g, loading 1.14mmol/g, 0.91mmol) was cleaved using 2% TFA/ DCM (3 x 10ml). The filtrate was evaporated to dryness at room temperature under reduced pressure to give an oily residue (200mg) which was purified by preparative HPLC to give compound **(24)** as the TFA salt. LCMS purity 95%, m/z 619 $[M^+ + H]^+$, ^1H NMR (400 MHz, DMSO), δ: 1.05-1.66 (16 H, m, 8 x CH_2), 1.79 (2 H, m, CH_2), 2.16-2.31 (2 H, m, 2.41-2.56 (2 H, m, CH_2), 3.60 (2 H, m, CH_2), 4.42 (2 H, s, CH_2), 4.49 (2 H, s, CH_2), 4.93 (1 H, m, CHOCO), 5.28 (1 H, m, OCOCHPh), 6.59 (1 H, d, Ar), 6.65 (1 H, s, Ar), 7.04 (1 H, d, Ar), 7.21 (5 H, m, Ar), 8.57 (1 H, m), 10.17 (1 H, s), 10.58 (1 H, s, Ar).

Stage 6: Saponification of cyclopentyl ester



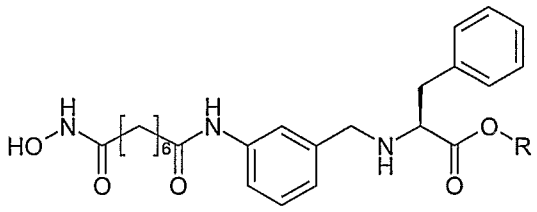
Stage 4 resin (1.0g, loading 1.14mmol/g, 1.14mmol) was saponified according to the the procedure described in Stage 3.

Stage 7: (S)-{2-[2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carbolin-6-yloxy]-acetylamino}-phenyl-acetic acid cyclopentyl ester **(25)**

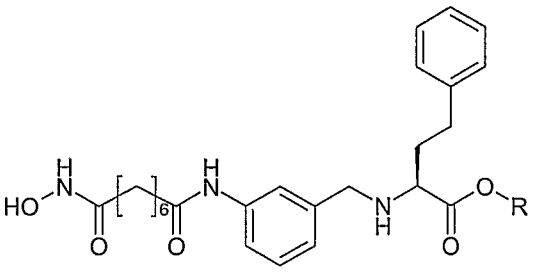


Stage 6 resin (1.0g, loading 1.14mmol/g, 1.14mmol) was cleaved and purified using the procedure detailed in stage 5. Compound **(25):** LCMS purity 97%, m/z 551 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.33-1.49 (4 H, m, 2 x CH_2), 1.58-1.75 (4 H, m, 2 x CH_2), 2.06-2.17 (2 H, m, CH_2), 2.51-2.60 (2 H, m, CH_2), 2.70-2.83 (2 H, m, CH_2), 3.85-3.96 (2 H, m, CH_2), 4.61 (2 H, m, CH_2), 4.78 (2 H, m, CH_2), 5.56 (1 H, s, OCOCHPh), 6.89 (1 H, m, Ar), 7.00 (1 H, s, Ar), 7.26 (1 H, m, Ar), 7.35 (5 H, m, Ar).

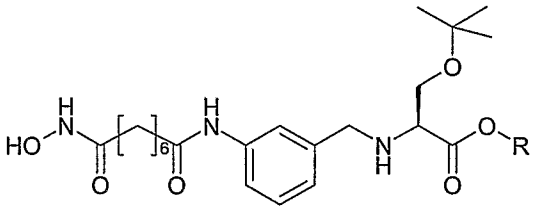
Synthesis of Compounds in Figure 2 as Exemplified by Compound (26) and Compound (27)



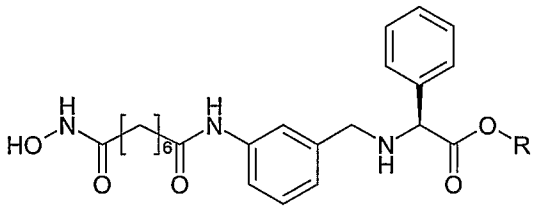
R= ethyl 26
R= H 27
R= cyclopentyl 28



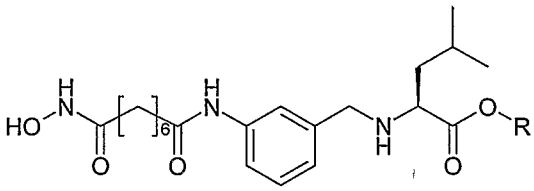
R= ethyl 29
R=H 30
R= cyclopentyl 31



R= ethyl 32
R= H 33
R= cyclopentyl 34



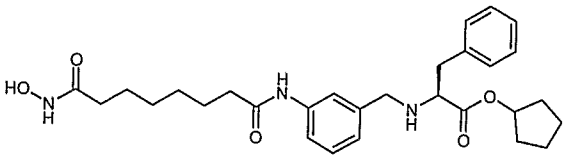
R = ^{t}Butyl 35
R= cyclopentyl 36
R= H 37



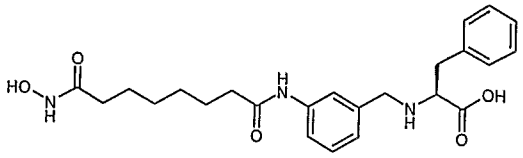
R= H 38
R= cyclopentyl 39

Figure 2

Synthesis of Compound (26) and Compound (27)

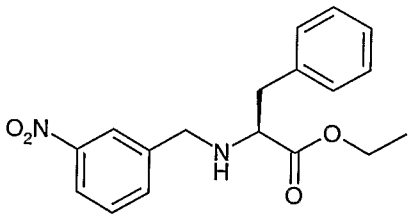


26



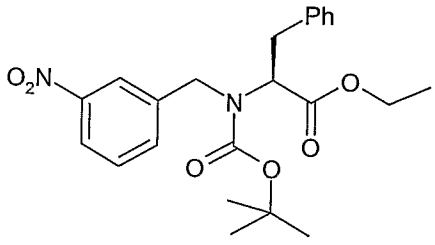
27

Stage 1: (S)-2-(3-Nitro-benzylamino)-3-phenyl-propionic acid ethyl ester



3-Nitrobenzyl bromide (10.0g, 46mmol) was dissolved in DMF (180ml) and potassium carbonate (12.7g, 92mmol) added, followed by L-phenylalanine ethyl ester hydrochloride (10.6g, 46mmol). The reaction was stirred for 17 h at room temperature before evaporating to dryness. The residue was re-dissolved in EtOAc (150ml) and washed with water (3X80ml), dried (Na_2SO_4) filtered and concentrated to dryness. After purification by flash column chromatography (30% EtOAc/ hexane) the product was obtained (3.7g, 24% yield). LCMS purity 86%, m/z 329 $[M^+ + H]^+$.

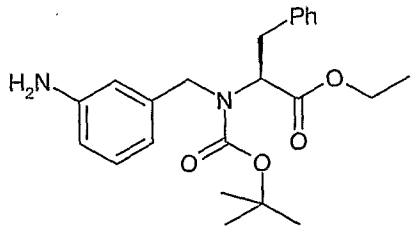
Stage 2: (S)-2-[tert-Butoxycarbonyl-(3-nitro-benzyl)-amino]-3-phenyl-propionic acid ethyl ester



Stage 1 amine (13.4g, 40.9mmol) was dissolved in THF (250ml) before addition of potassium carbonate (8.46g, 61.4mmol) and water (150ml). Di-^{t}butyl-dicarbonate (35.6. 163mmol) was added and the reaction mixture heated to 50°C for 18 h. DCM was added the resultant mixture washed consecutively with 0.1 M HCl (150ml), sat.

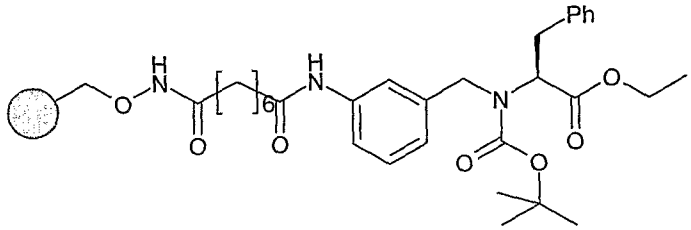
aq. $NaHCO_3$ and water (150 ml). The DCM layer was dried (Na_2SO_4), filtered and concentrated to dryness. After purification by flash column chromatography (5% EtOAc/ hexane) the product was isolated (9.4g, 54% yield). LCMS purity 95%, m/z 428 $[M^+H]^+$.

Stage 3: (S)-2-[(3-Amino-benzyl)-tert-butoxycarbonyl-amino]-3-phenyl-propionic acid ethyl ester



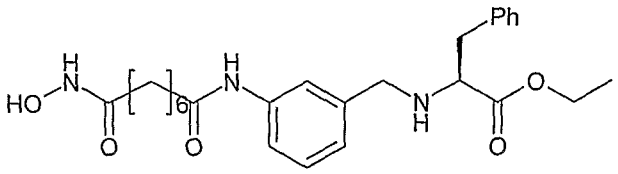
Stage 2 carbamate (4.92g, 11.5mmol) was dissolved in EtOAc (150ml) before addition of Pd/C (10% wet) catalyst (0.8g) and hydrogenated under balloon pressure at room temperature for 18 h. The reaction mixture was filtered through a pad of celite and evaporated to dryness to give a red solid (4.0g, 89% yield). LCMS purity 100%, m/z 399 $[M^+H]^+$.

Stage 4: Coupling to resin



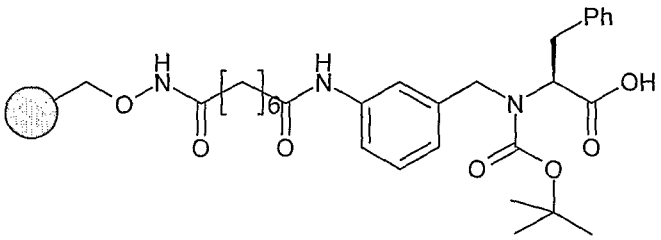
Hydroxylamine 2-chlorotrityl resin derivatized with suberic acid (1.0 g, loading 0.83mmol/g) was swollen in DMF (15ml) and PyBOP (1.36g, 2.61mmol) added, followed by DIPEA (1.5ml, 8.7mmol). Stage 3 aniline (1.04g, 2.61mmol) was dissolved in DCM (15ml) and added to the reaction mixture. The reaction was shaken for 24 h at room temperature. LCMS after a test cleave indicated 86% conversion, m/z 570 $[M^+H]^+$. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

Stage 5: (S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-3-phenyl-propionic acid ethyl ester **(26)**



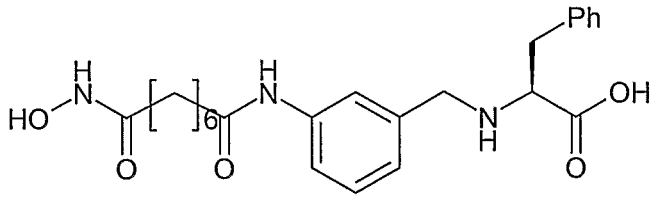
Stage 4 resin (1.3g, loading 0.83mmol) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and was filtered after 20mins. The combined filtrates were evaporated to dryness under reduced pressure at room temperature to give an oily residue. The residue was allowed to stand in 20% TFA/DCM for 40mins. After evaporation to dryness, also under reduced pressure at room temperature, the crude product was purified by preparative HPLC to yield compound **(26)**. LCMS purity 100%, m/z 470 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.08 (3 H, t, CH_3), 1.35-1.45 (4 H, m, 2 x CH_2), 1.60-1.80 (4 H, m, 2 x CH_2), 2.10 (2 H, t, CH_2), 2.40 (2 H, t, CH_2), 3.13 (1 H, dd, PhC<u>H</u>H), 3.40 (1 H, dd, PhCH<u>H</u>), 4.11 (2 H, q, C<u>H</u>$_2$CH$_3$), 4.14-4.22 (3 H, m), 7.20-7.48 (8 H, m, Ar), 7.92 (1 H, s, Ar).

Stage 6: Saponification



Stage 4 resin (1.4g, loading 0.83mmol) was suspended in THF (8.6ml) and methanol (8.6 ml) and 1.4M sodium hydroxide solution (8.6ml, 5.98mmol) was added. The mixture was shaken for 24 hours before test cleavage revealed 83% conversion to required acid, m/z 541 $[M^+ + H]$ The resin was filtered and washed with water x 2, MeOH x 2, followed by the standard wash procedure. The resin was dried under vacuum.

Stage 7: (S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-3-phenyl-propionic acid **(27)**



Stage 6 resin (1.44g, loading 0.83mmol) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and was filtered after 20mins. The combined filtrates were evaporated to dryness under reduced pressure at room temperature to give an oily residue. The residue was allowed to stand in 20% TFA/DCM for 40mins. After evaporation to dryness, under reduced pressure at room temperature, the crude product was purified by preparative HPLC to yield compound **(27)**. LCMS purity 100%, m/z 442 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.35-1.48 (4 H, m, 2 x CH_2), 1.60-1.78 (4 H, m, 2 x CH_2), 2.10 (2 H, t, CH_2), 2.40 (2 H, t, CH_2), 3.20 (1 H, dd, PhC<u>H</u>H), 3.28 (1 H, dd, PhCH<u>H</u>), 3.90 (1 H, t, OCOC<u>H</u>), 4.14 (2 H, m), 7.15 (1 H, d, Ar), 7.26 (6 H, m, Ar), 7.51 (1 H, d, Ar), 7.73 (1 H s, Ar).

The following compounds were prepared according to the procedure described for compound **(26)** and compound **(27)**

(S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-3-phenyl-propionic acid cyclopentyl ester (28)

LCMS purity 100%, m/z 510 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.00-1.61 (16 H, m, 8 x CH_2), 1.90 (2 H, t, CH_2), 2.20 (2 H, d, CH_2), 2.90 (1 H, dd, PhCH<u>H</u>), 3.20 (1 H, dd, PhCH<u>H</u>), 4.00-4.11 (3 H, m), 4.91 (1 H, m), 7.00-7.25 (8 H, m, Ar), 7.75 (1 H, s, Ar).

(S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-4-phenyl-butyric acid ethyl ester (29)

LCMS purity 100%, m/z 484 $[M^+H]^+$, ^1H NMR (400MHz, MeOD), δ: 1.23-1.29 (7 H, m, CH_3, 2 x CH_2), 1.53 (2 H, t, CH_2), 1.62 (2 H, t, CH_2), 1.99 (2 H, t, CH_2), 2.11-2.16 (2 H,

m, CH_2), 2.28 (2 H, t, CH_2), 2.53-2.61 (1 H, m, CH), 2.65-2.76 (1 H, m, CH), 3.80-3.90, (1 H, m, C<u>H</u>CO_2Et), 4.05 (2H, s, CH_2), 4.21 (2 H, q, CH_2), 7.05-7.15 (4 H, m, Ar), 7.15-7.22 (2 H, m, Ar), 7.25-7.39 (2 H, m, Ar), 7.75 (1 H, s, Ar).

(S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-4-phenyl-butyric acid (30)

LCMS purity 100%, m/z 456 $[M^+ + H]^+$, ^{1}H NMR (400MHz, MeOD), δ: 1.27-1.32 (4 H, m, 2 x CH_2), 1.53 (2 H, t, CH_2), 1.62 (2 H, t, CH_2), 1.99 (2 H, t, CH_2), 2.11-2.16 (2 H, m, CH_2), 2.29 (2 H, t, CH_2), 2.57-2.64 (1 H, m, CH), 2.69-2.77 (1 H, m, CH), 3.84-3.87 (1 H, m, C<u>H</u>CO_2H), 4.12 (2 H, q, CH_2), 7.09-7.11 (4 H, m, Ar), 7.16-7.20 (2 H, m, Ar), 7.27-7.35 (2 H, m, Ar), 7.78 (1 H, s, Ar).

(S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-4-phenyl-butyric acid cyclopentyl ester (31)

LCMS purity 100%, m/z 524 $[M^+ + H]^+$, ^{1}H NMR (400 MHz, MeOD), δ: 1.20-1.35 (4 H, m), 1.45-1.62 (10 H, m), 1.85 (2 H, m), 2.00 (2 H, t, CH_2), 2.10 (2 H, m), 2.28 (2 H, t, CH_2), 2.55 (1 H, m), 2.68 (1 H, m), 3.88 (1 H, t, OCOC<u>H</u>NH), 4.11 (2 H, s, C<u>H</u>$_2$Ph), 5.24 (1 H, m) 7.02-7.12 (4 H, m, Ar), 7.18 (2 H, m, Ar), 7.30 (2 H, m, Ar), 7.80 (1 H, s, Ar).

(S)-3-tert-Butoxy-2-[3-(7-hydroxycarbamoyl-heptanoylamino)-benzylamino]-propionic acid ethyl ester (32)

LCMS purity 90%, m/z 466 $[M^+ + H]^+$, ^{1}H NMR (400 MHz, MeOD), δ: 1.25 (9 H, s, C(C<u>H</u>$_3$)$_3$), 1.35 (3 H, t, CH_2C<u>H</u>$_3$), 1.35-1.45 (4 H, m, 2 x CH_2), 1.62-1.76 (4 H, m, 2 x CH_2), 2.12 (2 H, t, CH_2), 2.40 (2 H, t, CH_2), 3.89 (1 H, m), 3.98 (1 H, m), 4.20-4.40 (5 H, m), 7.25 (1 H, d, Ar), 7.39-7.50 (2 H, m, Ar), 7.90 (1 H, s, Ar).

(S)-3-tert-Butoxy-2-[3-(7-hydroxycarbamoyl-heptanoylamino)-benzylamino]-propionic acid (33)

LCMS purity 86%, m/z 438 $[M^+ + H]^+$, ^{1}H NMR (400 MHz, MeOD), δ: 1.20 (9 H, s, C(C<u>H</u>$_3$)$_3$), 1.38 (4 H, m, 2 x CH_2), 1.57-1.75 (4 H, m ,2 x CH_2), 2.10 (2 H, t, CH_2), 2.39 (2 H, t, CH_2), 3.78-3.85 (3 H, m), 4.26 (2 H, s, CH_2Ph), 7.21 (1 H, d, Ar), 7.39 (1 H, t, Ar), 7.50 (1 H, d, Ar), 7.80 (1 H, s, Ar).

(S)-3-tert-Butoxy-2-[3-(7-hydroxycarbamoyl-heptanoylamino)-benzylamino]-propionic acid cyclopentyl ester (34)

LCMS purity 94%, m/z 506 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.25 (9 H, s, $C(C\underline{H}_3)_3$), 1.33-1.50 (4 H, m, 2 x CH_2), 1.60-2.00 (12 H, m), 2.13 (2 H, t, CH_2), 2.42 (2 H, t, CH_2), 3.83-4.00 (2 H, m), 4.18 (1 H, m), 4.28 (2 H, s, CH_2Ph), 5.35 (1 H, m), 7.25 (1 H, m, Ar), 7.45 (2 H, m, Ar), 7.90 (1 H, s, Ar).

(S)-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-phenyl-acetic acid tert-butyl ester (35)

LCMS purity 97%, m/z 484 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.30 (13 H, m, 2 x CH_2, $C(C\underline{H}_3)_3$), 1.45-1.65 (4 H, m, CH_2 x 2), 1.93-2.05 (2 H, m, CH_2), 2.20-2.40 (2 H, m, CH_2), 3.99 (2 H, q, CH_2), 4.65-4.95 (1 H, m, CH, masked signal) 7.05 (1 H, d, Ar), 7.25-7.33 (2 H, m, Ar), 7.35-7.50 (5 H, m, Ar), 7.75 (1 H, s, Ar).

(S)-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-phenyl-acetic acid cyclopentyl ester (36)

LCMS purity 100%, m/z 496 $[M^+H]^+$, ^1H NMR (400MHz, MeOD), δ: 1.30-1.70 (16 H, m, 8 x CH_2), 2.00 (2 H, t, CH_2), 2.30 (2 H, t, CH_2), 4.05 (2 H, dd, $C\underline{H}_2NH$), 5.00 (1 H, m, OCOC<u>H</u>Ph), 5.15 (1 H, m, C<u>H</u>OCO), 7.05 (1 H, m, Ar), 7.30 (2 H, m, Ar), 7.40 (5 H, m, Ar), 7.75 (1H, m, Ar).

(S)-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-phenyl-acetic acid (37)

LCMS purity 100%, m/z 428 $[M^+H]^+$, ^1H NMR (400MHz, MeOD), δ: 1.20-1.35 (4 H, m, 2 x CH_2), 1.50-1.65 (4 H, m, 2 x CH_2), 2.00 (2 H, m, CH_2), 2.30 (2 H, m, CH_2), 4.00 (2 H, dd, $C\underline{H}_2NH$), 4.90 (1 H, m, OCOC<u>H</u>Ph), 7.05 (1 H, m, Ar), 7.25-7.50 (7 H, m, Ar), 7.70 (1 H, m, Ar).

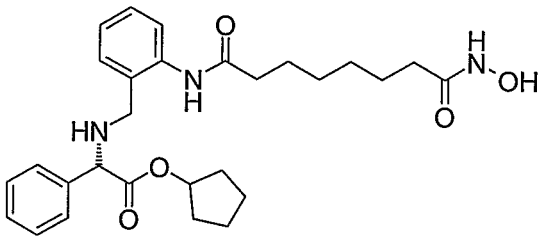
(S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-4-methyl-pentanoic acid (38)

LCMS purity 91%, m/z 408 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 0.78 (3 H, d, J = 6.6 Hz, CH_3), 0.84 Hz (3 H, d, J = 6.6 Hz, CH_3), 1.26 – 1.40 (6 H, m, alkyl), 1.49 - 1.70 (5 H, m, CH + 2 x CH_2), 1.95 (2 H, t, J = 7.32, CH_2), 2.25 (2 H, t, J = 7.36, CH_2), 3.00 (1 H, t, J = 6.88 Hz, NHC<u>H</u>CO), 3.42 (1 H, d, J = 12.7 Hz, CH), 3.68 (1 H, d, J = 12.5 Hz, CH), 7.00 (1 H, d, J = 7.6 Hz, Ar), 7.15 (1 H, t, J = 7.8 Hz, Ar), 7.30 (1 H, s. Ar), 7.47 (1 H, br d, Ar)

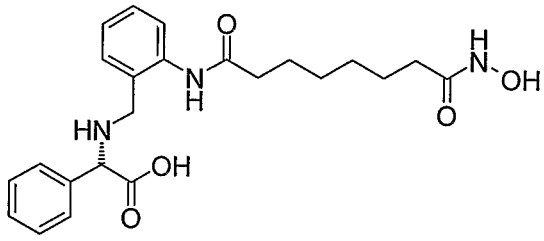
(S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-4-methyl-pentanoic acid cyclopentyl ester (39)

LCMS purity 100%, m/z 476 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 0.85 – 0.95 (6 H, 2 x d, 2 x CH_3), 1.30 (4 H, m, 2 x CH_2), 1.50 – 1.70 (13 H, m, alkyl), 1.75 (2 H, m, CH_2), 2.00 (2 H, t, CH_2), 2.30 (2 H, t, CH_2), 3.90 (1 H, NHCHCO), 4.10 (2 H, q, CH_2), 5.25, (1 H, m, CH), 7.10 (1 H, d, Ar), 7.30 (2 H, m), 7.80 (1 H, s, Ar)

Synthesis of Compound (40) and Compound (41)

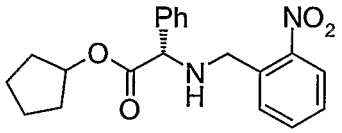


40



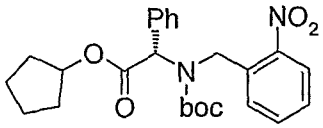
41

Stage 1: (S)-(2-Nitro-benzylamino)-phenyl-acetic acid cyclopentyl ester



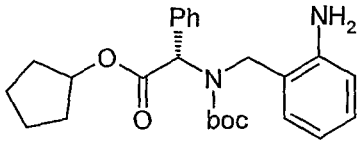
A mixture of 2-nitrobenzyl bromide (15g, 69.4mmol), L-phenylglycine cyclopentyl ester tosyl salt (27.2g, 69.4mmol) and potassium carbonate (19.2g, 138.8mmol) in DMF (300ml) was stirred at room temperature for 18 h. The reaction mixture was diluted with EtOAc (300ml) and washed with water (3 x 200ml). The EtOAc layer was isolated, dried (Na_2SO_4), filtered and concentrated to dryness yielding an orange coloured oil. A crude weight of 24g was isolated. LCMS purity 81%, m/z 355 $[M^+ + H]^+$. This product was used without further purification

Stage 2: (S)-[tert-Butoxycarbonyl-(2-nitro-benzyl)-amino]-phenyl-acetic acid cyclopentyl ester



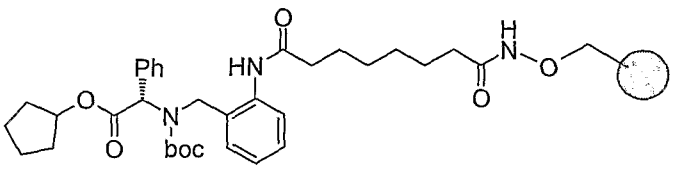
To a solution of (S)-(2-Nitro-benzylamino)-phenyl-acetic acid cyclopentyl ester (24.4g, 69.1mmol) in THF (150ml) was added K_2CO_3 (7.6g, 69.1mmol), followed by di-tert-butyl dicarbonate (30.1g, 138.1mmol). Water (150ml) was added and the reaction stirred at room temperature for 8 days with further di-tert-butyl dicarbonate (45.1g, 206.6mmol). The reaction mixture was evaporated to dryness. The residue was re-dissolved in EtOAc (300ml), washed with 0.1 M HCl (150ml), sat. aq.$NaHCO_3$ and water (150 ml). The EtOAc layer was dried (Na_2SO_4), filtered and concentrated to dryness yielding a yellow oil. After purification by column chromatography (20% EtOAc/ hexane) the product was obtained as clear yellow oil (15g, 48% yield).

Stage 3: (S)-[(2-Amino-benzyl)-tert-butoxycarbonyl-amino]-phenyl-acetic acid cyclopentyl ester



A mixture of stage 2 carbamate (4.44g, 9.78mmol) and 10% Pd/C (0.7g) in EtOAc (130ml) was hydrogenated at room temperature for 18 h under balloon pressure. The Pd/C catalyst was filtered off through a pad of celite. The filtrate was concentrated under reduced pressure to yield a white solid (4.25g). LCMS purity 100%, m/z 425 $[M^+ + H]^+$,

Stage 4: Coupling of stage 3 aniline

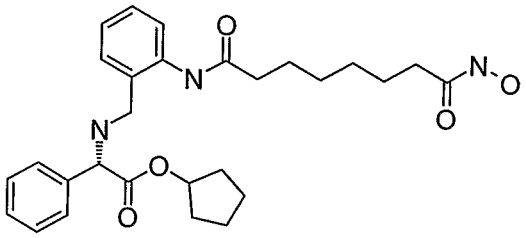


Hydroxylamine 2-chlorotrityl resin derivatized with suberic acid (1.6g, loading 0.83mmol) was swollen in anhydrous DCM (100ml). 1-Chloro-N,N-2-trimethylpropenylamine (Ghosez reagent)[1] (0.56ml, 3.3mmol, 3eq) was added at 0 °C under the atmosphere of N_2. The mixture was allowed to warm to room temperature

and gently shaken for 1-2 h. Stage 3 aniline (1.4g, 3.3mmol, 3eq) was added portionwise over 20min. Et_3N (0.76ml, 4.4mmol, 4eq) was added. The mixture was shaken for 1h. LCMS after a test cleave shows 97% conversion, m/z 596 $[M^+H]^+$. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

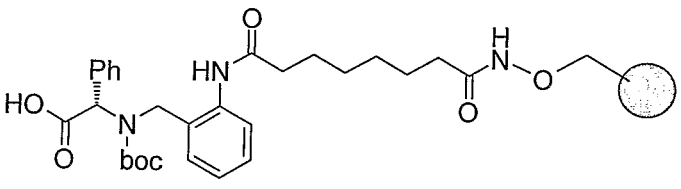
1. Ghosez et al, J. C. S. Chem. Comm., 1979, 1180.

Stage 5: (S)-[2-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-phenyl-acetic acid cyclopentyl ester **(40)**



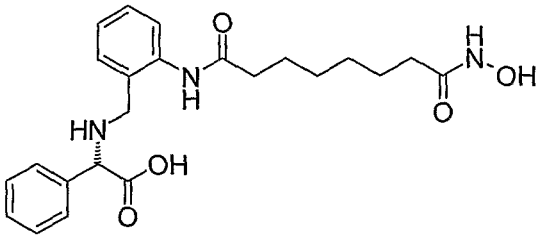
Stage 4 resin (1.34g, loading 0.83mmol) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was collected and evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and after 20mins filtered. The combined filtrates were evaporated to dryness under reduced pressure at room temperature to give a residue. This residue was allowed to stand in 20% TFA/DCM for 40mins. After evaporation to dryness, also under reduced pressure at room temperature, the residue was purified by preparative HPLC to yield compound **40** as the TFA salt, LCMS purity 100%, m/z 496 $[M^+H]^+$, ^1H NMR (400MHz, MeOD), δ: 1.40-2.00 (16 H, m, 8 x CH_2), 2.15 (2 H, m, CH_2), 2.45 (2 H, m, CH_2), 3.95 (1 H, d, $C\underline{H}_2NH$), 4.20 (1 H, d, $C\underline{H}_2NH$), 5.20 (1 H, m, OCOC<u>H</u>Ph), 5.35 (1 H, m, C<u>H</u>OCO), 7.25 (1 H, m, Ar), 7.40 (1 H, m, Ar), 7.50-7.60 (7 H, m, Ar).

Stage 6: Saponification



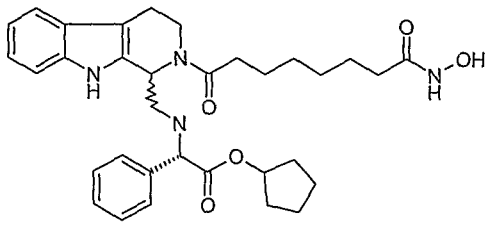
Stage 4 resin (2.0g, loading, 0.83mmol, 2.35mmol) was suspended in MeOH (6.1) and THF (6.1ml). 2.7 N NaOH (aq, 6.1ml) was added. The mixture was shaken for 5 days. LCMS of the test cleave confirmed the completion of reaction, m/z 528 $[M^+H]^+$. The resin was filtered and washed with water x 2, MeOH x 2, followed by the standard wash procedure. The resin was dried under vacuum.

Stage 7: (S)-[2-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-phenyl-acetic acid compound **(41)**

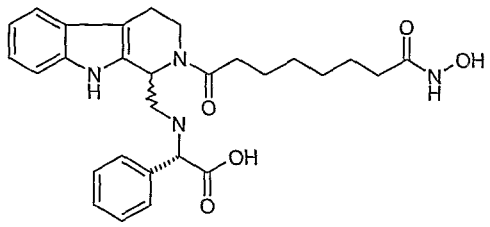


Stage 6 resin (2.0g, loading 0.83mmol) was cleaved and boc deprotected using the procedure outlined for stage 5. The crude product was purified by preparative HPLC yielding compound **(41)** as the TFA salt. LCMS purity 98%, m/z 428 $[M^+H]^+$, ^1H NMR (400MHz, MeOD), δ: 1.25-1.35 (4 H, m, 2 x CH_2), 1.50-1.65 (4 H, m, 2 x CH_2), 2.00 (2 H, m, CH_2), 2.30 (2 H, m, CH_2), 3.80 (1 H, d, $C\underline{H}_2NH$), 4.10 (1 H, d, $C\underline{H}_2NH$), 5.00 (1 H, m, $OCOC\underline{H}Ph$), 7.10 (1 H, m, Ar), 7.30 (1 H, m, Ar), 7.40-7.50 (7 H, m, Ar).

Synthesis of Compound (42) and Compound (43)

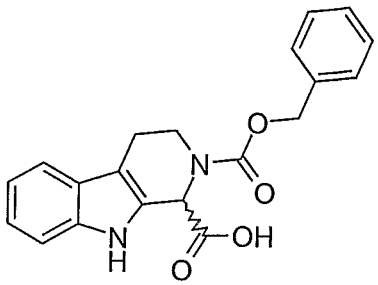


42



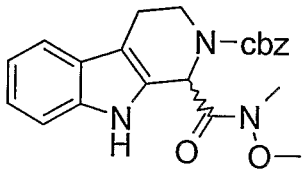
43

Stage 1: 1,3,4,9-Tetrahydro-beta-carboline-1,2-dicarboxylic acid 2-benzyl ester



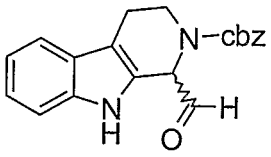
A solution of 1,2,3,4-tetrahydrocarboline-1-carboxylic acid (5g, 23.1mmol) in dioxane (25ml) and 2M NaOH (23ml, 46mmol) was cooled to 0°C. Benzyl chloroformate (3.95ml, 27mmol) was added slowly. After stirring at room temperature for 1 h further benzyl chloroformate (1.4ml, 9.5mmol) was added. After 2.5 h the reaction mixture was washed with ether. The aqueous layer was acidified to pH 2 and extracted with DCM, dried ($MgSO_4$), filtered and evaporated to dryness yielding a first crop of material as a yellow solid with LCMS purity of 79%, m/z 351 $[M^+H]^+$. The initial crop was used without further purification. A second crop of material was obtained following concentration of the ether layers to give further crude product. The crude material was purified by flash chromatography eluting with DCM to 20% 2M methanolic NH_3, 80% DCM yielding further Cbz-protected compound (yield 49%) at LCMS purity 82%, m/z 351 $[M^+H]^+$.

Stage 2: 1-(Methoxy-methyl-carbamoyl)-1,3,4,9-tetrahydro-beta-carboline-2-carboxylic acid benzyl ester



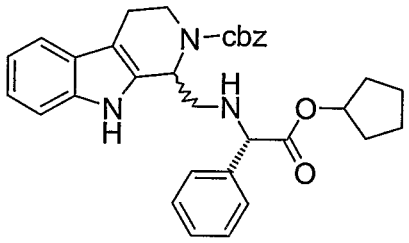
1,3,4,9-Tetrahydro-beta-carboline-1,2-dicarboxylic acid 2-benzyl ester (3g, 8.4mmol) was dissolved in anhydrous DCM (30ml) and triethylamine (5.22ml, 37.8mmol) added. To this solution was added HOBt (2.848g, 21.4mmol), EDCI (4.08g, 21.4mmol) and N, O-dimethylhydroxylamine hydrochloride (1.86g, 19.1.mmol). After stirring at room temperature for 2 h the reaction mixture was evaporated to dryness, re-dissolved in EtOAc and washed with saturated $NaHCO_3$ solution (2X100ml) and water (50ml). The EtOAc layer was dried (Na_2SO_4), filtered and evaporated to dryness. Purification by column chromatography using DCM to 3% methanol/DCM gave the required Weinreb amide (yield 40%). LCMS purity 85%, m/z 394 $[M^+H]^+$.

Stage 3: 1-Formyl-1,3,4,9-tetrahydro-beta-carboline-2-carboxylic acid benzyl ester



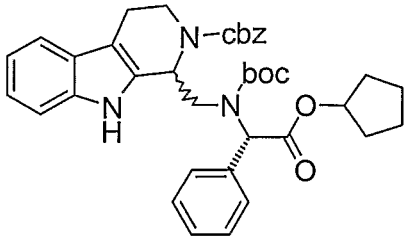
A solution of 1,3,4,9-tetrahydro-beta-carboline-1,2-dicarboxylic acid 2-benzyl ester (3.7mg, 9.4mmol) in THF (100ml) under N_2 was cooled to -78°C. 1.5M DIBAL in toluene solution (31.2ml, 47mmol) was added over 2 hours. After stirring for 4 hours the reaction mixture was quenched with methanol and water, extracted into EtOAc and washed with dilute aqueous HCl. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. LCMS purity 50%, m/z 335 $[M^+H]^+$ The material was used in the next stage without further purification.

Stage 4: 1-{[((S)-Cyclopentyloxycarbonyl-phenyl-methyl)-amino]-methyl}-1,3,4,9-tetrahydro-beta-carboline-2-carboxylic acid benzyl ester



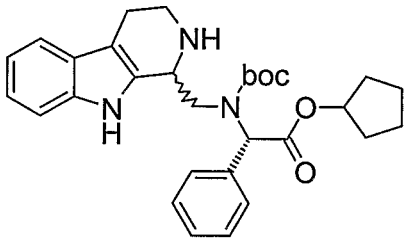
A mixture of 1-Formyl-1,3,4,9-tetrahydro-beta-carboline-2-carboxylic acid benzyl ester(1g, 3mmol), sodium acetate (0.68g, 87.4mmol), L-phenylglycine cyclopentyl ester tosyl salt (1.16g, 3mmol), sodium cyanoborohydride (0.26g, 4.2mmol) and molecular sieves in IPA (100ml) was stirred at room temperature for 1 hour. The reaction mixture was evaporated to dryness, re-dissolved in EtOAc and washed sequentially with saturated $NaHCO_3$ solution and brine. The EtOAc layer was dried over $MgSO_4$, filtered and evaporated to dryness. LCMS purity of 39%, m/z 538 $[M^+H]^+$. The crude material was taken to the next stage without further purification.

Stage 5: 1-{[tert-Butoxycarbonyl-((S)-cyclopentyloxycarbonyl-phenyl-methyl)-amino]-methyl}-1,3,4,9-tetrahydro-beta-carboline-2-carboxylic acid benzyl ester



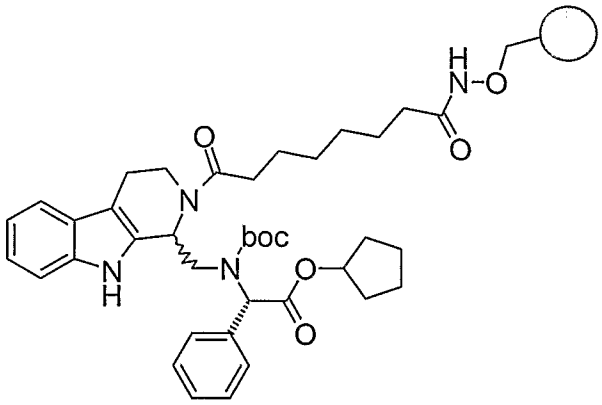
To a stirred solution of stage 4 amine (1.08g, 2.0mmol), in THF (20ml) was added potassium carbonate (0.42g, 3.0mmol) and di-tert-butyl dicarbonate (1.75g, 8.0mmol). The reaction mixture was stirred at 50°C for 96 hours and cooled to room temperature, diluted with DCM (50ml) and washed with 0.1M HCl solution (25ml), saturated $NaHCO_3$ solution (2X25ml) and water (15ml). The DCM layer was dried, Na_2SO_4, filtered and evaporated to dryness. Purification by column chromatography using 10% EtOAc/ heptane gave the product (0.89g 70% yield). LCMS purity of 79%, m/z 638 $[M^+ + H]^+$.

