PEPTIDE DERIVATIVES

The present invention relates to certain novel peptide derivatives which possess pharmacologically useful properties for use in treating autoimmune diseases or medical conditions, such as rheumatoid arthritis and other MHC class II dependent T-cell mediated diseases. The invention also includes pharmaceutical compositions of the novel peptide derivatives, processes for their manufacture, and their use in treating one or more of the aforementioned diseases or medical conditions and in the production of novel pharmaceuticals for use in such medical treatments.

Stimulation of the human immune response is dependent on the recognition of protein antigens by T cells. However T cells cannot respond to antigen alone and are only triggered by antigen when it is complexed with major histocompatibility complex (MHC) molecules on the surface of an antigen presenting cell, such as a B cell, macrophage or dendritic cell.

MHC class I molecules elicit a T-killer cell response which results in the destruction of the cell bearing the antigen. MHC class II molecules elicit a T-helper cell response which controls the expansion and maturation of selected B cells (i.e. generation of antigen-specific antibodies) and activation of macrophages.

A critical requirement of the immune system is the ability to differentiate between "self" and "non-self" (i.e. foreign) antigens. This discrimination is required to enable the immune system to mount a response to invading foreign pathogens whilst maintaining tolerance to self-proteins and thereby preventing damage to normal tissues. An autoimmune disease results when self-tolerance breaks down allowing the immune system to react against self-tissues such as the joints in rheumatoid arthritis. It is thought that the maintenance of tolerance and thus avoidance of autoimmune disease is critically dependent on the function of MHC molecules.

The observation that many autoimmune diseases are linked to the inheritance of particular MHC alleles suggests a key role for MHC molecules in the pathogenesis of autoimmune disease. For instance multiple sclerosis is linked to the inheritance of HLA-DR2, insulin dependent diabetes mellitus to HLA-DR3 and/or HLA-DR4 and Hashimoto's thyroiditis to HLA-DR5. In particular, an especially strong association exists between predisposition to development of the chronic inflammatory joint disease rheumatoid arthritis and the inheritance of HLA-DR4Dw4 and/or HLA-DR4w14 and/or HLA-DR1. It is thought

that the autoimmune disease associated MHC molecules bind to certain self-antigens and present them to T cells thus stimulating an autoimmune response. Other peptides which can bind to the autoimmune associated MHC molecules and/or either prevent the binding or displace already bound self-antigens and/or which inhibit T cell activation (especially the activity of pathogenic T cells (e.g. Th 1 cells)) and/or which increase the activity of protective T cells (e.g. Th 2 cells) or peptides which interact with MHC molecules by an alternative mechanism of action so as to prevent or modify stimulation of an autoimmune response mediated via said MHC molecules, may specifically suppress an autoimmune response.

An agent of this kind would offer therapy for the autoimmune disease whilst avoiding general suppression of the immune system, thus limiting deleterious side-effects. This kind of profile would have significant advantages over current therapy for diseases such as rheumatoid arthritis. It is contemporary practice to treat rheumatoid arthritis initially with symptom relief agents such as NSAIDs, which do not have any beneficial effect on disease progression and are often associated with unwanted side-effects. Treatment of more severe disease relies on the use of the so-called second-line agents. Often these are general cytotoxic compounds which are of limited efficacy and can cause severe toxicity problems. A rationally based, disease modifying agent, without associated non-specific cytotoxicity, would therefore offer significant benefits in the treatment of rheumatoid arthritis.

Peptides are disclosed in International Patent Application, Publication No's. WO 92/02543, WO 93/05011 and WO 95/07707 which bind to MHC molecules and inhibit T-cell activation.

Although a number of peptides have been discovered which inhibit HLA-DR restricted T cell activation by binding to HLA-DR molecules, there is a continuing need for alternative compounds which bind to such molecules and/or either prevent the binding or displace already bound self-antigens and/or inhibit T cell activation and/or increase the activity of protective T cells, or which interact with MHC molecules by an alternative mechanism of action, so as to prevent or modify stimulation of an autoimmune response that causes a disease or condition referred to above.

We have discovered that the peptide derivatives of the present invention (set out below) surprisingly possess such pharmacologically useful properties and this is a basis for the present invention.

According to one aspect of the invention there is provided a peptide derivative of the formula I (set out hereinafter), or a pharmaceutically acceptable salt thereof,

wherein

P is a hydrophobic residue;

AA^1, AA^2, AA^3, AA^4, AA^5, AA^6, AA^7 and AA^8 are L-amino acid residues in which 1, 2 or 3 of AA^1, AA^4 and AA^7 are selected from a residue of the L-amino acid of formula II (set out hereinafter)

wherein n is an integer 1, 2, 3 or 4;

X is -NH-CO-, -CO- (carbonyl) or -O.CO- (oxycarbonyl);

R^1 and R^2 are selected from (A), (B) and (C) wherein

(A) is a group of the formula $-(CH_2)_a-CO-N(R^3)(R^4)$ in which a is an integer 1 or 2 and R^3 and R^4 are independently selected from a group $-[(CH_2)_bO]_m-R^a$ in which R^a is methyl or ethyl and m is an integer 1, 2, 3, 4 or 5; when m is 1, b is 2 or 3 and when m is 2, 3, 4 or 5, the value of b in each $-(CH_2)_bO-$ unit is independently selected from 2 and 3;

(B) is a group of the formula $-(CH_2)_cO(CH_2)_d-CO-N(R^5)(R^6)$ in which c is an integer 2 or 3, d is an integer 1, 2 or 3, and R^5 and R^6 are independently selected from a group $-[(CH_2)_eO]_p-R^b$ in which R^b is methyl or ethyl and p is an integer 1, 2, 3, 4 or 5; when p is 1, e is 2 or 3 and when p is 2, 3, 4 or 5, the value for e in each $-(CH_2)_eO-$ unit is independently selected from 2 and 3; and

(C) is a group of the formula $-[(CH_2)_fO]_g-R^7$ in which R^7 is methyl or ethyl and g is an integer 1, 2, 3, 4 or 5; when g is 1, f is 2 or 3 and when g is 2, 3, 4 or 5, the value for f in each $-(CH_2)_fO-$ unit is independently selected from 2 and 3;

or AA^1, AA^2, AA^3, AA^6, AA^7, and AA^8 are L-amino acid residues in which one or both of AA^1 and AA^7 are selected from a residue of the L-amino acid of formula II as defined above and AA^4 together with AA^5 form a group of the formula III, IIIa, IV, IVa, V or Va (set out hereinafter) in which Ra, Rb and Rz are independently selected from hydrogen and (1-4C)alkyl, and A is oxygen or methylene;

or AA^1, AA^2, AA^3, AA^4, AA^5 and AA^8 are L-amino acid residues in which one or both of AA^1 and AA^4 are selected from a residue of the L-amino acid of formula II as defined above and AA^6 together with AA^7 form a group of the formula III, IIIa, IV, IVa, V or Va (set out hereinafter) in which Ra, Rb and Rz are independently selected from hydrogen and (1-4C)alkyl, and A is oxygen or methylene;

and Q is OH, NH_2, NRcRd wherein Rc is selected from (1-4C)alkyl, 2-carbamoylcyclopentyl, 2-pyridylmethyl, 4-carbamoylcyclohexyl, 4-carbamoylcyclohexylmethyl, 3-carbamoylphenyl, 4-carbamoylphenyl, 4-(carbamoylmethyl)phenyl, 4-(carboxymethyl)phenyl, 2-morpholinoethyl and a group of the formula $-A^1-G^1$ in which A^1 is (3-7C)alkylene or

A^1 is selected from

(1) a group of the formula $-A^2-B^2-$ in which A^2 is p-phenylene or 1,4-cyclohexylene and B^2 is (1-4C)alkylene or A^2 is methylene and B^2 is p-phenylene or 1,4-cyclohexylene; and

(2) a group of the formula $-A^3-B^3-C^3-$ in which A^3 is methylene, B^3 is p-phenylene or 1,4-cyclohexylene and C^3 is (1-3C)alkylene; and

G^1 is a group of the formula $-N=C[N(Rp)_2]_2$ in which each Rp is independently selected from hydrogen, methyl, ethyl and propyl; and Rd is hydrogen or (1-4C)alkyl; or Q is 1-piperazinyl, 4-methyl-1-piperazinyl, 4-(2-(2-hydroxyethoxy)ethyl)-1-piperazinyl, 4-amidino-1-piperazinyl, 1-piperidyl or 4-substituted-1-piperidyl wherein the 4-substituent is selected from carboxy, carbamoyl, N-(2-aminoethyl)carbamoyl and N-(4-aminobutyl)carbamoyl; or Q is a sequence of 1 to 6 amino acid residues or an amide thereof.

According to a further aspect of the invention there is provided a peptide derivative of the formula I (set out hereinafter), or a pharmaceutically acceptable salt thereof, wherein AA^1, AA^2, AA^3, AA^4, AA^5, AA^6, AA^7 and AA^8 are L-amino acid residues in which one of AA^1, AA^4 and AA^7 is selected from a residue of the L-amino acid of formula II (set out hereinafter) wherein n, X, R^1 and R^2 have any of the meanings defined above; or AA^1, AA^2, AA^3, AA^6, AA^7 and AA^8 are L-amino acid residues in which one of AA^1 and AA^7 is selected from a residue of the L-amino acid of formula II as defined above and AA^4 together with AA^5 form a group of the formula IIIa as defined above; or AA^1, AA^2, AA^3, AA^4, AA^5 and AA^8 are L-amino acid residues in which one of AA^1 and AA^4 is selected from a residue of the L-amino acid of formula II as defined above and AA^6 together with AA^7 form a group of the formula IIIa as defined above; and P and Q have any of the values defined above.

It is to be understood that an amino acid of Q may independently have the D- or L-stereochemistry. Furthermore, when Q is defined as hydroxy (OH), this will be understood to be the hydroxy group of the C-terminal amino acid AA^8. Similarly where Q is defined as NH_2, NRcRd, piperazinyl, piperidyl, etc., this means that the hydroxy group of the C-terminal

amino acid AA^8 is replaced by such a group. It is also to be understood that where an amino acid is referred to this means an alpha-amino acid. It is also to be understood that when an L-amino acid is referred to this also includes amino acids such as Gly, 2,2-diethylGly, aza-alanine and aza-glycine which have no chiral carbon atom. It is further to be understood that generic terms such as "alkyl" include both straight and branched chain variants when the carbon numbers permit. The same convention applies to other radicals.