Stage 6: (S)-[tert-Butoxycarbonyl-(2,3,4,9-tetrahydro-1H-beta-carbolin-1-ylmethyl)-amino]-phenyl-acetic acid cyclopentyl ester



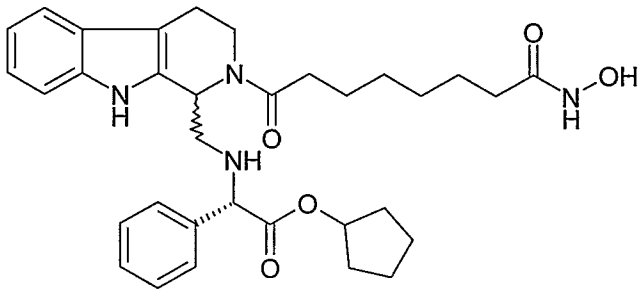
A solution of stage 5 dicarbamate (0.5g, 0.78mmol) in ethanol (40ml) was stirred under the atmosphere of hydrogen in the presence of 10% Pd/C (0.4g) for 2 h under balloon pressure. The reaction mixture was filtered through a pad of celite and evaporated to dryness yielding the required product (0.35g, 90%), 91% purity by LCMS, m/z 504 $[M^+ + H]^+$.

Stage 7: Coupling of stage 6 amine



Hydroxylamine 2-chlorotrityl resin derivatized with suberic acid (703mg, loading 0.83mmol/g) was swollen in DCM (12ml). PyBOP (912mg, 1.75mmol) was added, followed by stage 6 amine (325mg, 0.64mmol) and DIPEA (1.01ml, 5.8mmol). The reaction mixture was shaken for 18 hours. LCMS of material following test cleavage indicated 80% conversion m/z 675 $[M^+ + H]^+$. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

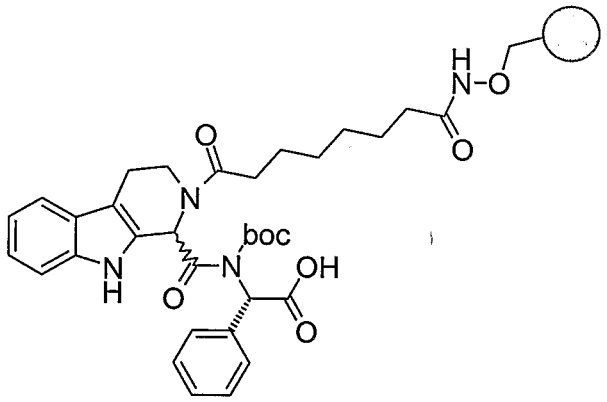
Stage 8: (S)-{[2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carbolin-1-ylmethyl]-amino}-phenyl-acetic acid cyclopentyl ester **(42)**



Stage 7 resin (135mg, loading 0.83mmol) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and was filtered after 20mins. The combined filtrates were evaporated to dryness under reduced pressure at room temperature to give an oily residue. The residue was allowed to stand in 20% TFA/DCM for 40mins. After evaporation to dryness, also under reduced pressure at room temperature, the crude product was purified by preparative HPLC to yield compound **(42)** as the TFA salt, LCMS purity 91%, m/z 575 $[M^+ + H]^+$, ^{1}H NMR (400MHz, MeOD), δ: 1.30-1.70 (16 H, m, 8 x CH_2), 2.00 (2 H, m, CH_2), 2.50 (2 H, m, CH_2), 2.75 (2 H, m, CH_2), 3.30-3.50 (2 H, m, CH_2), 4.15 (1 H, m,

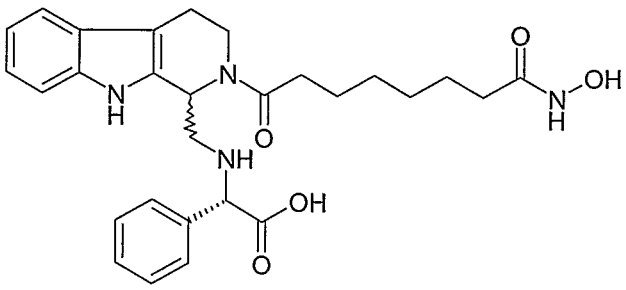
$CH_2C\underline{H}$), 4.80 (2 H, m, $C\underline{H_2}NH$, masked signal), 5.25 (1 H, m, $C\underline{H}OCO$), 6.00 (1 H, m, $OCOC\underline{H}Ph$), 6.90 (1 H, m, Ar), 7.00 (1 H, m, Ar), 7.20 (1 H, m, Ar), 7.30 (1H, m, Ar), 7.45 (5 H, m, Ar).

Stage 9: Saponification of cyclopentyl ester



Stage 7 resin (395mg, loading 0.83mmol) was suspended in THF (1.5ml) and methanol (1.5 ml) and 1.4M sodium hydroxide (aq) solution (1.17ml, 1.6mmol) was added. The mixture was shaken for 8 days. Test cleavage indicated 86% conversion to the acid, m/z 607 $[M^+H]$. The resin was filtered and washed with water x 2, MeOH x 2, followed by the standard wash procedure. The resin was dried under vacuum.

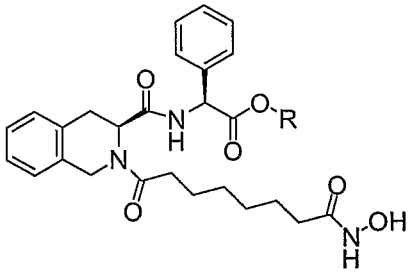
Stage 10: (S)-{[2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carbolin-1-ylmethyl]-amino}-phenyl-acetic acid **(43)**



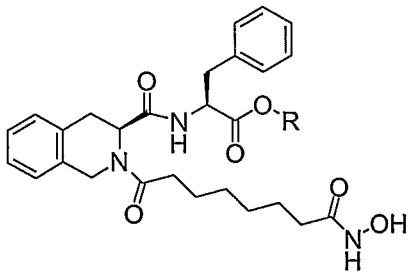
Stage 9 resin (100mg, loading 0.83mmol) was cleaved and boc deprotected using the procedure outlined for compound **(42)**. Purification by preparative HPLC afforded compound **(43)** as the TFA salt, LCMS purity 96%, m/z 507 $[M^+H]^+$, ^1H NMR (400MHz, MeOD), δ: 1.25-1.40 (4 H, m, 2 x CH_2), 1.50-1.65 (4 H, m, 2 x CH_2), 2.00 (2 H, m, CH_2), 2.50 (2 H, m, CH_2), 2.70 (2 H, m, CH_2), 3.40 (2 H, m, CH_2), 4.15 (1 H, m,

$CH_2C\underline{H}$), 4.80 (2 H, m, $C\underline{H_2}NH$, masked signal), 6.00 (1 H, m, OCOC<u>H</u>Ph), 6.90 (1 H, m, Ar), 7.00 (1 H, m, Ar), 7.20 (1 H, m, Ar), 7.30-7.50 (6 H, m, Ar).

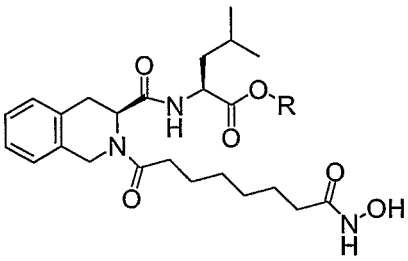
Synthesis of Compounds in Figure 3 as Exemplified by Compound (44) and Compound (45)



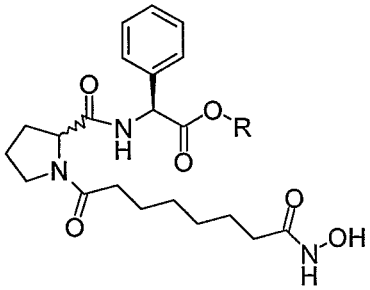
R= cyclopentyl 44
R=H 45
R = Et 46



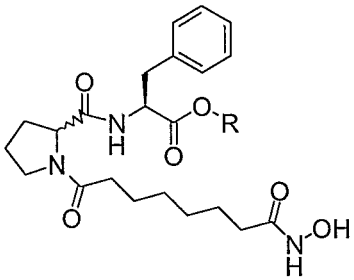
R= ethyl 47
R= cyclopentyl 48
R=H 49



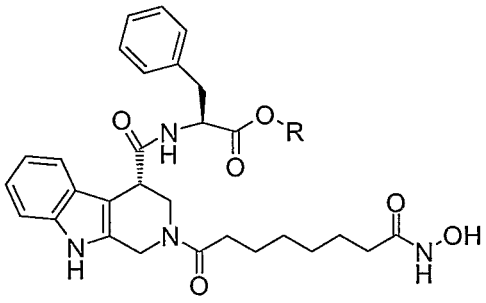
R= ethyl 50
R= cyclopentyl 51
R=H 52



R= cyclopentyl 53
R=H 54

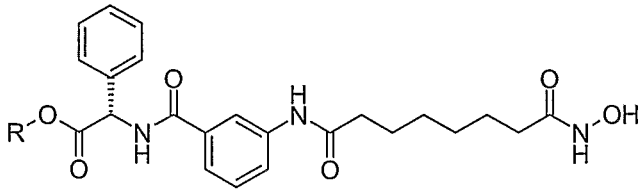


R= ethyl 55
R= cyclopentyl 56
R=H 57

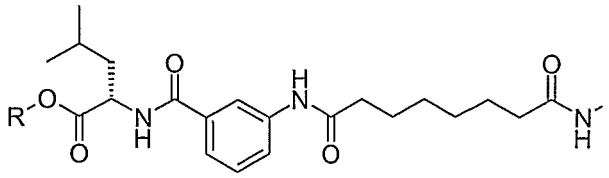


R= ethyl 58
R=H 59

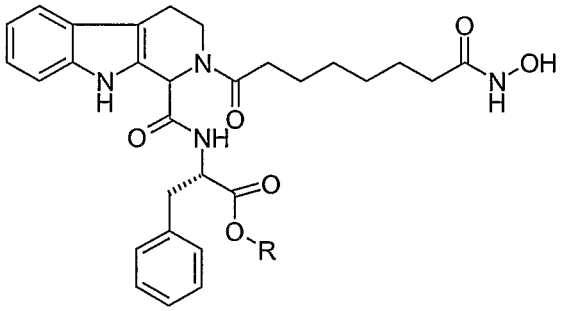
Figure 3



R= cyclopentyl 60
R= H 61



R= cyclopentyl 62
R= H 63

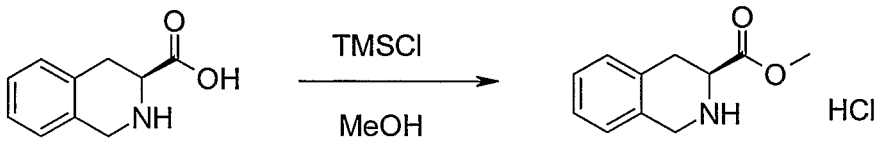


R= cyclopentyl 64
R= H 65

Figure 3 (continued)

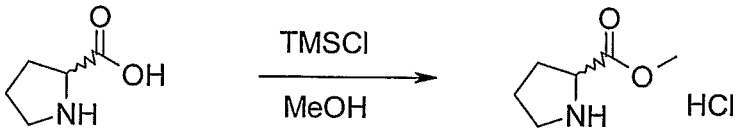
Preparation of Building Blocks H-L

Building block H



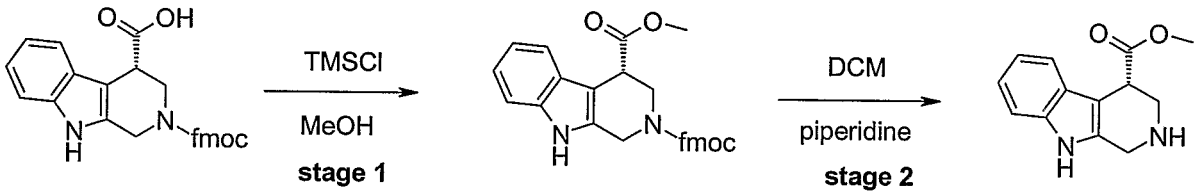
(S)-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid (10g, 56mmol), TMSCl (39ml, 310mmol) and methanol (500ml) were refluxed together (at 70°C) for 2 hours. The reaction mixture was evaporated to dryness and LCMS analysis indicated 100% conversion to (S)-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid methyl ester, m/z 192 $[M^{+}+H]^{+}$.

Building block I



DL-Proline (10g, 87mmol), TMSCl (51ml, 430 mmol) and methanol (500ml) were refluxed together (at 70°C) for 2 hours. The reaction mixture was evaporated to dryness and LCMS analysis indicated 100% conversion to desired product pyrrolidine-2-carboxylic acid methyl ester, m/z 130 $[M^+H]^+$.

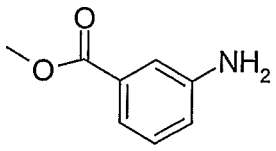
Building block J



Stages 1 & 2

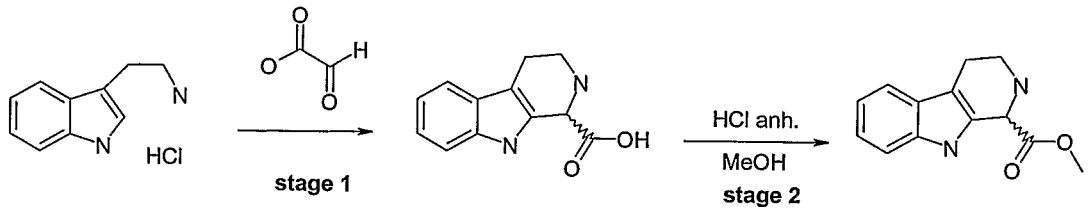
(R)-2-Fmoc-1,2,3,4-tetrahydronorharmane-3-carboxylic acid (2.0g, 9.25mmol) was added to solution of TMSCl (6ml, 47.17mmol) in methanol (100ml) and heated under reflux for 2 hours. The reaction mixture was evaporated to dryness to give 1.7g product (100% conversion by LCMS, m/z 453 $[M^+H]^+$). Stage 1 ester (1.7g) was treated with 20% piperidine in DCM (100ml) for 30 minutes to effect fmoc removal. The crude reaction mixture was evaporated to dryness, dissolved in DCM and washed with saturated $NaHCO_3$ solution. The DCM layer was isolated, dried (Na_2SO_4), filtered and concentrated to dryness. Purification by column chromatography was carried out using 3% MeOH/DCM to give (S)-2,3,4,9-tetrahydro-1H-beta-carboline-4-carboxylic acid methyl ester. LCMS 100%, m/z 231 $[M^+H]^+$.

Building block K



Meythl-3-aminobenzoate was obtained from commercial sources

Building block L



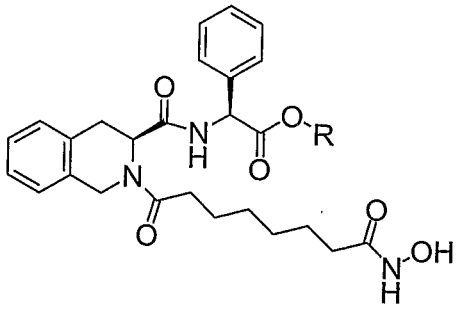
Stage 1

A solution of glyoxylic acid monohydrate (1.51g, 16.4mmol) in water (10ml) was added dropwise to a stirred solution of tryptamine.HCl (3.0g, 15.3mmol) in water (200ml). KOH (0.827g, 14.7mmol) in water (10ml) was added. The reaction mixture was stirred at room temperature for 1 h after which time precipitation occurred. Following filtration under reduced pressure the white precipitate was collected and washed with water to furnish 2,3,4,9-tetrahydro-1H-beta-carboline-1-carboxylic acid Yield 1.9g (58%); m/z 217 $[M^+ + H]^+$.

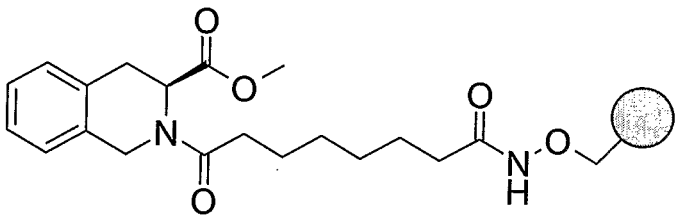
Stage 2

A solution of 1,2,3,4-tetrahydro-beta-carboline-1-carboxylic acid (7.4g) in MeOH (250ml) was saturated with HCl gas for 20min. The reaction mixture was gently stirred at room temperature for 18 h. The reaction mixture was re-treated with HCl gas and allowed to stir for a further 18h. Upon completion of the reaction the mixture was concentrated *in vacuo* to yield building block **L**, LCMS purity 95%, m/z 231 $[M^+ + H]^+$. The product (2,3,4,9-tetrahydro-1H-beta-carboline-1-carboxylic acid methyl ester) was used without further purification.

Synthesis of Compounds Outlined in Figure 3 Exemplified by Compound (44, R = cyclopentyl) and compound (45, R = H)

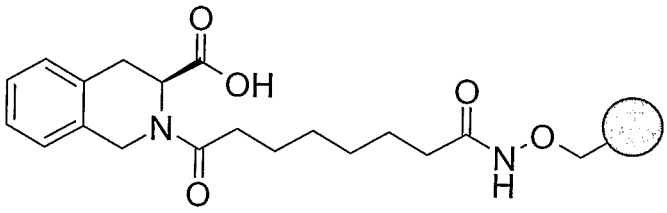


Stage 1: Loading of amine onto resin



Hydroxylamine 2-chlorotrityl resin derivatized with suberic acid (6.6g, loading, 0.83mmol) was swollen in anhydrous DCM (65ml). PyBOP (8.6g, 16.43mmol), amine building block A (3.7g, 16.43mmol) and DIPEA (9.5ml, 58.4mmol) were added. The reaction was shaken for 24 hours at room temperature. LCMS of test cleaved material indicated reaction completion. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

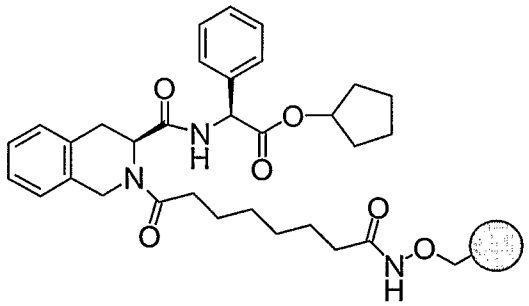
Stage 2: Saponification of methyl ester



Resin bound stage 1 ester (6.95g, loading 0.83mmol/g) was suspended in THF (25ml) and methanol (25ml). Sodium hydroxide, 1.4M aqueous solution (25ml) was added. The mixture was shaken for 48 hours and further sodium hydroxide (25ml) added after

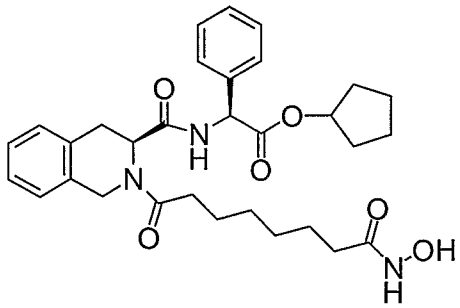
24 hours. LCMS of the test cleaved material indicated 65% conversion to the acid m/z 349 $[M^+H]^+$. The resin was filtered and washed with water x 2, MeOH x 2, followed by the standard wash procedure. The resin was dried under vacuum.

Stage 3: Coupling with L-phenylglycine cyclopentyl ester



Resin bound stage 2 carboxylic acid (2.2g, loading 0.83mmol/g) was swollen in anhydrous DCM (25ml). PyBOP (2.85g, 5.48mmol), L-phenylglycine cyclopentyl ester tosyl salt (2.14g, 5.48mmol) and DIPEA (3.17ml, 18.3mmol) were added. The mixture was shaken for 24 hours at room temperature. LCMS following test cleavage revealed 52% conversion, m/z 550 $[M^+H]^+$. The resin was filtered and washed using standard wash procedure. The resin was dried under vacuum

Stage 4: (S)-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-phenyl-acetic acid cyclopentyl ester **(44)**

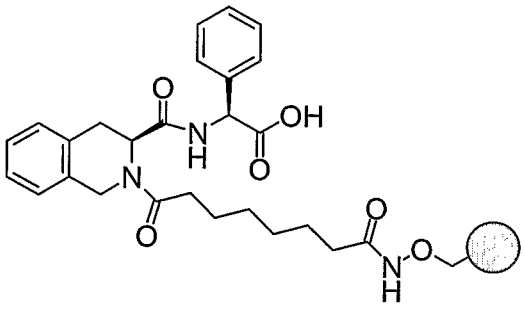


44

Stage 3 resin (2.2g, loading 0.83mmol) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was collected and evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and after 20mins filtered. The combined filtrates were evaporated to dryness

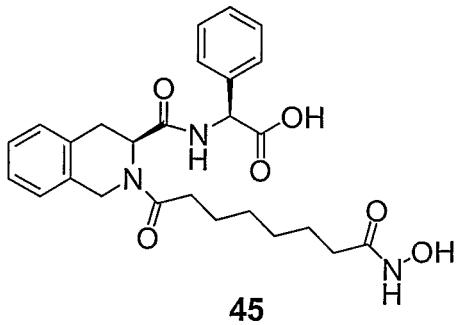
under reduced pressure at room temperature to give a residue. The residue was purified by preparative HPLC to yield compound **(44)** as the TFA salt. LCMS purity 95%, m/z 550 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.12-1.75 (16 H, m, 8 x CH_2), 1.92-2.02 (2 H, m, CH_2), 2.09-2.30 (1 H, m), 2.48 (1 H, m), 3.10 (2 H, m, CH_2), 4.58-4.66 (2 H, m, CH_2), 4.82 (1 H, m), 5.04 (1 H, m), 5.20 (1 H, s, OCOCHPh), 6.95-7.20 (9 H, m, Ar).

Stage 5- Saponification of cyclopentyl ester



Stage 3 resin (1.3g, 1.13mmol) was suspended in THF (4.6ml) and methanol (4.6 ml). Sodium hydroxide added as a 1.4M aqueous solution (4.6ml). The mixture was shaken for 24 hours. LCMS of the test cleaved material confirmed conversion to required acid. The resin was filtered and washed with water x 2, MeOH x 2, followed by the standard wash procedure. The resin was dried under vacuum.

Stage 6: (S)-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-phenyl-acetic acid **(45)**



45

Stage 5 resin (1.3g, loading 0.83mmol) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was collected and evaporated under

reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and after 20mins filtered. The combined filtrates were evaporated to dryness under reduced pressure at room temperature to give a residue. The residue was purified by preparative HPLC to yield compound **(45)**. LCMS purity 96%, m/z 482 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.12-1.38 (4 H, m, 2 x CH_2), 1.45-1.61 (4 H, m, CH_2), 1.98 (2 H, m, CH_2), 2.10-2.58 (2 H, m, CH_2), 3.04-3.20 (2 H, m, CH_2), 4.48-4.65 (2 H, m), 4.85 (1 H, m), 5.20 (1 H, m), 6.92-7.25 (9 H, m, Ar).

The following compounds were prepared according to the procedure described for compounds **(44)** and compound **(45)**

(S)-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-phenyl-acetic acid ethyl ester (46)

Building block H used

LCMS purity 97%, m/z 510 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.19 (3 H, t, CH_3), 1.32-1.48 (4 H, m, 2 x CH_2), 1.54-1.73 (4 H, m, 2 x CH_2), 2.02-2.15 (2 H, m, CH_2), 2.50-2.70 (2 H, m, CH_2), 3.10-3.30 (2 H, m, CH_2), 4.10 (2 H, m, CH_2), 4.70 (2 H, m), 4.95 (1 H, m), 5.35 (1 H, s, OCOCHPh), 7.10-7.40 (9 H, m, Ar).

(S)-2-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-3-phenyl-propionic acid ethyl ester (47)

Building block H used

LCMS purity 100%, m/z 524 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.20 (3 H, m, CH_3), 1.30-1.49 (4 H, m, 2 x CH_2), 1.55-1.70 (4 H, m, CH_2), 2.10 (2 H, m, CH_2), 2.60 (2 H, m), 2.88-3.25 (4 H, m), 4.08-4.20 (2 H, m, CH_2), 4.45-4.62 (2 H, m), 4.75 (1 H, m), 5.03 (1 H, m), 7.09-7.32 (9 H, m, Ar).

(S)-2-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-3-phenyl-propionic acid (48)

Building block H used

LCMS purity 100%, m/z 564 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.25-1.85 (16 H, m, 8 x CH_2), 2.10 (2 H, m, CH_2), 2.55 (2 H, t, CH_2), 2.85-3.20 (4 H, m), 4.40-4.60 (2 H, m), 4.75 (1 H, m), 4.95-5.15 (2 H, m), 7.05-7.30 (9 H, m, Ar).

(S)-2-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-3-phenyl-propionic acid (49)

Building block H used

LCMS purity 100%, m/z 496 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.10-1.31 (4 H, m, 2 x CH_2), 1.40-1.55 (4 H, m, 2 x CH_2), 1.98 (2 H, m, CH_2), 2.43 (2 H, m, CH_2), 2.75-3.10 (4 H, m), 4.30-4.75 (3 H, m), 4.90 (1 H, m), 6.90-7.15 (9 H, m, Ar).

(S)-2-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-4-methyl-pentanoic acid ethyl ester (50)

Building block H used

LCMS purity 98%, m/z 490 $[M^+ + H]^+$, ^1H NMR (400MHz, MeOD), δ: 0.60 (1 H, m, CH), 0.70-0.85 (6 H, m, 2 x CH_3), 1.25 (3 H, t, $CH_2C\underline{H_3}$), 1.38-1.65 (10 H, m, 5 x CH_2), 2.10 (2 H, m, CH_2), 2.60 (2 H, m, CH_2), 3.20 (2 H, m, CH_2), 4.10 (2 H, q, $C\underline{H_2}CH_3$), 4.35 (1 H, m, CH), 4.70-4.80 (2 H, m, CH_2), 4.95 (1 H, m, CH), 7.23-7.25 (4 H, m, Ar).

(S)-2-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-4-methyl-pentanoic acid cyclopentyl ester (51)

Building block H used

LCMS purity 96%, m/z 530 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 0.75 (3 H, d, CH_3), 0.88 (3 H, d, CH_3), 1.30-1.90 (19 H, m), 2.10 (2 H, t, CH_2), 2.60 (2 H, m, CH_2), 3.15-3.30 (2 H, m, CH_2), 4.30 (1 H, m), 4.65-4.85 (2 H, m), 4.95 (1 H, m), 5.10 (1 H, m), 7.15-7.28 (4 H, m, Ar).

(S)-2-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-4-methyl-pentanoic acid ethyl ester (52)

Building block H used

LCMS purity 100%, m/z 462 $[M^+ + H]^+$, ^1H NMR (400MHz, MeOD), δ: 0.60 (1 H, m, CH), 0.70-0.85 (6 H, m, 2 x CH_3), 1.38-1.65 (10 H, m, 5 x CH_2), 2.10 (2 H, m, CH_2), 2.40-2.60 (2 H, m, CH_2), 3.20 (2 H, m, CH_2), 4.35 (1 H, m, CH), 4.70-4.80 (2 H, m, CH_2), 4.95 (1 H, m, CH, masked signal), 7.23-7.25 (4 H, m, Ar).

(S)-{[1-(7-Hydroxycarbamoyl-heptanoyl)-pyrrolidine-2-carbonyl]-amino}-phenyl-acetic acid cyclopentyl ester (53)

Building block I used

LCMS purity 100%, m/z 488 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.30-2.45 (24 H, m), 3.50-3.70 (2 H, m, CH_2), 4.55 (1 H, m, CH), 5.18 (1 H, m, CH), 5.40 (1 H, m, CH), 7.40 (5 H, m, Ar).

(S)-{[1-(7-Hydroxycarbamoyl-heptanoyl)-pyrrolidine-2-carbonyl]-amino}-phenyl-acetic acid (54)

Building block I used

LCMS purity 90%, m/z 420 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.20-1.20 (4 H, m, 2 x CH_2), 1.45-1.56 (4 H, m, CH_2), 1.75-2.35 (8 H, m), 3.35-3.60 (2 H, m), 4.45 (1 H, m), 5.35 (1 H, m), 7.18-7.35 (5 H, m, Ar).

(S)-2-{[1-(7-Hydroxycarbamoyl-heptanoyl)-pyrrolidine-2-carbonyl]-amino}-3-phenyl-propionic acid ethyl ester (55)

Building block I used

LCMS purity 100%, m/z 462 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.20-2.20 (19 H, m), 2.94-3.20 (2 H, m, C$\underline{H}_2$Ph), 3.48-3.69 (2 H, m, C$\underline{H}_2$N), 4.10-4.25 (2 H, m, C$\underline{H}_2$$CH_3$), 4.33-4.49 (1 H, m), 4.60-4.79 (1 H, m), 7.20-7.35 (5 H, m, Ar).

(S)-2-{[1-(7-Hydroxycarbamoyl-heptanoyl)-pyrrolidine-2-carbonyl]-amino}-3-phenyl-propionic acid cyclopentyl ester (56)

Building block I used

LCMS purity 100%, m/z 502 $[M^+ + H]^+$, ^1H NMR (400MHz, MeOD), δ: 1.27-2.23 (22 H, m, 11 x CH_2), 2.35 (2 H, m, CH_2), 2.97-3.27 (2 H, m, C$\underline{H}_2$Ph), 3.53-3.63 (2 H, m, CH_2), 4.35-4.45 (1 H, m, CH), 4.60-4.70 (1 H, m, C$\underline{H}$$CH_2$Ph), 5.10-5.20 (1 H, m, C$\underline{H}$OCO), 7.23-7.30 (5 H, m, Ar).

(S)-2-{[1-(7-Hydroxycarbamoyl-heptanoyl)-pyrrolidine-2-carbonyl]-amino}-3-phenyl-propionic acid (57)

Building block I used

LCMS purity 90%, m/z 434 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.30-1.41 (4 H, m, 2 x CH_2), 1.55-1.69 (4 H, m, 2 x CH_2), 1.80-1.90 (8 H, m), 2.91-3.26 (2 H, m), 3.45-3.70 (2 H, m), 4.40 (1 H, m), 4.72 (1 H, m), 7.16-7.30 (5 H, m, Ar).

(S)-2-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-4-carbonyl]-amino}-3-phenyl-propionic acid cyclopentyl ester (58)

Building block J used

LCMS purity of 100%, m/z 563 $[M^+H]^+$, ^1H NMR (400 MHz MeOD), δ: 1.10-1.30 (3H, m, CH_3), 1.35-1.80 (8H, m, 4 X CH_2), 2.15 (2H, m, CH_2), 2.4-2.65 (2H, m, CH_2), 2.95-3.20 (3H, m), 4.0-4.2 (2H, m CH_2O), 4.3-5.0 (4H, m masked signal), 5.05-5.20 (1H, m CHOCO), 6.90-7.50 (9H, m, Ar).

(S)-2-{[(S)-2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-bet a-carboline-4-carbonyl]-amino}-3-phenyl-propionic acid (59)

Building block J used

LCMS purity of 100%, m/z 535 $[M^+H]^+$, ^1H NMR (400 MHz MeOD), δ: 1.20-1.40 (4H, m, 2 X CH_2), 1.45-1.65 (4H, m, 2 X CH_2), 1.90-2.10 (2H, m, CH_2), 2.30-2.50 (2H, m, CH_2), 2.70-3.15 (3H, m), 4.2-4.9 (4H, m masked signal), 5.00 (1H, m CHOCO), 6.75-7.40 (9H, m, Ar)

(S)-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzoylamino]-phenyl-acetic acid cyclopentyl ester (60)

Building block K used

LCMS purity 100%, m/z 510 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.28 (4 H, m, 2 x CH_2), 1.40-1.80 (12 H, m, 6 x CH_2), 1.98 (2 H, t, CH_2), 2.27 (2 H, t, CH_2), 5.12 (1 H, m), 5.50 (1 H, s, OCOCHPh), 7.21-7.32 (4 H, m, Ar), 7.36 (2 H, m, Ar), 7.45 (1 H, d, Ar), 7.61 (1 H, d, Ar), 7.90 (1 H, s, Ar).

(S)-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzoylamino]-phenyl-acetic acid (61)

Building block K used

LCMS purity 100%, m/z 442$[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.21-1.34 (4 H, m, 2 x CH_2), 1.48-1.63 (4 H, m, 2 x CH_2), 1.98 (2 H, t, CH_2), 2.26 (2 H, t, CH_2), 5.55 (1 H, s, OCOCHPh), 7.20-7.32 (4 H, m, Ar), 7.40 (2 H, d, Ar), 7.48 (1 H, d, Ar), 7.64 (1 H, d, Ar), 7.89 (1 H, s, Ar).

(S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzoylamino]-4-methyl-pentanoic acid cyclopentyl ester (62)

Building block K used

LCMS purity 93%, m/z 490 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 0.84 (3 H, d, $CH(C\underline{H}_3)$), 0.88 (3 H, d, $CH(C\underline{H}_3)$), 1.20-1.40 (4 H, m, 2 x CH_2), 1.40-1.85 (15 H, m, 6 x CH_2, $C\underline{H}(CH_3)_2$, $C\underline{H}_2CH(CH_3)_2$), 2.00 (2 H, t, CH_2), 2.25 (2 H, t, CH_2), 4.45 (1 H, m, $OCOC\underline{H}CH_2$), 5.10 (1 H, m, $C\underline{H}OCO$), 7.25 (1 H, m Ar), 7.40 (1 H, d, Ar), 7.60 (1 H, d, Ar), 7.90 (1 H, s, Ar).

(S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzoylamino]-4-methyl-pentanoic acid (63)

Building block K used

LCMS purity 97%, m/z 422 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.03 (3 H, d, $CH(C\underline{H}_3)$), 1.06 (3 H, d, $CH(C\underline{H}_3)$), 1.40-1.55 (4 H, m, 2 x CH_2), 1.65-1.95 (7 H, m, 2 x CH_2, $C\underline{H}(CH_3)_2$, $C\underline{H}_2CH(CH_3)_2$), 2.15 (2 H, t, CH_2), 2.45 (2 H, t, CH_2), 4.70 (1 H, m, $OCOC\underline{H}CH_2$), 7.45 (1 H, m, Ar), 7.60 (1 H, d, Ar), 7.80 (1 H, d, Ar), 8.05 (1 H, s, Ar).

(S)-2-{[2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-1-carbonyl]-amino}-3-phenyl-propionic acid cyclopentyl ester(64)

Building block L used

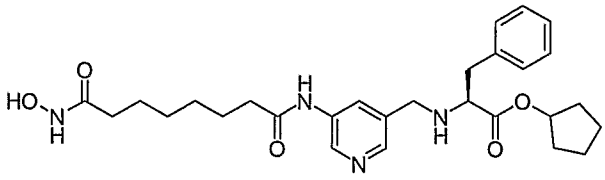
LCMS purity 100%, m/z 603 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.18-1.71 (16 H, m, 8 x CH_2), 2.00 (2 H, t, CH_2), 2.45 (2 H, m), 2.70 (2 H, m), 2.90-3.11 (2 H, m), 3.40 (1 H, m), 4.10 (1 H, m), 4.50 (1 H, m), 5.00 (1 H, m), 5.95 (1 H, m), 6.90-7.11 (7 H, m, Ar), 7.25 (1 H, d, Ar), 7.34 (1 H, d, Ar).

(S)-2-{[2-(7-Hydroxycarbamoyl-heptanoyl)-2,3,4,9-tetrahydro-1H-beta-carboline-1-carbonyl]-amino}-3-phenyl-propionic acid (65)

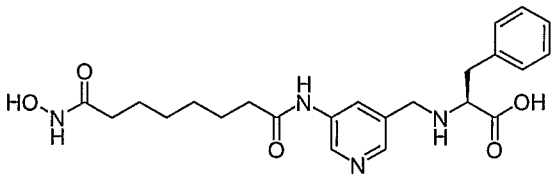
Building block L used

LCMS purity 91%, m/z 535 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD δ: 1.15-1.32 (4 H, m, 2 x CH_2), 1.40-1.60 (4 H, m, 2 x CH_2), 1.98 (2 H, t, CH_2), 2.41 (2 H, m), 2.69 (2 H, m), 2.90-3.11 (2 H, m), 3.30 (1 H, m), 4.06 (1 H, m), 4.60 (1H, m), 5.92 (1 H, m), 6.84 (7 H, m, Ar), 7.20 (1 H, d, Ar), 7.31 (1 H, d, Ar).

Synthesis of Compound (66) and Compound (67)

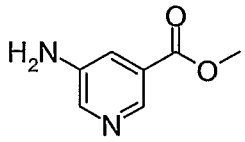


66



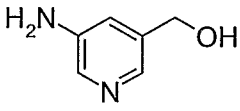
67

Stage 1: 5-Amino-nicotinic acid methyl ester



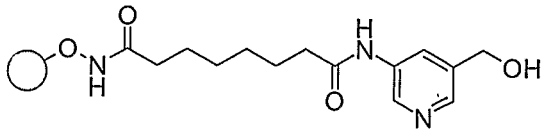
5-Aminonicotinic acid (1g,.7.2mmmol) was suspended in methanol (100ml) and thionyl chloride (4.22ml, 57.9mmol) added dropwise at 0°C. The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was evaporated to dryness and the resultant yellow oil was re-dissolved in methanol/ether (1:1) and afforded yellow crystals (HCl salt) which were collected by filtration, yield 1.2g (85%). LCMS purity 91%, m/z 153 $[M^+ + H]^+$,

Stage 2: (5-Amino-pyridin-3-yl)-methanol



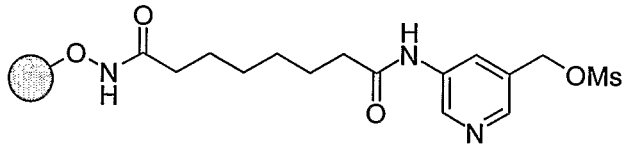
5-Amino-nicotinic acid methyl ester (5.7g, 30.2mmol) was dissolved in THF (150ml) and $LiAlH_4$ (1M in THF solution 133ml, 133mmol) added slowly at 0°C. The reaction mixture was stirred at room temperature for 21 h. The reaction mixture was quenched and acidified to pH 3 using dilute HCl, and basified (pH 8) using solid Na_2CO_3. Solvents were removed under reduced pressure. The residue was filtered through silica gel using 20% MeOH/DCM yielding the product 3.8g, (100%) with LCMS purity 97%, m/z 125 $[M^+ + H]^+$, by ELS.

Stage 3: Coupling of stage 2 acid onto resin



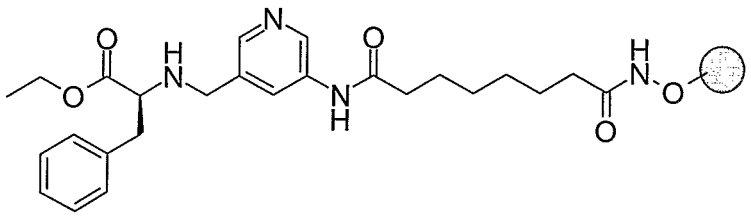
Hydroxylamine-2-chlorotrityl resin derivatized with suberic acid (0.49g, 0.86 mmol/g, 0.42mmol) was swollen in anhydrous DCM (6ml) and PyBOP (0.67g, 1.3mmol) added. Stage 2 aniline (0.16g, 1.3mmol) was added in DMF (6ml) followed by DIPEA (0.75ml, 4.2mmol). LCMS following test cleavage indicated 27% conversion, m/z 296 $[M^+H]^+$. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

Stage 4: Mesylation



Resin bound stage 3 alcohol (1.8g, 1.57mmol) was swollen in anhydrous DCM (30ml) and DIPEA (1.62ml, 9.42mmol) was added at 0°C followed by mesyl chloride (0.23ml, 3.14mmol). The reaction mixture was shaken at 0°C for 30 minutes. LCMS following test cleavage indicated 21% conversion, m/z 374 $[M^+H]^+$ and 9% by-product derived from chloride displacement of mesylate m/z 314 $[M^+H]^+$. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

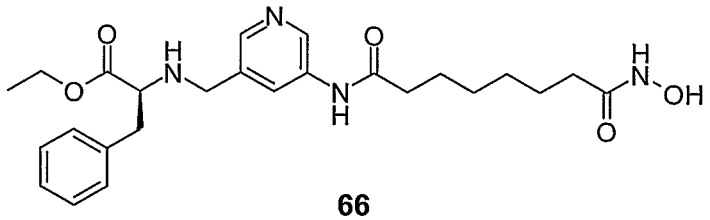
Stage 5: Displacement of mesylate with L-phenylalanine ethyl ester



Resin bound stage 4 product (0.5g, 0.43mmol) was swollen in anhydrous DMF (4ml) and sodium iodide (0.05g, 10%w/v) added. L-Phenylalanine ethyl ester hydrochloride salt (0.3g, 1.29mmol) in anhydrous DMF (4ml) was added followed by DIPEA (0.75ml,

4.3mmol). After shaking for 3 hours LCMS of test cleaved material indicated 35% conversion, m/z 471$[M^+H]^+$. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

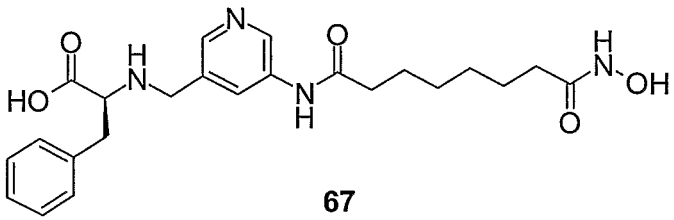
Stage 6: (S)-2-{[5-(7-Hydroxycarbamoyl-heptanoylamino)-pyridin-3-ylmethyl]-amino}-3-phenyl-propionic acid ethyl ester **(66)**



66

Stage 5 resin (2g, loading 0.87mmol) was gently shaken in 2% TFA/DCM (20ml) for 20mins. The resin was filtered. The filtrate was collected and evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (20ml) and filtered after 10 mins. The combined filtrates were evaporated to dryness under reduced pressure at room temperature to give a crude product. The crude was purified by preparative HPLC to yield compound **(66)** as the TFA salt. LCMS purity 100%, m/z 471 $[M^+H]^+$, ^1H NMR (400MHz, MeOD), δ: 1.10 (3H, t, $CO_2CH_2C\underline{H}_3$), 1.31-1.50 (4 H, m, 2 x CH_2), 1.58-1.80 (4 H, m, 2 x CH_2), 2.05-2.15 (1 H, m, CH), 2.24-2.38 (1 H, m, CH), 2.45 (2 H, t, CH_2), 3.10-3.20 (1 H, m, CH), 3.38-3.49 (1 H, m, CH), 4.12 (2 H, q, CH_2), 4.35 (3H, m, CH_2, CH) 7.20-7.40 (5 H, m, Ar), 8.30-9.00 (3 H, m, Ar).

Stage 7: (S)-2-{[5-(7-Hydroxycarbamoyl-heptanoylamino)-pyridin-3-ylmethyl]-amino}-3-phenyl-propionic acid **(67)**

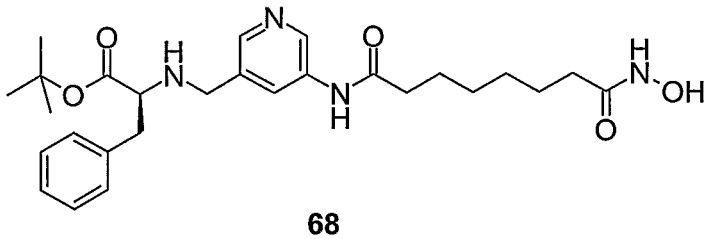


67

To a solution of Stage 6 product (30mg, loading 1.8mmol/g) in THF (1ml) was added 1.4M sodium hydroxide (1 ml). The reaction mixture was stirred for 30 minutes. LCMS showed 75% conversion, m/z 442$[M^+H]^+$. The reaction mixture was evaporated to

dryness and was purified by preparative HPLC to yield the desired compound as the TFA salt, compound **(67)**. LCMS purity 100%, m/z 443 $[M^+H]^+$. ^1H NMR (400 MHz, MeOD), δ: 1.30 (4 H, m, 2 x CH_2), 1.50-1.70 (4 H, m, 2 x CH_2), 2.00 (2 H, t, CH_2), 2.30 (2 H, t, CH_2), 3.20 (2 H, m, $C\underline{H}_2Ph$, masked signal), 4.20 (3 H, m), 7.20 (5 H, m, Ar), 8.30 (1 H, br s, Ar), 8.40 (1 H, s, Ar), 8.65 (1 H, br s, Ar).

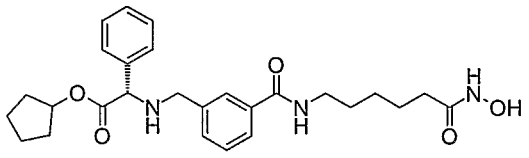
Synthesis of Compound (68)



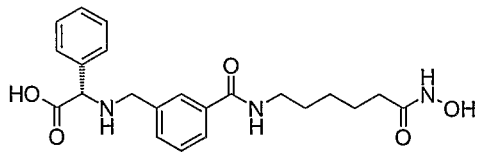
68

(S)-2-{[5-(7-Hydroxycarbamoyl-heptanoylamino)-pyridin-3-ylmethyl]-amino}-3-phenyl-propionic acid tert-butyl ester **(68)** (was prepared using the procedure outlined for the preparation of compound **(66):** LCMS purity 100%, m/z 499 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.20 (9 H, s, $C(C\underline{H}_3)_3$), 1.25-1.35 (4 H, m, 2 x CH_2), 1.49-1.65 (4 H, m, 2 x CH_2), 2.00 (2 H, t, CH_2), 2.35 (3 H, t, CH_2), 3.00 (1 H, m), 3.32 (1 H, m), 4.15-4.30 (3 H, m), 7.15-7.30 (5 H, m, Ar), 8.30 (1 H, br s, Ar), 8.45 (1 H, s, Ar), 8.65 (1 H, br s, Ar).

Synthesis of Compound (69) and Compound (70)

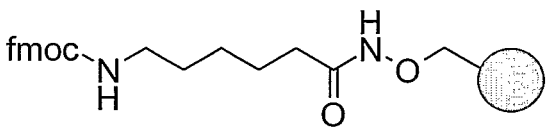


69



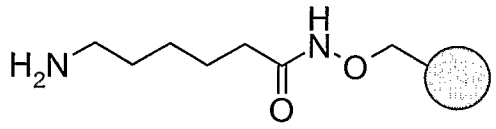
70

Stage 1: Loading of Fmoc amino caproic acid onto resin



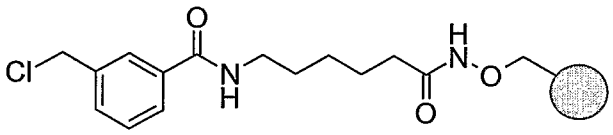
To a mixture of hydroxylamine 2-chlorotrityl resin (2.5g, loading 0.94mmol/g) in anhydrous DCM (10ml) was added a solution of 1,3-diisopropylcarbodiimide (1.1ml, 7.05mmol) and 6-(Fmoc-amino) caproic acid (2.5g, 7.05mmol) in anhydrous DCM (10ml). DMF (5ml) was added and the reaction shaken at room temperature for 1 h. Test cleavage revealed 96% conversion to required product. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

Stage 2: Fmoc deprotection



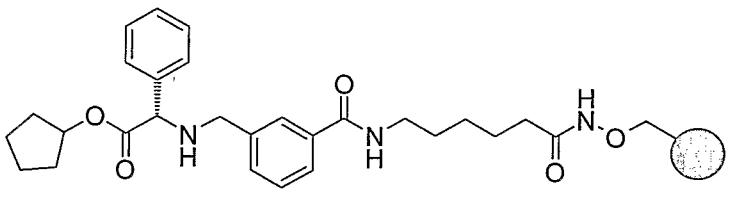
Stage 1 Fmoc protected amine resin (2.0g, loading 0.94mmol/g) was dissolved in a solution of 20% piperidine in DMF (25ml, excess) and shaken at room temperature for 30 minutes. A test cleavage indicated complete conversion by LCMS, 100% (ELS detection). The resin was filtered, washed using the standard wash procedure and dried under vacuum.

Stage 3: Coupling reaction



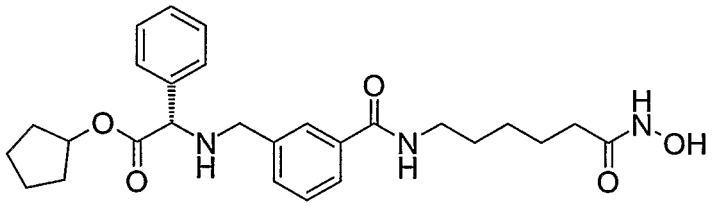
To resin bound stage 2 amine (2.0 g, loading 0.94mmol/g) in anhydrous DCM (10ml) and DMF (10ml) was added DIC (0.71ml, 5.64mmol) and 3- (chloromethyl) benzoic acid (0.96g, 5.64mmol). The mixture was shaken for 1 hour before test cleavage revealed 49% conversion by LCMS, m/z 219 $[M^+ + H]^+$. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

Stage 4: Chloride displacement with L-phenylglycine cyclopentyl ester



To resin bound stage 3 chloride (0.5g, 0.47mmol) in anhydrous DMF (5ml) was added L-phenylglycine cyclopentyl ester tosyl salt (0.57g, 1.41mmol), DIPEA (0.24ml, 1.41mmol) and a catalytic amount of sodium iodide. The reaction mixture was heated at 60°C for 1 hour. LCMS following test cleavage revealed 45% conversion, m/z 482 $[M^+H]^+$. The resin was filtered and washed using the standard wash procedure. The resin was dried under vacuum.

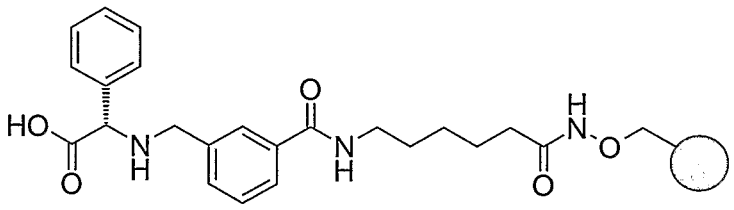
Stage 5: (S)-[3-(5-Hydroxycarbamoyl-pentylcarbamoyl)-benzylamino]-phenyl-acetic acid cyclopentyl ester **(69)**



69

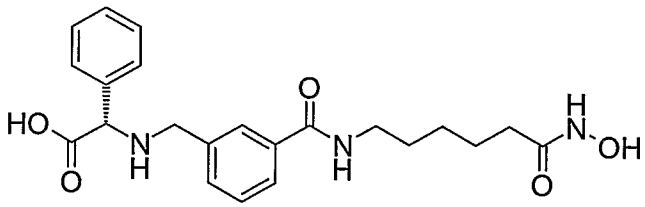
Stage 4 resin (1.0g, loading 0.94mmol/g) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was collected and evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and after 20mins filtered. The combined filtrates were evaporated to dryness under reduced pressure at room temperature to give a residue. The residue was purified by preparative HPLC to yield compound **(69)**. LCMS purity 89%, m/z 482 $[M^+H]^+$, ^{1}H NMR (400 MHz, MeOD), δ: 1.24-1.82 (14 H, m, 7 x CH_2), 2.03 (2 H, t, CH_2), 3.30 (2 H, t, CH_2), 4.08 (1 H, d, CHHPh), 2.20 (1 H, d, CHHPh), 5.09 (1 H, s, OCOCHPh), 5.18 (1 H, m, CHOCO), 7.39-7.54 (7 H, m, Ar), 7.77 (2 H, m, Ar).

Stage 6: Saponification of cyclopentyl ester



Stage 4 resin (1.35g, loading 0.94mmol/g) was suspended in THF (4.7ml) and methanol (4.7ml). 1.4M sodium hydroxide was added (9.4ml, 12.66mmol). The mixture was shaken for 48h. LCMS of the test cleave showed 49% conversion to the acid, m/z 414 $[M^+H]^+$. The resin was filtered and washed with water x 2, MeOH x 2, followed by the standard wash procedure. The resin was dried under vacuum

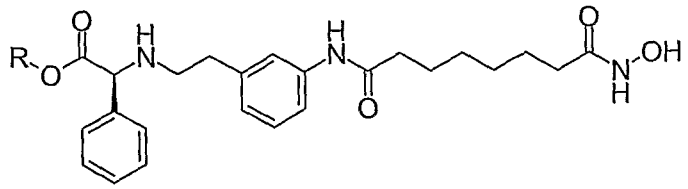
Stage 7: (S)-[3-(5-Hydroxycarbamoyl-pentylcarbamoyl)-benzylamino]-phenyl-acetic acid **(70)**



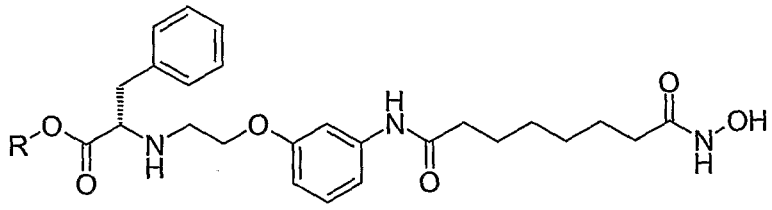
70

Stage 6 resin (1.35g, loading 0.94mmol/g) was gently shaken in 2% TFA/DCM (10ml) for 20mins. The resin was filtered. The filtrate was collected and evaporated under reduced pressure at room temperature. The resin was re-treated with 2% TFA/DCM (10ml) and after 20mins filtered. The combined filtrates were evaporated to dryness under reduced pressure at room temperature to give a residue. The residue was purified by preparative HPLC to yield compound **(70)**. LCMS purity 100%, m/z 414 $[M^+H]^+$, ^{1}H NMR (400 MHz, MeOD), δ: 1.30 (2 H, m, CH_2), 1.57 (4 H, m, 2 x CH_2), 2.20 (2 H, t, CH_2), 3.30 (2 H, t, CH_2), 4.05 (1 H, d, C<u>H</u>HPh), 4.18 (1 H, d, CH<u>H</u>Ph), 4.90 (1 H, s, OCOCHPh), 7.35-7.52 (7 H, m, Ar), 7.78 (2 H, m, Ar).

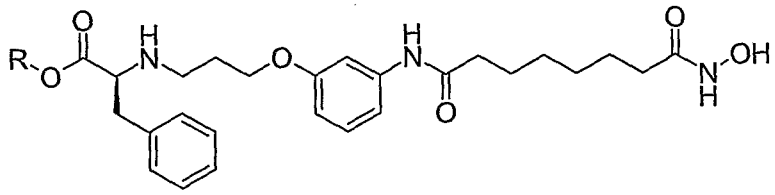
Synthesis of Compounds in Figure 4 Exemplified by Compound (71) and Compound (72)



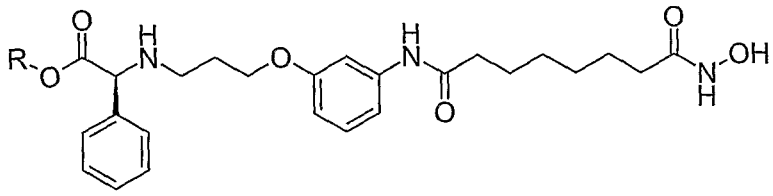
R= cyclopentyl 71
R= H 72



R= cyclopentyl 73
R= H 74



R= cyclopentyl 75
R= H 76

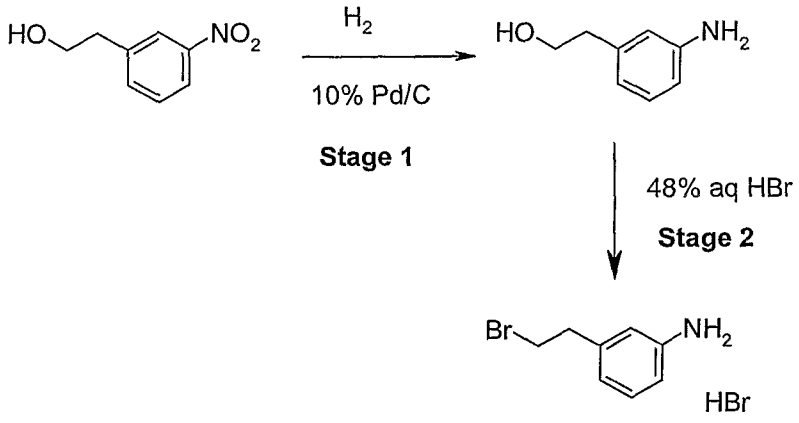


R= cyclopentyl 77
R= H 78

Figure 4

Preparation of building blocks M,N,O

Building block M



Building block M

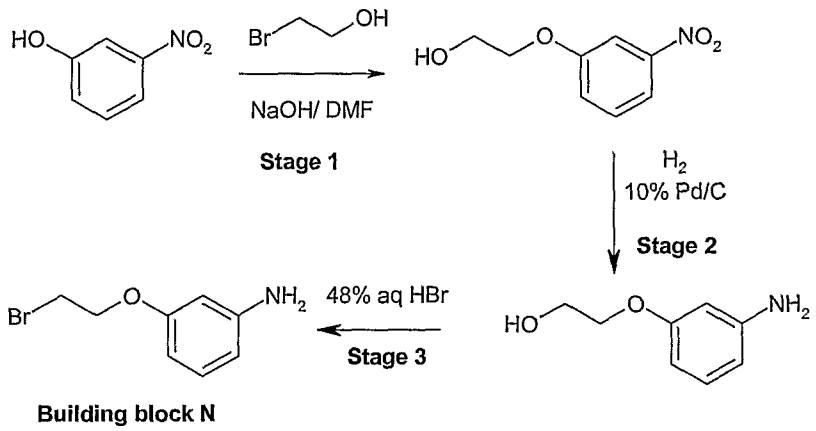
Stage 1: 2-(3-Amino-phenyl)-ethanol

A mixture of nitro phenethyl alcohol (8.0g, 0.047mol) and 10% Pd/C (0.6g) in ethanol (100ml) was stirred under a hydrogen atmosphere (balloon pressure) for 18 h. The reaction mixture was filtered through a pad of celite and the Pd/C catalyst removed. The filtrate was concentrated under reduced pressure to yield a light brown solid 6.1g (95% yield). LCMS purity 98%, m/z 138 $[M+H]^+$.

Stage 2: 3-(2-Bromo-ethyl)-phenylamine

A solution of 2-(3-Amino-phenyl)-ethanol (2.0g) in 48% aq HBr (20ml) was heated at 90 ^{0}C for 18 h. The mixture was cooled to room temperature, and the precipitate formed was collected by filtration. The solid was dried *in vacuo* yielding **Building block M**, 1.8g (61% yield). LCMS purity 90%, m/z 200/202 $[M+H]^+$.

Building block N



Building block N

Stage 1: 2-(3-Nitro-phenoxy)-ethanol

To a solution of 3-nitrophenol (10g, 71.9mmol) in DMF (40ml) was added NaOH pellets (3.16g, 79.1mmol) and 2-bromoethanol (5.6ml, 79.1mmol). The reaction mixture was heated at 60 ^{0}C for 18 h. LCMS indicated 65% conversion to the required product. The reaction mixture was diluted with water (10ml) and was slowly neutralised with 2M HCl. The reaction mixture was extracted with EtOAc (50ml) and washed with water (50ml). The EtOAc layer dried (Na_2SO_4), filtered and evaporated to dryness. Flash column chromatography purification eluting with 30% EtOAc/ heptane gave the required product (8.2g, 62% yield). LCMS purity 100%, m/z 184 $[M+H]^+$.

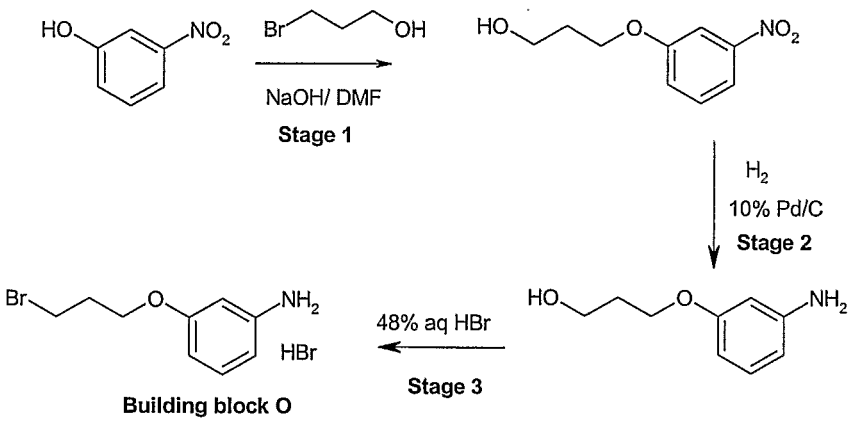
Stage 2: 2-(3-Amino-phenoxy)-ethanol

Reduction was carried out using the procedure outlined for Building block M.

Stage 3: 3-(2-Bromo-ethoxy)-phenylamine

Bromination was carried out using the procedure described for Building block M.

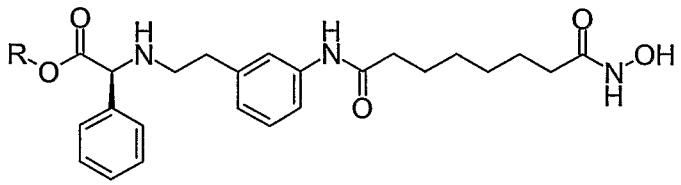
Building block O



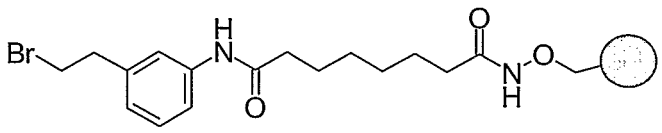
Building block O

Building block **O** was prepared as described for Building block **G** with 3-bromo-1-propanol used in place of 2-bromoethanol.

Synthesis of Compounds in Figure 4 Exemplified for Compound (71, R = cyclopentyl) and Compound (72, R =H)

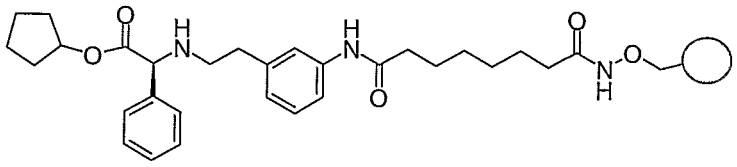


Stage 1: Coupling of aniline derivative to carboxylic acid functionalised resin



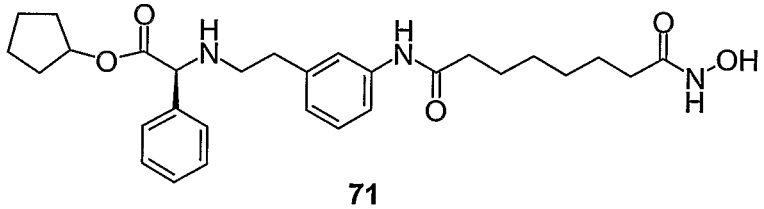
To a suspension of hydroxylamine 2-chlorotrityl resin derivatized with suberic acid (1.0g, 0.94mmol, loading 0.94mmol) in DCM/DMF (10ml/10ml) was added DIPEA (1.75ml) followed by building block **M**, 0.8g, 2.82mmol. PyBrOP (0.53g, 3.76mmol) was added and the suspension shaken for 18 h. The resin was washed using the standard wash procedure and was thoroughly dried.

Stage 2: Displacement of bromide with L-phenylglycine cyclopentyl ester



To a suspension of stage 1 resin (0.4g, 0.38mmol) in DMF (4ml) in a vial, was added L-phenylglycine cyclopentyl ester tosyl salt (0.44g, 1.12mmol) and DIPEA (0.67ml, 3.76mmol) followed by NaI (50mg). The reaction was allowed to stand at 65 °C for 8 h. The resin was thoroughly washed using the standard wash procedure.

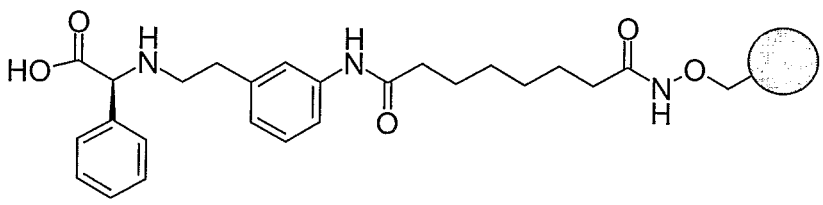
Stage 3: (S)-{2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenyl]-ethylamino}-phenyl-acetic acid cyclopentyl ester **(71)**



71

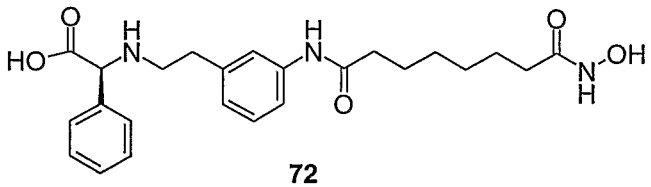
Stage 2 resin was cleaved with 2% TFA/ DCM (10ml x 3). The filtrate was concentrated to dryness and the residue purified by preparative HPLC to afford compound **(71)** as the TFA salt. Yield 21mg (11% overall), LCMS purity 99%, m/z 510 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.35-1.95 (16 H, m 8 x CH_2), 2.10 (2 H, t, CH_2), 2.38 (2 H, t, CH_2), 2.91-3.29 (4 H, m), 5.18 (1 H, s, OCOCHPh), 5.32 (1 H, m, CHOCO), 6.98 (1 H, m, Ar), 7.30 (2 H, m, Ar), 7.47-7.56 (5 H, m, Ar), 7.62 (1 H, s, Ar).

Stage 4: Saponification



Stage 2 resin (1.2g, 1.12mmol) was suspended in THF/ MeOH (12ml/12ml). 2.7M NaOH solution added and mixture was shaken for 18 h at room temperature Upon completion of reaction the resin was thoroughly washed (Standard wash procedure).

Stage 5: (S)-{2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenyl]-ethylamino}-phenyl-acetic acid **(72)**



72

Stage 4 resin (0.8g, 0.76mmol) was cleaved with 2% TFA/ DCM (10ml x 3). Filtrate was concentrated to dryness and residue purified by preparative HPLC to give compound **(72)** as a TFA salt, yield 40mg (10% overall). LCMS purity of 100%, m/z 442 $[M^+H]^+$, ^1H NMR (400 MHz MeOD), δ: 1.20-1.35 (4 H, m, 2 x CH_2), 1.45-1.70 (4 H, m, 2 x CH_2), 1.95 (2 H, t, CH_2), 2.25 (2 H, t, CH_2), 2.80-3.20 (4 H, m $C\underline{H}_2NH$, $C\underline{H}_2Ph$), 5.00 (1 H, s, $C\underline{H}COOH$), 6.85 (1 H, m, Ar), 7.15 (2 H, m, Ar), 7.40 (5 H, s, Ar), 7.50 (1 H, s, Ar).

The following compounds were prepared according to the procedure described for compounds **(71)** and compound **(72)**

(S)-2-{2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenoxy]-ethylamino}-3-phenyl-propionic acid cyclopentyl ester (73)

Building block N used

LCMS purity 94%, m/z 540 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.26-1.82 (16 H, m, 8 x CH_2), 2.11 (2 H, t, CH_2), 2.39 (2 H, t, CH_2), 3.15 (1 H, dd, $C\underline{H}HPh$), 3.44 (1 H, dd, $CH\underline{H}Ph$), 3.56 (2 H, m, CH_2), 4.30 (2 H, t, CH_2), 4.40 (1 H, m), 5.13 (1 H, m, $C\underline{H}OCO$), 6.76 (1 H, d, Ar), 7.00 (1 H, d, Ar), 7.24-7.41 (6 H, m, Ar), 7.57 (1 H, s, Ar).

(S)-2-{2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenoxy]-ethylamino}-3-phenyl-propionic acid (74)

Building block N used

LCMS purity 100%, m/z 472 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.35-1.46 (4 H, m, 2 x CH_2), 1.61-1.77 (4 H, m, 2 x CH_2), 2.11 (2 H, t, CH_2), 2.40 (2 H, t, CH_2), 3.28-3.40 (2 H, m, CH_2, masked signal), 3.53 (2 H, t, CH_2), 4.29 (2 H, t, CH_2), 4.38 (1 H, t, OCOC<u>H</u>CH_2), 6.75 (1 H, d, Ar), 7.00 (1 H, d, Ar), 7.25 (1 H, t, Ar), 7.30-7.41 (5 H, m, Ar), 7.53 (1 H, s, Ar).

(S)-2-{3-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenoxy]-propylamino}-3-phenyl-propionic acid cyclopentyl ester (75)

Building block O used

LCMS purity 100%, m/z 554 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.29-1.87 (16 H, m, 8 x CH_2), 2.10 (2 H, t, CH_2), 2.21 (2 H, m, CH_2), 2.37 (2 H, t, CH_2), 3.12 (1 H, dd, C<u>H</u>HPh), 3.25-3.43 (3 H, m, CH<u>H</u>Ph, CH_2), 4.11 (2 H, t, CH_2), 4.33 (1 H, m, OCOC<u>H</u>CH_2), 5.18 (1 H, m, C<u>H</u>OCO), 6.78 (1 H, d, Ar), 7.00 (1 H, d, Ar), 7.22 (1 H, t, Ar), 7.24-7.39 (5 H, m, Ar), 7.44 (1 H, s, Ar).

(S)-2-{3-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenoxy]-propylamino}-3-phenyl-propionic acid (76)

Building block O used

LCMS purity 100%, m/z 486 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.34-1.47 (4 H, m, 2 x CH_2), 1.60-1.75 (4 H, m, 2 x CH_2), 2.10 (2 H, t, CH_2), 2.19 (2 H, m, CH_2), 2.38 (2 H, t, CH_2), 3.25-3.40 (4 H, m, 2 x CH_2, masked signal), 4.09 (2 H, t, CH_2), 4.35 (1 H, OCOC<u>H</u>CH_2), 6.75 (1 H, d, Ar), 6.98 (1 H, d, Ar), 7.20 (1 H, t, Ar), 7.28-7.39 (5 H, m, Ar), 7.41 (1 H, s, Ar).

(S)-{3-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenoxy]-propylamino}-phenyl-acetic acid cyclopentyl ester (77)

Building block O used

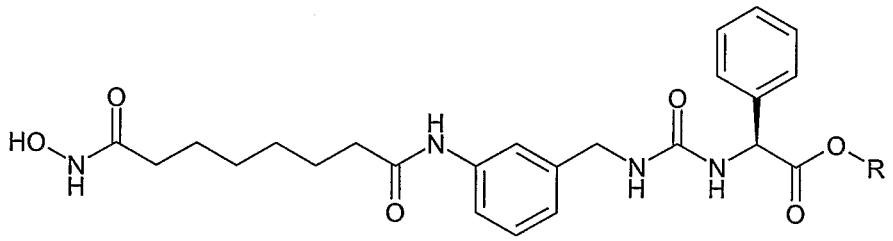
LCMS purity of 100%, m/z 540 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.20-1.50 (8 H, m, 4 x CH_2), 1.50-1.80 (8 H, m, 4 x CH_2), 1.80-1.90 (2 H, m, $NHCH_2C\underline{H_2}$), 2.00 (2 H, t, CH_2), 2.25 (2 H, t, CH_2), 2.55-2.70 (2 H, m, $NHC\underline{H_2}$), 3.90 (2 H, t, $CH_2C\underline{H_2}O$), 4.25 (1H, s, OCOC<u>H</u>Ph), 5.05 (1 H, m, C<u>H</u>OCO), 6.55 (1 H, m, Ar), 6.95 (1 H, m, Ar), 7.00-7.10 (1 H, m, Ar), 6.15-6.35 (6 H, m, Ar)

(S)-{3-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenoxy]-propylamino}-ph enyl-acetic acid cyclopentyl ester (78)

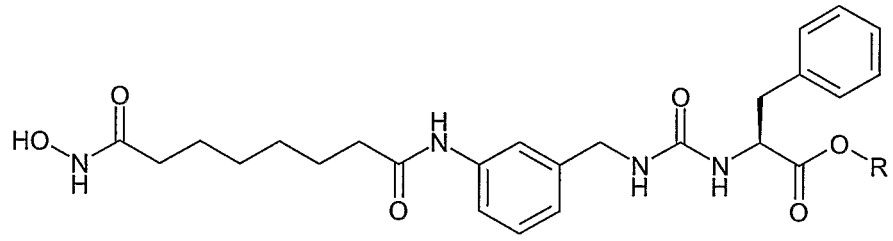
Building block O used

LCMS purity of 100%, m/z 472 $[M^+H]^+$, ^1H NMR (400 MHz, DMSO), δ: 1.20-1.40 (4 H, m, 2 x CH_2), 1.45-1.65 (4 H, m, 2 x CH_2), 1.90 (2 H, m, $NHCH_2C\underline{H}_2$), 2.00-2.20 (2 H, m, CH_2), 2.20-2.35 (2 H, m, CH_2), 2.80-3.10 (2 H, m, $NHC\underline{H}_2$ masked signal), 3.90-4.00 (2 H, m, $CH_2C\underline{H}_2O$), 4.60-4.85 (1 H, br s, $OCOC\underline{H}Ph$), 6.55 (1 H, d, Ar), 7.05 (1 H, d, Ar), 7.15 (1 H, m, Ar), 7.30-7.60 (6 H, m, Ar), 8.50-8.85 (1H, br s), 9.85 (1 H, s), 10.35 (1 H, s).

Synthesis of Compounds in Figure 5 Exemplified by Compound 79 and Compound 80



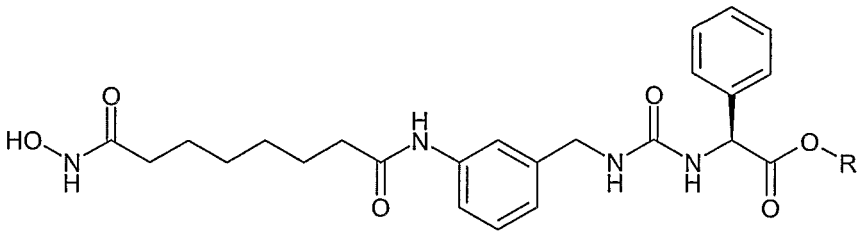
R = cyclopentyl 79
R = H 80



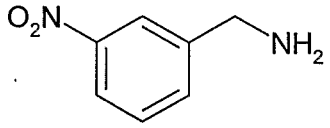
R = Cyclopentyl 81
R = H 82

Figure 5

Synthesis of Compound 79 (R = cyclopentyl) and Compound 80 (R = H)

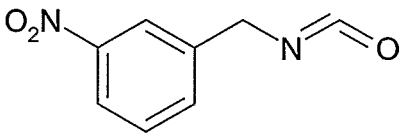


Stage 1: 3-Nitro-benzylamine



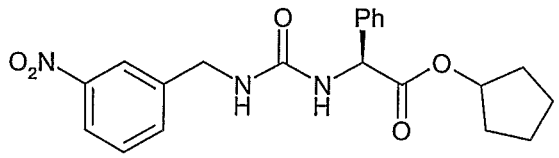
3-Nitrobenzyl bromide (10g, 46.3mmol) was dissolved in ethanol (200ml) and stirred at room temperature A solution of conc.NH_3 (aq) (200ml) in ethanol (300ml) was added dropwise to the reaction over 30 minutes. The reaction was stirred for 18 h at room temperature before evaporating to dryness. Water (350ml) was added to the residue and the solution was washed with EtOAc (2x 200ml). The aqueous layer was basified with 1M NaOH and extracted with EtOAc (2x 200ml). The organic extracts of the basic layer were combined, dried (Na_2SO_4) and evaporated to dryness. The product was obtained as an orange oil (4.6g, 65% yield). LCMS purity 100%, m/z 153 $[M^+ + H]^+$.

Stage 2: 1-Isocyanatomethyl-3-nitro-benzene



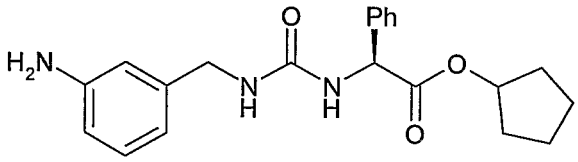
3-Nitro-benzylamine (2.3g, 15.1mmol) was dissolved in anhydrous dioxane (50ml) under N_2 atmosphere. Diphosgene (2.2ml, 18.2mmol) was added, a precipitate formed which dissolved upon heating to 75°C. The reaction was stirred at 75°C for 3 h, cooled and evaporated to dryness giving 3.4g of crude material which was used in the next step without further purification.

Stage 3: (S)-[3-(3-Nitro-benzyl)-ureido]-phenyl-acetic acid cyclopentyl ester



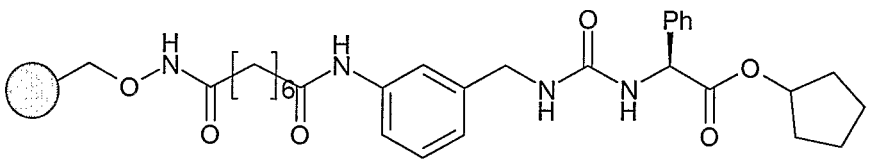
L-phenylglycine cyclopentyl ester tosyl salt (7.47g, 19.1mmol) was dissolved in DMF (70ml). Triethylamine (5.8ml, 42.0mmol) was added and the mixture was cooled to 0°C. A solution of 1-Isocyanatomethyl-3-nitro-benzene (3.4g, 19.1mmol in 30ml of DMF) was added slowly to the reaction mixture under a N_2 atmosphere. Stirring was continued for 18 h allowing the reaction to warm to room temperature The mixture was diluted with water (200ml) and extracted with EtOAc (2x 200ml). The organic extracts were washed with water (3x 100ml) and brine (100ml), dried (Na_2SO_4) and evaporated to dryness. The crude urea was purified by column chromatography (1% MeOH/ DCM) to yield a pale yellow oil (4.6g, 65% yield). LCMS purity 85%, m/z 398 $[M^+ + H]^+$.