It will further be appreciated that when R^1 and R^2 are both groups of the formula (A), the values of a, R^3 and R^4 in R^1 may be the same or different to those in R^2. Similarly when R^1 and R^2 are both groups of the formula (B), the values of c, d, R^5 and R^6 in R^1 may be the same or different to those in R^2, and when R^1 and R^2 are both groups of the formula (C), the values of f, g and R^7 in R^1 may be the same or different to those in R^2.

It is well known in the art that compounds having a chiral centre may exist in the form of a racemate (or a mixture of diastereoisomers where there is more than one chiral centre) or as an optically active enantiomer or diastereoisomer. It is also well known in the art that a particular biological activity associated with a racemic or diastereomeric mixture may result largely or solely from a single optically active isomer. It will therefore be understood that the invention concerns any form of a peptide derivative of formula I which possesses the aforementioned pharmaceutically useful properties. It is well known in the art how to obtain a single optically active isomer, for example by separation from a racemic or diastereomeric mixture containing the isomer using conventional techniques, such as chromatography, or by chiral synthesis using an appropriate optically active starting material or intermediate, as exemplified herein. It is also well known in the art how to determine the pharmacological properties of such racemic or diastereomeric mixtures, and the individual optically active isomers, for example by using the assays described herein. The person skilled in the art is therefore easily able to obtain the particular isomers of the peptide derivatives of formula I having the beneficial pharmacological properties referred to herein. It is to be understood that the present invention also encompasses any polymorphic form, any tautomer or any solvate, or any mixture thereof, of a peptide derivative of formula I which possesses the beneficial pharmacological properties referred to herein.

Suitable independent values for the α-amino acid residues AA^1, AA^2, AA^3, AA^4, AA^5, AA^6, AA^7 and AA^8, when they are not a residue of the amino acid of formula II or form part of a group of the formula III, IIIa, IV, IVa, V or Va, include, for example, those of the 20

naturally occurring amino acids encoded by the genetic code, particularly alanine (Ala), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), lysine (Lys), asparagine (Asn), glutamine (Gln), arginine (Arg), threonine (Thr), valine (Val) and proline (Pro). Residues of amino acids such as sarcosine (Sar), 3,3,3-trifluoroalanine, 2,2-diethylglycine, 2,3-diaminopropanoic acid (Dap), 2,4-diaminobutanoic acid (Dab), 2-aminobutanoic acid (Abu), homoarginine, homophenylalanine, trans-4-hydroxyproline (Hyp), aza-alanine [H_2N-$N(CH_3)$-COOH; Azala], aza-glycine [H_2N-NH-COOH; Azgly], 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic), octahydroindole-2-carboxylic acid (Oic), decahydroisoquinoline-3- carboxylic acid (Dic) are also suitable. (Where Dic is referred to this means the forms in which the ring-junctions both have the R configuration or both have the S configuration.) Residues of corresponding N^2-methylated amino acids may also be used, as well as residues of corresponding amino acids in which a free side-chain carboxylic acid function is esterified (for example as an (1-6C) alkyl or benzyl ester) and a free side-chain amino group is alkylated (for example, methylated), acetylated or converted to a carbamate (for example, an alkyl (such as methyl or ethyl), phenyl or benzyl carbamate). Other suitable values include, for example, residues of 2-substituted glycine in which the 2-substituent is a group of the formula $-(CH_2)_s NH_2$ wherein s is 1 to 3, or a group of the formula $-(CH_2)_pN(Re)_3^+.X^-$ wherein p is 2 to 4 and X^- is a counter ion (such as acetate, trifluoroacetate, hydroxide or chloride), or a group of the formula $-(CH_2)_qN(Re)_2$ wherein q is 0 to 4 or a group of the formula $-(CH_2)_rN=C[N(Re)_2]_2$ wherein r is 1 to 4 and wherein in which last three groups each Re is independently selected from hydrogen and (1-4C)alkyl (such as methyl or ethyl).

A suitable value for the hydrophobic residue P (which it will be appreciated is attached to the amino group of the N-terminal amino acid AA^1) includes, for example, an organic hydrophobic group such as a hydrophobic aliphatic, aromatic, heteroaromatic or mixed aliphatic/aromatic or aliphatic/heteroaromatic organic group of from 5 to 20 carbon atoms (and 1, 2 or 3 heteroatoms selected from oxygen, sulphur and nitrogen for heteroaryl-containing groups), for example a group of the formula R-, R.CO-, $R.SO_2$-, R.O.CO-, R.NHCO-, R.O.CS-, R.S.CO-, R.NHCS-, R.S.CS- and R.CS-, in which R includes, for example, (5-10C)alkyl, aryl, heteroaryl, aryl(2-10C)alkyl, heteroaryl(2-10C)alkyl, diaryl(2-8C)alkyl, aryl(2-10C)alkenyl, arylcyclopropyl, (5-10C)cycloalkyl, (5-10C)cycloalkyl(2-6C)alkyl, 3-biphenyl, 4-biphenyl, 4-cyclohexylphenyl, 2-naphthyloxymethyl, 3-naphthyloxymethyl, phenoxyphenyl and tetrahydronaphthyl, an aryl or heteroaryl group of

which values of R may bear one or more (1-4C)alkyl, halogeno, cyano or (1-4C)alkoxy substituents. One particular embodiment of the invention includes, for example, compounds of the formula I in which P is R.CO- as defined above. A further particular embodiment of the invention includes, for example, peptide derivatives of the formula I wherein P is a hydrophobic aliphatic, aromatic or aliphatic/aromatic organic group of from 5 to 20 carbon atoms.

Particular values for R include, for example, when it is (5-10C)alkyl: pentyl, isopentyl, tert-pentyl, 2-methylpentyl, hexyl, isohexyl, 5-methylhexyl and octyl; when it is aryl: phenyl, naphthyl and indenyl; when it is heteroaryl: 2-, 3-, 5- or 6-indolyl, 2-, 3-, 5- or 6-indolinyl, 2-, 3-, 5- or 6- benzo[b]thiophenyl, thienyl, 2-, 4- or 5-benzothiazolyl, 2-, 4- or 5-benzoxazolyl, 2-, 4- or 5-benzimidazolyl, 1,4-benzodioxanyl attached at the 2-, 3-, 6- or 7-position and 2-, 3-, 5- or 6-benzofuranyl; when it is aryl(2-10C)alkyl: aryl(2-6C)alkyl (where the aryl portion includes, for example, any of the specific values for aryl given above and the (2-6C)alkyl portion includes, for example, methylene, ethylene, trimethylene, tetramethylene and pentamethylene) such as 2-phenylethyl, 3-phenylpropyl, 4-phenylbutyl and 5-phenylpentyl; when it is heteroaryl(2-10C)alkyl: heteroaryl(2-6C)alkyl (where the heteroaryl portion includes, for example, any of the specific values for heteroaryl given above and the (2-6C)alkyl portion includes, for example, methylene, ethylene, trimethylene, tetramethylene and pentamethylene) such as in 2-(2-cyanobenzo[b]thiophen-5-yl)ethyl; when it is diaryl(2-8C)alkyl: diaryl(2-6C)alkyl such as 2,2-diphenylethyl, 3,3-diphenylpropyl and 4,4-diphenylbutyl; when it is aryl(2-10C)alkenyl: aryl(2-6C)alkenyl such as styryl, 3-phenylpropen-2-yl and 4-phenylbuten-1-yl; when it is arylcyclopropyl: phenylcyclopropyl, 1-naphthylcyclopropyl and 2-naphthylcyclopropyl; when it is (5-10C)cycloalkyl: cyclopentyl, cyclohexyl and 1-adamantyl; and when it is (5-10C)cycloalkyl(2-6C)alkyl: 2-(cyclohexyl)ethyl, 3-(cyclohexyl)propyl and 4-(cyclohexyl)butyl. A particular value for a substituent on an aryl group of R includes, for example, methyl, ethyl, chloro, bromo, iodo, methoxy, ethoxy and cyano.

The hydrophobic residue P also includes, for example, a hydrophobic L-amino acid, such as phenylalanine (Phe) and hydrogenated analogues thereof such as cyclohexylalanine (Cha), para-chloroPhe, 3-(2-thienyl)alanine, tyrosine (Tyr), Tyr(Omethyl), tryptophan (Trp), biphenylalanine, 3-(1-naphthyl)alanine, 3-(2-naphthyl)alanine and hydrogenated analogues thereof, 3-(1-adamantyl)alanine (Ada), Glu(OBenzyl), 3-(benzyloxy)Ala, 3-

(benzylsulfanyl)Ala and 9-fluorenylGly, each of which may optionally bear on the N-terminus a hydrophobic aliphatic, aromatic, heteroaromatic or mixed aliphatic/aromatic or aliphatic/heteroaromatic organic group as defined or exemplified above. Alternatively, the hydrophobic amino acid may optionally bear, for example, a further sequence of 1 to 3 amino acids selected from any of the suitable independent values for AA^1 to AA^8 defined above. For example P includes the particular sequences Ala-Cha, Ala-Ala-Cha, Tyr-Ala-Ala-Cha, Tyr-Ala-Ala-Phe, Ala-Phe-Phe-Phe and Ala-Ala-Ala-Phe. The first amino acid of such further sequence of 1 to 3 amino acids (as read from left to right) may be an L- or D-amino acid and may also optionally bear a hydrophobic aliphatic, aromatic, heteroaromatic or mixed aliphatic/aromatic or aliphatic/heteroaromatic organic group as defined or exemplified above.