Stage 4: (S)-[3-(3-Amino-benzyl)-ureido]-phenyl-acetic acid cyclopentyl ester



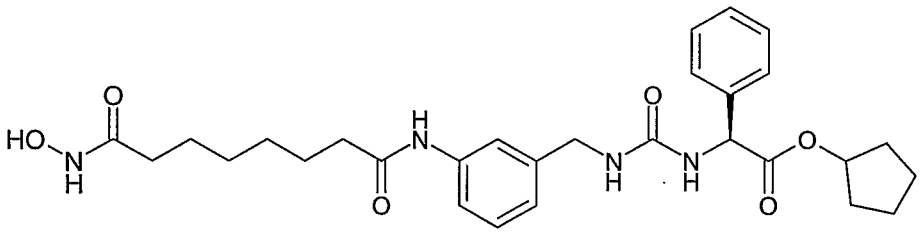
(S)-[3-(3-Nitro-benzyl)-ureido]-phenyl-acetic acid cyclopentyl ester (3.6g, 9.0mmol) was dissolved in ethanol (50ml) Pd/C (10% wet) catalyst (100mg) was added and the mixture was stirred under H_2 atmosphere (balloon pressure) for 18h. The reaction mixture was filtered through a celite plug and evaporated to dryness to give a purple oil (2.34g, 71% yield). LCMS purity 90%, m/z 368 $[M^+ + H]^+$.

Stage 5: Coupling to resin



Hydroxylamine 2-chlorotrityl resin derivatized with suberic acid (1.5g, loading 0.94mmol/g) was swollen in DMF (15ml) and PyBOP (2.2g, 4.23mmol) added, followed by DIPEA (2.4ml, 14.1mmol). Stage 4 aniline (1.3g, 3.53mmol) was dissolved in DCM (15ml) and added to the reaction mixture. The reaction was shaken for 42 h at room temp before standard resin wash and drying.

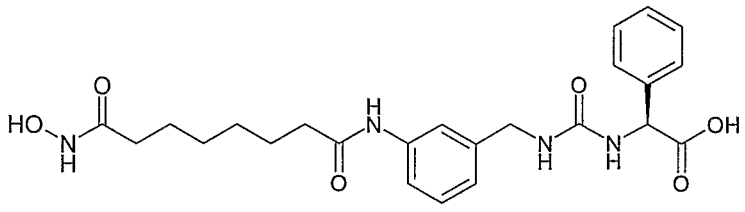
Stage 6: (S)-{3-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzyl]-ureido}-phenyl-acetic acid cyclopentyl ester **(79)**



79

Stage 5 resin bound cyclopentyl ester (1.75g) was shaken with 2%TFA/ DCM (15ml) for 10min before filtering the resin and evaporating the solvent under reduced pressure at room temperature This process was repeated (x3) and the combined crude product was purified by preparative HPLC to yield compound **(79)**. LCMS purity 100%, m/z 539 $[M^+ + H]^+$, ^{1}H NMR (400 MHz, MeOD), δ: 1.34-1.90 (16 H, m, 8 x CH_2), 2.11 (2 H, t, CH_2), 2.38 (2 H, t, CH_2), 4.30 (2 H, s, CH_2), 5.18 (1 H, m, CHOCO), 5.30 (1 H, s, OCOCHPh), 7.05 (1 H, d, Ar), 7.26 (1 H, t, Ar), 7.34-7.40 (5 H, m, Ar), 7.47 (2 H, m, Ar).

Stage 7: (S)-{3-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzyl]-ureido}-phenyl-acetic acid **(80)**



80

Compound **(79)** (75mg) was dissolved in THF (1ml) and 2M NaOH (aq,1ml) added. The reaction was stirred at room temperature for 2 h. THF was removed under a

stream of N_2 and the aqueous layer (~1ml) was purified by preparative HPLC to yield compound **(80)**. LCMS purity 99%, m/z 471 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.20-1.35 (4 H, m, 2 x CH_2), 1.45-1.65 (4 H, m, 2 x CH_2), 2.00 (2 H, t, CH_2), 2.25 (2 H, t, CH_2), 4.20 (2 H, s $C\underline{H}_2NH$), 5.25 (1 H, s $C\underline{H}Ph$), 6.90 (1 H, d, Ar), 7.10 (1 H, t, Ar), 7.15-7.40 (7 H, m, Ar).

The following compounds were prepared according to the procedure described for compounds **(79)** and compound **(80)**

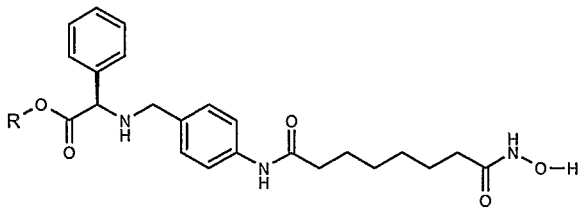
(S)-2-{3-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzyl]-ureido}-3-phenyl-propionic acid cyclopentyl ester (81)

LCMS purity 95%, m/z 553 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.20-1.40 (4 H, m, 2 x CH_2), 1.40-1.80 (12 H, m, 6 x CH_2), 2.00 (2 H, t, CH_2), 2.25 (2 H, t, CH_2), 2.90 (2 H, m, $CHC\underline{H}_2Ph$), 4.15 (2 H, s $C\underline{H}_2NH$), 4.40 (1 H, m, $OCOC\underline{H}CH_2$), 5.00 (1 H, m, $C\underline{H}OCO$), 6.85 (1 H, d, Ar), 7.00-7.25 (6 H, m, Ar), 7.35 (2 H, br s, Ar).

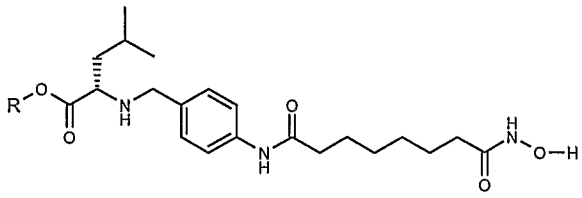
(S)-2-{3-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzyl]-ureido}-3-phenyl-propionic acid (82)

LCMS purity 94%, m/z 485 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ: 1.30-1.50 (4 H, m, 2 x CH_2), 1.60-1.80 (4 H, m, 2 x CH_2), 2.10 (2 H, t, CH_2), 2.35 (2 H, t, CH_2), 2.95-3.25 (2 H, m, $CHC\underline{H}_2Ph$), 4.25 (2 H, s, $C\underline{H}_2NH$), 4.60 (1 H , m $OCOC\underline{H}CH_2$), 7.00 (1 H, d, Ar), 7.15-7.35 (6 H, m, Ar), 7.45 (2 H, m, Ar)

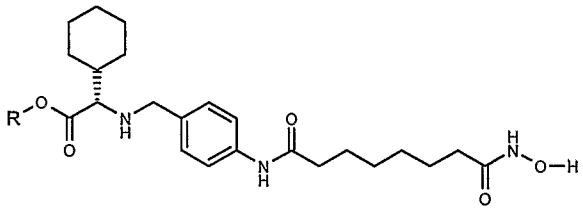
Synthesis of Compounds Outlined in Figure 6 Exemplified by Compound (83) and Compound (84)



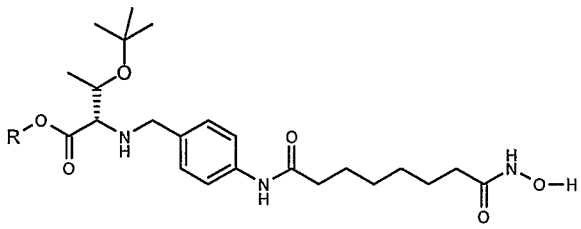
R= cyclopentyl 83
R= H 84



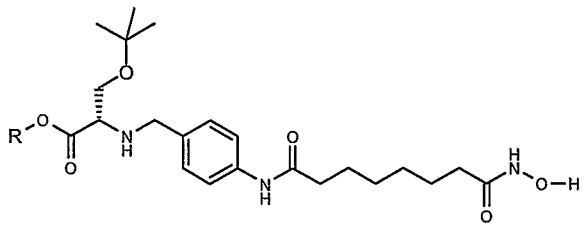
R= cyclopentyl 85



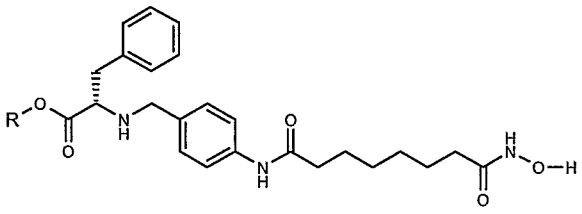
R= cyclopentyl 86
R= H 87



R= cyclopentyl 88



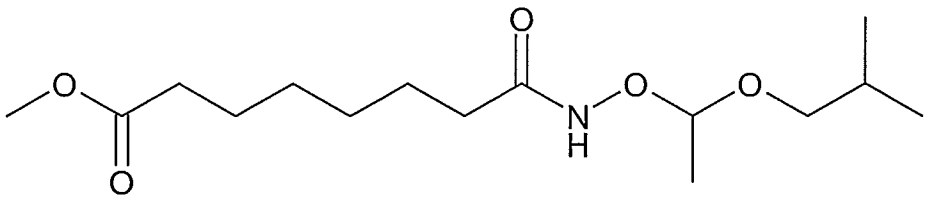
R= cyclopentyl 89
R= H 90



R= cyclopentyl 91

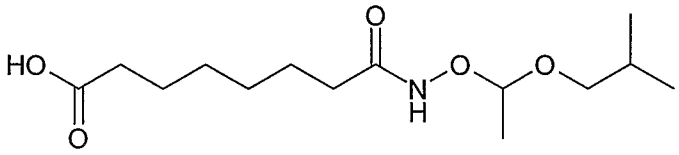
Figure 6

Stage 1: 7-(1-Isobutoxy-ethoxycarbamoyl)-heptanoic acid methyl ester



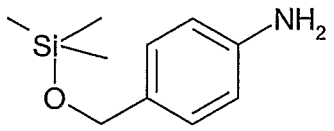
Monomethyl suberate (25.0 g, 13.3 mmol, 1.0 eq) was dissolved in THF (300 mL) and DCM (300 mL). EDC.HCl (25.46 g, 13.3 mmol, 1.0 eq) was added to the stirred solution, followed by HOBt (17.95 g, 13.3 mmol, 1.0 eq) and triethylamine (48.5 mL, 34.5 mmol, 2.6 eq). *O*-(1-Isobutoxy-ethyl)-hydroxylamine (21.9 mL, 15.9 mmol, 1.2 eq) was added to the viscous solution and the reaction allowed to stir overnight at room temperature. The reaction mixture was concentrated under vacuum, DCM (350 mL) was added and washed with water (250 mL) and brine (200 mL). The organic layer was isolated, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was obtained as a white solid (36.6 g, 91% yield) LCMS purity 88%, m/z 302 $(M^+ + H)^+$. This was used in the next step without further purification.

Stage 2: 7-(1-Isobutoxy-ethoxycarbamoyl)-heptanoic acid



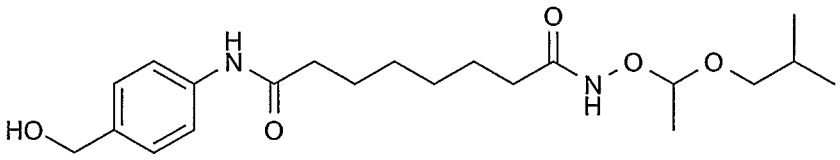
7-(1-Isobutoxy-ethoxycarbamoyl)-heptanoic acid methyl ester (36.6 g, 12.1 mmol, 1.0 eq) was stirred in THF (200 mL) and water (200 mL) in the presence of lithium hydroxide (8.68 g, 36.2 mmol, 3.0 eq) for 3 h at 50°C. THF was evaporated under vacuum and to the mixture water (100 mL) and ethyl acetate (200 mL) were added. The mixture was acidified cautiously to pH 3 by addition of 1N HCl. The organic phase was isolated and the aqueous layer re-extracted with ethyl acetate (150 mL). The organic phases were combined, dried ($MgSO_4$), filtered and concentrated in vacuo. The product was obtained as a white solid (29.0 g, 83% yield), m/z 288 $[M^+ + H]^+$ and used in stage 4 without further purification.

Stage 3: 4-Trimethylsilanyloxymethyl-phenylamine



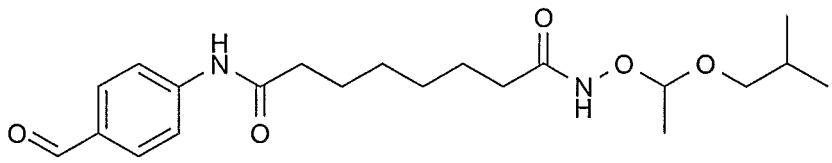
To a stirred solution of 4-amino benzyl alcohol (16.0 g, 13.0 mmol, 1.0 eq) in THF (400 mL), was added triethylamine (18.9 mL, 13.6 mmol, 1.05 eq) followed by trimethylchlorosilane (17.2 mL, 13.6 mmol, 1.05 eq). The reaction mixture was stirred under a nitrogen atmosphere overnight at room temperature. THF was evaporated under vacuum and the mixture partitioned with ethyl acetate (300 mL) and water (300 mL). The organic phase was isolated and the aqueous layer re-extracted with ethyl acetate (2 x 100 mL). The combined organic phases were washed with brine (2 x 150 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was obtained as a yellow oil (24.0 g, 95% yield) and used in stage 4 without further purification.

Stage 4: Octanedioic acid (4-hydroxymethyl-phenyl)-amide (1-isobutoxyethoxy)-amide



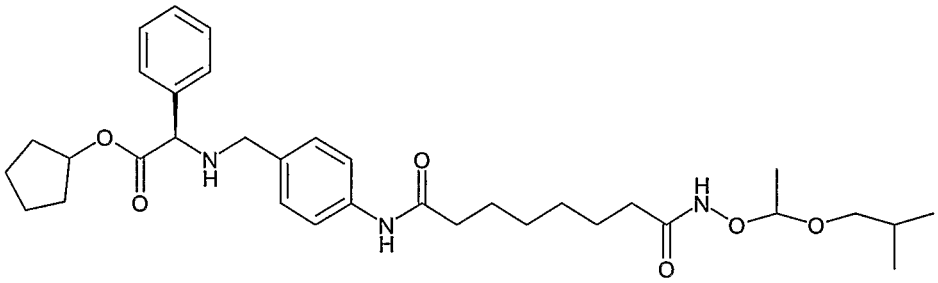
7-(1-Isobutoxy-ethoxycarbamoyl)-heptanoic acid (5.0 g, 1.72 mmol, 1.0 eq) and 4-trimethylsilanyloxymethyl-phenylamine (3.38 g, 1.72 mmol, 1.0 eq) were stirred together in DMF (140 mL). To the mixture was added PyBroP (10.5 g, 2.25 mmol, 1.3 eq) and DiPEA (3.9 mL, 2.25 mmol, 1.3 eq). The reaction was stirred under a nitrogen atmosphere overnight at room temperature. Ethyl acetate (200 mL) and water (200 mL) were added. The aqueous phase was isolated and re-extracted with ethyl acetate (2 x 100 mL). The combined organic phases were washed with water (2 x 50 mL) and brine (50 mL), then dried ($MgSO_4$), filtered and concentrated in vacuo. The crude product was dissolved in the minimum of ethyl acetate and purified by passing through a pad of silica. The product was washed through the silica using ethyl acetate and collected in 100 mL conical flasks until elution ceased by LCMS analysis. Purification gave a yellow oil (3.59 g, 53% yield). LCMS purity 61%, m/z 417 $[M^+ + Na]^+$

Stage 5: Octanedioic acid (4-formyl-phenyl)-amide (1-isobutoxy-ethoxy)-amide



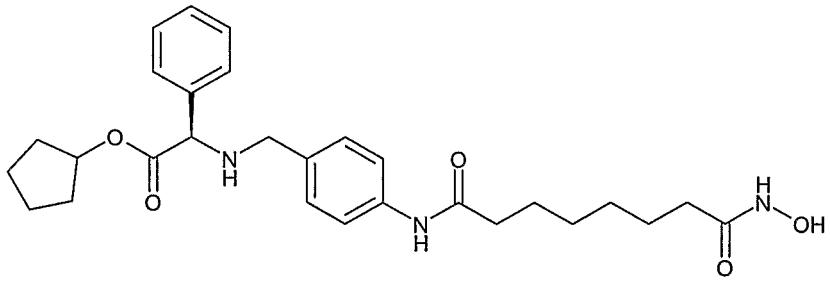
Octanedioic acid (4-hydroxymethyl-phenyl)-amide (1-isobutoxyethoxy)-amide (100 mg, 0.025 mmol, 1.0 eq) was dissolved in DCM (5 mL). To the reaction mixture, was added MnO_2 (286 mg, 0.33 mmol, 13.0 eq) and was stirred at room temperature for 1.5 h. The reaction mixture was filtered over Celite and washed through with DCM, followed by evaporation of solvent to give a yellow oil (78.6 mg, 79% yield) LCMS purity 53%, m/z 415 $[M^+ + Na]^+$. The product was used in the subsequent steps without further purification.

Stage 6: (R)-{4-[7-(1-Isobutoxy-ethoxycarbamoyl)-heptanoylamino]-benzylamino}-phenyl-acetic acid cyclopentyl ester



Octanedioic acid (4-formyl-phenyl)-amide (1-isobutoxy-ethoxy)-amide (276 mg, 0.70 mmol, 1.0 eq) and *D*-phenylglycine cyclopentyl ester (170 mg, 0.77 mmol, 1.1 eq) were stirred in DCE (15 mL) for 10 min. Acetic acid (65 µL) was added and stirred for 2 min. Sodium triacetoxyborohydride (448 mg, 0.21 mmol, 3.0 eq) was introduced and the reaction mixture stirred under a nitrogen atmosphere, at room temperature for 1 h. Sodium hydrogen carbonate was added to quench the reaction. DCM was then added and the organic phase isolated. The aqueous layer was re-extracted with DCM, organic layers combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give crude product (100 mg, 24%) LCMS purity 94.0%, m/z 496 $[m^+ + H]^+$ which was taken on without further purification.

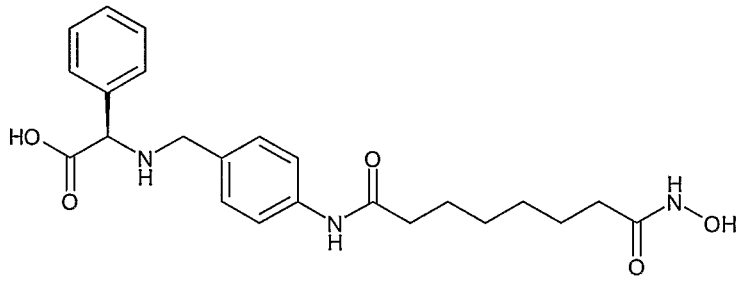
Step 7: (R)-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-phenyl-acetic acid cyclopentyl ester **(83)**



83

(R)-{4-[7-(1-Isobutoxy-ethoxycarbamoyl)-heptanoylamino]-benzylamino}-phenyl-acetic acid cyclopentyl ester (50 mg, 0.08 mmol, 1 eq) was dissolved in DCM (0.5 mL) and stirred with 4M HCl in dioxane (0.2 mL) for 30 min. The resulting salt was concentrated, dissolved in methanol and purified by preparative HPLC to yield compound **(83)**. LCMS purity 94%, m/z 496 $[M^+ + H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.41-1.95 (16H, m, 8 x CH_2), 2.15-2.17 (2H, m, CH_2), 2.40 (2H, t, J = 7.2 Hz, CH_2), 4.16 (2H, q, J = 13.5 Hz, CH_2), 5.12 (1H, s, CH), 5.27-5.30 (1H, m, CH), 7.40 (2H, d, J = 8.7 Hz, Ar-H), 7.50-7.56 (5H, m, Ar-H), 7.67 (2H, d, J = 8.7 Hz, Ar-H).

Stage 8: (R)-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-phenyl-acetic acid **(84)**



84

To a solution of CHR-003644 (50 mg, 0.008 mmol, 1.0 eq) in THF (2 mL) and water (2 mL), was added LiOH (8.0 mg, 0.033 mmol, 4.0 eq). The reaction was stirred under a nitrogen atmosphere at 40°C overnight. THF was evaporated under vacuum and the remaining aqueous reaction solvent washed with ethyl acetate. The solution was acidified to pH 3 and the product concentrated in vacuo. The resulting salts were

dissolved in methanol and the product purified by preparative HPLC to yield compound **(84)**. LCMS purity 97%, m/z 428 $[M^+H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.39-1.41 (4H, m, 2 x CH_2), 1.62-1.74 (4H, m, 2 x CH_2), 2.13-2.15 (2H, m, CH_2), 2.40 (2H, t, J = 7.5 Hz, CH_2), 4.14 (2H, q, J = 12.9 Hz, CH_2), 5.06 (1H, s, CH), 7.39 (2H, d, J = 8.4 Hz, Ar-H), 7.54 (5H, s, Ar-H), 7.67 (2H, d, J = 8.7 Hz, Ar-H)

The following compound was prepared in a similar manner to Compound (83) and Compound (84) using the appropriate intermediates.

(S)-2-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-4-methyl-pentanoic acid cyclopentyl ester (85)

LCMS purity 97%, m/z 476 $[M^+H]^+$, ^1H NMR (300 MHz, MeOD), δ:0.98-1.03 (6H, m, 2 x CH_3), 1.41-1.42 (4H, m, 2 x CH_2), 1.71-1.96 (14H, m, 7 x CH_2), 2.10-2.15 (2H, m, CH_2), 2.40 (2H, t, J = 7.2 Hz, CH_2), 3.96-4.01 (1H, m, CH), 4.15-4.26 (2H, m, CH_2), 4.81 (1H, s, CH), 5.31-5.34 (1H, m, CH), 7.44 (2H, d, J = 8.7 Hz, Ar-H), 7.70 (2H, d, J = 8.7 Hz, Ar-H)

(S)-Cyclohexyl-[4-(7-hydroxycarbamoyl-heptanoylamino)-benzylamino]-acetic acid (87)

LCMS purity 95%, m/z 434 $[M^+H]^+$, ^1H NMR (300 MHz, MeOD), δ:1.23-1.96 (18H, m, 9 x CH_2), 2.10-2.15 (2H, m, CH_2), 2.39 (2H, m, CH_2), 3.71 (1H, m, CH), 4.12 (2H, q, J = 7.2 Hz, CH_2), 4.80 (1H, s, CH), 7.43 (2H, d, J = 8.4 Hz, Ar-H), 7.68 (2H, d, J = 8.7 Hz, Ar-H)

(S)-3-tert-Butoxy-2-[4-(7-hydroxycarbamoyl-heptanoylamino)-benzylamino]-butyric acid cyclopentyl ester (88)

LCMS purity 83%, m/z 520 $[M^+H]^+$, ^1H NMR (300 MHz, MeOD), δ:1.19 (9H, s, 3 x CH_3), 1.29 (3H, d, J = 7.8 Hz, CH_3), 1.37-1.41 (4H, m, CH_2), 1.64-1.89 (12H, m, CH_2), 2.09-2.15 (2H, m, CH_2), 2.40 (2H, t, J = 7.2 Hz, CH_2), 3.36-3.37 (1H, m, CH), 3.70-3.71 (1H, m, CH), 4.24-4.27 (2H, m, CH_2), 5.17-5.19 (1H, m, CH), 7.42 (2H, d, J = 6.9 Hz, Ar-H), 7.68 (2H, d, J = 8.4 Hz, Ar-H)

(S)-3-tert-Butoxy-2-[4-(7-hydroxycarbamoyl-heptanoylamino)-benzylamino]-propionic acid cyclopentyl ester (89)

LCMS purity 95%, m/z 506 $[M^+H]^+$, ^1H NMR (300 MHz, MeOD), δ:1.23 (9H, s, $C(CH_3)_3$), 1.36-2.09 (16H, m, 8 x CH_2), 1.66 (2H, t, J=7.7Hz, CH_2), 1.75 (2H, t,

J=7.4Hz, CH_2), 2.11 (2H, t, J=7.4Hz, CH_2), 2.40 (2H, t, J=7.3Hz, CH_2), 3.92 (2H, m, CH_2), 4.16 (1H, m, CH), 4.26 (2H, s, CH_2), 5.32 (1H, m, CH), 7.45 (2H, d, J=8.5Hz, ArH), 7.67 (2H, dd, J=3.2, 8.3Hz, ArH).

(S)-3-tert-Butoxy-2-[4-(7-hydroxycarbamoyl-heptanoylamino)-benzylamino]-propionic acid (90)

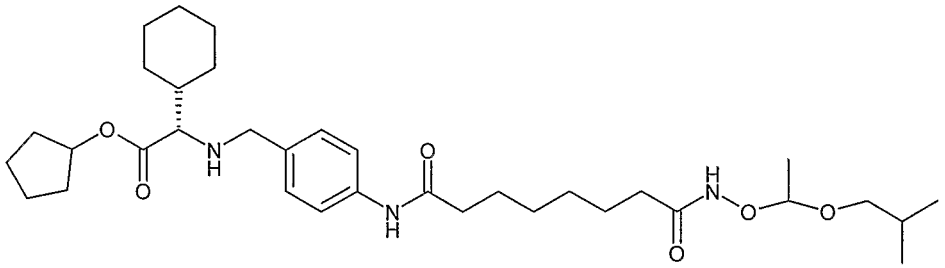
LCMS purity 95%, m/z 438 $[M^+H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.25 (9H, s, $C(CH_3)_3$), 1.39-1.42 (4H, m, 2 x CH_2), 1.62-1.69 (4H, m, 2 x CH_2), 2.08-2.17 (2H, m, CH_2), 2.40 (2H, t, J = 7.5 Hz, CH_2), 3.85-3.96 (2H, m, CH_2), 4.01-4.04 (1H, m, CH), 4.26 (2H, s, CH_2), 7.46 (2H, d, J = 8.4 Hz, Ar-H), 7.68 (2H, d, J = 8.4 Hz, Ar-H)

(S)-2-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzylamino]-3-phenyl-propionic acid cyclopentyl ester (91)

LCMS purity 95%, m/z 510 $[M^+H]^+$, ^1H NMR (300 MHz, MeOD), δ:1.17- 2.43 (22H, m, 11 x CH_2), 4.19- 4.30 (2H, m, CH_2), 5.08 (1H, s, CH), 5.20- 5.26 (1H, m, CH), 7.24 - 7.71 (9H, m, Ar-H)

Compound (86) was prepared was prepared via alternative methodology the modified conditions are detailed below

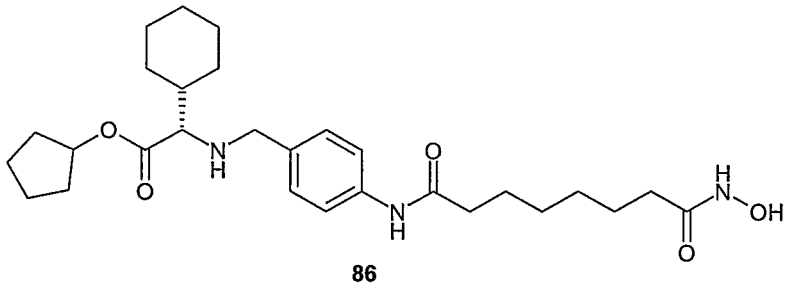
Step 6b: (S)-Cyclohexyl-{4-[7-(1-isobutoxy-ethoxycarbamoyl)-heptanoylamino]-benzylamino}-acetic acid cyclopentyl ester



Octanedioic acid (4-formyl-phenyl)-amide (1-isobutoxy-ethoxy)-amide (220 mg, 0.056 mmol, 1.0 eq) and *L*-cyclohexyl-glycine cyclopentyl ester (138.9 mg, 0.062 mmol, 1.1 eq) were stirred in methanol (8 mL) overnight at room temperature. Sodium borohydride (31.8 mg, 0.084 mmol, 1.5 eq) was introduced and the reaction mixture stirred for 15 min. The reaction mixture was transferred to an ice bath and 2 drops of sodium hydroxide (2M) were added. Diethyl ether was added and the organic phase

isolated. The aqueous layer was re-extracted with diethyl ether, organic layers combined and washed with brine. The organic phase was then dried ($MgSO_4$) filtered and concentrated *in vacuo* to give crude material which was taken to the next step without further purification.

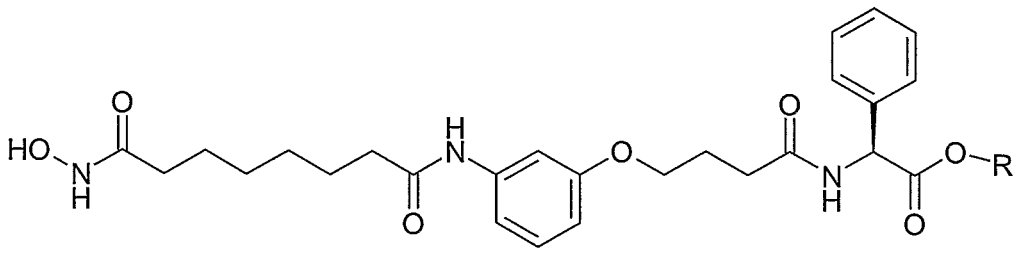
Step 7b: (S)-Cyclohexyl-[4-(7-hydroxycarbamoyl-heptanoylamino)-benzylamino]-acetic acid cyclopentyl ester **(86)**



86

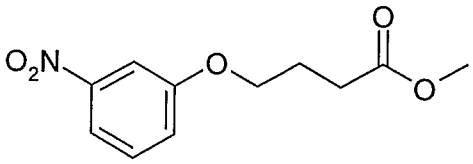
Material from step 6b (50 mg, 0.083 mmol, 1 eq) was dissolved in DCM/methanol (2 mL : 2 mL) and stirred with TFA (1.0 mL) for 2 h. The resulting salt was concentrated, dissolved in methanol and purified by preparative HPLC to yield compound **(86)**. LCMS purity 100%, m/z 502 $[M^+ + H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.28-1.98 (26H, m, 13 x CH_2), 2.10-2.15 (2H, m, CH_2), 2.40 (2H, t, J = 7.8 Hz, CH_2), 3.81 (1H, d, J = 3.9 Hz, CH), 4.21 (2H, m, CH_2), 5.01 (1H, s, CH), 5.23-5.25 (1H, m, CH), 7.43 (2H, d, J = 8.4 Hz, Ar-H), 7.68 (2H, d, J = 8.7 Hz, Ar-H)

Synthesis of 92 and 93



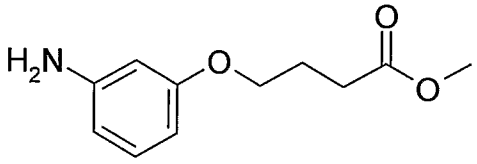
R = cyclopentyl 92
R = H 93

Stage 1: 4-(3-Nitro-phenoxy)-butyric acid methyl ester



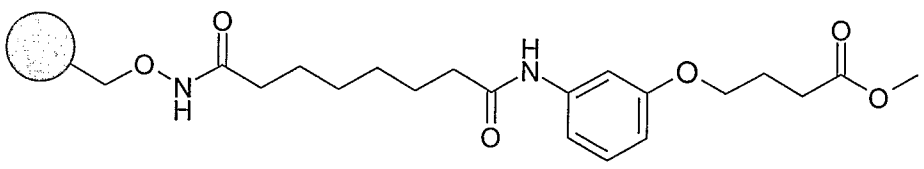
To a solution of 3-nitrophenol (8.35g, 60mmol), in DMF (50ml) was added K_2CO_3 (16.56g, 120mmol) and methyl 1, 4 - bromobutyrate (11.95g, 66mmol). The reaction was stirred at room temperature for 16 h. The reaction was diluted with ethyl acetate and water. The organic phase was separated and washed with water (2 x 200ml). The organic phase was dried with Na_2SO_4 and concentrated *in vacuo*. The required ether was isolated following chromatography (ethyl acetate : heptane, 1 : 9) as a pale yellow solid (12.2g, 85% yield). LCMS purity 100%, m/z 240 $[M^+H]^+$.

Stage 2: 4-(3-Amino-phenoxy)-butyric acid methyl ester



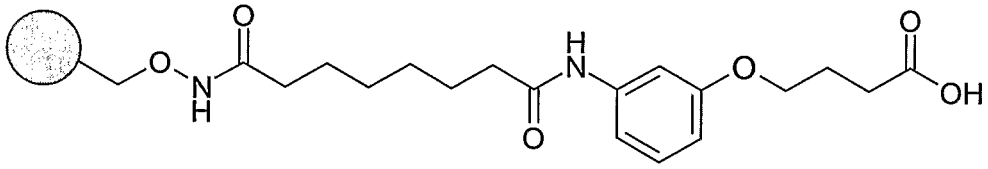
Stage 1 nitro ester (250mg, 1mmol) was dissolved in ethanol (3ml). Pd/carbon (40mg) was added and the reaction stirred under a hydrogen atmosphere (balloon pressure) for 16 h. The reaction mixture was filtered through celite. The celite pad was washed with ethanol and the combined organic fractions concentrated *in vacuo* to give the required product as an orange oil (210mg, 100% yield). LCMS purity 89%, m/z 210 $[M^+H]^+$.The aniline was used in the next stage without further purification.

Stage 3: Coupling to resin



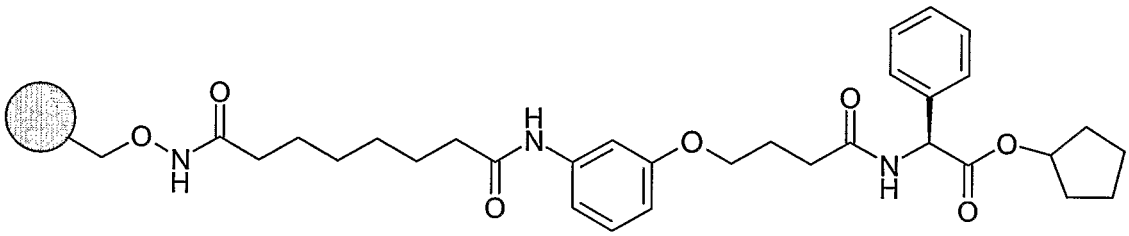
Suberic acid derivatised hydroxylamine 2-chlorotrityl resin (8g, 7.52mmol, loading, 0.94 mmol/g) was swollen in DCM/DMF (80ml/80ml). PyBOP (11.8g, 22.6mmol) and diisopropylethylamine (13.1ml, 75.2mmol) were added to the flask followed by 4-(3-amino-phenoxy)-butyric acid methyl ester (4.73g, 22.6mmol). The reaction was shaken at room temperature for 72 h before standard wash and drying.

Stage 4: Ester hydrolysis



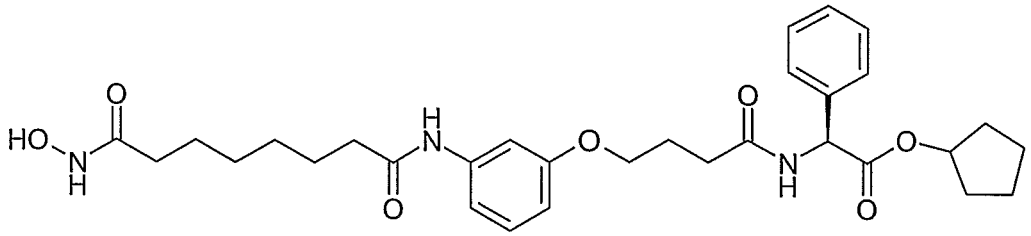
Stage 3 resin (9.5 g), was suspended in THF/MeOH (34 ml/34ml). NaOH (1.4 M, aq, 34 ml) was added and the reaction shaken for 16 h at room temperature. The resin was washed using the standard wash procedure before air drying.

Stage 5: Amino acid ester coupling



Stage 4 resin (2.1g), was suspended in DCM/DMF (20ml/20ml). PyBOP (3.1g, 5.92mmol), N-phenlglycine cyclopentyl ester (2.4g, 5.92mmol) and diisopropylethylamine (3.4ml, 19.7mmol) were added sequentially and the reaction shaken at room temperature for 72 hours. The resin was submitted to standard wash and dried.

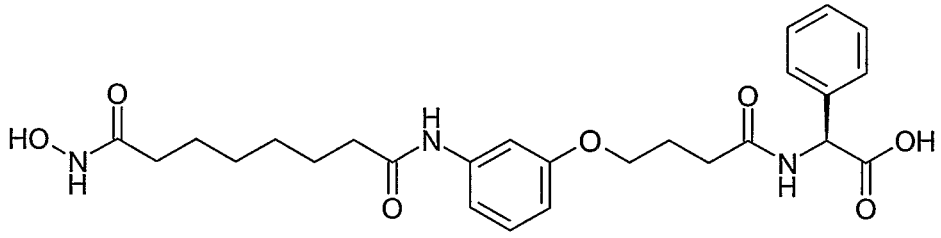
Stage 6: (S)-{4-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenoxy]-butyrylamino}-phenyl-acetic acid cyclopentyl ester **(92)**



92

Stage 5 resin bound cyclopentyl ester (1.1g) was shaken with 2%TFA/DCM (10ml) for 10 minutes before filtering the resin and evaporating the solvent under reduced pressure at room temperature. The process was repeated (x3) and the combined crude product purified by preparative HPLC to yield compound **(92)** (114mg). LCMS purity 99%, m/z 568 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ:1.40 (4 H, m, 2 x CH_2), 1.45 – 1.90 (13 H, m, alkyl), 2.10 (4 H, m, 2 x CH_2), 2.40 (2 H, t, CH_2), 2.50 (2 H, m, CH_2), 4.00 (2 H, m, CH_2), 5.15 (1 H, m,), 5.40 (1 H, s, NHCHCO), 6.65 (1 H, m, Ar) 7.15 (1 H, m, Ar), 7.20 (1 H, t, Ar), 7.30 (1 H, s, Ar), 7.40 (5 H, s, Ar).

Stage 7: (S)-{4-[3-(7-Hydroxycarbamoyl-heptanoylamino)-phenoxy]-butyrylamino}-phenyl-acetic acid **(93)**

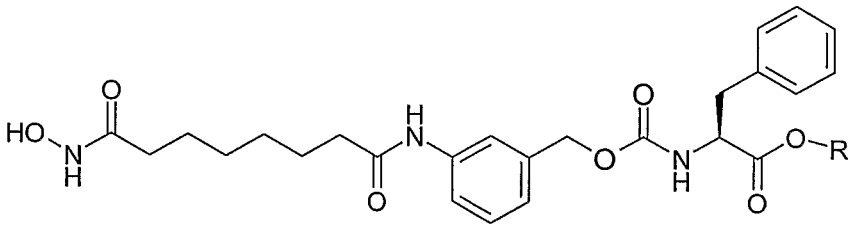


93

Stage 6 cyclopentyl ester resin (500mg) was suspended in THF (15ml). To the suspension was added NaOH (1.4M aq., 1.6 ml) and the reaction shaken for 16 hr at room temperature. The filtrate was removed and the resin washed and dried before cleavage. Cleavage was effected by shaking with 2%TFA/DCM (5ml) for 10 minutes before filtering the resin and evaporating the solvent under reduced pressure at room temperature. The process was repeated (x3) and the combined crude product purified by preparative HPLC to yield compound **(93)** (62mg). LCMS purity 99%, m/z 500 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ:1.40 (4 H, m, 2 x CH_2), 1.60 – 1.80 (4 H, m, alkyl), 2.10 (4 H, m, 2 x CH_2), 2.40 (2 H, t, CH_2), 2.50 (2 H, t, CH_2), 4.00 (2 H, m, CH_2),

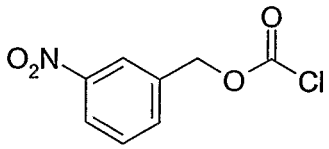
5.45 (1 H, s, NHCHCO), 6.65 (1 H, d, Ar) 7.10 (1 H, m, Ar), 7.20 (1 H, t, Ar), 7.25 (1 H, s, Ar), 7.30-7.45 (5 H, m, Ar).

Synthesis of 94 and 95



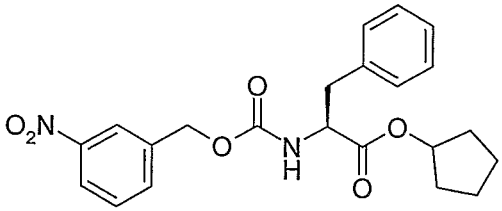
R = cyclopentyl 94
R = H 95

Stage 1: 3-nitro-benzyl-chroroformate formation



To a solution of 3-nitro benzyl alcohol (10g, 65mmol), in dioxane (anhydrous, 100ml) was added trichloromethyl chloroformate (9.47ml, 78mmol). The reaction was heated at 75°C under nitrogen for 16 h. The solvent was evaporated and the residue resuspended in dioxane and evaporated. The procedure was repeated (x 3). The crude chloroformate was used in the next stage without further purification.

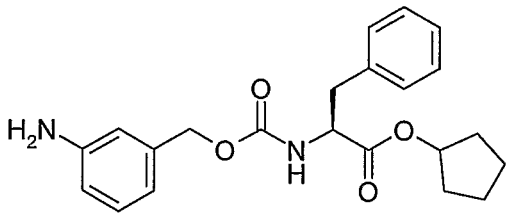
Stage 2: (S)-2-(3-Nitro-benzyloxycarbonylamino)-3-phenyl-propionic acid cyclopentyl ester



L-Phe-cyclopentyl ester.TsOH salt (9.42g, 23mmol) was suspended in DCM (40ml). Triethylamine (6.5ml, 47mmol) was added and the reaction stirred at room

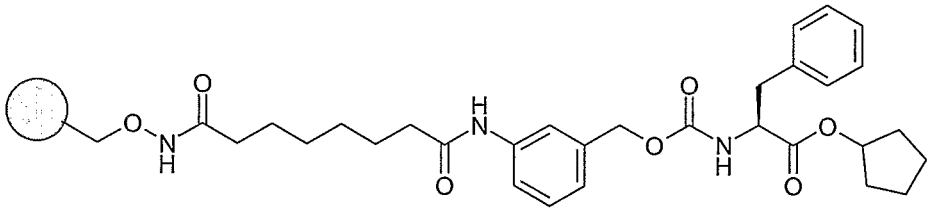
temperature for 5 min. Stage 1 chloroformate (5g, 23mol) dissolved in DCM (10ml) was added to the reaction mixture added dropwise with cooling (ice bath). The reaction was stirred for 16 h at room temperature. The solvent was removed and the residue dissolved in EtOAc (100ml) washed with water (50ml x 3) and dried (Na_2SO_4) before concentration *in vacuo.* The crude material was purified by chromatography (EtOAc : heptane, 1 : 9) to give the required carbamate (6.4g, 67% yield). m/z 413 $[M^+ + H]^+$

Stage 3: (S)-2-(3-Amino-benzyloxycarbonylamino)-3-phenyl-propionic acid cyclopentyl ester



Stage 2 nitro carbamate (6.4g, 15.5mmol) was dissolved in ethanol (64ml). Tin chloride dihydrate (17.5g, 77mmol) was added and the reaction stirred for 16 h at room temperature. The solvent was evaporated and the residue dissolved in EtOAc (60ml). A saturated solution of sodium potassium tartrate (60ml) was added followed by a solution of saturated sodium hydrogen carbonate (120ml). The biphasic solution was stirred for 15 min. The organic layer was separated and the aqueous phase extracted with EtOAc (60ml x1). The organic layers were combined, dried and the solvent evaporated to give the crude product which was purified by chromatography (EtOAc : heptane 1 : 3 → 1 : 1). The required product was isolated (3.5g, 59% yield). LCMS purity 100%, m/z 383 $[M^+ + H]^+$.

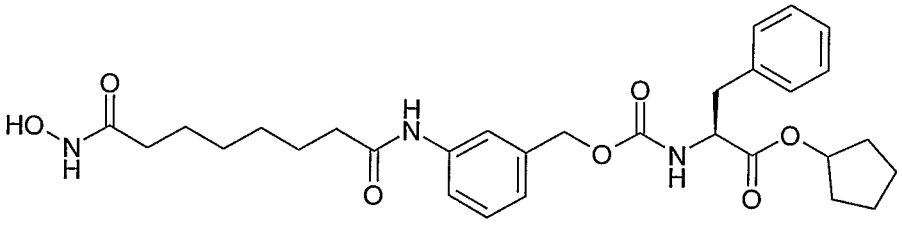
Stage 4: Coupling to resin



Suberic acid derivatised hydroxylamine 2-chlorotrityl resin (2.0g, 1.88mmol, loading, 0.94 mmol/g) was swollen in DMF (20ml). PyBOP (2.93g, 5.64mmol) and diisopropyl

ethylamine (3.25ml, 18.8mmol) were added. Stage 3 anilino carbamate (1.8g, 4.7mmol) dissolved in DCM (20ml) was added and the reaction shaken for 4 d before filtrate removal and standard wash of the resin which was dried under air.

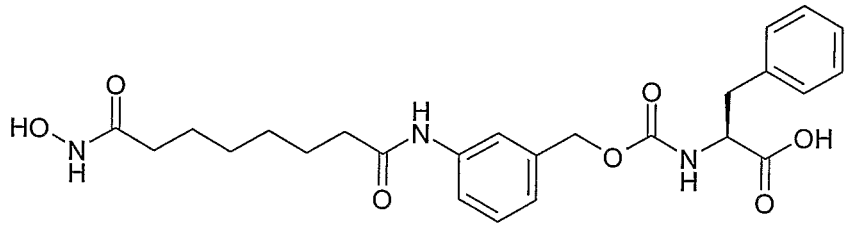
Stage 5: (S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzyloxycarbonylamino]-3 -phenyl-propionic acid cyclopentyl ester **(94)**



94

Stage 4 resin bound cyclopentyl ester (2g) was shaken with 2%TFA/DCM (15ml) for 10 minutes before filtering the resin and evaporating the solvent under reduced pressure at room temperature. The process was repeated (x3) and the combined crude product purified by preparative HPLC to yield compound **(94)**. LCMS purity 95%, m/z 554 $[M^+ + H]^+$, ^{1}H NMR (400 MHz, MeOD), δ:1.30 (4 H, m, 2 x CH_2), 1.40 – 1.90 (13 H, m, alkyl), 2.00 (2 H, t, CH_2), 2.30 (2 H, t, CH_2), 2.90 (2 H, ddd, CH_2), 3.80 (1 H, m), 4.25 (1 H, dd, NHCHCO), 4.90 (2 H, s, CH_2), 5.05 (1 H, m), 5.35 (1 H, m), 6.95 (1 H, d, Ar) 7.05-7.25 (6 H, m, Ar), 7.35 -7.75 (2 H, m, Ar).

Stage 6: (S)-2-[3-(7-Hydroxycarbamoyl-heptanoylamino)-benzyloxycarbonylamino]3 -phenyl-propionic acid **(95)**

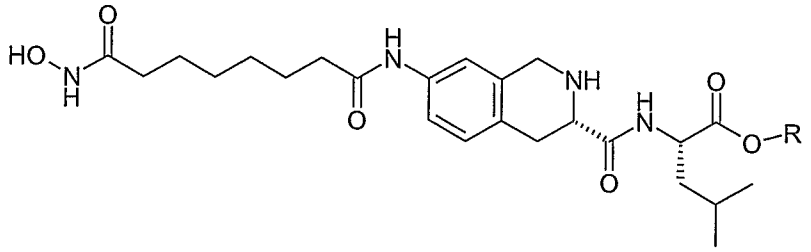


95

Compound **(94)** (100mg, 0.18mmol) was dissolved in THF (1ml) and 2M NaOH (1ml) added. The reaction vial was shaken for 4 h before THF removal via a stream of nitrogen. The aqueous residue was purified by preparative HPLC to yield compound **(95)** (62mg). LCMS purity 95%, m/z 486 $[M^+ + H]^+$, ^{1}H NMR (400 MHz, MeOD), δ:1.35

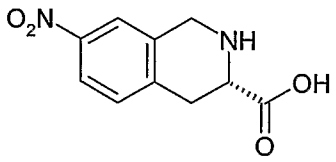
(4 H, m, 2 x CH_2), 1.55 – 1.75 (4 H, m, alkyl), 2.10 (2 H, t, CH_2), 2.35 (2 H, t, CH_2), 2.95 (2 H, dd, CH), 3.20 (1 H, dd, CH), 4.45 (1 H, dd, NHCHCO), 5.00 (2 H, s, CH_2), 7.05 (1 H, d, Ar), 7.20-7.35 (6 H, m, Ar), 7.55 (2 H, m, Ar).

Synthesis of Compound (96) and Compound (97)



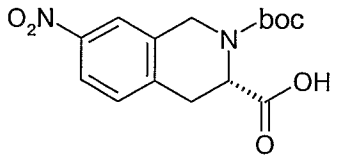
R = cyclopentyl 96
R = H 97

Stage 1: (S)-7-Nitro-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid



Prepared as described in Tett Letts 42, 2001, 3507.

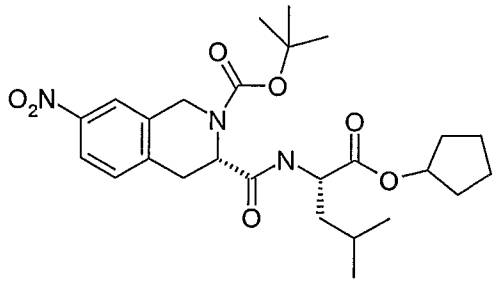
Stage 2: (S)-7-Nitro-3,4-dihydro-1H-isoquinoline-2,3-dicarboxylic acid-2-tert-butyl ester



(S)-7-Nitro-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (7g, 31.5mmol) was dissolved in THF : water (1 :1, 350 ml). K_2CO_3 was added (5.2g, 37mmol) followed by boc anhydride (13.7g, 63 mmol) and the solution heated at 40°C for 1 h. THF was removed by evaporation and the aqueous layer adjusted to pH = 7 before extraction with EtOAc. The organic layer was washed 0.1 M HCl (x3) and dried over Na_2SO_4 before concentration *in vacuo.* The required N-protected product was obtained

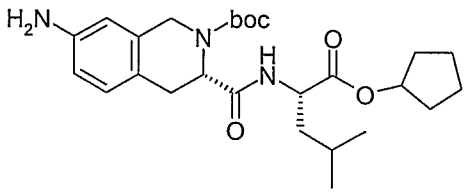
following column chromatography (EtOAc : heptane 2 : 3 → EtOAc), (7.5g, 74%), LCMS purity 92%, molecular ion not observed

Stage 3: (S)-3-((S)-1-Cyclopentyloxycarbonyl-3-methyl-butylcarbamoyl)-7-nitro-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester



(S)-7-Nitro-3,4-dihydro-1H-isoquinoline-2,3-dicarboxylic acid-2-tert-butyl ester (2.5g, 7.76mmol) was dissolved in DCM (100ml). HOBt (1.16g, 8.53mmol) was added, L-leucine cyclopentyl ester (3.19g, 8.53mmol) was added followed by triethylamine (2.38ml, 17.1mmol). EDCI.HCl (1.46g, 8.5mmol) was added and the reaction stirred at room temperature for 16 h. To the reaction was added DCM (100ml) and the organic layer washed with water (3 x 300ml), dried with Na_2SO_4 and the solvent removed *in vacuo*. The crude product was purified by chromatography (EtOAc : heptane 1 : 2 → EtOAc) to give the required product 3.14g (82% yield), LCMS purity 100%, m/z 504 $[M^+ + H]^+$

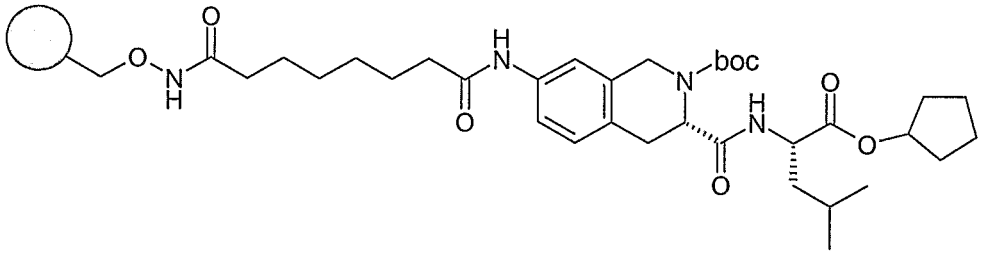
Stage 4: (S)-7-Amino-3-((S)-1-cyclopentyloxycarbonyl-3-methyl-butylcarbamoyl)-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester



(S)-3-((S)-1-Cyclopentyloxycarbonyl-3-methyl-butylcarbamoyl)-7-nitro-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester (3.14g, 6.24mmol) and Pd/carbon (0.4g) were suspended in EtOAc (20ml). The reaction was stirred under a hygrogen atmosphere (balloon pressure) for 16 h. The solution was filtered through a pad of

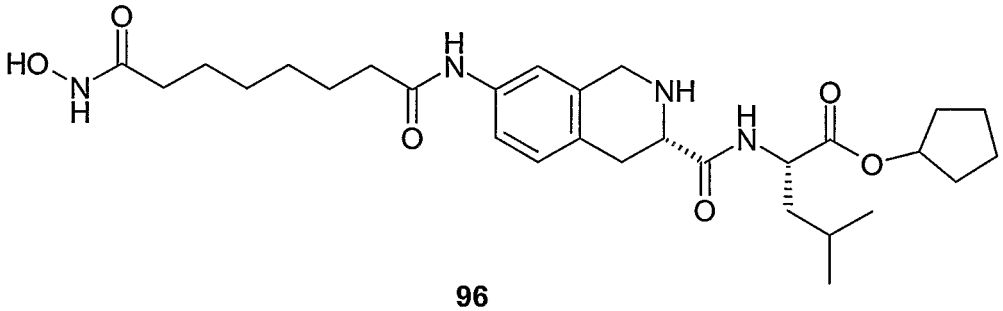
celite and the solvent removed. The crude product (3.04g) was used in the next step without further purification. LCMS purity 83%, m/z 474 $[M^+ + H]^+$

Stage 5: Coupling to resin



Suberic acid derivatised hydroxylamine 2-chlorotrityl resin (2.2g, loading, 0.94mmol/g) was swollen in DCM/DMF (1:1, 80ml). PyBOP (3.20g, 6.15mmol) and diisopropylethylamine (3.54ml, 20.7mmol) were added. Stage 3 anilino amide (3.04g, 6.43mmol) dissolved in DMF (40ml) was added and the reaction shaken for 3 days before filtrate removal and standard wash of the resin which was dried under air.

Stage 6: (S)-2-{[(S)-7-(7-Hydroxycarbamoyl-heptanoylamino)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-4-methyl-pentanoic acid cyclopentyl ester **(96)**

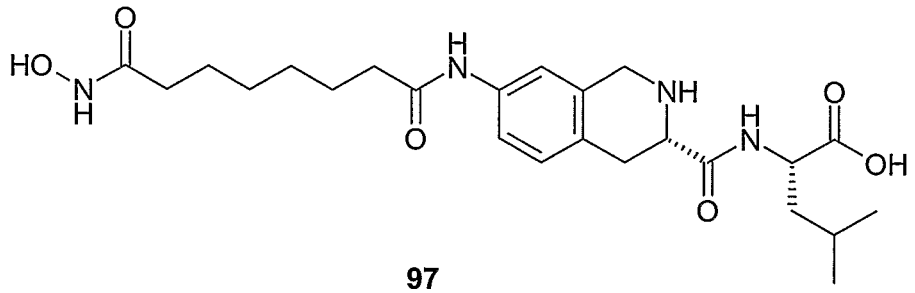


96

Stage 5 resin bound cyclopentyl ester (600mg) was shaken with 2%TFA/DCM (8ml) for 30 minutes before filtering the resin and evaporating the solvent under reduced pressure at room temperature. The crude product was purified by preparative HPLC to yield (S)-2-{[(S)-7-(7-Hydroxycarbamoyl-heptanoylamino)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-4-methyl-pentanoic acid cyclopentyl ester (17.5mg). The boc group is removed in addition to resin cleavage. LCMS purity 98%, m/z 545 $[M^+ + H]^+$, ^{1}H NMR (400 MHz, MeOD), δ:0.85 – 0.88 (6 H, 2 x d, J = 6.4 Hz, J = 6.5 Hz, 2 x CH_3), 1.30 (4 H, m, alkyl), 1.50 – 1.65 (13 H, m, alkyl), 1.80 (2 H, m, CH_2), 1.95 (2 H, t, CH_2), 2.25 (2 H, t, CH_2), 3.00 (1H, m, CH), 3.25 (1H, m, CH), 4.10 (1 H, m, CH),

4.25 (2H, s, CH_2), 4.29 (1H, m, CH), 5.10 (1 H, m, CH), 7.11 (1H, d, J = 8.4 Hz, Ar), 7.25 (1H, d, J = 8.3 Hz, Ar), 7.55 (2H, m, Ar).

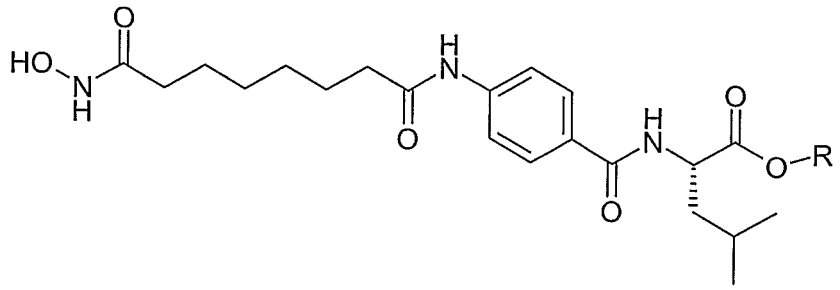
Stage 7: (S)-2-{[(S)-7-(7-Hydroxycarbamoyl-heptanoylamino)-1,2,3,4-tetrahydro-isoquinoline-3-carbonyl]-amino}-4-methyl-pentanoic acid **(96)**



97

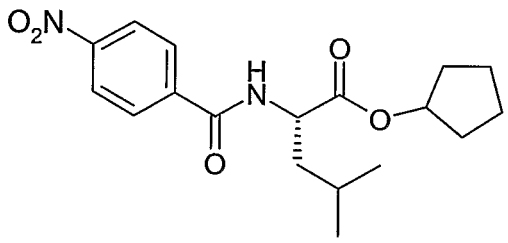
Stage 5 cyclopentyl ester resin (1.55g) was suspended in THF/MeOH (10ml /10ml). To the suspension was added NaOH (1.4 M aq.,5ml) and the reaction shaken for 16 hr at r.t. The filtrate was removed and the resin washed (standard) and dried before cleavage. Cleavage (600mg of resin) was effected by shaking with 2%TFA/DCM (8ml) for 30 minutes before filtering the resin and evaporating the solvent under reduced pressure at room temperature. The crude product purified by preparative HPLC to yield Compound **(97)** (73.4mg). The boc group is removed in addition to resin cleavage. LCMS purity 96%, m/z 477 $[M^+ + H]^+$, ^{1}H NMR (400 MHz, MeOD), δ:0.98 – 1.02 (6 H, 2 x d, J = 6 Hz, J = 6.1 Hz, 2 x CH_3), 1.40 (4 H, m, alkyl), 1.60 – 1.80 (7 H, m, alkyl), 2.10 (2 H, t, J = 7.4 Hz, CH_2), 2.39 (2 H, t, 7.6 Hz, CH_2), 3.15 (1 H, dd, J = 12.5 Hz, J = 16.6 Hz, CH), 3.45 (1 H, dd, J = 4.9 Hz, J = 17 Hz, CH) 4.00 (1 H, s, CH), 4.20 (1 H, dd, J = 4.7 Hz, J = 12.2 Hz, CH), 4.40 (2 H, m, CH_2), 4.55 (1 H, dd, J = 4.7 Hz, J = 10 Hz, CH), 7.25 (1 H, d, J = 8.4 Hz, Ar), 7.25 (1 H, d, J = 8 Hz, Ar), 7.55 (1 H, J = 7 Hz, Ar).

Synthesis of Compound (98) and Compound (99)



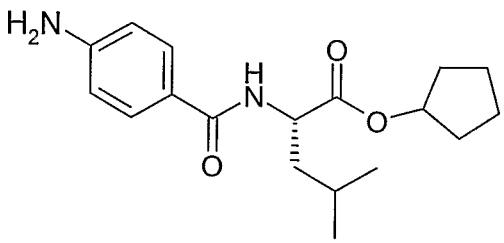
R = cyclopentyl 98
R = H 99

Stage 1: (S)-4-Methyl-2-(4-nitro-benzoylamino)-pentanoic acid cyclopentyl ester



L-leucine cyclopentyl ester. TsOH salt (7.98g, 21.51mmol) was dissolved in THF (40ml) and triethylamine (6ml, 21.5mmol) added. 4-Nitrobenzoyl chloride (4g, 21.5mmol) was added portionwise with cooling, ice bath. The reaction was stirred at room temperature for 16 h before evaporation to dryness. The residue was dissolved in DCM (100ml) and washed with saturated sodium hydrogen carbonate (3x100ml), 1 M HCl (3x100ml) and brine, dried (Na_2SO_4), and the solvent removed *in vacuo.* to give the required product 5.25g (70% yield) which was used in the next step without further purification, LCMS purity 100%, m/z 349 $[M^+ + H]^+$

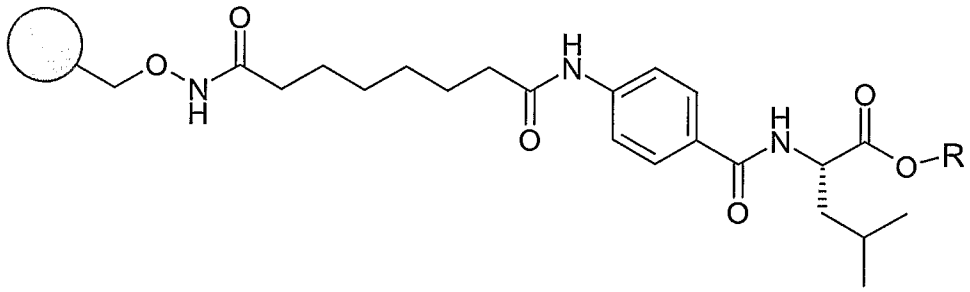
Stage 2: (S)-2-(4-Amino-benzoylamino)-4-methyl-pentanoic acid cyclopentyl ester



Stage 1 nitro amide (5.25g, 15.1mmol) was dissolved in ethanol (100ml). Pd/carbon (200mg) was added and the reaction stirred for 16 h at room temperature under

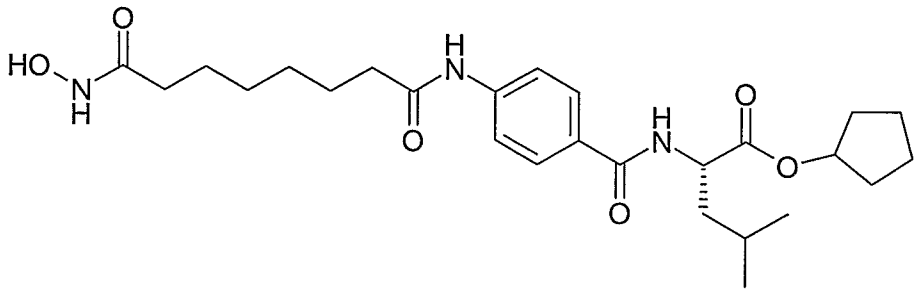
hydrogen (balloon pressure). The reaction mixture was filtered through celite and evaporated to give the required amine amide 3.9g (81% yield) which was used in the next step without further purification, LCMS purity 100%, m/z 319 $[M^+ + H]^+$

Stage 3: Coupling to resin



Suberic acid derivatised Wang hydroxylamine resin (1.6g, loading, 1.8mmol/g) was swollen in DCM (anhydrous, 20ml). 1-Chloro-N,N-2-trimethylpropenylamine (1.15ml, 8.64mmol) was added dropwise before shaking at room temperature for 1 h. (S)-2-(4-Amino-benzoylamino)-4-methyl-pentanoic acid cyclopentyl ester (2.75g, 8.64mmol) was added followed by triethylamine (2.4ml, 17.63mmol) and the reaction shaken at room temperature for 16 h. The resin was washed (standard) and air dried.

Stage 4: (S)-2-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzoylamino]-4-methyl-pentanoic acid cyclopentyl ester **(98)**

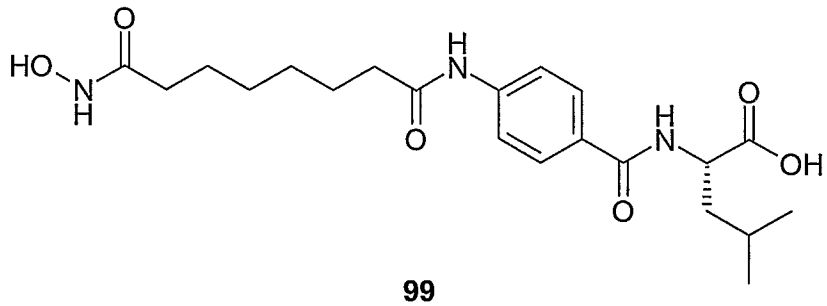


98

Stage 3 resin bound cyclopentyl ester was shaken with 2%TFA/DCM (10ml) for 10 minutes before filtering the resin and evaporating the solvent under reduced pressure at room temperature. The process was repeated (x3) and the combined crude product purified by preparative HPLC to yield compound **(98)** (36mg). LCMS purity 91%, m/z 490 $[M^+ + H]^+$, ^{1}H NMR (400 MHz, MeOD), δ:0.80 – 0.95 (6 H, 2 x CH_3), 1.25

(4 H, m, alkyl), 1.40 – 1.85 (15 H, m, alkyl), 2.00 (2 H, t, CH_2), 2.30 (2 H, t, CH_2), 4.45 (1 H, m, CH), 5.05 (1 H, m, CH), 7.60 (2 H, d, Ar), 7.75 (2 H, d, Ar).

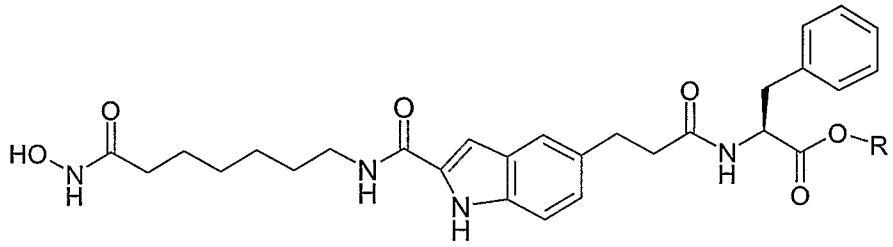
Stage 5: (S)-2-[4-(7-Hydroxycarbamoyl-heptanoylamino)-benzoylamino]-4-methyl-pentanoic acid **(99)**



99

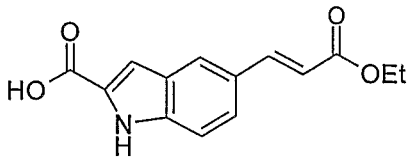
Compound **(98)** (21mg, 0.043mmol) was dissolved in THF (1ml) and 2M NaOH (1ml) added. The reaction vial was shaken for 16 h before THF removal by blowing a stream of N_2 gas at the surface of the solution. The aqueous residue was purified by preparative HPLC to yield compound **(99)** (5.2mg). LCMS purity 92%, m/z 422 $[M^+H]^+$, ^1H NMR (400 MHz, MeOD), δ:0.95 – 1.05 (6 H, m, 2 x CH_3), 1.30 – 1.50 (4 H, m, alkyl), 1.55 – 1.85 (7 H, m, alkyl), 2.10 (2 H, t, CH_2), 2.40 (2 H, t, CH_2), 4.65 (1 H, m, CH), 7.65 (2 H, d, Ar), 7.80 (2 H, d, Ar).

Synthesis of Compound (100) and Compound (101)



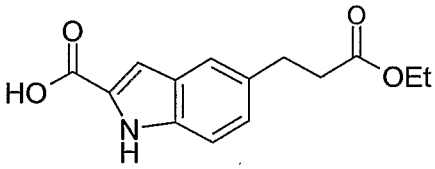
R = cyclopentyl 100
R = H 101

Stage 1: 5-((E)-2-Ethoxycarbonyl-vinyl)-1H-indole-2-carboxylic acid



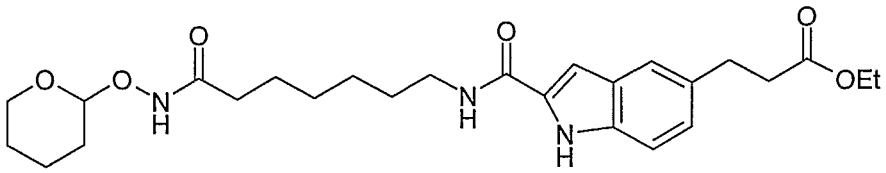
5-Bromoindole-2-carboxylic acid (400mg, 1.66mmol) and tri-*O*-tolyl phosphine (96mg, 0.32mmol) were added to a microwave tube. Ethyl acrylate (0.56ml, 5.6mmol), Et_3N (0.92ml, 6.6mmol), acetonitrile (2.5ml) and $Pd(OAc)_2$ (40mg, 0.18mmol) were added. The reaction was placed in a CEM microwave at 150W, 90°C for 30 minutes with 5 min ramp time. EtOAc was added and the reaction mixture filtered through celite. The celite pad was washed with DCM and the combined organic fractions removed to give a yellow solid. The solid was redissolved in DCM and extracted into saturated sodium hydrogen carbonate. The aqueous layer was washed with DCM and diethyl ether. The aqueous basic layer was acidified with 2M HCl (pH=5) and the product extracted into EtOAc. The solvent was removed to give the required product (370 mg, 86% yield). LCMS purity 86%, m/z 260 $[M^+ + H]^+$

Stage 2: 5-((E)-2-Ethoxycarbonyl-vinyl)-1H-indole-2-carboxylic acid



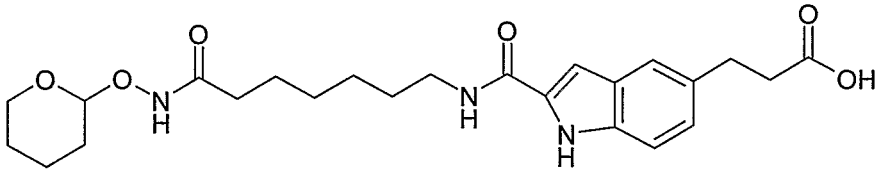
5-((E)-2-Ethoxycarbonyl-vinyl)-1H-indole-2-carboxylic acid (430mg, 1.66mmol) was dissolved in EtOAc (100ml). Pd/carbon (100mg) was added and the reaction stirred under a hydrogen atmosphere (balloon pressure) for 18 h. The reaction mixture was filtered through a pad of celite and washed with EtOAc. The solvent was removed to give the required product which was used in the next step without further purification (0.47g). LCMS purity 92%, m/z 262 $[M^+ + H]^+$

Stage 3: 3-{2-[6-(Tetrahydro-pyran-2-yloxycarbamoyl)-hexylcarbamoyl]-1H-indol-5-yl}-propionic acid ethyl ester



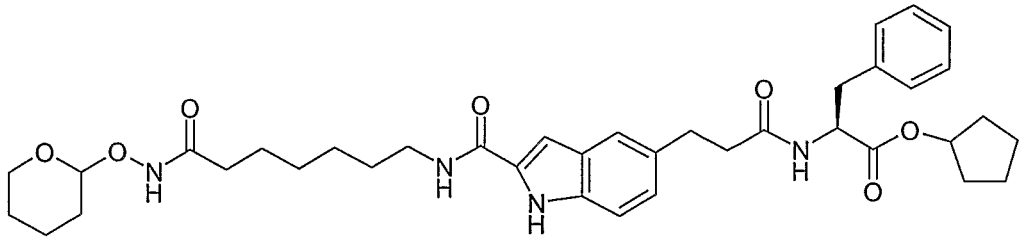
5-((E)-2-Ethoxycarbonyl-vinyl)-1H-indole-2-carboxylic acid (0.427g, 1.6mmol) was dissolved in anhydrous DMF (20ml). EDCI.HCl (0.38g, 2mmol), Et_3N (0.59 ml, 4.3mmol), HOBt (0.27 g, 2mmol) and 7 amino heptanoic acid (tetrahydropyran-2-yloxy) amide * (0.4 g, 1.6mmol in anhydrous DMF 20ml) were added and the reaction stirred at room temperature for 16 h under nitrogen. Water was added, the reaction mixture acidified to pH = 6-7 (10% citric acid) and extracted with DCM. The organic layer was washed with 10% citric acid and saturated sodium hydrogen carbonate (x2). The solvent was removed in vacuo to give crude product which was purified by chromatography (EtOAc : hexane 1 : 2 → EtOAc) to give the required product as a yellow solid (550mg, 69% yield). LCMS purity 93%, m/z 488 $[M^+ + H]^+$

Stage 4: 3-{2-[6-(Tetrahydro-pyran-2-yloxycarbamoyl)-hexylcarbamoyl]-1H-indol-5-yl}-propionic acid



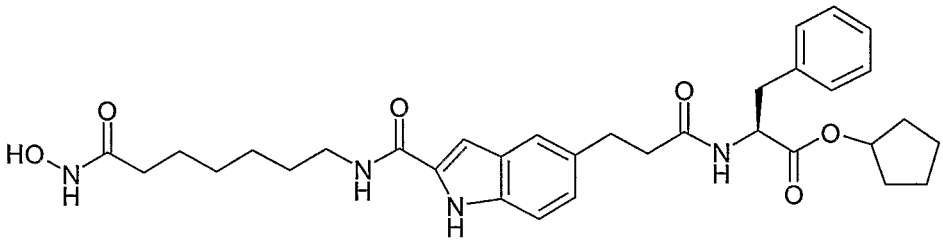
3-{2-[6-(Tetrahydro-pyran-2-yloxycarbamoyl)-hexylcarbamoyl]-1H-indol-5-yl}-propionic acid ethyl ester (550mg, 1.13mmol) was dissolved in THF/methanol (50ml/ 25ml). 1.4 M NaOH solution (50ml) was added and the reaction stirred at room temperature for 4 h. The solvent was reduced to ~ 50% volume and 1M HCl added to pH 6-7. The mixture was extracted with DCM and further extracted with EtOAc. The combined organic layer was dried, Na_2SO_4 and the solvent removed *in vacuo* to give the required product 357mg (69% yield) as a yellow powder which was used in the next step without further purification. LCMS purity 94%, m/z 460 $[M^+ + H]^+$

Stage 5: (S)-3-Phenyl-2-(3-{2-[6-(tetrahydro-pyran-2-yloxycarbamoyl)-hexyl-carbamoyl]-1H-indol-5-yl}-propionylamino)-propionic acid cyclopentyl ester



3-{2-[6-(Tetrahydro-pyran-2-yloxycarbamoyl)-hexylcarbamoyl]-1H-indol-5-yl}-propionic acid (0.357g, 0.78mmol) was dissolved in DCM/DMF (20ml/20ml). EDCI.HCl (0.163mg, 0.86mmol), triethylamine (0.24ml, 1.7mmol), HOBt (0.116mg, 0.88mmol) and L-phenylalanine cyclopentyl ester.TsOH salt (0.346 mg, 0.88mmol) were added and the reaction mixture stirred for 16 h at room temperature under nitrogen. The solvent volume was reduced (~10ml), DCM was added and the organic layer washed with water (x3). The organic layer was dried (Na_2SO_4) and the solvent removed to give the required product (500mg, 95% yield) which was used without further purification. LCMS purity 77%, m/z 675 $[M^+ + H]^+$

Stage 6: (S)-2-{3-[2-(6-Hydroxycarbamoyl-hexylcarbamoyl)-1H-indol-5-yl]-propionylamino}-3-phenyl-propionic acid cyclopentyl ester **(100)**

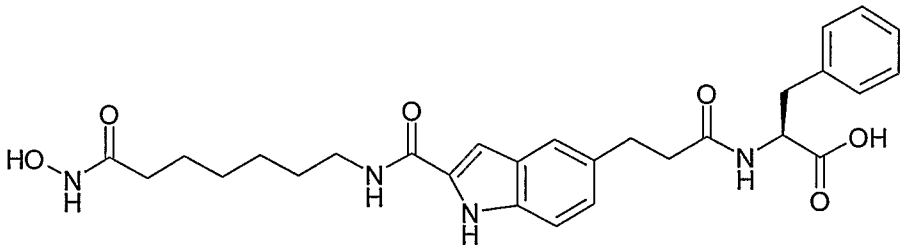


100

(S)-3-Phenyl-2-(3-{2-[6-(tetrahydro-pyran-2-yloxycarbamoyl)-hexyl-carbamoyl]-1H-indol-5-yl}-propionylamino)-propionic acid cyclopentyl ester (200mg. 0.297mmol) was stirred at room temperature for 3.5 h in TFA/DCM/MeOH (1.5ml/15ml/15ml). Further TFA (0.3 ml) was added and the reaction stirred for a further 30 minutes. The solution was concentrated *in vacuo,* resuspended in DCM and the solvent removed (x3). The crude material was purified by prep HPLC to give pure compound **(100)** (19.3mg), LCMS purity 100%, m/z 591 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ:1.27 – 1.70 (16

H, m, alkyl), 1.97 (2 H, t, CH_2), 2.40 (2 H, t, J = 7.84 Hz, CH_2), 2.76 – 2.85 (4 H, m, 2 x CH_2), 3.25 (2 H, t, J = 7 Hz, CH_2), 4.41 (1 H, m, NHCHCO, 4.95 (1 H, m, CH), 6.85 (1 H, s, CH), 6.95 (3 H, m, Ar), 7.05 (3 H, m, Ar), 7.20 – 7.26 (2 H, s + d, J = 8.5 Hz, Ar)

Stage 7: (S)-2-{3-[2-(6-Hydroxycarbamoyl-hexylcarbamoyl)-1H-indol-5-yl]-propionylamino}-3-phenyl-propionic acid **(101)**

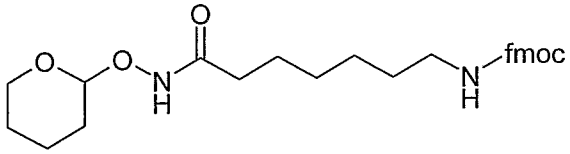


101

Compound **(100)** (80mg, 0.14mmol) was dissolved in THF/MeOH (1ml/ 0.5ml) and 1.4 M NaOH (0.5ml) added. The reaction was stirred at room temperature for 2 h. THF was removed by blowing a stream of N_2 gas at the surface of the solution and the residual material purified by preparative HPLC to give compound **(101)** (34.9mg), LCMS purity 95%, m/z 523 $[M^+ + H]^+$, ^{1}H NMR (400 MHz, MeOD), δ:1.35 – 1.50 (4 H, m, alkyl), 1.60 – 1.75 (4 H, m, alkyl), 2.15 (2 H, br t, CH_2), 2.55 (2 H, br t, CH_2), 2.95 (3 H, m, CH + CH_2), 3.10 (1H, dd, CH), 4.65, (1 H, m, NHCHCO), 7.00 – 7.15 (7 H, m, Ar), 7.35 – 7.41 (2 H, m, Ar)

*Preparation of 7 amino heptanoic acid (tetrahydropyran-2-yloxy) amide

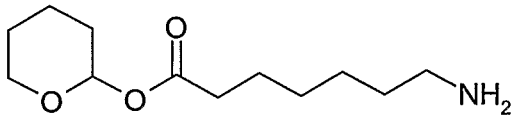
Stage 1: 6-(Tetrahydro-pyran-2-yloxycarbamoyl)-hexyl]-carbamic acid 9H-fluoren-9-ylmethyl ester



To a solution of 7-(9H-fluoren-9-yloxycarbonylamino) heptanoic acid (1g, 2.72 mmol) in anhydrous DCM/THF (15ml/15ml) was added EDCI. HCl (627mg, 3.27mmol), HOBt (442mg, 3.27mmol) and O-(tetrahydro-pyran-2-yl)-hydoxylamine (383mg, 3.27mmol)

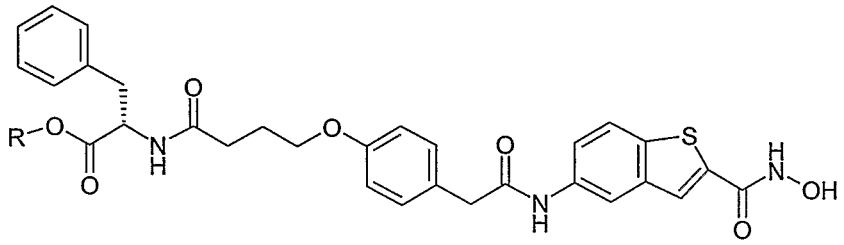
which was stirred under nitrogen for 48 h. EDCI. HCl (260mg, 1.36mmol), HOBt (184mg, 1.36mmol) and O-(tetrahydro-pyran-2-yl)-hydoxylamine (159mg, 1.36mmol) were added and the reaction continued for a further 24 h. The reaction mixture was diluted with DCM (100 ml), washed with water (3 x 100ml), brine (100ml), dried (Na_2SO_4), filtered and concentrated *in vacuo.* Purification by chromatography (MeOH : DCM 2 : 98) gave a white solid (1.03g, 81%).