Further particular values for P include, for example, 3-(benzyloxycarbonyl)propionyl-Phe, 3-(benzyloxycarbonyl)propionyl-Cha, 4-(benzyloxycarbonyl)butyryl-Phe, 4-(benzyloxycarbonyl)butyryl-Cha, (5-oxo-pyrrolidin-2-yl)carbonyl-Phe-Tyr, , (5-oxo-pyrrolidin-2-yl)carbonyl-Glu(OBenzyl)-Tyr, acetyl-Glu(OBenzyl)-Tyr, diphenylmethyl.CONH.CH_2CH_2.CO-Cha, diphenylmethyl.CONH.CH_2CH_2.CO-Tyr, diphenylmethyl.CONH.$CH_2CH_2CH_2$.CO-Cha, diphenylmethyl.CONH.$CH_2CH_2CH_2$.CO-Tyr, diphenylmethyl.NHCO.$CH_2CH_2CH_2$.CO-Cha, diphenylmethyl.NHCO.$CH_2CH_2CH_2$.CO-Tyr, benzyl.NHCO.CH_2CH_2.CO-Cha, benzyl.NHCO.CH_2CH_2.CO-Tyr, N-acetyl-4-chloro-beta-hydroxyPhe, 4-phenoxyphenyl.NHCO-, benzyl.NHCO.CH_2CH_2.CO.(N-methylPhe), benzyl.NHCO.CH_2CH_2.CONH.CH($CHPh_2$).CO, benzyl.NHCO.CH_2CH_2.CO-Tyr, 3,3-diphenylpropionyl, trans-cinnamoyl, 5-phenylvaleryl and 3-(2-cyanobenzo[b]thiophen-5-yl)propionyl.

A value for P of particular interest includes, for example, Ph.$(CH_2)_4$.CO-(5-phenylvaleryl (Phv)), Ph.$(CH_2)_4$.CS- and 3-(2-cyanobenzo[b]thiophen-5-yl)-propionyl.

A preferred value for the hydrophobic residue P includes, for example, 3-(2-cyanobenzo[b]thiophen-5-yl)propionyl and 5-phenylvaleryl (Phv), especially the latter.

When Rc is a group of the formula -A^1-G^1, a particular value for A^1 when it is alkylene includes, for example, methylene, ethylene, propylene and butylene; a particular value for B^2 when is is (1-4C)alkylene includes, for example, methylene, ethylene and propylene; and a particular value for C^3 when it is (1-3C)alkylene includes, for example, methylene, ethylene and propylene.

A particular value for -A^1-G^1 includes, for example, 3-guanidinopropyl, 4-(2-guanidinoethyl)phenyl, 4-(2-morpholinoethyl.NH.CO.CH_2)phenyl and 4-(4-[2-(2-hydroxyethoxy)ethyl]piperazin-1-yl.CO.CH_2)phenyl.

A particular value for Q when it is a sequence of 1 to 6 amino acid residues includes, for example, a sequence of L- amino acid residues independently selected from any of the suitable independent values for AA^1 to AA^6 defined above, (such as Ala-Thr-Gly-OH), or their D- analogues, or a sequence containing both D- and L- amino acids, or an amide thereof, such as an amide derived from ammonia, an (1-4C)alkylamine (such as methylamine) or a di(1-4C)alkylamine (such as dimethylamine).

A preferred value for Q includes, for example, 4-carbamoyl-1-piperidyl (the residue of piperidine-4-carboxamide (Pip-NH_2)), 4-carboxy-1-piperidyl (the residue of piperidine-4-carboxylic acid (Pip-OH)), 4-(carbamoylmethyl)anilino (the residue of 4-aminophenylacetamide (Papa-NH_2)), 4-(carboxymethyl)anilino (the residue of 4-aminophenylacetic acid (Papa-OH)), and 4-(2-guanidinoethyl)anilino (the residue of 2-(4-aminophenyl)ethylguanidine (Pape-NHC(=NH)NH_2). Pip-NH_2 and Papa-NH_2 are particularly preferred values for Q, and especially Papa-NH_2.

Particular novel peptide derivatives of the invention include, for example, peptide compounds of the formula I as defined above wherein:

(1) in formula II, n = 1 and X = -CO-;

(2) in formula II, n = 4 and X = -NHCO-;

(3) in formula II, R^1 and R^2 are groups (the same or different) of formula (A) wherein a = 1;

(4) in formula II, R^1 and R^2 are groups (the same or different) of formula (A) wherein R^3 and R^4 are groups (the same or different) of formula -$[(CH_2)_bO]_m$-R^a wherein b = 2;

(5) in formula II, R^1 and R^2 are groups (the same or different) of formula (A) wherein R^3 and R^4 are groups (the same or different) of formula -$[(CH_2)_bO]_m$-R^a wherein m = 1, 2 or 3;

(6) in formula II, R^1 and R^2 are groups (the same or different) of formula (B) wherein c = 2;

(7) in formula II, R^1 and R^2 are groups (the same or different) of formula (B) wherein d = 2;

(8) in formula II, R^1 and R^2 are groups (the same or different) of formula (B) wherein R^5 and R^6 are groups (the same or different) of formula $-[(CH_2)_eO]_p-R^e$ wherein e = 2;

(9) in formula II, R^1 and R^2 are groups (the same or different) of formula (B) wherein R^5 and R^6 are groups (the same or different) of formula $-[(CH_2)_eO]_p-R^e$ wherein p = 1;

(10) in formula II, R^1 and R^2 are groups (the same or different) of formula (C) wherein f = 2; and

(11) in formula II, R^1 and R^2 are groups (the same or different) of formula (C) wherein g = 3;

and wherein, unless otherwise stated, AA^1, AA^2, AA^3, AA^4, AA^5, AA^6, AA^7, AA^8, P and Q have any of the meanings defined hereinbefore.

Particular values for R^3, R^4, R^5 and R^6, and R^1 and R^2 when they are selected from a group of the formula $-[(CH_2)_fO]_g-R^7$, include, for example, $-CH_2CH_2OCH_3$, $-CH_2CH_2CH_2OCH_3$, $-CH_2CH_2OCH_2CH_2OCH_3$, $-CH_2CH_2OCH_2CH_2CH_2OCH_3$, $-CH_2CH_2CH_2OCH_2CH_2OCH_3$, $-CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$, $-CH_2CH_2OCH_2CH_2CH_2OCH_2CH_2OCH_3$ and $-CH_2CH_2OCH_2CH_2CH_2OCH_2CH_2CH_2OCH_3$.

Further particular novel peptide derivatives of the invention include, for example, the following peptide derivatives of the formula I:

(i) $P-AA^1-AA^2-AA^3-II-AA^5-AA^6-AA^7-AA^8-Q$;

(ii) $P-II-AA^2-AA^3-AA^4-AA^5-AA^6-AA^7-AA^8-Q$;

(iii) $P-AA^1-AA^2-AA^3-IIIa-AA^6-II-AA^8-Q$;

(iv) $P-AA^1-AA^2-AA^3-AA^4-AA^5-AA^6-II-AA^8-Q$;

(v) $P-AA^1-AA^2-AA^3-II-AA^5-IIIa-AA^8-Q$; and

(vi) $P-II-AA^2-AA^3-AA^4-AA^5-IIIa-AA^8-Q$;

wherein II represents a residue of the L-amino acid of the formula II as defined hereinbefore and IIIa represents a group of the formula IIIa as set out hereinafter, and wherein, unless otherwise stated, AA^1, AA^2, AA^3, AA^4, AA^5, AA^6, AA^7, AA^8, P and Q have any of the meanings, including the particular and preferred values, defined herein. (It will be appreciated that in groups (i) to (vi), where AA^1, AA^2, AA^3, AA^4, AA^5, AA^6, AA^7 or AA^8 appear they are L-amino acid residues and the presence and position of a group of the formula II or IIIa is

specifically indicated.) Groups (i) to (vi) are further independent aspects of the present invention.

Peptide derivatives of particular interest within (i) to (vi) include, for example, those in which, in formula II, n = 1 or 2, X = carbonyl and R^1 and R^2 are both groups of the formula (C) (especially wherein f = 2, g = 3 and/or R^7 = methyl, such as $-CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$) or are both groups of the formula (A) (especially wherein a = 1, b = 2, m = 1 or 3 and/or R^8 = methyl, such as $-CH_2CON(CH_2CH_2OCH_3)_2$) or $-CH_2CON[(CH_2CH_2O)_3CH_3]_2$. Of groups (i) to (vi), peptide derivatives of groups (i) and (v) are of particular interest. Preferred peptide derivatives include, for example, compounds of group (v) in which, in formula II, n = 1, X = carbonyl and R^1 and R^2 are both groups of the formula (A).

Preferred values for AA^1 to AA^8 (when AA^1, AA^4 or AA^7 is not a residue of the amino acid of formula II and when AA^4 together with AA^5 or AA^6 together with AA^7 are not groups of the formula III, IIIa, IV, IVa, V or Va) include, for example,

AA^1 selected from Ala, Ile, Tyr, Val, Glu, Lys, Arg, Gly, Gap, $GapMe_4$ and 3,3,3-trifluoroalanine, particularly Ala, Ile, Arg, Gap, $GapMe_4$, especially Ala, Arg and Ile, and more especially Ala and Arg;

AA^2 selected from Ala, Lys, Glu, Sar, Val, Arg, Gly, Pro, Ile, Tic, 3,3,3-trifluoroalanine and N^6-diethylLys, particularly Ala, Arg, Ile, Lys and Tic, especially Ala, Arg, Ile and Tic and more especially Ala and Arg;

AA^3 selected from Ala, His, Gln, Val, Thr, Glu, Gly, Asp, Asn and N^3-diethylDap, particularly Ala, His, Asp and Asn, especially Ala and Asn and more especially Ala;

AA^4 selected from Ala, Lys, Asn, Arg, Thr, Glu, Sar, Gly, Pro, His and N^6-diethylLys, particularly Ala, Arg, Lys and His, especially Ala, Arg and His and more especially Ala;

AA^5 selected from Thr, Val, Ala, Gly, Dap, Dab, Pro, Hyp, Asn, Ser and N^3-diethylDap, particularly Thr, Val and Dap, and especially Thr and Val;

AA^6 selected from Gly, Leu, Lys, Ala, Pro, Glu, Sar, His and Dap (especially Ala and Pro);

AA^7 selected from Pro, Ala, Lys, Arg, Glu, Sar, Gly, Oic and Dic (especially Ala and Arg); and

AA^8 selected from from Ala, Gly, Dap, azaalanine and azaglycine, particularly Ala, Gly and azaglycine and especially Ala and Gly. (Where Gap is referred to this means the residue $-NH.CH[CH_2NH.C(=NH).NH_2].CO-$ and where $GapMe_4$ is referred to this means the residue

-NH.CH(CH_2N=C[N(CH_3)$_2$]$_2$).CO-.)