Stage 2: 7-Amino-heptanoic acid tetrahydro-pyran-2-yl ester



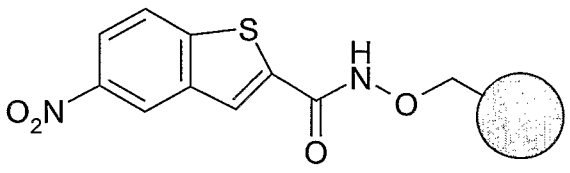
6-(Tetrahydro-pyran-2-yloxycarbamoyl)-hexyl]-carbamic acid 9H-fluoren-9-ylmethyl ester (300mg, 0.644mmol) was dissolved in 20% piperidine/DCM (30ml) and the reaction stirred for 0.5 h. The reaction was evaporated to dryness, redissolved in DCM and evaporated (x3). The required product was obtained following chromatography (MeOH : DCM : NH_3), 120mg. LCMS purity 98%, m/z 245 $[M^+ + H]^+$.

Synthesis of Compound (102) and Compound (103)



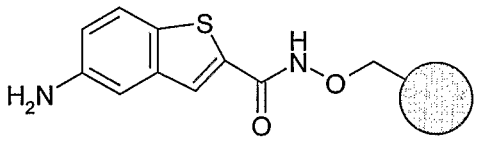
R = cyclopentyl 102
R = H 103

Stage 1: Resin loading



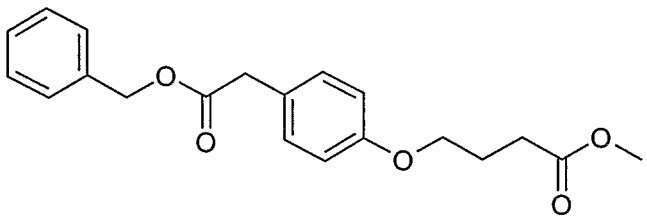
Wang hydroxylamine resin (3.72g, 1.8 mmol/g) was swollen in DMF (50ml). HATU (7.5g, 19.7mmol), 5–nitro-1-benzothiophene-2-carboxylic acid (3g, 13.45mmol, dissolved in DMF 150ml) and diisopropylethylamine (4.65ml, 26.7 mmol) were added and the resin shaken at room temperature for 4 d. The resin was filtered and washed using the standard washing procedure and air dried.

Stage 2: Nitro reduction



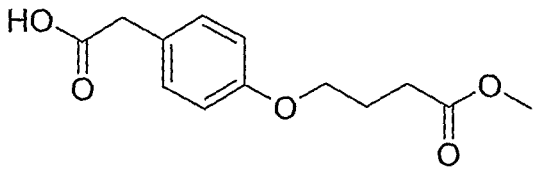
Stage 1 resin (4.9g, 1.8mmol/g), was swollen in DMF (200ml) and tin chloride dihydrate (19.9g, 88mmol) added. The reaction was shaken at room temperature for 16 h. The resin was filtered and washed using the standard washing procedure and air dried.

Stage 3: 4-(4-Benzyloxycarbonylmethyl-phenoxy)-butyric acid methyl ester



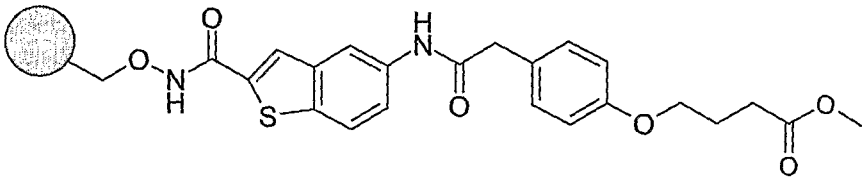
Benzyl 4-hydroxyphenyl acetate (9g, 37mmol) was dissolved in DMF (300ml). Ground sodium hydroxide (2.23g, 56mmol) and 4-methyl bromo butyrate (6.4 ml, 56mmol) were added and the reaction heated at 60 °C for 16 h. Water was added to the cooled reaction mixture and the solution acidified (pH = 5/6) with 1M HCl. The aqueous layer was extracted with EtOAc and the organic layer washed with water (x2), dried over Na_2SO_4, filtered and evaporated to dryness. The required diester was obtained following chromatography (EtOAc : heptane 1 : 2), (9.56g, 75%) LCMS purity 90%, m/z 343 $[M^+ + H]^+$.

Stage 4: 4-(4-Carboxymethyl-phenoxy)-butyric acid methyl ester



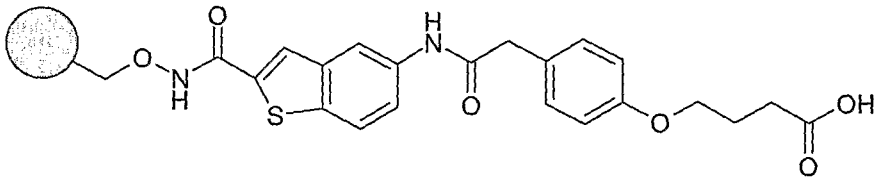
4-(4-Benzyloxycarbonylmethyl-phenoxy)-butyric acid methyl ester (1.4g, 4.09mmol) was dissolved in EtOAc (60ml). Pd/carbon (100mg) was added and the reaction stirred under a hydrogen atmosphere (balloon) for 16 h at room temperature. The reaction mixture was filtered through a pad of celite and the pad washed with EtOAc. The filtrate was evaporated to dryness to give a white solid (1.03g, 100% yield). LCMS purity 93%, m/z 253 $[M^+ + H]^+$.

Stage 5: Coupling to resin



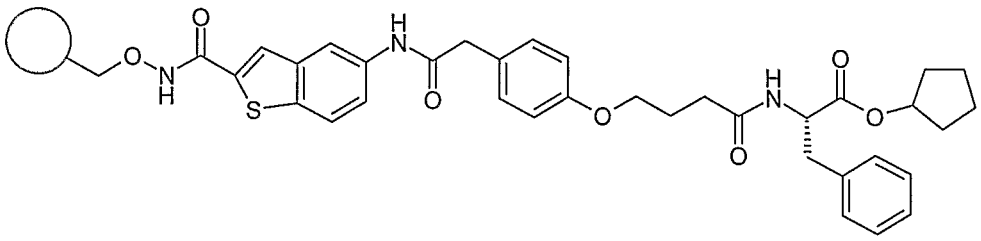
Stage 2 resin (0.18 g, 1.8mmol/g) was swollen in DMF (5ml). HATU (0.37g, 0.96mmol), 4-(4-Carboxymethyl-phenoxy)-butyric acid methyl ester (0.247g, 0.96mmol dissolved in DMF – 10ml) and diisopropylamine (0.56ml, 3.3mmol) were added and the reaction shaken at room temperature for 16 h. The reaction was filtered and the resin washed using the standard wash procedure and air dried.

Stage 6: Ester hydrolysis



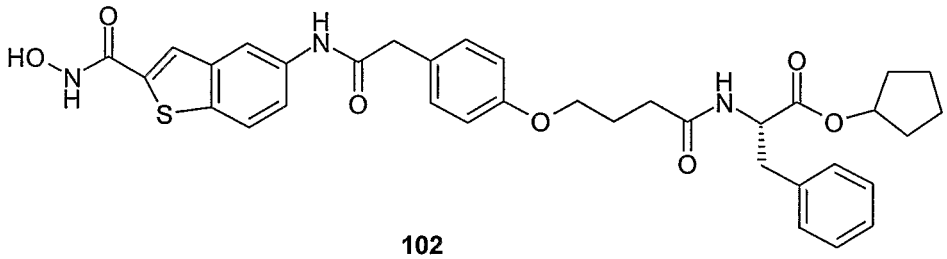
Stage 5 methyl ester (280mg, 1.8mmol/g) was dissolved in THF/MeOH (4ml/ 4ml) and 1.4 M NaOH (8ml) added. The reaction was shaken at r.t. for 16 h. The resin was filtered and washed using the standard wash and air dried.

Stage 7: Amino acid coupling



Stage 6 resin (1.6g, 1.8mmol/g) was swollen in anhydrous DMF (120ml). HATU (3.3g, 8.6mmol), L-phenylalanine cyclopentyl ester. TsOH salt (3.4g, 8.6mmol) and diisopropylamine (5ml, 2.9mmol) were added and the reaction shaken at room temperature for 16 h. The reaction was filtered and the resin washed using the standard wash procedure and air dried.

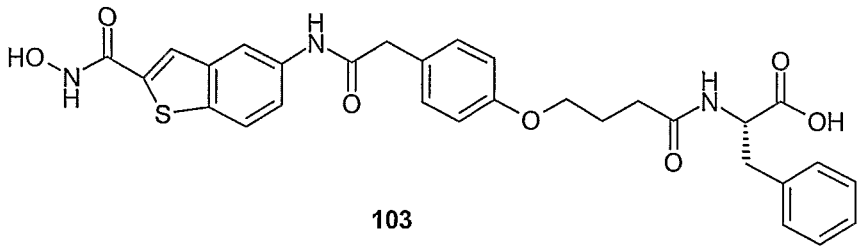
Stage 8: (S)-2-(4-{4-[(2-Hydroxycarbamoyl-benzo[b]thiophen-5-ylcarbamoyl)-methyl]-phenoxy}-butyrylamino)-3-phenyl-propionic acid cyclopentyl ester **(102)**



102

Stage 7 resin bound cyclopentyl ester was shaken with 2%TFA/DCM (10ml) for 10 minutes before filtering the resin and evaporating the solvent under reduced pressure at room temperature. The process was repeated (x3) and the combined crude product purified by preparative HPLC to yield compound **(102)** (22.5mg). LCMS purity 99%, m/z 644 $[M^+ + H]^+$, ^1H NMR (400 MHz, MeOD), δ:1.45 – 1.80 (6 H, m, alkyl), 1.95 (2 H, pent, CH_2), 2.34 (2 H, t, J = 7.3 Hz, CH_2), 2.90 (1 H, dd, CH), 3.04 (1 H, dd, CH), 3.62 (2 H, s, CH_2), 3.86, (2 H, m, CH_2), 4.55 (1 H, m, NHCHCO), 5.07 (1 H, br s, CH), 6.83 (2 H, d, J = 8.3 Hz, Ar), 7.14 – 7.18 (5 H, m, Ar), 7.25 (2 H, d, J = 8 Hz, Ar), 7.47 (1 H, d, J = 9 Hz, Ar), 7.73 (1 H, s, Ar), 7.81 (1 H, d, J = 8.8 Hz), 8.25 (1 H, s, Ar)

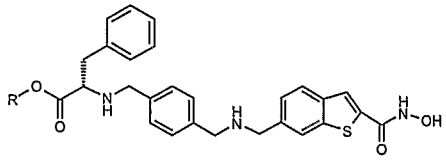
Stage 9: (S)-2-(4-{4-[(2-Hydroxycarbamoyl-benzo[b]thiophen-5-ylcarbamoyl)-methyl]-phenoxy}-butyrylamino)-3-phenyl-propionic acid **(103)**



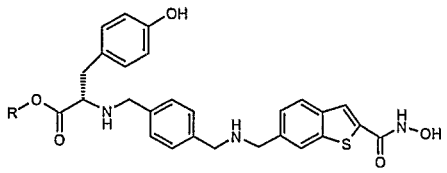
103

Stage 7 cyclopentyl ester resin (200mg) was swollen in THF/MeOH (2ml/2ml) and 1.4 M NaOH (2ml) added. The reaction was shaken at room temperature for 16 h. The resin was filtered and washed using the standard wash. Resin bound carboxylic acid was shaken with 2%TFA/DCM (3ml) for 10 minutes before filtering the resin and evaporating the solvent under reduced pressure at room temperature. The process was repeated (x3) and the combined crude product purified by preparative HPLC to yield compound **(103)** (33.7mg). LCMS purity 88%, m/z 576 $[M^+ + H]^+$, ^{1}H NMR (400 MHz, d6-DMSO), δ:1.93 (2 H, m, CH_2), 2.30 (2 H, m, CH_2), 2.91 (1 H, dd, J = 9.9 Hz, J = 13.8 Hz, CH), 3.13 (1 H, dd, J = 4.8 Hz, J = 13.9 Hz, CH), 3.67 (2 H, s, CH_2), 3.91, (2 H, m, CH_2), 4.50 (1 H, m, NHCHCO), 6.92 (2 H, d, J = 8.7 Hz, Ar), 7.24 – 7.34 (7 H, m, Ar), 7.61 (1 H, m), 7.92 (1 H, br s, Ar), 8.00 (1 H, d, J = 8.8 Hz, Ar), 8.31 (1 H, d, J = 8.1 Hz, Ar), 8.39 (1 H, s), 9.36 (1 H, br s), 10.36 (1 H, s), 11.52 (1 H, s), 12.76 (1 H, br s)

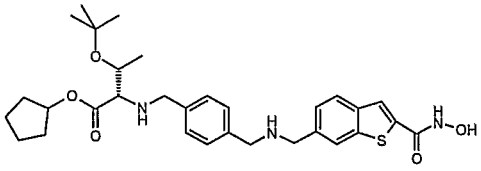
Synthesis of Compounds in Figure 7 as Exemplified for Compound (104) and Compound (105)



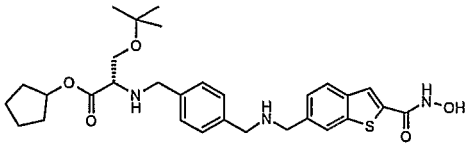
R= cyclopentyl 104
R=H 105



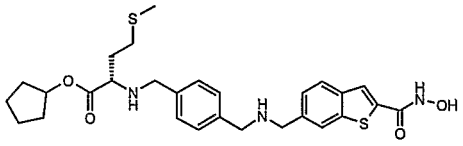
R= cyclopentyl 106
R=H 107



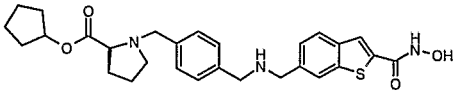
108



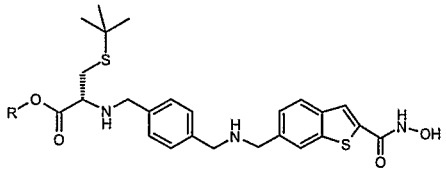
109



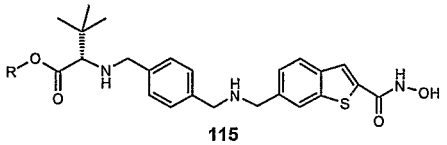
110



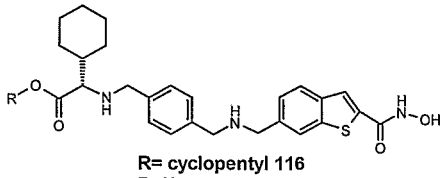
R= cyclopentyl 111
R=H 112



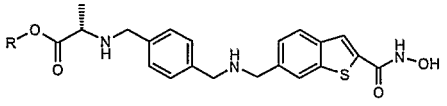
R= cyclopentyl 113
R=H 114



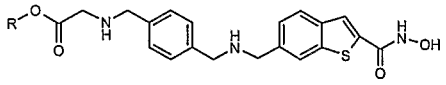
115



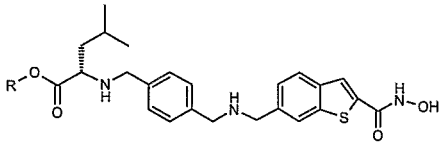
R= cyclopentyl 116
R=H 117



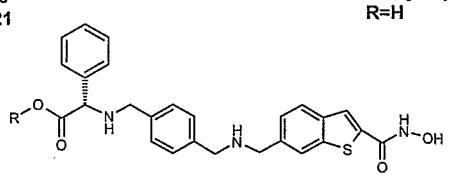
R= cyclopentyl 118
R=H 119



R= cyclopentyl 120
R=H 121



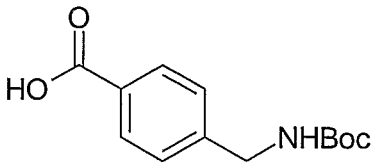
R= cyclopentyl 122
R=H 123



R= cyclopentyl 124
R=H 125

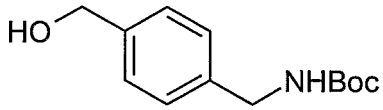
Figure 7

Step 1: 4-(tert-Butoxycarbonylamino-methyl)-benzoic acid



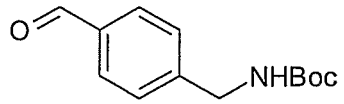
4-Aminomethylbenzyl alcohol (1.0 g, 6.60 mmol) was slurried in a mixture of THF (10 mL) and water (10 mL). A solution of saturated sodium hydrogen carbonate was added until the pH of the solution was > pH 9. The mixture was cooled to 0 °C and di-tert-butyldicarbonate (2.89 g, 13.23 mmol) added. The reaction was allowed to stir overnight then THF removed under vacuum. The aqueous mixture was extracted with EtOAc (20 mL) and then acidified to pH 3 by addition of 1N HCl. This was extracted with EtOAc (2 x 10 mL), the organic layers combined, dried ($MgSO_4$) and evaporated to dryness to afford the desired product (1.60 g, 97%). m/z 252 $[M^+ + H]^+$

Step 2: (4-Hydroxymethyl-benzyl)-carbamic acid tert-butyl ester



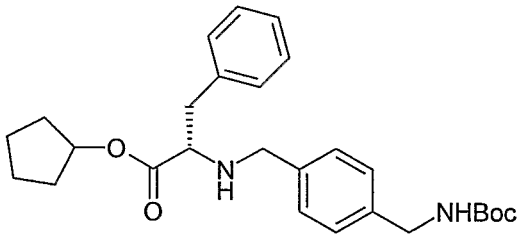
$LiAl_4$ (227 mg, 5.97 mmol) was slurried in a mixture of THF (5 mL) and dioxane (5 mL) and cooled to 0 °C under an atmosphere of N_2. 4-(tert-Butoxycarbonylamino-methyl)-benzoic acid was dissolved in a mixture of THF (5 mL) and dioxane (5 mL) and added to the chilled solution drop-wise over 15 min. The reaction mixture was allowed to warm to r.t and stirred for 16 h. Water (1 mL) was added to the reaction mixture which was then filtered through celite. The filtrate was evaporated to dryness and the residue partitioned between EtOAc (25 mL) and water (25 mL). The aqueous layer was extracted with EtOAC (2 x 25 mL), the organic layers combined, dried (Na_2SO_4) and evaporated to dryness to afford the desired product (460 mg, 100%). m/z 260 $[M^+ + Na]^+$

Step 3: (4-Formyl-benzyl)-carbamic acid tert-butyl ester



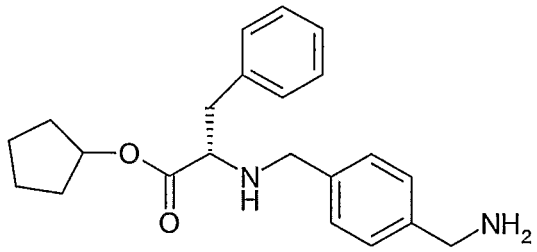
(4-Hydroxymethyl-benzyl)-carbamic acid tert-butyl ester (480 mg, 0.71 mmol) was dissolved in DCM (3 mL) and cooled to -78 °C (dry ice / acetone). Dess-Martin periodinane (331 mg, 0.78 mmol) was added to the reaction which was allowed to warm to r.t and stir for 3 h. A 1:1 solution of saturated sodium bicarbonate and sodium sulfite (20 mL) was added and the reaction mixture stirred vigorously for 15 min. The organic layer was isolated, washed with saturated sodium bicarbonate (10 mL), dried (Na_2SO_4) and evaporated to dryness to afford the desired compound (480 mg, 100%). m/z 258 $[M^+ + Na]^+$

Step 4: (S)-2-[4-(tert-Butoxycarbonylamino-methyl)-benzylamino]-3-phenyl-propionic acid cyclopentyl ester



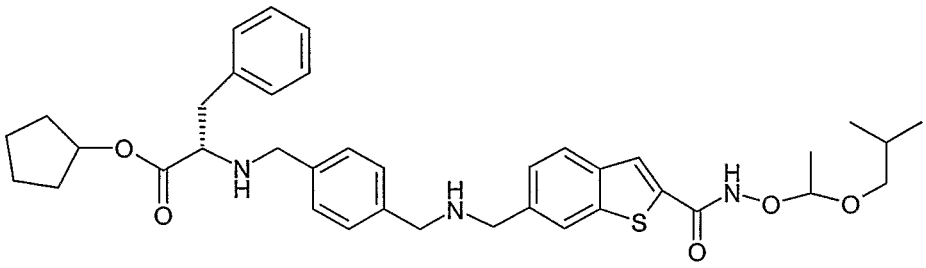
(4-Formyl-benzyl)-carbamic acid tert-butyl ester (200 mg, 0.85 mmol) was dissolved in DCE (10 mL) and to this was added phenyl alanine cyclopentyl ester (214 mg, 0.94 mmol). The reaction was stirred at r.t. for 15 min. Sodium triacetoxyborohydride (538 mg, 2.55 mmol) and acetic acid (60 uL) were added and the reaction stirred for a further 1h. Saturated sodium bicarbonate (10 ml) was added and the solution diluted with DCM (20 mL). The organic layer was isolated and concentrated to afford the desired product which was taken onto the next step without further purification. m/z 453 $[M^+ + H^+$

Step 5: (S)-2-(4-Aminomethyl-benzylamino)-3-phenyl-propionic acid cyclopentyl ester



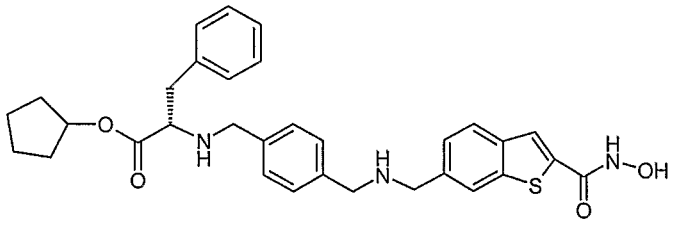
(S)-2-[4-(tert-Butoxycarbonylamino-methyl)-benzylamino]-3-phenyl-propionic acid cyclopentyl ester was treated with 4M HCl in dioxane (1 mL, 0.25 mmol) and stirred at r.t. for 1 h. The mixture was evaporated to dryness and partitioned between EtOAc (20 mL) and water (20 mL). Saturated sodium bicarbonate (20 mL) was added to the aqueous layer which was then extracted with EtOAc (3 x 20 mL). The organic layers were combined, dried (Na_2SO_4) and evaporated to dryness to give the desired product (263 mg, 79% over 2 steps). m/z 353 $[M^+ + H]^+$

Step 6: (S)-2-[4-({[2-(1-Isobutoxy-ethoxycarbamoyl)-benzo[b]thiophen-6-ylmethy l]-amino}-methyl)-benzylamino]-3-phenyl-propionic acid cyclopentyl ester



6-formyl-benzo[b]thiophene-2-carboxylic acid (1-isobutoxy-ethoxy) amide (Scheme 7) (220 mg, 0.68 mmol) and (S)-2-(4-Aminomethyl-benzylamino)-3-phenyl-propionic acid cyclopentyl ester (263 mg, 0.75 mmol) were dissolved in DCE (10 mL) under an atmosphere of N_2. Sodium triacetoxyborohydride (430 mg, 2.04 mmol) and acetic acid (50 µL) were added and the reaction stirred at r.t for 3 h. Sodium hydrogen carbonate (20 mL) was added and the reaction mixture extracted with dichloromethane (3 x 50 mL). The organic layers were combined and concentrated. The residue was purified by column chromatography (50%-100% EtOAc/heptane) to give the protected compound (80 mg, 21%). m/z 658 $[M^+ + H]^+$

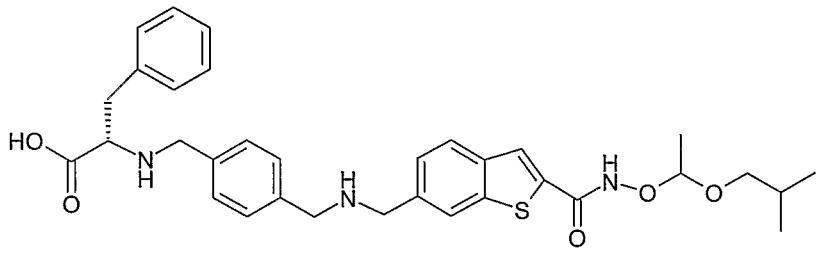
Step 7: (S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-met hyl}-benzylamino)-3-phenyl-propionic acid cyclopentyl ester **(104)**



104

(S)-2-[4-({[2-(1-Isobutoxy-ethoxycarbamoyl)-benzo[b]thiophen-6-ylmethyl]-amino}-methyl)-benzylamino]-3-phenyl-propionic acid cyclopentyl ester was dissolved in DCM (2 mL) and MeOH (2 mL) and treated with TFA (1 mL). The mixture was stirred for 1 h at r.t then concentrated to dryness and DCM (5 mL) and heptane (5 mL) added. The mixture was evaporated to dryness. This process was repeated three times to yield compound **(104)** (20 mg, 59%) as a oil. LCMS purity 95%, m/z 558 $[M^+H]^+$, 1H NMR (300 MHz, MeOD), δ: 1.25-1.91 (8H, m, 4xCH2), 3.12-3.47 (2H, m, CH2), 4.26 (1H, m, CH), 4.33 (2H, d, J=5.5 Hz, CH2), 4.36 (2H, s, CH2), 4.43 (1H, s, CH2), 5.16 (1H, s, CH), 5.13 (1H, m, CH), 7.25-7.37 (6H, m, ArH), 7.54-7.63 (4H, m, ArH), 7.94 (2H,m, ArH), 8.09 (1H, s, ArH).

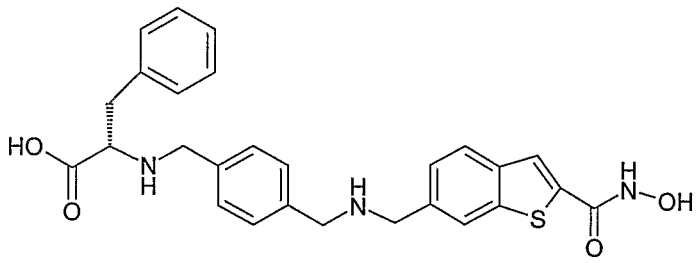
Step 8: (S)-2-[4-({[2-(1-Isobutoxy-ethoxycarbamoyl)-benzo[b]thiophen-6-ylmethy l]-amino}-methyl)-benzylamino]-3-phenyl-propionic acid



(S)-2-[4-({[2-(1-Isobutoxy-ethoxycarbamoyl)-benzo[b]thiophen-6-ylmethyl]-amino}-methyl)-benzylamino]-3-phenyl-propionic acid cyclopentyl ester (40 mg, 0.06 mmol) was dissolved in THF (2 mL) and water (2 mL). LiOH (8 mg, 0.30 mmol) was added and the reaction mixture heated to 50 °C for 36 h. THF was removed by evaporation and the residue partitioned between water (10 mL) and EtOAc (10 mL). The aqueous

layer was isolated and the pH adjusted to 3 by addition of 1M HCl. This was extracted with EtOAc (3 x 20 mL), the organic layers combined and evaporated to dryness.

Step 9: (S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-met hyl}-benzylamino)-3-phenyl-propionic **(105)**



105

(S)-2-[4-({[2-(1-Isobutoxy-ethoxycarbamoyl)-benzo[b]thiophen-6-ylmethyl]-amino}-methyl)-benzylamino]-3-phenyl-propionic acid was dissolved in MeOH (2 mL) and THF (2 mL). TFA (1 mL) was added at the mixture stirred for 1 h at r.t. The reaction mixture was concentrated to dryness and DCM (5 mL) and heptane (5 mL) added. The mixture was evaporated to dryness. This process was repeated three times to yield compound **(105)** (17 mg, 57%) as a pink solid. LCMS purity 90%, m/z 490 $[M^+H]^+$, 1H NMR (300 MHz, MeOD), δ: 3.33 (2H, m, CH2), 4.19 (1H, m, CH2), 4.29 (2H, s, CH2), 4.35 (2H, s, CH2), 4.42(2H, s, CH2), 7.29-7.39 (5H, m, ArH), 7.54-7.72 (5H, m, ArH), 7.88 (1H, s, ArH), 8.00 (1H, d J=8.0Hz, ArH), 8.08 (1H, s, ArH)

The following compounds were prepared according to the procedure described for compound **(104)** and compound **(105)**

(S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-3-(4-hydroxy-phenyl)-propionic acid cyclopentyl ester (106)

LCMS purity 98%, m/z 574 $[M^+H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.30-1.87 (8H, m, 4 x CH_2), 2.97-3.35 (2H, m, CH_2), 4.17 (1H, m, CH), 4.31 (2H, d, J = 5.4 Hz, CH_2), 4.36 (2H, s, CH_2), 4.42 (2H, s, CH_2), 5.11-5.16 (1H, m, CH), 6.77 (2H, d, J = 8.4 Hz, Ar-H), 7.06 (2H, d, J = 8.4 Hz, Ar-H), 7.54-7.65 (5H, m, Ar-H), 7.87 (1H, s, Ar-H), 7.99 (1H, d, J = 8.4 Hz, Ar-H), 8.08 (1H, s, Ar-H)

(S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-3-(4-hydroxy-phenyl)-propionic acid (107)

LCMS purity 90%, m/z 505 $[M^+ + H]^+$, ^1H NMR (300 MHz, MeOD), δ: 3.09-3.27 (2H, m, CH2), 4.03 (1H, m, CH), 4.24 (2H, s , CH2), 4.34 (2H, s, CH2), 4.42 (2H, s, CH2), 6.76 (2H, d J = 8.3 Hz, Ar-H), 7.11 (2H, d J = 8.5 Hz, Ar-H), 7.56-7.59 (5H, m, Ar-H), 7.89 (1H, s, Ar-H), 8.02 (1H, d, J = 8.4 Hz, Ar-H), 8.08 (1H, s, Ar-H)

(S)-3-tert-Butoxy-2-(4-{[(2-hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-butyric acid cyclopentyl ester (108)

LCMS purity 99%, m/z 568 $[M^+ + H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.20 (9H, s, 3 x CH_3), 1.29 (3H, d, J = 6.6 Hz, CH_3), 1.67-1.94 (8H, m, 4 x CH_2), 3.75 (1H, d, J = 2.7 Hz, CH), 4.29-4.32 (1H, m, CH), 4.36 (4H, d, J = 2.4 Hz, 2 x CH_2), 4.43 (2H, s, CH_2), 5.22-5.25 (1H, m, CH), 7.54-7.65 (5H, m, Ar-H), 7.88 (1H, s, Ar-H), 8.01 (1H, d, J = 8.1 Hz, Ar-H), 8.08 (1H, s, Ar-H)

(S)-3-tert-Butoxy-2-(4-{[(2-hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-propionic acid cyclopentyl ester (109)

LCMS purity 95%, m/z 554 $[M^+ + H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.23 (9H, s, 3x CH_3), 1.67-1.98 (8H, m, 4 x CH_2), 3.87-3.98 (2H, m, CH_2), 4.19-4.22 (1H, m, CH), 4.34-4.36 (4H, m, 2xCH_2), 4.42 (2H, s, CH_2), 5.31-5.35 (1H, m, CH), 7.54-7.62 (5H, m, Ar-H), 7.87 (1H, s, Ar-H), 8.00 (1H, d, J = 8.1 Hz, Ar-H), 8.08 (1H, s, Ar-H)

(S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-4-methylsulfanyl-butyric acid cyclopentyl ester (110)

LCMS purity 90%, m/z 541 $[M^+ + H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.70-1.98 (8H, m, 4 x CH_2), 2.11 (3H, s, CH_3), 2.17-2.34 (2H, m, CH_2), 2.54-2.73 (2H, m, CH_2), 4.19-4.23 (1H, m, CH), 4.27-4.42 (6H, m, 3 x CH_2), 5.35-5.39 (1H, m, CH), 7.54-7.63 (5H, m, Ar-H), 7.87 (1H, s, Ar-H), 7.97-8.00 (1H, m, Ar-H), 8.08 (1H, s, Ar-H)

(S)-1-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzyl)-pyrrolidine-2-carboxylic acid cyclopentyl ester (111)

LCMS purity 96%, m/z 508 $[M^+ + H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.66-2.29 (12H, m, 6 x CH_2), 3.59-3.67 (2H, m, CH_2), 4.38-4.46 (6H, m, 3 x CH_2), 4.62 (1H, d, J = 12.3 Hz, CH), 5.21 (1H, m, CH), 7.61-7.68 (5H, m, Ar-H), 7.88 (1H, s, Ar-H), 8.00-8.02 (1H, m, Ar-H), 8.13 (1H, s, Ar-H)

(S)-1-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzyl)-pyrrolidine-2-carboxylic acid (112)

LCMS purity 100%, m/z 440 $[M^+ + H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.96-2.09 (2H, m, CH_2), 2.14-2.23 (2H, m, CH_2), 2.52-2.65 (1H, m, CH_2), 3.56-3.67 (1H, m, CH_2), 4.19-4.25 (1H, m, CH), 4.36-4.57 (6H, m, 3 x CH_2), 7.55-7.64 (5H, m, Ar-H), 7.88 (1H, s, Ar-H), 7.99-8.01 (1H, m, Ar-H), 8.09 (1H, s, Ar-H)

(R)-3-tert-Butylsulfanyl-2-(4-{[(2-hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-propionic acid cyclopentyl ester(113)

LCMS purity 97%, m/z 570 $[M+H]^+$, ^1H NMR (300MHz, MeOD), δ: 1.36(9H, s), 1.79 (8H, m), 3.16 (2H, d,5.3Hz), 4.25(2H, t, J=5.6Hz), 4.31(2H, s), 4.36(2H,s), 4.42(2H, s), 5.34(1H, m), 7.56(1H, d, J=8.1Hz), 7.62(4H, s), 7.89(1H, s), 8.09 (1H, s), 8.51(1H, d, J=8.1Hz),

(R)-3-tert-Butylsulfanyl-2-(4-{[(2-hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-propionic acid (114)

LCMS purity 97%, m/z 502 $[M]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.35 (9H, s, 3 x CH_3), 3.09 (2H, m, CH_2), 3.22 (2H, m, CH_2), 3.83 (1H, t, J = 8.8 Hz, CH), 4.34 (2H, s, CH_2), 4.42 (2H, s, CH_2), 7.57 (1H, d, J = 10.0 Hz, ArH), 7.62 (4H, s, ArH x 4), 7.89 (1H, s, ArH), 8.02 (1H, d, J = 8.1 Hz, ArH), 8.09 (1H, s, ArH).

(S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-3,3-dimethyl-butyric acid cyclopentyl ester (115)

LCMS purity 94%, m/z 546 $[M+Na]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.08 (9H, s, 3 x CH_3), 1.80 (8H, m, 4 x CH_2), 3.49 (1H, s, CH), 4.29 (2H, d, J = 13.5 Hz, CH_2), 4.29 (2H, d, J = 13.5 Hz, CH_2), 4.36 (2H, s, CH_2), 4.44 (2H, s, CH_2), 5.19 (1H, t, J = 5.7Hz), 7.59 (5H, m, ArH x 5), 7.88 (1H, ArH), 8.00 (1H, d, J = 8.3Hz), 8.09 (1H, s, ArH).

(S)-Cyclohexyl-(4-{[(2-hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-acetic acid cyclopentyl ester (116)

LCMS purity 100%, m/z 550 $[M+H]^+$, ^1H NMR (300 MHz, MeOD), δ: 0.86-1.95 (18H, m, 9xCH2), 3.73 (1H, m, CH), 4.11 (1H, d J = 5.7 Hz, CH), 4.19 (2H, s, CH2), 4.26 (2H, s, CH2), 4.36 (2H, s, CH2), 7.53 (5H, m, ArH), 7.77 (1H, s, CH), 7.82 (1H, d J = 11.6 Hz, ArH), 8.02 (1H, s, ArH)

(S)-Cyclohexyl-(4-{[(2-hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)- amino]-methyl}-benzylamino)-acetic acid (117)

LCMS purity 100%, m/z 482 $[M+H]^+$, ^1H NMR (300 MHz, MeOD), δ: 0.72-1.60 (10H, m, 9xCH2), 3.89 (1H, m, CH), 4.11 (3H, m, CH), 4.23 (2H, s, CH2), 4.31 (2H, s, CH2), 7.48 (5H, m, ArH), 7.76 (1H, s, CH), 7.88 (1H, d J = 11.6 Hz, ArH), 7.98 (1H, s, ArH).