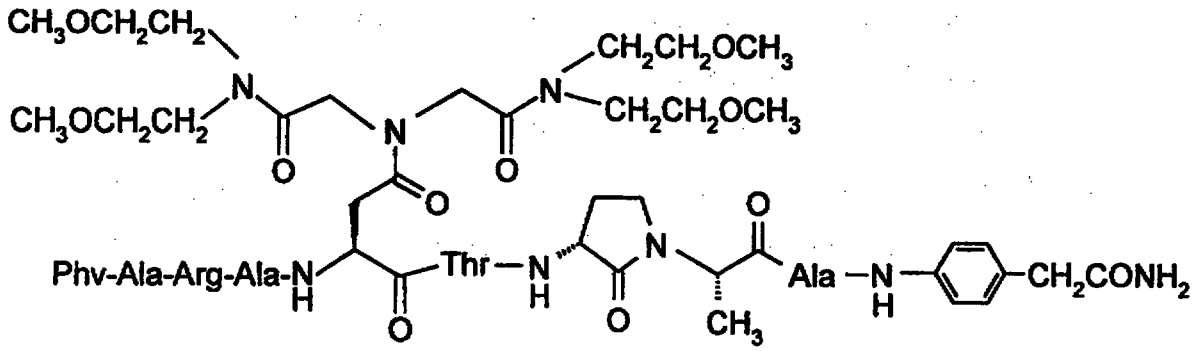
Further particular independent groups of peptide derivatives of the invention include, for example, peptide derivatives of formula I wherein 1 or 2 of AA^1, AA^4 and AA^7 are selected from a residue of the L-amino acid of formula II as defined hereinbefore and the remainder of AA^1, AA^2, AA^3, AA^4, AA^5, AA^6, AA^7 and AA^8 are residues of L-amino acids (including the suitable, specific and preferred values defined above).

When a peptide derivative of formula I contains a group of formula III, IV, IVa or V, particular values for Ra in formula III and Rb in formula IV and IVa and Rz in formula V include, when they are alkyl, methyl, ethyl, propyl and isopropyl. Of the peptide derivatives containing a group of formula III, IIIa, IV, IVa, V or Va, those containing a group of formula IIIa are preferred.

A further preferred aspect of the present invention comprises peptide derivatives of the formula I which contain an arginine residue, particularly compounds in which AA^1 or AA^2 is arginine (such as compounds in which the partial sequence AA^1-AA^2-AA^3 is Ala-Arg-Ala or Arg-Ala-Ala).

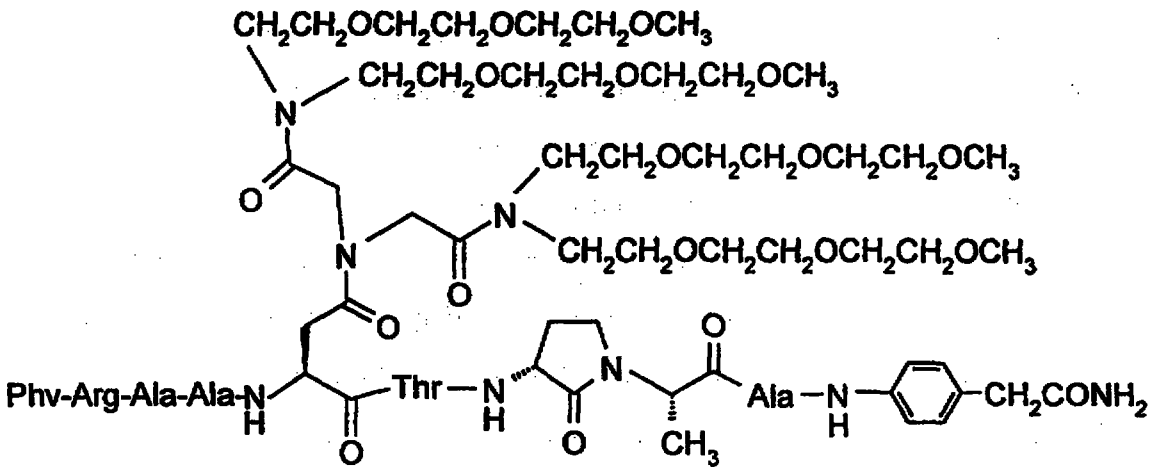
Peptide derivatives of the invention which are of particular interest include, for example, the specific embodiments set out hereinafter in the accompanying Examples. Of these, the peptide derivatives of Examples 3 and 6 are of special importance and these compounds, or a pharmaceutically acceptable salt thereof, are provided as further features of the invention.

(SEQ ID NO: 3)



Example 3

(SEQ ID NO : 6)



Example 6

A further aspect of the present invention comprises a protected (for example with Fmoc) or unprotected amino acid of the formula II wherein n, X, R^1 and R^2 have any of the values, including the particular and preferred values, defined above.

Pharmaceutically acceptable salts include, for example, for peptide derivatives that are sufficiently basic, for example those having a free amino group, salts with acids forming physiologically acceptable anions, such as salts with mineral acids, for example, hydrogen halides (such as hydrogen chloride and hydrogen bromide), sulphonic and phosphonic acids, and with organic acids such as acetic, oxalic, tartaric, mandelic, p-toluenesulphonic, methanesulphonic, trifluoroacetic acids and the like, and for peptide derivatives that are sufficiently acidic, for example those having a free carboxylic acid group, salts with bases forming physiologically acceptable cations, such as salts with alkali metal (such as sodium and potassium), alkaline earth metal (such as magnesium and calcium), aluminium and ammonium salts, as well as salts with suitable organic bases such as ethanolamine, methylamine, diethylamine, isopropylamine, trimethylamine and the like.

As stated above, the peptide derivatives of formula I, or a pharmaceutically acceptable salt thereof, will have beneficial pharmacological effect in warm-blooded animals (including man) in a range of autoimmune diseases or medical conditions, to treat symptoms or as a disease modifying agent or as a prophylactic treatment. Such diseases may include, for

example, rheumatoid arthritis, multiple sclerosis, Goodpasture's syndrome, idiopathic thrombocytopenic purpura, juvenile rheumatoid arthritis, coeliac disease, systemic lupus erythematosus, ankylosing spondylitis, Sjogren syndrome, myasthenia gravis, Type 1 (insulin dependent) diabetes, Hashimoto's disease, Grave's disease, Addison's disease, scleroderma, polymyositis, dermatomyositis, pemphigus vulgaris, bullous pemphigoid, autoimmune haemolytic anaemia, pernicious anaemia, glomerulonephritis, graft rejections and such like, especially rheumatoid arthritis and multiple sclerosis.

The utility of the peptide derivatives of the formula I, or a pharmaceutically acceptable salt thereof, may be assessed using a variety of standard tests and clinical studies, including those described in International Patent Application, Publication Nos. WO92/02543, WO93/05011 and WO95/07707 (or modifications thereof) and those described below. The peptide derivatives of formula I show significant activity in one or more of such tests or studies.

Test A: Purified HLA-DR peptide in vitro binding assay. (This assay may be used to demonstrate the binding of the peptide derivatives of formula I to disease-associated MHC class II molecules.) 30µl of biotin-FHA $_{307-320}$ (FHA (307-320) peptide, derivatised with long-chain biotin at the N-terminus, Biotin-Ahx-Pro-Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-Gly-OH) at 800nM in phosphate buffered saline solution (PBS)) is incubated with 30µL of purified HLA-DR4Dw4 at a concentration between 0.5 and 5 µg/ml in V-welled micro-titre plates (Nunc) for 48 hours with or without inhibitor peptides. At the end of the incubation period 100ul of the incubate is transferred to Enzyme Linked ImmunoSorbant Assay (ELISA) plates (Nunc) previously coated with an anti-MHC antibody (L243 - American Type Culture Collection (ATCC) HB55 as described in Lampson and Levy (1980) J. Immunol. 125, 293-299) at a concentration of 10 µg/ml for 1 hour at room temperature and blocked thereafter for 1 hour with 1% bovine serum albumin (BSA) in PBS and 0.05% Tween 20. After a further 1 hour period the unbound peptide is washed away and a 1/4,000 dilution of streptavidin peroxidase (Sigma) in PBS with 0.01% of a suitable detergent such as NP-40 (Sigma) added for 2 hours at room temperature. After further washing tetramethylbenzidene (TMB) substrate solution (1 TMB tablet (Sigma) in 10 mls of 0.1M citrate/acetate buffer, pH 6.0 with 36 µl urea hydrogen peroxide (UHPO) (Fluka)) is added to each of the plates. The reaction is stopped by adding 2M sulphuric acid (10µl per well) and the absorbance read at

450nm to quantify the amount of peptide bound. The inhibitory activity of peptides is obtained by plotting absorbance against concentration.

The purified HLA-DR4Dw4 may be obtained as follows:-

(i) Expression of HLA-DR in the Baculovirus system

The expression of recombinant proteins in insect cells from baculovirus vectors is an established procedure to obtain high yields of recombinant protein [Luckow, VA & Summers, MD (1988) Biotechnology, 6,47-551]. To enable the expression of the heterodimeric HLA-DR, eg. HLA-DR4Dw4, from a single recombinant baculovirus vector (as opposed to having separate recombinant viruses for the α and β chains and then doing a co-infection), a double-recombinant baculovirus is constructed which carries both the α and β chains.