(S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-4-methyl-pentanoic acid cyclopentyl ester (122)

LCMS purity 94%, m/z 524 $[M^+H]^+$, 1H NMR (300 MHz, MeOD), δ: 1.01 (6H, s, 2xCH3), 1.28 (1H, m, CH), 1.56-1.95 (10H, m, 4xCH2, CH2), 4.00 - 4.43 (6H, m, 3xCH2), 4.88 (1H, m, CH), 5.36 (1H, br s, CH), 7.47-7.62 (5H, m, ArH), 7.94 (2H, t, ArH), 8.08 (1H, s, ArH).

(S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-4-methyl-pentanoic acid (123)

LCMS purity 98%, m/z 456 $[M^+H]^+$, 1H NMR (300 MHz, MeOD), δ: 1.00 (6H, m, 2xCH3), 1.86 (2H, m , CH2), 3.86 (1H, m, CH), 4.29 (2H, s, CH2), 4.36 (2H, s, CH2), 4.43 (2H, s, CH2), 7.56 (1H, m, ArH), 7.89 (1H, s, CH), 8.02 (1H, d J=8.2 Hz, ArH), 8.09 (1H, s, ArH).

(S)-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-phenyl-acetic acid cyclopentyl ester (124)

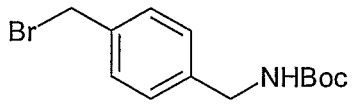
LCMS purity 90%, m/z 544 $[M^+H]^+$, 1H NMR (300 MHz, MeOD), δ: 1.31-1.91 (10H, m, 4xCH2, CH2), 4.22 (2H, dd J=13.1 Hz, CH2), 4.35 (2H, s, CH2), 4.42 (1H, s, CH2), 5.16 (1H, s, CH), 5.30 (1H, m, CH), 7.47-7.62 (9H, m, ArH), 7.94 (2H,m, ArH), 8.08 (1H, s, ArH).

(S)-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-phenyl-acetic acid (125)

LCMS purity 100%, m/z 476 $[M^+H]^+$, 1H NMR (300 MHz, MeOD), δ: 4.08 - 4.24 (3H, m, CH, CH2), 4.35 (2H, s, CH2), 4.43 (1H, s, CH2), 7.46-7.75 (10H, m, ArH), 7.89 (1H, s, ArH), 8.01 (1H, d J=7.9 Hz, ArH), 8.09 (1H, s, ArH)

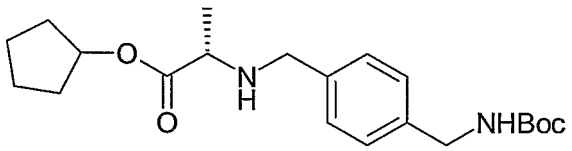
The following compounds were prepared according to the procedure described for compound **(104)** and compound **(105)** using alternatives for step 3 and 4 as outlined below

Step 3b: (4-Bromomethyl-benzyl)-carbamic acid tert-butyl ester



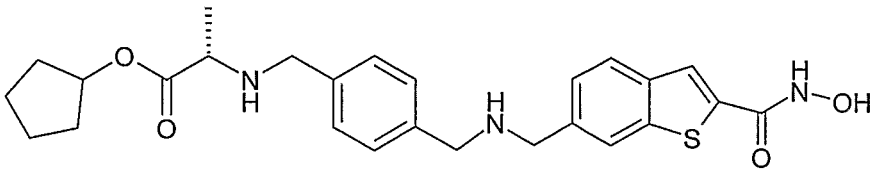
N-Bromosuccinimide (5.13 g, 28.8 mmol) was dissolved in DCM (80 mL) and cooled to 0 °C. A solution of triphenylphosphine (7.18 g, 27.0 mmol) in DCM (20 mL) was prepared and added to the chilled solution followed by pyridine (1.0 mL, 1.26 mmol). Material from step 2 (2.14 g, 9.0 mmol) was dissolved in DCM (20 mL) and added and the reaction allowed to warm to r.t. and stirred for 16 h. The mixture was concentrated and the residue purified by column chromatography (50%/50% EtOAc/heptane) to afford the desired compound (864 mg, 32%). m/z 301 $[M^+ + Na]^+$

Step 4b: (S)-2-[4-(tert-Butoxycarbonylamino-methyl)-benzylamino]-propionic acid cyclopentyl ester



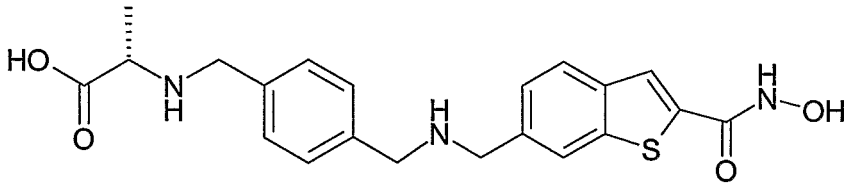
L-Alanine cyclopentyl ester (463 mg, 1.41 mmol) was dissolved in DMF (9 mL) and to this was added DIPEA (0.74 mL, 4.24 mmol). The mixture was stirred at r.t. for 15 min and then a solution of material from step 3b (212 mg, 0.706 mmol) in DMF (5 mL) added dropwise over 1 hr. The reaction was then allowed to stir at r.t. for 16 hr and was then diluted with water (50 mL) and EtOAc (50 mL). The organic layer was washed with brine (2 x 50 mL), dried and concentrated to give crude material (0.26 g, 100%) which was taken to the next step without further purification. m/z 377 $[M^+ + Na]^+$

Steps 5 – 9 were as described for compound **(104)** and compound **(105)**



(S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-propionic acid cyclopentyl ester (118)

LCMS purity 95%, m/z 482 $[M^+ + H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.61 (3H, d, J = 7.2 Hz, CH_3), 1.71-1.97 (8H, m, 4 x CH_2), 4.13 (1H, q, J = 7.2 Hz, CH), 4.30 (2H, s, CH_2), 4.36 (2H, s, CH_2), 4.43 (2H, s, CH_2), 5.33-5.36 (1H, m, CH), 7.54-7.63 (5H, m, Ar-H), 7.88 (1H, s, Ar-H), 8.00 (1H, d, J = 8.1 Hz, Ar-H), 8.08 (1H, s, Ar-H)

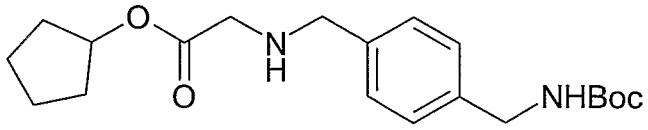


(S)-2-(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-propionic acid (119)

LCMS purity 95%, m/z 414 $[M^+ + H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.64 (3H, d, J = 7.1 Hz,CH_3), 4.06 - 4.14(1H, m,CH), 4.31 (2H, s,CH_2), 4.36 (2H, s, CH_2), 4.43 (2H, s, CH_2), 7.55-7.63 (5H, m, Ar-H), 7.88 (1H, s, Ar-H), 8.00 (1H, d, J = 8.1 Hz, Ar-H), 8.09 (1H, s, Ar-H)

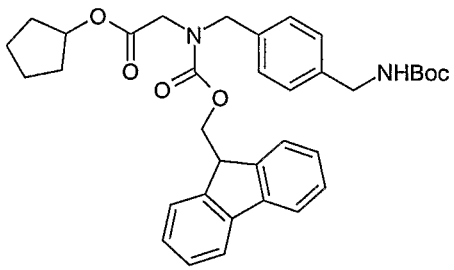
The following compounds were prepared according to the procedures outlined for compound **(118)** and compound **(119)** incorporating the following alternative/ additional steps

Step 4b: [4-(tert-Butoxycarbonylamino-methyl)-benzylamino]-acetic acid cyclopentyl ester



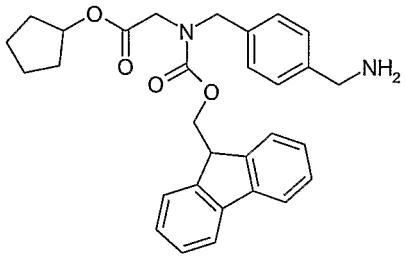
Procedure as in step 4a (using the HCl salt of the cyclopentyl ester)
Product: m/z 363 $[M^+ + H]^+$

Step 4c: [[4-(tert-Butoxycarbonylamino-methyl)-benzyl]-(9H-fluoren-9-ylmethoxy-carbonyl)-amino]-acetic acid cyclopentyl ester



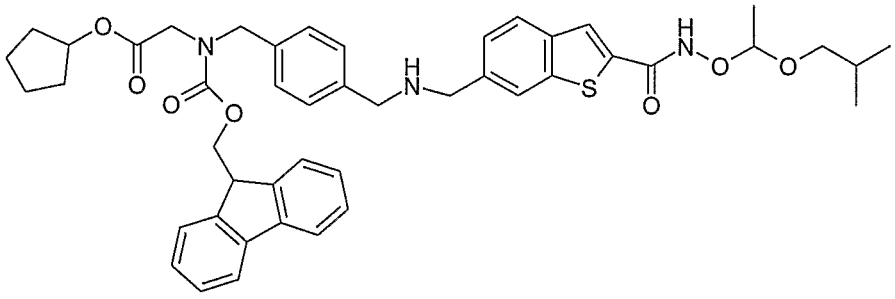
To a solution of [4-(tert-Butoxycarbonylamino-methyl)-benzylamino]-acetic acid cyclopentyl ester (0.2 g, 0.55 mmol) and 1 M Na_2CO_3 (1.1 mL, 1.1 mmol) in DCM (2 mL), was added slowly with stirring and ice bath cooling, a solution of 9-Fluorenylmethyl chloroformate (0.14 g, 0.55 mmol) in dioxane (1.4 mL). The mixture was stirred in the ice bath for 4 h and at room temperature overnight. The mixture was poured into water (90 mL) and extracted with diethyl ether. The organic extracts were combined, dried ($MgSO_4$) and evaporated to dryness to afford the desired product (0.32 g, 100%). m/z 607 $[M^+ + Na]^+$

Step 5a: [(4-Aminomethyl-benzyl)-(9H-fluoren-9-ylmethoxycarbonyl)-amino]-acetic acid cyclopentyl ester



Procedure as described in step 5.
Product m/z 485 $[M^+ + H]^+$

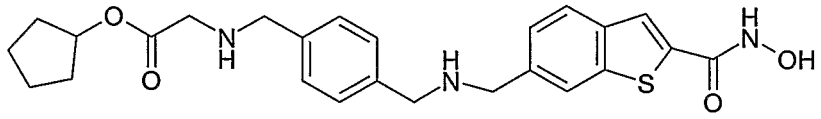
Step 6a: {(9H-Fluoren-9-ylmethoxycarbonyl)-[4-({[2-(1-isobutoxy-ethoxycarbamoyl)-benzo[b]thiophen-6-ylmethyl]-amino}-methyl)-benzyl]-amino}-acetic acid cyclopentyl ester



Procedure as described in step 6.

Product m/z 790 $[M^+ + H]^+$

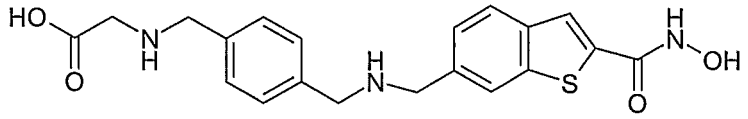
Step 7a: (4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-acetic acid cyclopentyl ester **(120)**



120

{(9H-Fluoren-9-ylmethoxycarbonyl)-[4-({[2-(1-isobutoxy-ethoxycarbamoyl)-benzo[b]thiophen-6-ylmethyl]-amino}-methyl)-benzyl]-amino}-acetic acid cyclopentyl ester (0.11 g, 0.14 mmol) was dissolved in acetonitrile (3 mL) and to it was added piperidine (1.5 mL). The resulting mixture was stirred at room temperature for 1 h. The solvent was evaporated to dryness and the product separated into 2 portions. One portion was taken through to hydrolysis of the cyclopentyl ester, while the second portion was dissolved in DCM (1.5 mL) and stirred with 4M HCl in dioxane (1.0 mL) for 2 h. The solvent was evaporated to dryness and the product purified by preparative HPLC to afford the desired product as a TFA salt. LCMS purity 99%, m/z 468 $[M^+ + H]^+$, ^{1}H NMR (300 MHz, MeOD), δ: 8.09 (1H, s, ArH), 8.00 (1H, d, J = 8.3 Hz, ArH), 7.88 (1H, s, ArH), 7.54-7.65 (5H, m, ArH), 5.31-5.35 (1H, m, CH), 4.43 (2H, s, CH_2), 4.35 (2H, s, CH_2), 4.31 (2H, s, CH_2), 3.96 (2H, s, CH_2), 1.65-1.93 (8H, m, 4 x CH_2)

Step 9a: (4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-benzylamino)-acetic acid **(121)**

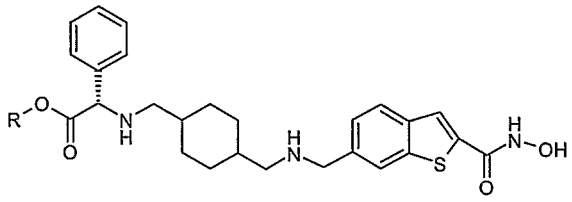


121

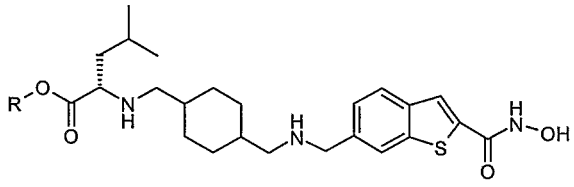
Procedure as described in step 9.

LCMS purity 99%, m/z 400 $[M^+ + H]^+$, ^1H NMR (300 MHz, MeOD), δ: 8.09 (1H, s, ArH), 8.00 (1H, d, J = 8.3 Hz, ArH), 7.89 (1H, s, ArH), 7.54-7.62 (5H, m, ArH), 4.43 (2H, s, CH_2), 4.35 (2H, s, CH_2), 4.32 (2H, s, CH_2), 3.93 (2H, s, CH_2)

Synthesis of Compounds in Figure 8 Exemplified by Compound (126) and Compound (127)



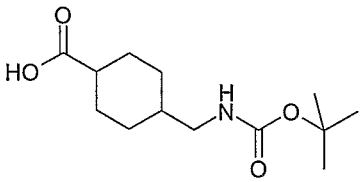
R= cyclopentyl 126
R= H 127



R= cyclopentyl 128
R= H 129

Figure 8

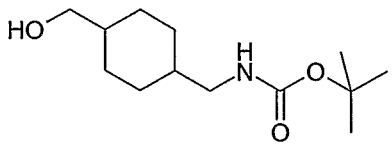
Stage 1: 4-(tert-Butoxycarbonylamino-methyl-cyclohexanecarboxylic acid



A solution of trans-4-(aminomethyl)cyclohexane carboxylic acid (1g, 6.4mmol) and sodium hydroxide (256mg, 6.4mmol) in 40ml of dioxane and 40ml of water was cooled in an ice-water bath while stirring. Di-*tert*-butyl dicarbonate (1.39g, 6.4mmol)

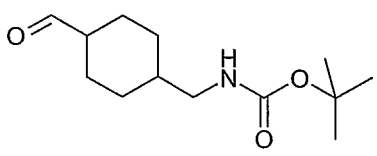
was added and the mixture stirred at r.t. for 5 hours and left standing overnight. The solution was concentrated *in vacuo* and acidified with 2N HCl to pH 2. The acidified aqueous layer was extracted 3 times with EtOAc. The organic layers were pooled and washed with brine. The organic layer was dried over magnesium sulfate and evaporated to dryness. The product was obtained as a white solid (1.1g, 64% yield). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 0.86 - 1.07 (2 H, m, CH_2), 1.34 - 1.53 (11 H, m, boc and CH_2), 1.84 (2 H, dd, *J*=13.0, 2.3 Hz, CH_2), 2.05 (2 H, dd, CH_2), 2.18 - 2.35 (1 H, m, C<u>H</u>CH_2), 2.99 (2 H, t, *J*=6.3 Hz, C<u>H</u>$_2$NH), 4.59 (1 H, br. s, C<u>H</u>COOH), 11.0 (1 H, br. s, COOH).

Stage 2: (4-Hydroxymethyl-cyclohexylmethyl)-carbamic acid tert-butyl ester



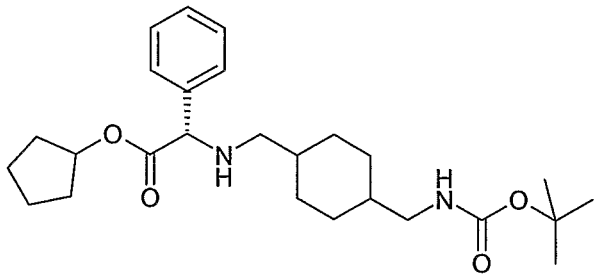
Lithium aluminium hydride (465mg, 12.2mmol) was suspended in anhydrous THF (10ml) and cooled down to 0°C under N_2 atmosphere. A solution of 4-(tert-butoxy-carbonyl-amino-methyl-cyclohexanecarboxylic acid (1.1g, 4.1mmol) in THF and dioxane (10ml, 1:1) was added slowly and the mixture was stirred overnight at room temperature. Excess lithium aluminium hydride was quenched by adding water dropwise. The cake was filtered and washed with THF (10ml) and MeOH (10ml). The filtrate was concentrated *in vacuo* and acidified with 1N HCl to pH 2. The aqueous was extracted twice with EtOAc. The organic layer was dried over magnesium sulfate, filtered and evaporated to dryness to yield 964mg of product (97% yield). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 0.81-1.08 (4 H, m, 2 x CH_2), 1.33-1.60 (10 H, m, boc and CH), 1.82 (4 H, d, *J*=5.7 Hz, 2 x CH_2), 2.98 (2 H, t, *J*=6.4 Hz, C<u>H</u>$_2$NH), 3.46 (2 H, d, *J*=6.4 Hz, C<u>H</u>$_2$OH), 4.60 (1 H, br. s, CH)

Stage 3: (4-Formyl-cyclohexylmethyl)-carbamic acid tert-butyl ester



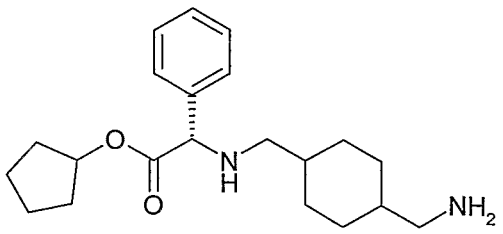
(4-Hydroxymethyl-cyclohexylmethyl)-carbamic acid tert-butyl ester (965mg, 4.0mmol) was dissolved in DCM (20ml) and cooled down to -78°C. Dess Martin reagent (2.52g, 6.0mmol) was dissolved in DCM (30ml) and added slowly to the stage 2 alcohol in solution. The reaction mixture was then stirred at r.t. for 3h. The resulting solution was poured into a vigorously stirred saturated $NaHCO_3$ and $Na_2S_2O_3$ solution (1:1, 100ml). The organic layer was separated and washed with brine, dried over magnesium sulfate and evaporated to dryness to yield the product (786mg, 82% yield). ^1H NMR (300 MHz, $CDCl_3$), δ: 0.83 - 1.01 (2 H, m, CH_2), 1.15 - 1.24 (2 H, m, CH_2), 1.34 (9 H, s, Boc), 1.75 - 1.88 (2 H, m, CH_2), 1.90 - 2.00 (2 H, m, CH_2), 2.05 - 2.18 (1 H, m, CH), 2.93 (2 H, t, *J*=6.4 Hz, $C\underline{H}_2NH$), 4.53 (1 H, br. s, $C\underline{H}CHO$), 9.55 (1 H, s, CHO)

Stage 4: (S)-{[4-(tert-Butoxycarbonylamino-methyl)-cyclohexylmethyl]-amino}-phenyl-acetic acid cyclopentyl ester



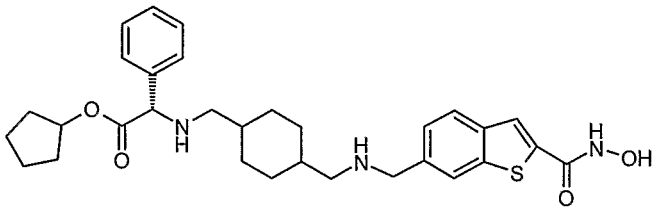
(4-Formyl-cyclohexylmethyl)-carbamic acid tert-butyl ester (390mg, 1.6mmol) and (S)-amino-phenyl-acetic acid cyclopentyl ester (394mg, 1.8mmol) were stirred in DCE (6ml) at r.t. for 25min. Acetic acid (9.6ul, 0.16mmol) and sodium triacetoxy-borohydride (1.0g, 4.8mmol) were added and the resulting mixture was stirred for 1h30 at r.t. DCM (10ml) and a saturated solution of $NaHCO_3$ (10ml) were added and phases were separated. Aqueous were extracted with EtOAc (2 x 10ml), the organics were dried over magnesium sulfate, filtered and evaporated to dryness. The crude product was purified by column chromatography (8:2 heptane / EtOAc) to yield 223mg of the pure amine (31% yield). LCMS purity 100%, m/z 445 $[M^+ + H]^+$.

Stage 5: (S)-[(4-Aminomethyl-cyclohexylmethyl)-amino]-phenyl-acetic acid cyclopentyl ester



(S)-{[4-(tert-Butoxycarbonylamino-methyl)-cyclohexylmethyl]-amino}-phenyl-acetic acid cyclopentyl ester (223mg, 0.5mmol) was stirred in DCM (4ml), TFA (1ml) was added and the mixture was stirred at r.t, for 2h. The solution was concentrated *in vacuo*, taken up in DCM, washed twice with a saturated solution of $NaHCO_3$ and once with a saturated solution of brine.The organic phase was dried over magnesium sulfate, filtered and evaporated to yield the expected amine as a yellow oil (130mg, 75% yield). LCMS purity 100%, m/z 345 $[M^+ + H]^+$.

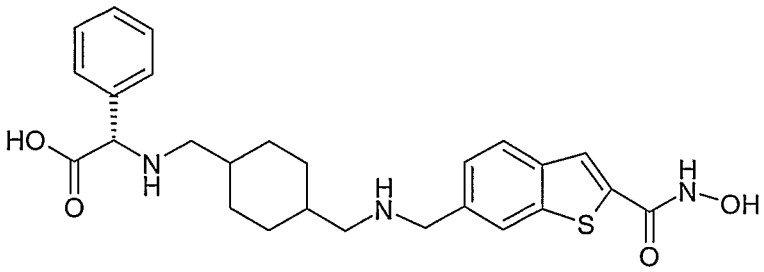
Stage 6: (S)-[(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-cyclohexylmethyl)-amino]-phenyl-acetic acid cyclopentyl ester **(126)**



126

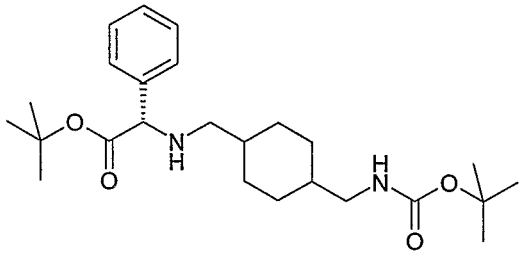
Stage 5 amine (130mg, 0.38mmol) was stirred with 6-formyl-benzo[b]thiophene-2-carboxylic acid (1-isobutoxy-ethoxy) amide (Scheme 7) (110mg, 0.34mmol) in DCE for 30 min at r.t.. Acetic acid (2.1ul, 0.03mmol) and sodium triacetoxyborohydride (218mg, 1.0mmol) were added and the resulting mixture was stirred overnight at r.t. The mixture was concentrated *in vacuo*, taken up in EtOAc, washed with a saturated solution of $NaHCO_3$ (10ml) and brine (10ml). The organics were dried over magnesium sulfate, filtered and evaporated to dryness. The crude product (167mg) was purified by preparative HPLC to yield compound **(126)** as a light pink solid. LCMS purity 86%, m/z 550 $[M^+ + H]^+$, *ca.* 10% carboxylic acid. ^1H NMR (300 MHz, MeOD), δ: 1.08 (4 H, m, 2 x CH_2), 1.76 (14 H, m, 6 x CH_2 and 2 x CH), 2.81 (4 H, m, 2 x $C\underline{H}_2NH$), 4.35 (2 H, s, $C\underline{H}_2NH$), 5.12 (1 H, s, $C\underline{H}NH$), 5.30 (1 H, m, OCH), 7.51 (6 H, m, Ar), 7.85 (1 H, s, Ar), 7.96 (1 H, d, Ar), 8.08 (1 H, s, Ar).

Synthesis of ((S)-[(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-cyclohexylmethyl)-amino]-phenyl-acetic acid (127)



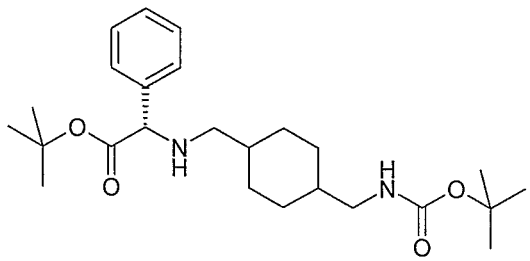
127

Stage 1: (S)-{[4-(tert-Butoxycarbonylamino-methyl)-cyclohexylmethyl]-amino}-phenyl-acetic acid tert-butyl ester



(4-Formyl-cyclohexylmethyl)-carbamic acid *tert*-butyl ester (899mg, 3.7mmol) and (S)-*tert*-butyl phenylglycine ester (850mg, 4.1mmol) were stirred in DCE (20ml) for 30min. Acetic acid (20ul, 0.37mmol) and sodium triacetoxyborohydride (2.37g, 11.1mmol) were added and the reaction mixture was stirred at r.t. for 3h. DCM (10ml) and a saturated solution of $NaHCO_3$ (20ml) were added and phases were separated. The aqueous phase was extracted with EtOAc (20ml). The organics were dried over magnesium sulfate, filtered and concentrated under vacuum. The crude product (2.4g) was purified on column chromatography (7:3 heptane / EtOAc) to yield the expected product (385mg, 24% yield). LCMS purity 100%, m/z 433 $[M^+ + H]^+$.

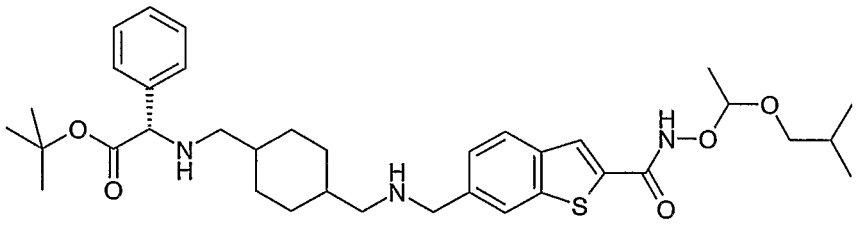
Stage 2: (S)-{[4-(tert-Butoxycarbonylamino-methyl)-cyclohexylmethyl]-amino}-phenyl-acetic acid tert-butyl ester



(S)-{[4-(tert-Butoxycarbonylamino-methyl)-cyclohexylmethyl]-amino}-phenyl-acetic acid tert-butyl ester (385mg, 0.9mmol) was stirred in DCM (5ml) and TFA (2ml) was added and the mixture was stirred at r.t. for 30min. The solution was concentrated *in*

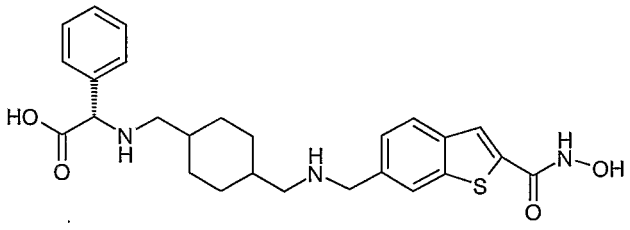
vacuo, taken up in EtOAc (5ml), washed twice with a saturated solution of $NaHCO_3$ (2 x 5ml) and once with a saturated solution of brine (5ml). The organic phase was dried over magnesium sulfate, filtered and evaporated to yield the expected amine as a yellow oil (290mg, 97% yield). LCMS purity 100%, m/z 333 $[M^+H]^+$.

Stage 3: (S)-{[4-({[2-(1-Isobutoxy-ethoxycarbamoyl)-benzo[b]thiophen-6-ylmethyl]-amino}-methyl)-cyclohexylmethyl]-amino}-phenyl-acetic acid tert-butyl ester



(S)-{[4-(tert-Butoxycarbonylamino-methyl)-cyclohexylmethyl]-amino}-phenyl-acetic acid tert-butyl ester (290mg, 0.9mmol) and 6-formyl-benzo[b]thiophene-2-carboxylic acid (1-isobutoxy-ethoxy) amide (Scheme 7) (255mg, 0.8mmol) were stirred in DCE (8ml) for 30min. Acetic acid (4ul, 0.08mmol) and sodium triacetoxyborohydride (504mg, 2.4mmol) were added and the reaction mixture was stirred at r.t. for 1h30. DCM (5ml) and a saturated solution of $NaHCO_3$ (10ml) were added and phases were separated. The aqueous phase was extracted with EtOAc (15ml). The organics were dried over magnesium sulfate, filtered and concentrated under vacuum. The crude product (543mg) was purified on column chromatography (5 to 10% MeOH in DCM) to yield the expected pure product (172mg, 34% yield). LCMS purity 100%, m/z 638 $[M^+H]^+$.

Stage 4: (S)-{[4-({[2-(1-Isobutoxy-ethoxycarbamoyl)-benzo[b]thiophen-6-ylmethyl]-amino}-methyl)-cyclohexylmethyl]-amino}-phenyl-acetic acid tert-butyl ester **(127)**



127

(S)-{[4-({[2-(1-Isobutoxy-ethoxycarbamoyl)-benzo[b]thiophen-6-ylmethyl]-amino}-methyl)-cyclohexylmethyl]-amino}-phenyl-acetic acid tert-butyl ester (172mg, 0.27mmol) was stirred in 4M HCl in dioxane solution (2ml) at r.t. for 30min. The solution was evaporated to dryness to yield compound **(127)** as a beige solid (123mg, 95% yield). LCMS purity 98%, m/z 482 $[M^+H]^+$. ^1H NMR (300 MHz, MeOD), δ: 1.10 (4 H, m, 2 x CH_2), 1.80 (6 H, m, 2 x CH_2 and 2 x CH), 2.88 (2 H, dd, C$\underline{H_2}$NH), 2.94 (2 H, d, C$\underline{H_2}$NH), 4.36 (2 H, s, C$\underline{H_2}$NH), 5.07 (1 H, s, CH), 7.53 (6 H, m, Ar), 7.87 (1 H, s, Ar), 7.97 (1 H, d, Ar), 8.12 (1 H, s, Ar).

The following compounds were prepared according to the procedure described for Compound **(126)** and Compound **(127)**

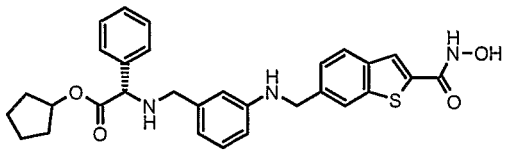
(S)-2-[(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-cyclohexylmethyl)-amino]-4-methyl-pentanoic acid cyclopentylester (128)

LCMS purity 86%, m/z 530 $[M^+H]^+$, ^1H NMR (300 MHz, MeOD), δ: 0.99 (6 H, t, J= 6Hz, 2 x CH_3), 1.11 (4 H, t, J= 8.1 Hz, 2 x CH_2), 1.76 (20 H, m, 2 x CH and 9 x CH_2), 2.80 (1 H, m, CH), 2.97 (4 H, m, 2 x C$\underline{H_2}$NH), 3.95 (1 H, m, CH), 4.35 (2 H, s, C$\underline{H_2}$NH), 5.32 (1 H, m, OCH), 7.54 (1 H, d, J= 6 Hz, Ar), 7.84 (1 H, s, Ar), 7.94 (1 H, d, J=9 Hz, Ar), 8.08 (1 H, s, Ar).

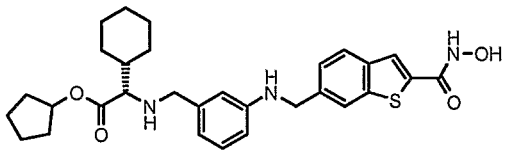
(S)-2-[(4-{[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-methyl}-cyclohexylmethyl)-amino]-4-methyl-pentanoic acid (129)

LCMS purity 95%, m/z 462 $[M^+H]^+$, ^1H NMR (300 MHz, MeOD), δ: 1.00 (6 H, t, J=6.3 Hz, 2 x CH_3), 1.10 (4 H, m, 2 x CH_2), 1.85 (8 H, m, 2 x CH and 3 x CH_2), 2.94 (4 H, m, 2 x C$\underline{H_2}$NH), 3.91 (1 H, m, C$\underline{H}$NH), 4.36 (2 H, s, C$\underline{H_2}$NH), 7.54 (1 H, d, J=7.8 Hz, Ar), 7.85 (1 H, s, Ar), 7.95 (1H, d, J=8.1 Hz, Ar), 8.08 (1H, s).

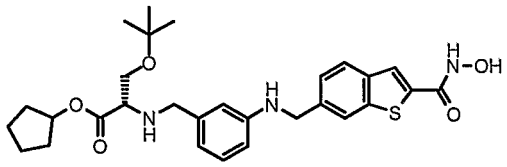
Synthesis of Compounds in Figure 9 as Exemplified for Compound (130)



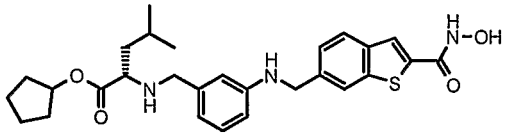
130



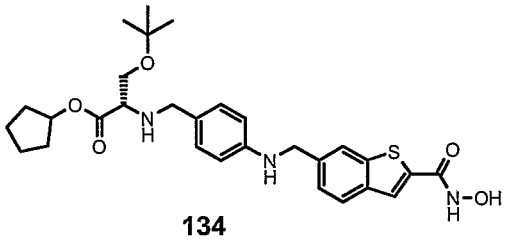
131



132

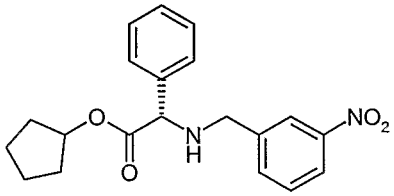


133



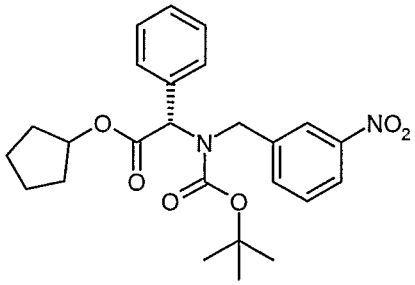
134

Stage 1: (S)-[(3-nitro-benzyl) -amino]-phenyl-acetic acid cyclopentyl ester



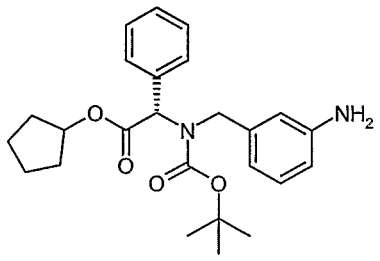
To a solution of phenylglycine cyclopentyl ester tosic acid salt (3.08 g, 7.8 mmol) in DCE (120 ml) was added 3-nitrobenzaldehyde (1.01 g, 6.7 mmol) then sodium triacetoxy-borohydride (3.03 g). The mixture was stirred for 3.5h, then quenched by addition of saturated sodium bicarbonate solution (200 ml). Product was extracted with DCM (250 ml) and the organic extract was dried ($MgSO_4$). The product was carried forward without further purification.