A cDNA encoding the sequence of the α polypeptide is cloned into the transfer vector pacYM1 [Matsuura, Y; Possee, RD; Overton, HA & Bishop, DHL (1987) J. Gen. Virol., 68, 1233-1250] to place expression of the protein under the control of the polyhedrin promoter. The unit is inserted into the baculovirus genome by homologous recombination in Sf21 insect cells to create a single recombinant baculovirus for the α chain. The techniques for the culture and infection of insect cells, for the homologous recombination and detection/isolation of recombinant viruses are all fully described by Summers, MDD & Smith GE (1987) [A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures; Texas Agricultural Experiment Station, Bulletin No. 1555]. The molecular genetic techniques used to construct the recombinant vectors are likewise readily available in the literature and are most fully described by Sambrook, J; Fritsch, EF & Maniatis T, (1989) [Molecular Cloning. A Laboratory Manual. 2nd Edition. Cold Spring Harbor Laboratory Press].

To create the double-recombinant baculovirus, a cDNA encoding the β chain is cloned into the transfer vector pAcUW1 [Weyer, U; Knight, S & Possee, RD (1990) J. Gen. Virol., 71, 1525-1534] to place expression of the protein under the control of the P10 promoter. The unit is then inserted into the genome of the single recombinant baculovirus carrying the α chain. Double-recombinant viruses are detected by spotting insect cells, infected with randomly picked viruses from the transfection, onto membranes and reacting them with a monoclonal antibody, e.g. L243, which specifically recognises the HLA-DR heterodimer. Binding of the antibody to Sf21 insect cells is detected using standard flow cytometry

techniques, readily available in the literature. Stable, double-recombinant baculovirus expressing HLA-DR are plaque-purified.

(ii) Purification of HLA-DR from insect cells

The method used is a modification of that described by Gorga et al 1987. (Gorga et al 1987. J. Biol. Chem. 262, 16087-16094). HLA-DR expressing baculovirus/Sf21 cells (10L which is approximately equal to 2 x 10^{10} cells) are solubilised in 100ml of 5mM EDTA (sodium salt), 50mM Tris-HCL pH 8.5, 2% NP40, 150nM NaCl, 1mM iodoacetamide, 1mM PMSF by homogenisation with 10 strokes of a teflon glass homogeniser. The homogenate is spun at 100,000g for 1 hour and the supernatant collected. The anti-HLA-DR monoclonal antibody LB3.1 (Gorga et al 1986, Cell. Immunol. 103, 160-172) covalently coupled at a ratio of 50mg of L243 to 10ml of Protein A-Sepharose fast flow (Pharmacia) and pre-incubated with 10mM Tris-HCl, pH 8.0, 0.1% NP-40 is incubated overnight with the supernatant. The resin is then put into a column and washed with 10mM Tris-HCl, pH 8.0, 0.1% NP-40 (20 column volumes) followed by 0.15 M NaCl, 50nM Na_2HPO_4, pH 7.0 1% octylglucoside (20 column volumes). The HLA-DR is eluted with 50mM diethylamine pH 11.0, 0.15 M NaCl, 1% octylglucoside. Column fractions are immediately neutralised with 1 M Tris-HCl pH 8.0 and concentrated by ultracentrifugation through a centricon-10 membrane. Protein content is determined by a BCA protein assay (Pierce) and purity by SDA-PAGE electrophoresis.

In general, the peptide derivatives of formula I as defined above which were tested in test A showed significant inhibition at a concentration of about 10μM or much less.

A further preferred aspect of the present invention comprises a peptide derivative of the formula I, or a pharmaceutically acceptable salt thereof, which does not bind to HLA-DR3 but binds to HLA-DR1 and/or HLA-DR4Dw4 and/or HLA-DR4Dw14. HLA-DR3 is a common HLA-DR allele which is not associated with rheumatoid arthritis. Accordingly, in rheumatoid arthritis patients who carry HLA-DR3 as one of their alleles (which is approximately one third of the total rheumatoid arthritis patients), such a peptide derivative of the formula I will not interfere with the normal role of HLA-DR3 in the host-defense function. The use of such a peptide derivative is therefore particularly advantageous for treating rheumatoid arthritis patients as it will result in less immunosuppression than would occur with a non-selective DR binder.

As a variant to test A, the ability of a peptide of the invention to bind to one or more HLA-DR molecules was assessed as follows:

(i) Purification of HLA-DR Types from Cell lines

The method used was a modification of that described by Gorga et al, 1987, J.Biol.Chem. 262, 16087-16094. Human HLA-DR antigens were purified from various cell lines by immunoaffinity chromatography. Briefly, $1x10^9$ - $5 x 10^9$ pelleted cells of the appropriate cell line selected from Hom 2 (source of DR1), BBF (source of DR2), AVL-B (source of DR3), JAH (source of DR4Dw4), JHAF (source of DR4Dwl3) or PE117 (source of DR4Dwl4) were solubilised at approximately 4°C in 50 ml of 5 mM EDTA (sodium salt), 50 mM Tris-HCL pH 7.4, 2% NP40, 150 mM NaCl, 1 mM iodoacetamide, 1 mM PMSF, by homogenisation with 10 strokes of a teflon glass homogeniser. The homogenate was spun at 100,000 g for 1 hour and the supernatant collected. The anti-HLA-DR monoclonal antibody LB3.1 (Gorga et al, 1986, Cell.Immunol., 103, 160-173) covalently coupled to CNBr-Sepharose 4B (Pharmacia) was pre-equilibrated with 150 mM NaCl, 50 mM Tris-HCL, pH 7.4, 0.1% NP-40 and incubated overnight with the supernatant. The resin was then packed in a column and washed with 0.15 M NaCl, 50 mM Tris-HCL, pH 7.4, 1% octylglucoside (20 column volumes). The HLA-DR was eluted with 50 mM diethylamine pH 11.0, 0.15 M NaCl, 1% octylglucoside. Column fractions were immediately neutralised with 0.5 M HEPES NaOH pH 7.4. Protein content was determined by a Biorad protein assay and purity by SDS-PAGE electrophoresis.

(ii) Peptide Selectivity Binding Assays

200nM biotin-FHA $_{307-320}$ in phosphate buffered saline (PBS) was incubated with either purified HLA-DR1, DR2, DR4Dw4, DR4Dw13 or DR4Dwl4 (2-20µg/ml) in V-well microtitre plates (Nunc) with or without inhibitor peptides in assay buffer (PBS, 0.01% NP40 (Sigma.)) For DR3 inhibition, 400nM Biotin-Ahx-(D)Ala-Ala-Ala-Che-Ile-Ala-Ala-Ala-Thr-Leu-Lys-Ala-Ala-(D)Ala-OH was incubated with purified DR3 (20µg/ml.), and incubated as above. After 48 hours, the incubates were treated, and absorbance readings taken as described in Test A. The inhibitory activity of peptides, expressed as IC_{50} values, was calculated using Microcal Origin software on a PC.

Test B: Inhibition of T cell activation in vitro. (This assay may be used to demonstrate the ability of the peptide derivatives of formula I to inhibit a T cell immune response mediated by or through an MHC class II molecule).

Inhibitor peptides were tested for the ability to block stimulation of the B52.24 murine T cell hybridoma line which responds to the $FHA_{307-320}$ peptide (H-Pro-Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-Gly-OH) presented by HLA-DR4Dw4 molecules. B52.24 was produced by the fusion of lymph node T cells taken from $FHA_{307-320}$ immunised HLA-DR4Dw4 transgenic mice (International Patent Application, Publication No WO95/03331) with the BW5147 murine T cell lymphoma line (White et al (1989) J. Immunol. 143, 1822) as outlined in Woods et al (1994) J. Exp. Med. 180, 173-181 and following the general methods for the generation of T cell hybridomas given in Current Protocols in Immunology, Volume 2, 7.21.

Inhibitor peptides at concentrations between 100 and 0.1μM (or lower) were mixed with the antigenic peptide $FHA_{307-320}$ in either varying concentrations between 100 and 0.1 μM or at a fixed concentration of 10 μM by dilution in RPMI-1640 culture media (Gibco) in a 96-well mictrotitre plate (Nunc) in a final volume of 100 μl. HLA-DR4Dw4 expressing B cells such as the JAH EBV transformed lymphoblastoid cell line (European Culture Collection ECACC 85102909) or B cells taken from an HLA-DR4Dw4 homozygous individual and transformed with Epsten Barr virus according to the method described in Current Protocols in Immunology 7.22.1 were fixed using gluteraldehyde by suspension in 1% gluteraldehyde (Sigma) at 4 x 10^6 cells/ml for 30 seconds, after which an equal volume of 200mM lysine (Sigma) was added for 3 minutes. The cells were recovered by centrifugation at 300g, washed in RPMI-1640 and added to the microtitre plates containing antigen and inhibitor compounds at a concentration of 2 x 10^5 cells per well. The microtitre plates were incubated for 2 hours at 37°C and 5% CO_2.

The microtitre plates were then washed in RPMI-1640 by centrifugation at 300g and aspirated twice before the addition of the B52.24 T cell hybridoma line at a concentration of 10^5 cells per well in culture medium (RPMI-1640, 10% foetal calf serum (Gibco) and 2mM glutamine (Gibco)). The microtitre plates were then incubated for a further 2 days at 37°C and 5% CO_2. The plates were then centrifuged at 300g for 10 minutes and 150μl of supernatant removed from all wells to be frozen at -20°C prior to bioassay for IL-2 content.

The culture plates containing supernatants to be assayed were left at room temperature to thaw and 100ml of supernatant was transferred to fresh 96 round bottomed well plates. 1:1 serial dilutions of IL-2 were carried out using culture media (RPMI-1640 (Gibco), 10% foetal calf serum (Advanced Protein Products), 100 μg/ml streptomycin and 100 U/ml penicillin

(Gibco), 2mM L-glutamine (Gibco) and 50 μM 2-mercaptoethanol (Sigma)), to produce a standard curve of 250 units/ml to 0.04 units/ml IL-2 final. An IL-2 dependent cell line such as CTLL-2 cells (Nature (1977) 268 154-156) or HT-2 cells (J. Immunol. Methods (1987) 94-104) were harvested and washed twice using culture media prior to resuspension at 5×10^4 cells/ml. 100μl of IL-2 dependent cell suspension was added to each well of the standard curve and test samples. The culture plates were incubated for 72hrs at 37°C and 5% CO_2. After which, 20μl (1mCi) of 3H-Thymidine (Amersham International) was added to each well and the plates returned to the incubator for a further 16hrs. The contents of each plate were harvested onto glass fibre filter mats and the radioactivity measured using a betaplate scintillation counter.

In general, the peptide derivatives of formula I as defined above which were tested in test B showed significant inhibition at a concentration of about 10μM or much less.

Test C: Peptide stimulated DTH (delayed type hypersensitivity) in BALB/C mice. (The assay may be used to demonstrate in vivo activity of peptide derivatives of formula I in an animal model). Balb/c female mice (18-20g), 5 per group, were immunised sub-cutaneously on the flank with 0.1ml of an emulsion of ovalbumin (Sigma) (2mg/ml in saline) mixed 1:1 (v/v) with complete Freunds adjuvant (Sigma). Seven days later footpad thickness was detemined using a dual caliper micrometer followed by a challenge in one hind footpad with a 30μl sub-plantar injection of 1% heat-aggregated ovalbumin protein in saline. Twenty-four hours after antigen challenge, footpads were measured and the DTH response calculated as the percentage increase in footpad thickness in the injected footpad compared to contralateral control. Inhibitors were adminstered by 3-day osmotic mini-pumps (Alzet) implanted 24 hours prior to antigen challenge at doses ranging from 10mg/kg/day to 0.1 μg/kg/day. The degree of inhibition was calculated by substracting the value for swelling of inhibitor treated footpads from that of the vehicle dosed controls, dividing by the control value and multiplying by 100%.

In general , the peptide derivatives of formula I as defined above which were tested in Test C showed significant inhibition at a dose of about 1mg/kg/day or much less, without any overt toxicological or other untoward pharmacological effect.

Test D: (This assay may be used to demonstrate in vivo activity of peptide derivatives of formula I in an animal model of arthritis).

Balb/c female mice (19-21g, 5-10/group) are immunised on day 0 and boosted on day 7 with a sub-cutaneous injection of 0.1ml of an emulsion containing equal volumes of 2mg/ml methylated bovine serum albumin (met-BSA, Sigma) in saline and complete Freund's adjuvant (Sigma) supplemented with 2.5mg/ml Mycobacterium tudercolosis (MTB, strains C, DT and PN, MAFF, Weybridge, Surrey) thus giving a final MTB concentration of 3.5 mg/ml. An additional 0.1 ml i.p injection of 10^9 Bordetella pertussis organisms (Wellcome Pertussis vaccine) in saline is given at the same time. Fourteen days later, animals are challenged into one knee joint with a 10µl intra-articular injection containing 100ug of met-BSA in saline using a 30G needle and hamilton syringe. The contralateral knee is injected with a similar volume of saline and serves as a control. The degree of inflammation/swelling associated with both knees is determined 13 days later by measuring using a dual-caliper micrometer. This is achieved by making an incision with blunt-ended scissors and forceps into the skin approximately 5mm above and below the knee and continuing along the side of the knee to form a flap which is then carefully cut away to expose the underlying joint. Measurements are made across the widest part of the knee, in the horizontal plane, on the flexed limb held in a fixed position. Percentage increase in inflammation in the antigen-injected knee compared to control is calculated according to the formula: [antigen-injected knee thickness - saline-injected knee thickness/saline-injected knee thickness] x 100. Inhibitors are administered using 14 day osmotic mini-pumps (Alzet) implanted 24 hrs before antigen challenge at does ranging from 10mg/kg/day to 0.1ug/kg/day. The percentage inhibition of inflammation/swelling is calculated from the thickness measurements by subtracting the value for swelling in the inhibitor-treated group from that of the vehicle dosed controls, dividing by the control value and multiplying by 100. Additional assessments of disease involve 1) the histological evaluation of inflammation, synovitis and cartilage/bone erosions carried out on fixed knee sections stained with haemotoxylin and eosin and 2) the determination of levels of acute phase reactants in serum, serum amyloid P and/or haptoglobin.

Peptide derivative of formula I as defined above may show in Test D significant inhibition at a dose of about 10 mg/kg/day or much less.

By way of illustration of the pharmacological activity of particular peptide derivatives of the formula I, the compounds of Examples 3 and 6 showed significant binding to HLA-

DR4Dw4 in Test A (or the variant to test A described hereinbefore) at a concentration of 0.1 micromolar or less, and were active at <0.1 mg/kg/day in Test C. The compound of Example 3 also showed significant binding to HLA-DR4Dw14, but no significant binding (IC_{50} >100 micromolar) to HLA-DR1, HLA-DR2 or HLA-DR3 was found with the compounds of Example 3 or Example 6. Both compounds showed good aqueous stability at pH3 and pH7.6 and, in the form of an extracted polymer depot formulation, showed minimal loss due to degradation on extrusion and minimal degradation on release from such a depot formulation.

A peptide derivative of formula I may be prepared by any process well known in the art of peptide chemistry to be applicable to the synthesis of analogous peptides.

A peptide derivative of formula I may be obtained, for example, by procedures analogous to those disclosed in "Solid Phase Peptide Synthesis: A practical approach" by Atherton and Sheppard (published by IRL press at Oxford University Press, 1989). "Solid Phase Peptide Synthesis" by Stewart and Young (published by the Pierce Chemical Company, Illinois, 1984), "Principles of Peptide Synthesis" (published by Springer-Verlag, Berlin, 1984), and a series of books "Amino Acids, Peptides and Proteins" (volumes 1 - 25; volume 25 published in 1994) (published by the Royal Society of Chemistry, Cambridge, UK).

Preferably, a peptide derivative of formula I is prepared by solid phase sequential synthesis. Using this technique, the amino acid which is to become the C-terminus amino acid of the peptide is protected at the alpha-amino group, and, if necessary, in the side chain and coupled to a solid support, for example a resin, such as 2-chlorotritylchloride resin or Merrifield resin (chloromethylpolystyrene-divinylbenzene) if a free carboxylic acid is required after cleavage, or Rink Amide resin (4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin) or Rink Amide MBHA resin (N-(4-[2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucyl)-4-methyl benzhydrylamine resin (all available from Calbiochem-Novabiochem) if a carboxamide is required after cleavage, whereafter the protecting group on the alpha-amino group is removed. The amino acid which is to be attached to the C-terminus amino acid is protected at the alpha-amino group and, if necessary, in the side chain and coupled to the C-terminus amino acid which remains attached to the solid support. The stepwise process of deprotection of the alpha-amino group and coupling to the next amino acid is repeated to give a protected or unprotected polypeptide attached to the solid support. A protected amino acid of formula II may be obtained, for example, as described in the Examples or as illustrated in Scheme 1 hereinafter, or by analogy therewith,

using a symmetrical or unsymmetrical amine of the formula R^1R^2NH. Amines of the formula R^1R^2NH may be obtained by methods well known in the art, for example, as described in the Examples or as illustrated in Scheme 2 hereinafter, or by analogy therewith. The reagents and conditions used for carrying out the reaction steps of Schemes 1 and 2, such as alkylation of alcohols and amines, coupling of amines and carboxylic acids to form amides, formation of ureas and carbamates, and protection and deprotection of protecting groups, are all well known in the art and described in standard textbooks. The group of formula III or IV is incorporated into the sequence by using an appropriately protected (3-amino-2-oxo-pyrrolidin-1-yl)alkanoic acid (for a peptide compound containing III in which A = methylene) or a corresponding oxa analogue obtained as described in J. Med. Chem., 1993, 36, 256-263 or by analogy therewith (for a peptide compound containing III in which A is oxygen) or an (6-oxo-1,7-diazaspiro[4.4]non-7-yl)alkanoic acid (for a peptide compound containing IV) in place of a protected amino acid. The group of formula V may be incorporated into the sequence using an appropriately protected 3-amino-2-oxoperhydroazepine-1-alkanoic acid obtained as described in J. Med. Chem., 1993, 36, 256-263 (or by analogy therewith) or as described in the Examples hereinafter (or by analogy therewith). The protected or unprotected polypeptide is released from the solid support by standard procedures, for example using a mixture of trifluoroacetic acid, triethylsilane and water.

It will be appreciated that a side-chain protecting group may be cleaved under the conditions used to release the peptide from the solid support, or may be cleaved as a separate step prior or subsequent to release of the peptide from the solid support. It will also be appreciated that the procedure to build up the polypeptide may be modified by using a sequence of two or more suitably protected amino acids in a particular coupling step. The synthesis may use manual techniques or be carried out automatically, employing for example, an Applied Biosystems 431A or 430A peptide synthesiser, an Advanced Chemtech ACT357 peptide synthesiser or similar automatic peptide synthesiser, or a combination of both techniques may be used.

During the assembly of the peptides, the amino acid functional groups not taking part in the reaction are protected by various functional groups. For example, the N-terminal and side chain amino groups may be protected by using 9-fluorenylmethoxycarbonyl (Fmoc), t-butoxycarbonyl (Boc), biphenylisopropoxycarbonyl (Bpoc), 2-[3,5-dimethoxyphenyl]propyl-2-oxycarbonyl (Ddz), adamantyloxycarbonyl (Adoc), allyloxycarbonyl (Aloc), 2,2,2-

trichloroethoxycarbonyl (Troc), benzyloxycarbonyl and various substituted benzyloxycarbonyl groups. These protecting groups can be cleaved when required by the standard techniques (e.g. acid or base treatment, catalytic hydrogenolysis and Pd(0) treatment or zinc/acetic acid treatment).

Suitable protecting groups used for the protection of the side chain guanidino group in the peptides containing an arginine residue include a nitro, adamantyloxycarbonyl, 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr), 2,2,5,7,8-pentamethylchroman-6-sulphonyl (Pmc) and (especially) 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulphonyl (Pbf) group.

Suitable protecting groups used for the protection of a side chain hydroxy group include t-butyl, benzyl and trityl (Trt). Suitable protecting groups used for the side chain imidazole group in the peptides containing a histidine residue, include a trityl, benzyl, tosyl, dinitrophenyl, Adoc, Boc or Fmoc group.

Suitable protecting groups used for the protection of a side chain carboxyl group include various esters (e.g. methyl, ethyl, t-butyl, benzyl, nitrobenzyl, allyl and 9-fluorenylmethyl).