Stage 2: (S)-[(3-nitro-benzyl)-tert-butoxycarbonyl-amino]-phenyl-acetic acid cyclopentyl ester



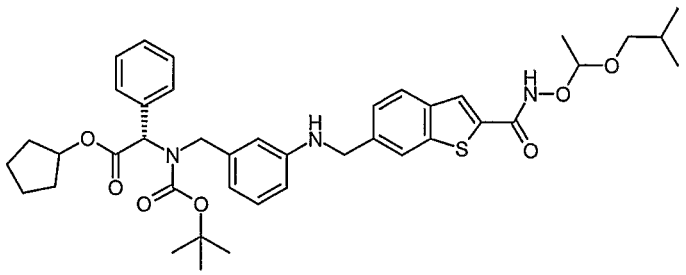
To the crude mixture of (S)-[(3-nitro-benzyl) -amino]-phenyl-acetic acid cyclopentyl ester in DCM (50 ml) was added di-ter-butyl dicarbonate (3.38 g, 15.6 mmol). The mixture was heated at 50°C overnight, then cooled to rt. N,N,N'-trimethylethylene diamine (2 ml) was then added and the mixture stirred for 2h. The mixture was then poured into ethyl acetate (150 ml) and washed with 1M HCl (3 times 50 ml), dried ($MgSO_4$) and concentrated to yield the desired product as a colourless oil (1.509 g, 42% yield). LCMS purity 98%, m/z 477 ($M+Na^+$). ^1H NMR (300MHz, d6-DMSO), δ: 7.97 (1H, dd, J=2.1, 9 Hz), 7.15-7.45 (8H, m), 5.60-6.00 (1H, m), 5.20-5.35 (1H, m), 4.65-4.82 (1H, m), 4.21 (1H, d, J=16 Hz), 1.30-1.95 (17H, m)

Stage 3: (S)-[(3-Amino-benzyl)-tert-butoxycarbonyl-amino]-phenyl-acetic acid cyclopentyl ester



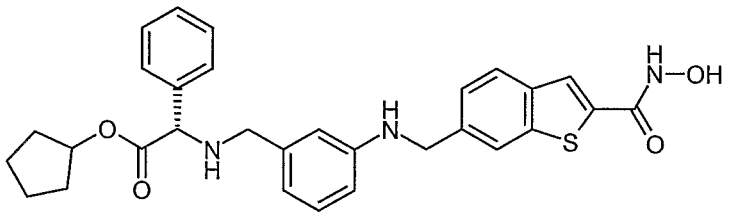
To a solution of (S)-[(3-nitro-benzyl)-tert-butoxycarbonyl-amino]-phenyl-acetic acid cyclopentyl ester (1.509 g, 3.32 mmol) in ethanol (10 ml) was added palladium on carbon (10%, 0.38g, 0.36 mmol). The flask was evacuated and back-filled with hydrogen gas. The mixture was stirred overnight, then filtered through Celite, washed with ethanol (150 ml) and then concentrated to yield the desire product as a colourless oil (1.351 g, 96% yield). ^1H NMR (300 MHz, $CDCl_3$), δ:7.19-7.42 (5H, m), 6.97 (1H, t, J=7.5 Hz), 6.46 (2H, dd, J=8.1, 16.5 Hz), 6.29 (1H, br s), 5.58 (1H,br s), 5.29 (1H, br s), 4.69 (1H, br s), 4.00 (1H, d, J=15.9 Hz), 3.74 (1H, q, J=6.9 Hz), 3.51 (2H, br s), 1.20-2.00 (17H, m)

Stage 4: (S)-[tert-Butoxycarbonyl-(3-{[2-(1-isobutoxy-ethoxycarbamoyl)-benzo[b]-thiophen-6-ylmethyl]-amino}-benzyl)-amino]-phenyl-acetic acid cyclopentyl ester



To (S)-[(3-Amino-benzyl)-tert-butoxycarbonyl-amino]-phenyl-acetic acid cyclopentyl ester (0.317g, 0.75 mmol) was added 6-Formyl-benzo[b]thiophene-2-carboxylic acid (1-isobutoxy-ethoxy)-amide (Scheme 7) (0.210 g, 0.65 mmol) in DCE (8 ml). 2 drops of glacial acetic acid were added, and then sodium triacetoxyborohydride (0.170 g, 0.8 mmol). The mixture was stirred for 2h and then poured into DCM (150 ml). The solution was washed with saturated sodium bicarbonate (50 ml), then dried ($MgSO_4$), concentrated and purified by flash column chromatography to yield the desired product as a pale yellow foam (0.346 g, 73% yield). ^1H NMR (300 MHz, $CDCl_3$), δ: 8.35-8.43 (1H, m), 7.56-8.05 (2H, m), 7.01-7.41 (8H, m), 6.90-7.01 (1H, m), 6.42 (1H, dd, J=2.6, 7.9 Hz), 5.25-5.31 (1H, m), 5.12 (1H, q, J=5.2 Hz), 4.40 (1H, d, J 5.4 Hz), 4.00 (1H, dd, J=3.1, 15.8 Hz), 3.60-3.70 (1H, m), 3.35-3.40 (1H, m), 1.33-1.94 (21H, m), 0.98 (3H, d, J=6.6 Hz), 0.97 (3H, d, J=6.6 Hz)

Stage 5: (S)-{3-[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-benzyl-amino}-phenyl-acetic acid cyclopentyl ester **(130)**



130

To a solution of (S)-[tert-Butoxycarbonyl-(3-{[2-(1-isobutoxy-ethoxycarbamoyl)-benzo[b]thiophen-6-ylmethyl]-amino}-benzyl)-amino]-phenyl-acetic acid cyclopentyl ester (0.100 g, 0.14 mmol) in DCM/MeOH (1ml:1ml) was added TFA (8 ml). The solution was stirred for 2h, then diluted with DCM (200 ml). The solution was washed with saturated sodium bicarbonate (100 ml). The solution was dried (Na_2SO_4), concentrated and purified by reverse phase HPLC to yield the desired product (8.1

mg, 11% yield). LCMS purity 98%, m/z 531 $(M+H)^+$ 300 MHz, DMSO, δ:1.26-1.85 (8H, m), 3.47 (2H, s), 4.21 (1H, s), 4.38 (2H, d, J 5.8 Hz), 5.02-5.07 (1H, m), 6.32 (1H, t, J 6.0 Hz), 6.45 (2H, d, J 7.9 Hz), 6.57 (1H, s), 6.97 (1H, t, J 7.8 Hz), 7.23-7.35 (5H, m), 7.42 (1H, dd, J 1.2, 8.3 Hz), 7.84 (1H, s), 7.87 (1H, s), 7.93 (1H, s), 9.23 (1H, br s), 11.4 (1H, br s)

The following compounds were prepared according to the procedures described for compound **(130)**

(S)-{3-[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-benzylamino}-cyclohexyl-acetic acid cyclopentyl ester (131)

LCMS purity >98%, m/z 536.25 $(M+H)^+$, ^1H NMR (300 MHz, d6-DMSO), δ: 0.7-1.25 (8H, m), 1.50-1.95 (14H, m), 3.69 (1H, br s), 3.98 (2H, br s), 4.43 (2H, br s), 5.14 (1H, t, J 5.4 Hz), 6.60-6.71 (2H, m), 7.05-7.28 (3H, m), 7.43 (1H, d, J 8.5 Hz), 7.83-7.98 (2H, m), 9.25 (1H, br s), 11.45 (1H, br s)

(S)-3-tert-Butoxy-2-{3-[(2-hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-benzylamino}-propionic acid cyclopentyl ester (132)

LCMS purity >98%, m/z 540.25 $(M+H)^+$, ^1H NMR (300 MHz, d6-DMSO), δ: 1.05 (9H, s), 1.50-1.78 (8H, m), 3.15-3.67 (5H, m), 4.38 (2H, d, J 5.8 Hz), 5.05-5.15 (1H, m), 6.31 (1H, t, J 5.9 Hz), 6.40-6.48 (2H, m), 6.56 (1H, s), 6.96 (1H, t, J 7.8 Hz), 7.42 (1H, d, J 8.2 Hz), 7.87 (1H, s), 7.93 (1H, s), 9.24 (1H, br s), 11.43 (1H, br s)

(S)-2-{3-[(2-Hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-benzylamino}-4-methyl-pentanoic acid cyclopentyl ester (133)

LCMS purity 98%, m/z 510.25 $(M+H)^+$, ^1H NMR (300 MHz, d6-DMSO), δ: 0.87 (6H, d, J 6.4 Hz), 1.50-1.94 (10H, m), 3.57-4.20 (3H, m), 4.44 (2H, s), 5.20 (1H, t, J 5.8 Hz), 6.58-6.74 (3H, m), 7.11 (1H, t, J 7.7 Hz), 7.43 (1H, d J 8.3 Hz), 7.85-7.96 (3H, m), 9.38 (2H, br s), 11.46 (1H, br s)

(S)-3-tert-Butoxy-2-{4-[(2-hydroxycarbamoyl-benzo[b]thiophen-6-ylmethyl)-amino]-benzylamino}-propionic acid cyclopentyl ester (134)

LCMS purity 98%, m/z 540.25 $(M+H)^+$,^1H NMR (300 MHz, d4-MeOD), δ: 7.64-7.77 (2H, m), 7.28 (1H, d, J=7.2 Hz), 6.99 (2H, d, J=6.6 Hz), 6.53 (2H, d, J=6.6 Hz), 5.06-5.08 (1H, m), 4.35 (2H, s), 3.40-3.75 (5H, m), 1.50-1.82 (8H, m), 1.04 (9H, s)

Measurement of biological activities

Histone deacetylase activity

The ability of compounds to inhibit histone deacetylase activities was measured using the commercially available HDAC fluorescent activity assay from Biomol. In brief, the *Fluor de Lys™* substrate, a lysine with an epsilon-amino acetylation, is incubated with the source of histone deacetylase activity (HeLa nuclear extract) in the presence or absence of inhibitor. Deacetylation of the substrate sensitises the substrate to *Fluor de Lys™* developer, which generates a fluorophore. Thus, incubation of the substrate with a source of HDAC activity results in an increase in signal that is diminished in the presence of an HDAC inhibitor.

Data are expressed as a percentage of the control, measured in the absence of inhibitor, with background signal being subtracted from all samples, as follows:-

$$\% \text{ activity} = ((S^i - B) / (S^\circ - B)) \times 100$$

where S^i is the signal in the presence of substrate, enzyme and inhibitor, S° is the signal in the presence of substrate, enzyme and the vehicle in which the inhibitor is dissolved, and B is the background signal measured in the absence of enzyme.

IC50 values were determined by non-linear regression analysis, after fitting the results of eight data points to the equation for sigmoidal dose response with variable slope (% activity against log concentration of compound), using Graphpad Prism software.

Histone deacetylase activity from crude nuclear extract derived from HeLa cells was used for screening. The preparation, purchased from 4C (Seneffe, Belgium), was prepared from HeLa cells harvested whilst in exponential growth phase. The nuclear extract is prepared according to Dignam JD1983 Nucl. Acid. Res. 11, 1475-1489, snap frozen in liquid nitrogen and stored at -80°C. The final buffer composition was 20 mM Hepes, 100 mM KCl, 0.2 mM EDTA, 0.5 mM DTT, 0.2 mM PMSF and 20 % (v/v) glycerol.

IC50 results were allocated to one of 3 ranges as follows:

Range A: IC50<100nM,

Range B: IC50 from 101nM to 1000nM;

Range C: IC50 >1001nM.

NT = Not tested

Results of testing the compounds of the examples in this assay are given in the second column of Table 2 below.

Cell inhibition Assays

The corresponding cancer cell lines (Hela, U937 and HUT) growing in log phase were harvested and seeded at 1000 cells/well (200ul final volume) into 96-well tissue culture plates. Following 24h of cell growth cells were treated with compounds (final concentration of 20uM). Plates were then re-incubated for a further 72h before a sulphorhodamine B (SRB) cell viability assay was conducted according to Skehan 1990 J Natl Canc Inst 82, 1107-1112.

Data were expressed as a percentage inhibition of the control, measured in the absence of inhibitor, as follows:-

$$\% \text{ inhibition} = 100-((S^i/S^o)x100)$$

where S^i is the signal in the presence of inhibitor and S^o is the signal in the presence of DMSO.

IC50 values were determined by non-linear regression analysis, after fitting the results of eight data points to the equation for sigmoidal dose response with variable slope (% activity against log concentration of compound), using Graphpad Prism software.

IC50 results were allocated to one of 3 ranges as follows:

Range A: IC50<330nM,

Range B: IC50 from 330nM to 3300nM;

Range C: IC50 >3301nM.

NT= Not tested

Results of testing the compounds of the examples in this assay are given in the third-fifth columns of Table 2 below.

Table 2

Example No.	HDAC Activity	Hela	U937	HUT
1	B	C	B	B
2	B	C	B	B
3	C	C	B	C
4	B	C	C	C
5	B	B	B	B
6	A	C	C	C
7	B	C	B	B
8	B	C	C	C
9	B	B	B	B
10	A	C	C	C
11	B	B	B	B
12	B	C	C	C
13	B	C	B	B
14	B	C	C	C
15	C	C	B	B
16	C	C	C	C
17	B	C	B	B
18	B	C	C	C
19	B	B	B	B
20	B	B	A	B
21	B	C	C	C
22	B	B	B	B
23	B	C	C	C
24	B	C	C	B
25	B	C	C	C
26	A	B	B	B
27	B	C	NT	NT
28	B	B	B	B
29	B	B	B	B
30	B	C	NT	NT

Example No.	HDAC Activity	Hela	U937	HUT
31	B	B	A	B
32	A	B	NT	B
33	B	C	NT	NT
34	B	B	A	B
35	B	B	B	B
36	A	B	A	B
37	B	C	C	C
38	B	NT	NT	NT
39	B	NT	A	B
40	C	C	C	C
41	C	C	C	C
42	C	B	B	B
43	B	C	C	C
44	B	C	B	B
45	C	C	NT	NT
46	C	C	B	B
47	B	C	B	B
48	C	C	B	B
49	B	C	NT	NT
50	C	C	B	B
51	B	C	B	B
52	C	C	NT	NT
53	C	C	C	C
54	C	C	NT	NT
55	C	C	C	C
56	C	C	C	C
57	C	C	NT	NT
58	B	B	B	B
59	A	C	NT	NT
60	B	B	B	B
61	B	C	NT	NT

Example No.	HDAC Activity	Hela	U937	HUT
62	B	C	C	B
63	B	NT	NT	NT
64	B	B	NT	B
65	A	C	NT	NT
66	B	C	C	C
67	B	C	NT	NT
68	B	C	B	B
69	B	B	NT	B
70	B	C	NT	NT
71	A	B	A	B
72	A	NT	NT	NT
73	A	B	A	B
74	B	NT	NT	NT
75	A	B	A	A
76	A	NT	NT	NT
77	A	B	A	A
78	A	NT	NT	NT
79	A	NT	C	C
80	B	NT	NT	NT
81	A	C	C	B
82	B	NT	NT	NT
83	B	B	B	B
84	B	NT	NT	NT
85	B	C	A	B
86	B	C	A	B
87	A	NT	NT	NT
88	B	B	A	B
89	B	C	A	B
90	B	NT	NT	NT
91	B	C	B	B
92	A	B	B	B

Example No.	HDAC Activity	Hela	U937	HUT
93	A	NT	NT	NT
94	B	NT	B	B
95	B	NT	NT	NT
96	B	C	B	B
97	B	NT	NT	NT
98	B	B	B	B
99	C	NT	NT	NT
100	A	C	B	B
101	A	NT	NT	NT
102	B	C	B	C
103	A	NT	NT	NT
104	B	B	A	B
105	A	NT	NT	NT
106	A	B	A	A
107	NT	NT	NT	NT
108	B	B	B	B
109	A	B	A	B
110	A	B	A	A
111	A	A	A	A
112	B	NT	NT	NT
113	B	B	B	B
114	B	NT	NT	NT
115	B	B	B	B
116	B	C	B	C
117	A	NT	NT	NT
118	A	A	A	A
119	A	NT	NT	NT
120	A	NT	A	A
121	A	NT	NT	NT
122	A	B	A	B
123	A	NT	NT	NT

Example No.	HDAC Activity	Hela	U937	HUT
124	B	B	A	B
125	A	NT	NT	NT
126	B	NT	A	C
127	B	NT	NT	NT
128	B	B	A	A
129	B	NT	NT	NT
130	C	C	B	C
131	C	C	B	C
132	B	C	B	B
133	B	B	B	C
134	B	NT	B	C

Broken Cell Carboxyesterase Assay

Preparation of cell extract

U937 or Hct116 tumour cells (~ 10^9 were washed in 4 volumes of Dulbeccos PBS (~ 1litre) and pelleted at 160g for 10mins at 4□C. This was repeated twice and the final cell pellet was then resuspended in 35ml of cold homogenising buffer (Trizma 10mM , NaCl 130mM, $CaCl_2$ 0.5mM PH 7.0) at 25°C. Homogenates were prepared by nitrogen cavitation (700psi for 50min at 4°C). The homogenate was kept on ice and supplemented with a cocktail of inhibitors designed to give final concentrations of

Leupeptin 1μM

Aprotinin 0.1μM

E64 8μM

Pepstatin 1.5μM

Bestatin 162μM

Chymostatin 33μM

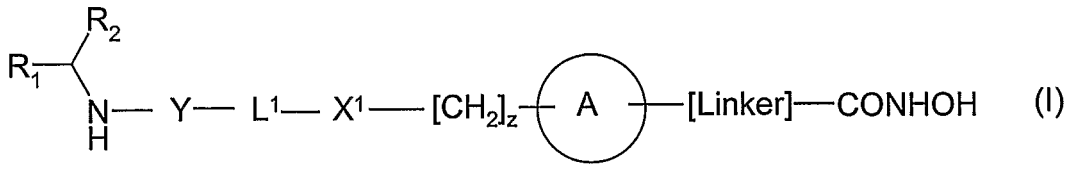
After clarification of the cell homogenate by centrifugation at 360 rpm for 10min, the resulting supernatant was used as a source of esterase activity and could be stored at -80°C until required.

Measurement of ester cleavage

Hydrolysis of ester to the corresponding carboxylic acid can be measured using this cell extract. To this effect cell extract (~30ug / total assay volume of 0.5ml) was incubated at 37°C in a Tris- HCl 25mM,125mM NaCl, buffer, PH 7.5 at 25°C. At zero time the relevant ester (substrate), at a final concentration of 2.5μM was then added and samples incubated at 37°C for the appropriate time (Usually zero or 80 minutes). Reactions were stopped by the addition of 3x volumes of Acetonitrile. For zero time samples the acetonitrile was added prior to the ester compound. After centrifugation at 12000g for 5minutes, samples were analysed for the parent ester and its corresponding carboxylic acid at room temperature by LCMS (Sciex API 3000, HP1100 binary pump, CTC PAL). Chromatographic conditions used were based on an AceCN (75*2.1mm) column and a mobile phase of 5-95% acetonitrile in water /0.1% formic acid.

Claims:

1. A compound of formula (I) or a salt, N-oxide, hydrate or solvate thereof:



(I)

wherein

R_1 is a carboxylic acid group (-COOH), or an ester group which is hydrolysable by one or more intracellular carboxyesterase enzymes to a carboxylic acid group;

R_2 is the side chain of a natural or non-natural alpha amino acid;

Y is a bond, –C(=O)-, -S(=O)$_2$-, -C(=O)O-, -C(=O)NR$_3$-, -C(=S)-NR$_3$, -C(=NH)NR$_3$ or -S(=O)$_2$NR$_3$- wherein R_3 is hydrogen or optionally substituted C_1-C_6 alkyl;

L^1 is a divalent radical of formula –(Alk^1)$_m$(Q)$_n$(Alk^2)$_p$- wherein

m, **n** and **p** are independently 0 or 1,

Q is (i) an optionally substituted divalent mono- or bicyclic carbocyclic or heterocyclic radical having 5 - 13 ring members, or (ii), in the case where both m and p are 0, a divalent radical of formula –X^2-Q^1- or –Q^1-X^2- wherein X^2 is –O-, S- or NR^A- wherein R^A is hydrogen or optionally substituted C_1-C_3 alkyl, and Q^1 is an optionally substituted divalent mono- or bicyclic carbocyclic or heterocyclic radical having 5 - 13 ring members,

Alk^1 and **Alk^2** independently represent optionally substituted divalent C_3-C_7 cycloalkyl radicals, or optionally substituted straight or branched, C_1-C_6 alkylene, C_2-C_6 alkenylene ,or C_2-C_6 alkynylene radicals which may optionally contain or terminate in an ether (–O-), thioether (-S-) or amino (–NR^A-) link wherein R^A is hydrogen or optionally substituted C_1-C_3 alkyl;

X^1 represents a bond; -C(=O); or -S(=O)$_2$-; –NR$_4$C(=O)-, -C(=O)NR$_4$-,–NR$_4$C(=O)NR$_5$-, -NR$_4$S(=O)$_2$-, or -S(=O)$_2$NR$_4$- wherein R$_4$ and R$_5$ are independently hydrogen or optionally substituted C$_1$-C$_6$ alkyl;

z is 0 or 1;

A represents an optionally substituted mono-, bi- or tri-cyclic carbocyclic or heterocyclic ring system wherein the radicals R$_1$R$_2$NH-Y-L^1-X^1-[CH$_2$]$_z$- and HONHCO-[LINKER]- are attached different ring atoms; and

-[Linker]- represents a divalent linker radical linking a ring atom in A with the hydroxamic acid group CONHOH, the length of the linker radical, from the terminal atom linked to the ring atom of A to the terminal atom linked to the hydroxamic acid group, is equivalent to that of an unbranched saturated hydrocarbon chain of from 3-10 carbon atoms.

2. A compound as claimed in claim 1 wherein the said length of the linker radical is equivalent to that of an unbranched saturated hydrocarbon chain of from 4 to 9 carbon atoms.

3. A compound as claimed in claim 1 or claim 2 wherein –[Linker]- represents a divalent radical of formula –(CH$_2$)$_x$-Z-L^2- wherein

x is 0 or 1;

Z is a bond, –NR$_3$C(=O)-, -C(=O)NR$_3$-,–NR$_4$C(=O)-NR$_3$-, -C(=S)-NR$_3$, -C(=NH)-NR$_3$ –NR$_3$S(=O)$_2$-, or -S(=O)$_2$NR$_3$- wherein R$_3$ is hydrogen or C$_1$-C$_6$ alkyl; -C(=O); or -S(=O)$_2$-; and

L^2 represents an optionally substituted, straight or branched, C$_4$-C$_7$ alkylene, C$_4$-C$_6$ alkenylene or C$_4$-C$_6$ alkynylene radical which may optionally contain or terminate in an ether (–O-), thioether (-S-) or amino (–NR^A-) link wherein R^A is hydrogen or optionally substituted C$_1$-C$_3$ alkyl.

4. A compound as claimed in claim 2 or claim 3 wherein x is 0.

5. A compound as claimed in any of claims 2 to 4 wherein Z is –C(=O)-,

-NHC(=O)- or –C(=O)NH-.

6. A compound as claimed in any of claims 2 to 5 wherein L^2 is $–(CH_2)_5$-, $–(CH_2)_6$-, or $–(CH_2)_7$-.

7. A compound as claimed in claim 1 wherein –[Linker]- represents a divalent radical of formula $–(CH_2)_x$- L^3-Ar^1-L^4- wherein

x is 0 or 1;

L^3 is Z or L^2 or Z-L^2 wherein Z is as defined in claim 3 and and L^2 is a bond or an optionally substituted divalent C_1-C_3 alkylene radical;;

Ar^1 is a divalent phenyl radical or a divalent mono-, or bi-cyclic heteroaryl radical having 5 to 13 ring members, and

L^4 is a bond or optionally substituted $–CH_2$- or –CH=CH-,

8. A compound as claimed in claim 7 wherein x is 0.

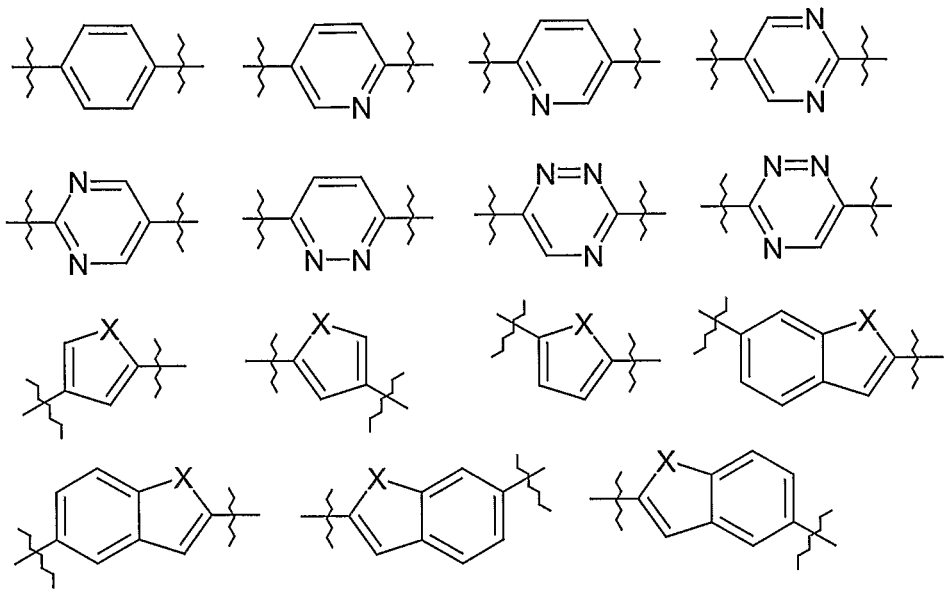
9. A compound as claimed in claim 7 or claim 8 wherein L^3 is Z or Z-L^2, wherein Z is –NH-, $-NHS(=O)_2$-, $–S(=O)_2NH$- or $–S(=O)_2$-; L^2 is $–CH_2$- and L^4 is a bond or $–CH_2$-;

10. A compound as claimed in claim 7 or claim 8 wherein L^3 is L^2, wherein L^2 is a straight chain C_3-C_5 alkylene radical which may optionally contain an ether (–O-), thioether (-S-) or amino ($–NR^A$-) link wherein R^A is hydrogen or optionally substituted C_1-C_3 alkyl, for example hydroxyethyl.

11. A compound as claimed in any of claims 7 to 10 wherein L^4 is –CH=CH-.

12. A compound as claimed in any of claims 7 to 10 wherein L^4 is $–CH_2$-.

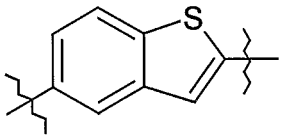
13. A compound as claimed in any of claims 7 to 12 wherein Ar^1 is divalent radical selected from the following:



wherein X is O, S or NH.

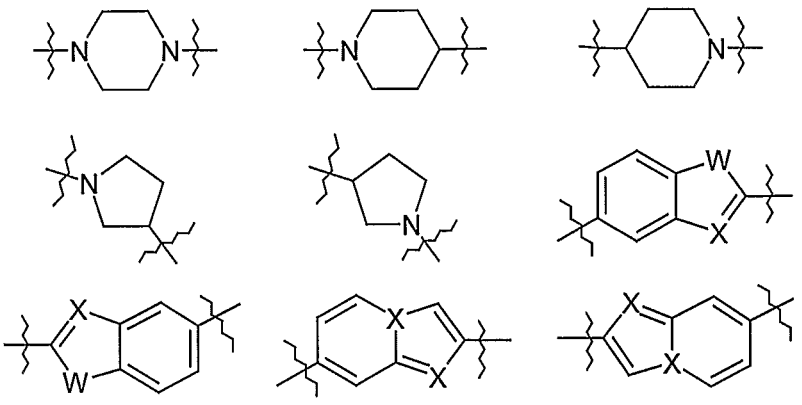
14. A compound as claimed in claim 7 wherein x is 0, L^3 and L^4 are bonds, and Ar^1 is a divalent phenyl radical or a divalent bicyclic heteroaryl radical having 9 to13 ring members.

15. A compound as claimed in claim 7 wherein Ar^1 is a divalent phenyl radical.or a divalent benzo[b]thiophenyl radical of formula:



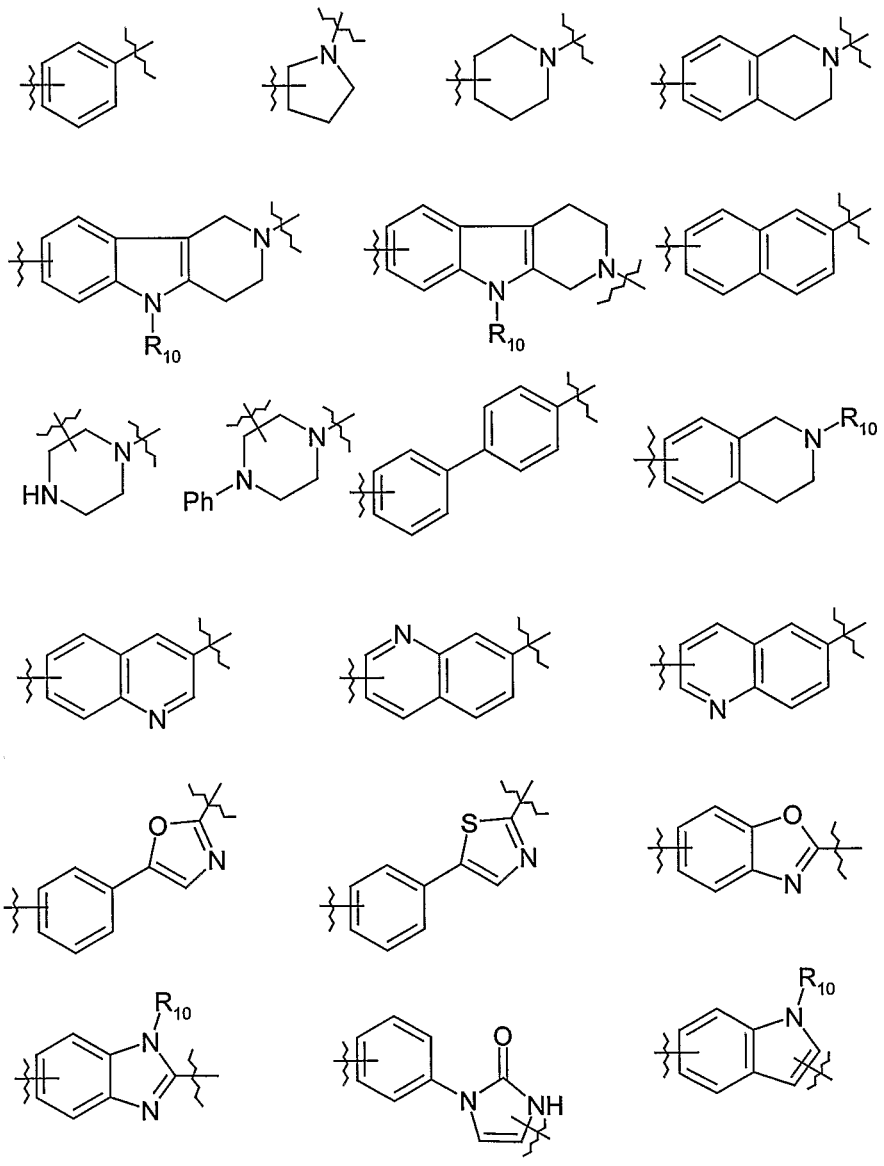
16. A compound as claimed in claim 1 wherein –[Linker]- represents a divalent radical of formula $-(CH_2)_x-L^3-B-Ar^1-L^4-$ wherein x, Ar^1, L^3 and L^4 are as defined in any of claims 7 to 13; and B is a mono- or bi-cyclic heterocyclic ring system.

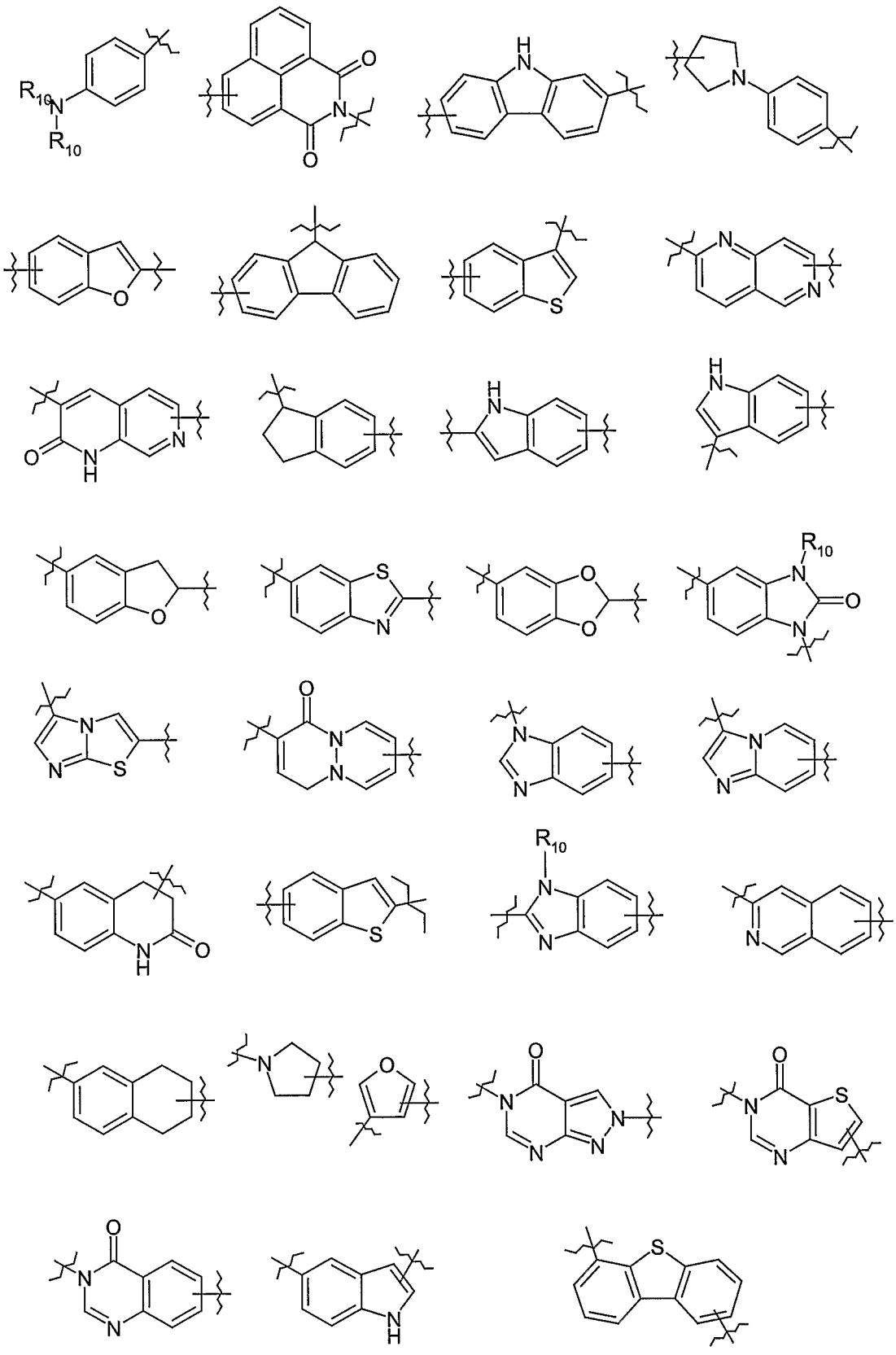
17. A compound as claimed in claim 16 wherein B is selected from the following:



wherein X is N and W is NH, O or S.

18. A compound as claimed in any of the preceding claims wherein A is one of the following, optionally substituted:





wherein R_{10} is hydrogen or optionally substituted C_1-C_6 alkyl, the bond intersected by the wavy lines shown as connected to a fixed atom connects to the Linker radical in the compounds (I), and the other bond shown as floating links any convenient ring atom of the ring system shown to the grouping $R_1R_2CHNHYL^1X^1[CH2]_z$.

19. A compound as claimed in any of the preceding claims wherein z is 0.

20. A compound as claimed in any of the preceding claims wherein Y is $-S(=O)_2-$, $-C(=S)-NR_3$, $-C(=NH)-NR_3$ or $-S(=O)_2NR_3-$ wherein R_3 is hydrogen or C_1-C_6 alkyl.

21. A compound as claimed in any of claims 1 to 19 wherein Y is a bond.

22. A compound as claimed in any of the preceding claims wherein, in the radical L^1, Alk^1 and Alk^2, when present, are selected from $–CH_2-$, $–CH_2CH_2-$, $–CH_2CH_2CH_2-$, and divalent cyclopropyl, cyclopentyl and cyclohexyl radicals.

23. A compound as claimed in any of the preceding claims wherein, in the radical L^1, Q is a divalent phenyl radical or a mono-, or bi-cyclic heteroaryl radical having 5 to13 ring members,

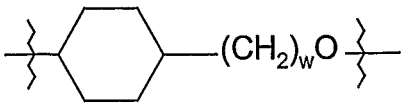
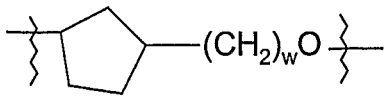
24. A compound as claimed in claim 22 wherein Q is 1,4-phenylene.

25. A compound as claimed in any of the preceding claims wherein, in the radical L^1, m and p are 0.

26. A compound as claimed in any of claims 1 to 24 wherein, in the radical L^1, n and p are 0 and m is 1.

27. A compound as claimed in any of claims 1 to 24 wherein, in the radical L^1, m, n and p are all 0.

28. A compound as claimed in any of the preceding claims wherein the radical $–Y-L^1-X^1-[CH_2]_z-$ is selected from $–C(=O)-$, $–C(=O)NH-$, $-(CH_2)_v-$, $-(CH_2)_vO-$, $-C(=O)-(CH_2)_v-$, $-C(=O)-(CH_2)_vO-$, $-C(=O)-NH-(CH_2)_w-$, $-C(=O)-NH-(CH_2)_wO-$

 and 

wherein v is 1, 2, 3 or 4 and w is 1, 2 or 3.

29. A compound as claimed in any of claims 1 to 27 wherein the radical $-Y-L^1-X^1-[CH_2]_z-$, is $-CH_2-$, $-CH_2O-$, $-C(=O)-CH_2-$, $-C(=O)-CH_2O-$, $-C(=O)-NH-CH_2-$, or $-C(=O)-NH-CH_2O-$.

30. A compound as claimed in any of the preceding claims wherein R_1 is an ester group of formula $-(C=O)OR_9$ wherein R_9 is

(i) R_7R_8CH- wherein R_7 is optionally substituted (C_1-C_3)alkyl-$(Z^1)_a-(C_1-C_3)$alkyl- or (C_2-C_3)alkenyl-$(Z^1)_a-(C_1-C_3)$alkyl- wherein a is 0 or 1 and Z^1 is –O-, -S-, or –NH-, and R_8 is hydrogen or (C_1-C_3)alkyl- or R_7 and R_8 taken together with the carbon to which they are attached form an optionally substituted C_3-C_7 cycloalkyl ring or an optionally substituted heterocyclic ring of 5- or 6-ring atoms; or

(ii) optionally substituted phenyl or monocyclic heterocyclic having 5 or 6 ring atoms,

31. A compound as claimed in claim 30 wherein R_9 is methyl, ethyl, n- or iso-propyl, n- or sec-butyl, cyclohexyl, allyl, phenyl, benzyl, 2-, 3- or 4-pyridylmethyl, N-methylpiperidin-4-yl, tetrahydrofuran-3-yl or methoxyethyl.

32. A compound as claimed in claim 30 wherein R_9 is cyclopentyl.

33. A compound as claimed in any of the preceding claims wherein R_2 is cyclohexylmethyl, cyclohexyl, pyridin-3-ylmethyl, sec-butyl, tert-butyl, 1-benzylthio-1-methylethyl, 1-methylthio-1-methylethyl, or 1-mercapto-1-methylethyl.

34. A compound as claimed in any of claims 1 to 32 wherein R_2 is hydrogen

35. A compound as claimed in any of claims 1 to 32 wherein R_2 is phenyl, benzyl, phenylethyl, tert-butoxymethyl or iso-butyl.

36. A compound as claimed in claim 1 having the structure of any of the compounds of the specific Examples herein.

37. A pharmaceutical composition comprising a compound as claimed in any of the preceding claims, together with a pharmaceutically acceptable carrier.

38. The use of a compound of formula (I) as claimed in any of claims 1 to 36 in the preparation of a composition for inhibiting the activity of an HDAC enzyme.

39. The use as claimed in claim 38 for the inhibition of HDAC1 activity, *ex vivo* or *in vivo*.

40. A method of inhibiting the activity of an HDAC enzyme comprising contacting the enzyme with an amount of a compound as claimed in any of claims 1 to 36 effective for such inhibition.

41. A method for the selective inhibition of the activity of an HDAC enzyme in macrophages and/or monocytes relative to other cell types, comprising contacting the enzyme with an amount, effective for such inhibition, of a compound as claimed in any of claims 1 to 36 wherein Y is a bond, $-S(=O)_2$ or $-S(=O)_2NR_3-$ wherein R_3 is hydrogen or optionally substituted C_1-C_6 alkyl.

42. A method as claimed in claim 40 or claim 41 for the inhibition of HDAC1 activity, *ex vivo* or *in vivo*.

43. A method for the treatment of cell-proliferation disease, polyglutamine disease, neurodegenerative disease, autoimmune disease, inflammatory disease, organ transplant rejection, diabetes, haematological disorders and infection, which comprises administering to a subject suffering such disease an effective amount of a compound of formula (I) as claimed in any of claims 1 to 36.

44. A method as claimed in claim 43 for the treatment of cancer cell proliferation, Huntingdon disease, or Alzheimer disease.

45. A method as claimed in claim 43 for the treatment of rheumatoid arthritis.

46. A method for the selective inhibition of the activity of an aurora kinase enzyme in macrophages and/or monocytes relative to other cell types, comprising contacting the enzyme with an amount, effective for such inhibition, of a compound as claimed in any of claims 1 to 36 wherein Y is a bond, $-S(=O)_2$ or $-S(=O)_2NR_3-$ wherein R_3 is hydrogen or optionally substituted C_1-C_6 alkyl.

47. A pharmaceutical composition as claimed in claim 37 which is adapted for topical administration and wherein, in the compound as claimed in any of claims 1 to 36, R_2 is linked to the carbon atom to which it is attached through a methylene radical $-CH_2-$.