The protecting group cleavage reactions can be performed at temperatures in the range of 4°C to 40°C (preferably at or about ambient temperature) and over a period of time in the range of 10 minutes to 24 hours.

Suitable coupling methods used for the coupling of the individual amino acids include the commonly used azide, symmetrical anhydride, mixed anhydride and various active esters and carbodiimides. In the case of various carbodiimides (e.g. dicyclohexyl- or diisopropyl-carbodiimides), a number of additives (e.g. 1-hydroxybenzotriazole (HOBT) and N-hydroxysuccinimde) may also be added. In addition, the amino acid couplings can also be achieved by using a number of other reagents, e.g. 1H-benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBOP), (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (HBTU). The coupling reactions can be performed at temperatures in the range of -20°C and 40°C and over a period of time in the range of 10 minutes to 24 hours. A suitable medium for carrying out the coupling reactions includes, for example, N,N-dimethylformamide (DMF). A particularly suitable method includes the use of HBTU, HOBT and diisopropylethylamine in DMF.

These and other methods of peptide synthesis are exemplified in the International Patent Applications referred to herein. A hydrophobic residue P which is a group of the formula R-, R.CO-, R.SO_2-, R.O.CO-, R.NHCO-, R.O.CS-, R.S.CO-, R.NHCS-, R.S.CS- and R.CS- (or such a group present as a substituent on a terminal amino group of P where P is a hydrophobic amino acid or a hydrophobic amino acid bearing further amino acids) may be incorporated, for example, as a final step by alkylation, acylation or other standard functional group modification of a terminal amino group. When C-terminus modifications are required (to obtain a particular value for Q), they may be performed after the peptide is synthesised, using conventional functional group modification. Alternatively a particular value for Q may be obtained by appropriate choice of the initial starting resin and/or the protected entity first coupled to the resin (for example by using a suitably protected group of the formula H-Q). Typical examples of the preparation of peptide compounds of formula I are provided in the examples hereinafter.

A typical procedure for measuring the stability of a peptide derivative of the present invention is as follows, in which, to minimize microbial contamination and degradation, all equipment that is used to prepare peptide solutions is sterilized in an autoclave and all material transfers carried out in a Class II laminar flow cabinet. Approximately 20ml of McIlvaine's citric acid-phosphate buffer solution at pH 3 or 7.6 containing 0.02% sodium azide is filtered into a 50ml bottle using a sterile 0.22μm filter unit and a 20ml syringe. Approximately 1.2 mg of peptide is accurately weighed in a capped vial. Using a sterile pipette tip, sufficient buffer solution is added to the peptide in the vial to give a peptide concentration of 0.1 mg/ml. The vial is capped and shaken to dissolve the peptide. Using a sterile pipette tip, aliquots of approximately 1ml of the peptide solution are transferred to 10 HPLC vials, which are then capped. 5 vials are stored at -18 and 37°C. The area of the peptide peak for the solution is determined by HPLC using appropriate standards initially and after storage at -18 and 37°C for 1, 2, 3 and 4 weeks, using a fresh vial at each time point with duplicate sample injections. The percentage of peptide remaining after storage at 37°C at each time point is determined from the ratio of the area of the peptide peak at each time point to the initial area. Preferred peptide derivatives of the present invention have greater than 90%, and preferably greater than 95%, of peptide remaining after storage at 37°C at both pH 3 and 7.6.

The peptide derivative of formula I will generally be administered for therapeutic or

prophylactic purposes to warm-blooded animals (including man) requiring such treatment in the form of a pharmaceutical composition, as is well known in the pharmaceutical art.

According to a further feature of the invention there is provided a pharmaceutical composition which comprises a peptide derivative of the formula I, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable diluent or carrier.

The composition may be in a form suitable for oral use, for example a tablet, capsule, aqueous or oily solution, suspension or emulsion; for nasal use, for example a snuff, nasal spray or nasal drops; for vaginal or rectal use, for example a suppository; for administration by inhalation, for example as a finely divided powder or a liquid aerosol; for sub-lingual or buccal use, for example a tablet or capsule; or for parenteral use (including intravenous, subcutaneous, intramuscular, intravascular or infusion), for example a sterile aqueous or oily solution or suspension. The composition may be in a form suitable for topical administration such as for example creams, ointments and gels. Skin patches are also contemplated. Formulation in general is described in Chapter 25.2 of Comprehensive Medicinal Chemistry, Volume 5, Editor Hansch et al, Pergamon Press 1990.

In general the above compositions may be prepared in a conventional manner using conventional excipients. However, in the case of a composition for oral administration, it may be convenient for the composition to include a coating to protect the polypeptide active ingredient from the actions of enzymes in the stomach.

A preferred composition of the invention is one suitable for oral adminstration in unit dosage form for example a tablet or capsule which contains from 2.5 to 500mg, and preferably 10 to 100 mg, of polypeptide in each unit dose, or one suitable for parenteral administration which contains from 0.5 to 100mg of polypeptide per ml, and preferably 1 to 10mg of polypeptide per ml of solution.

A parenteral composition is preferably a solution in isotonic saline or isotonic dextrose buffered if necessary to a pH of 5 to 9. Alternatively, the parenteral composition may be one designed for slow release in which case the amount of polypeptide per unit dose is in general greater than that required when a conventional injectable formulation is used. A preferred slow release formulation is a continuous release formulation, for example a formulation of the type described in European Patent Specification No. 58481 or, for peptide derivatives of formula I containing at least one basic group, a formulation as described in International Patent Application, Publication No. WO93/24150. Certain peptide derivatives of the present

invention possess solubility characteristics which make them particularly suitable for the manufacture and processing of slow release parenteral formulations, particularly formulations containing biodegradeable polyesters such as polylactides, and for providing slow release formulations with beneficial release profiles. Furthermore, peptide derivatives of the present invention containing one or more basic groups, particularly arginine, can also form peptide-polymer salts with acid-ended polyesters, such as polylactides, and such peptides and peptide-polymer salts constitute a further aspect of the present invention. Certain such salts possess solubility characteristics which make them particularly suitable for the manufacture and processing of slow release parenteral formulations, for example as described in WO93/24150, and for providing slow release formulations with beneficial release profiles and storage stability charateristics. A preferred slow release parenteral formulation contains from 1 to 100 mg (such as 5 to 50 mg) of polypeptide per unit dose. A preferred slow release parenteral formulation is also one designed for slow release over a period of at least 5 days.

Preferred peptide derivatives of the present invention include those which, when in the form of an extruded polymer depot formulation, show minimal loss due to degradation on extrusion or which show minimal degradation on release from such a depot formulation. Typical procedures for measuring the level of degradation of a peptide of the present invention are as follows:-

<u>Preparation of extruded polymer depot formulation of peptide</u>

About 20 mg of peptide is accurately weighed and sufficient polymer (50/50% molar poly(D,L-lactic acid/glycolic acid) copolymer of approximate weight average molecular weight 20kD and approximate polydispersity of 1.7 as determined by size exclusion chromatography relative to polystyrene standards) added to produce an approximate 20% w/w mixture. This is dissolved in anhydride-free glacial acetic acid to produce an approximate 10% w/v solution. The solution is freeze dried and the resulting freeze dried product is stored under vacuum prior to use.

About 100mg of freeze dried material is loaded into the barrel of a small laboratory extruder and the plunger pushed down to consolidate the sample. The extruder is heated to between 90 and 95 °C and held at this temperature for 10 minutes before the freeze dried material is extruded under pressure to give a cylindrical extrudate of approximately 1mm in diameter.

Analysis of peptide content of extruded polymer depot formulation of peptide

Two approximate 5mm lengths of extruded polymer depot containing peptide are accurately weighed and each dissolved in 1ml of anhydride-free glacial acetic acid in separate 25ml volumetric flasks. After about 1.5 hours the volume of each is made up to 25ml with distilled water, causing the polymer to precipitate. The solids are filtered off using a 0.5μm Millex PTFE filter and the solutions, A, collected.

A series of standard solutions are prepared from a stock solution of peptide in distilled water at 0.5mg/ml and a stock solution of polymer in anhydride-free glacial acetic acid at 2.5mg/ml as follows with each solutions made up to 10ml with distilled water:

Concentration of peptide(μg/ml)	Volume of stock polymer solution (μl)	Volume of stock peptide solution (μl)
50	1000	1000
40	1000	800
30	1000	600
20	1000	400
10	1000	200
5	1000	100
0	1000	0

Each standard is filtered through a 5μm Millex PTFE filter and an aliquot of filtrate, together with aliquots of the solutions A, analysed by HPLC using duplicate sample injections. The peptide content of the extruded polymer depot formulation of peptide is calculated from the concentration of peptide in solutions A, which is determined by comparing the area of the peptide peak in solutions A with the area of the peptide peak from the standard solutions. Preferred peptide derivatives of the present invention show minimal loss due to degradation on extrusion and thus the peptide content of the extruded polymer depot formulation is close to the approximate theoretical value of 20% w/w.

Degradation of peptide on *in vitro* release from an extruded polymer depot

A solution of McIlvaine's citric acid-phosphate buffer solution at pH 7.6 containing 0.02% sodium azide, is filtered through a 0.22μ filter and stored at 4°C. Approximately 10 mg of extruded polymer depot containing peptide is placed in two small vials and 2ml of the buffer solution added. The vials are then capped and stored in a water bath at 37°C for a month. At suitable time points over a month, three 0.6ml aliquots of release medium are removed from each vial and either analysed by HPLC or stored frozen in an HPLC vial at -18°C prior to analysis by HPLC. 1.8ml of the buffer solution is added to each vial containing the depot to replace the release medium that has been removed at each time point.

The average amount of intact peptide in the release medium at each time point is determined by HPLC using duplicate sample injections by comparing the area of the peptide peak in the release media with the area of the peptide peak from standard buffer solutions of peptide at known concentrations. The approximate average amount of peptide degradation products in the release media at each time point is determined by HPLC by comparing the area of additional new peaks in the release media with the area of the peptide peak from standard buffer solutions of peptide at known concentrations and assuming the extinction coefficient has not changed. The average cumulative *in vitro* release profile of intact peptide and total peptide (intact peptide and peptide degradation products) is determined from the amounts of intact peptide and peptide degradation products in the release medium at each time point. Preferred peptide derivatives of the present invention show minimal degradation on *in vitro* release and thus show total peptide degradation products of less than 10% and preferably less than 5% of total peptide after a month of *in vitro* release into McIlvaine's buffer solution at pH 7.6 at 37°C.

The composition of the invention will generally be administered to man such that, for example, a daily dose will be from 10 micrograms to 5000mg, preferably 0.1 to 100mg, for a 70kg patient, given in divided doses as necessary. The precise amount of composition administered and the route and form of administration may depend on the size, age and sex of the person being treated and on the particular disease or medical condition being treated and its severity, according to principles well know in the medical art.

A peptide derivative of formula I, or a pharmaceutically acceptable salt thereof, may also be advantageously administered for therapeutic or prophylactic purposes together with

one or more other pharmacological agents known in the general art to be of value in treating or relieving the symptoms of (or to be a disease modifying agent of) one or more of the diseases or medical conditions referred to hereinabove, such as a NSAID (such as ibuprofen or piroxicam), an analgesic (such as paracetamol), a corticosteroid, a muscle relaxant, a lipoxygenase inhibitor, methotrexate, azathioprine, D-penicillamine, Cyclosporin A or a monoclonal antibody therapy (such as anti-CD4 or anti-TNF). In diabetes the peptide compound may be co-administered with insulin or other therapies for diabetes or diabetes complications, (such as an aldose reductase inhibitor). It is to be understood that such combination therapy consitutes a further aspect of the invention.

According to a further aspect of the present invention there is provided a method for treating a MHC class II dependent T-cell mediated autoimmune or inflammatory disease, for example one or more of the diseases or medical conditions referred to herein, which comprises administering to a warm-blooded mammal (including man) in need of such treatment an effective amount of a peptide derivative of formula I, or a pharmaceutically acceptable salt thereof. The invention also provides the use of a peptide derivative of formula I, or a pharmaceutically acceptable salt thereof in the production of a novel medicament for use in the treatment of a MHC class II dependent T-cell mediated autoimmune or inflammatory disease.

In addition to their aforesaid use in therapeutic medicine in humans, the peptide derivatives of formula I are also useful in the veterinary treatment of similar conditions affecting commercially valuable warm-blooded animals, such as dogs, cats, horses and cattle. In general for such treatment, the peptide derivative of the formula I will be administered in an analogous amount and manner to those described above for administration to humans. The peptide derivatives of formula I are also of value as pharmacological tools in the development and standardisation of test systems for the evaluation of the effects of MHC class II molecules in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the continuing search for new and improved therapeutic agents, or as diagnostic reagents.

The invention will now be illustrated by the following non-limiting Examples in which, unless otherwise stated:-

(i) concentrations and evaporations were carried out by rotary evaporation in vacuo;

(ii) operations were carried out at room temperature, that is in the range 18-26°C;

(iii) yields, when given, are intended for the assistance of the reader only and are not necessarily the maximum attainable by diligent process development;

(iv) the following abbreviations are used (and in Schemes 1 and 2):

Phv = 5-phenylvaleryl; Boc = tert-butoxycarbonyl; tBu = tert-butyl; DMF = N,N-dimethylformamide; HOBT = 1-hydroxy-benzotriazole; Met = methionine; Fmoc = 9-fluorenylmethyloxycarbonyl; Fmoc-Pip-OH = N-(9-fluorenylmethoxycarbonyl)piperidine-4-carboxylic acid; Fmoc-Papa-OH = 4-[N-(9-fluorenylmethoxycarbonyl)amino]phenylacetic acid; Z or CbZ = benzyloxycarbonyl; Pmc=2,2,5,7,8-pentamethylchroman-6-sulphonyl; Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulphonyl; Trt = trityl; THF = tetrahydrofuran; DMSO = dimethylsulfoxide; HBTU = 2-(1H-benzotriazol-1yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate; DIPEA = diisopropylethylamine; TFA = trifluoroacetic acid; HPLC = high pressure liquid chromatography; and RP-HPLC = reverse phase high pressure liquid chromatography (which unless otherwise stated was carried out on a Vydac C18 column 218TP54, 4.6 x 250mm);

(v) flash chromatography and chromatography on silica was performed on Merck Kieselgel 60 (Art No. 9385) obtained from E Merck, Darmstadt, Germany;

(vi) ^{1}H NMR spectra were determined at 200 Mhz in $CDCL_3$ or d_6-dimethylsulphoxide (d_6-DMSO) using tetramethylsilane (TMS) as an internal standard, and are expressed as chemical shift (delta) values in parts per million relative to TMS using conventional abbreviations for designation of major peaks: s, singlet; m, multiplet; t, triplet; br, broad, d, doublet;

(vii) the following Fmoc-protected amino acids were used for the introduction of a Lys, Thr, Arg or His residue:

for Lys: Fmoc-Lys(Boc)-OH; for Thr: Fmoc-Thr(OtBu)-OH; for Arg: Fmoc-Arg(Pmc)-OH or Fmoc-Arg(Pbf)-OH; and for His: Fmoc-His(Trt)-OH;

(viii) where -II- appears in a formula, this means a residue of the L-amino acid of formula II as set out hereinafter, the particular values for n, X, R^1 and R^2 being given; and

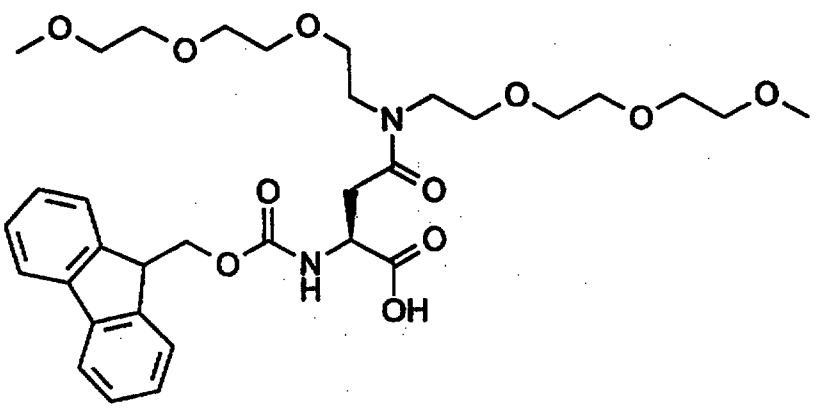
(ix) where -IIIa- appears in a formula, this means a group of the formula IIIa as set out hereinafter.

Example 1

Preparation of Phv-Ala-Ala-Ala-II-Val-Ala-Ala-Ala-Pip-NH_2 (SEQ ID NO : 1)

(Formula II: n = 1; X = carbonyl; $R^1 = R^2 = -CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$)

1.1 Preparation of N^2-(9-fluorenylmethyloxycarbonyl)-N^4,N^4-bis(2-[2-(2-methoxyethoxy)ethoxy]ethyl)-L-asparagine



(a) 1-Bromo-2-(2-methoxyethoxy)ethane (30 g) was added to a stirred soln. of N-benzyldiethanolamine (10g) in tetrahydrofuran (100ml) under nitrogen. Sodium hydride (5.3g of a 60% dispersion in mineral oil) was then added in portions whilst cooling in a water-bath. After addition was complete, the mixture was stirred for 2 hours and further sodium hydride (4.4 g of a 60% dispersion in mineral oil) was added in portions. The mixed was then allowed to stir at ambient temperature for 16 hours. Water (50ml) was cautiously added to the reaction mixture to destroy excess sodium hydride and the mixture was evaporated to remove the organic solvent. Water (100ml) was added to the residue and the mixture was acidified to about pH2 with concentrated hydrochloric acid and then extracted with diethyl ether (2 x 100ml). The extracts were discarded. The aqueous layer was then adjusted to about pH12 by addition of concentrated sodium hydroxide solution and extracted with diethyl ether (2 x 150ml). The ether extracts were combined, washed with brine, dried ($MgSO_4$) and evaporated to dryness. The residue was purified by chromatography on silica using a gradient of chloroform increasing to 5% methanol/chloroform saturated with concentrated aqueous ammonia. The appropriate fractions were collected and evaporated to dryness to give a gum (12g). Methanol (100ml) was added, followed by 10% palladium on carbon (2.5g) and ammonium formate (7.2g), and the mixture was stirred under nitrogen for 1hour at 60°C. The

mixture was then cooled, filtered and evaporated to dryness. The residue purified by chromatography on silica using a gradient of chloroform increasing to 10% methanol/chloroform saturated with concentrated aqueous ammonia. The appropriate fractions were collected, combined and evaporated to dryness to give N,N-bis(2-[2-(2-methoxyethoxy)ethoxy]ethyl)amine as a gum (7.1g).; NMR ($CDCl_3$): 2.8 (m, 4H), 3.3 (s, 6H), 3.45 (m, 4H), 3.6 (m, 16H).

(b) N-Methylmorpholine (1.6g), N-tert-butyloxycarbonyl-L-aspartic acid α-benzyl ester (2.6g), hydroxybenztriazole (2.16g) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.68g) was added to N,N-bis(2-[2-(2-methoxyethoxy)ethoxy]ethyl)amine (2.5 g) in dimethylformamide (12 ml) with stirring. The mixture was stirred for 16 hours and then added to 2% acetic acid in water (50 ml). The mixture was extracted with diethyl ether (2 x 50ml) and the combined organic extract was washed with sodium bicarbonate solution and dried ($MgSO_4$). Volatile material was removed by exaporation to give N^2-tert-butyloxycarbonyl-N^4,N^4-bis(2-[2-(2-methoxyethoxy)ethoxy]ethyl)-L-asparagine α-benzylester as a gum (2.8g); NMR ($CDCl_3$):1.4 (s, 9H), 2.9 (m, 1H), 3.2 (m, 1H), 3.4 (s, 6H), 3.6 (m, 24H), 4.6 (m, 1H), 5.2 (q, 2H), 5.8 (d, 1H), 7.4 (s, 5H).

(c) 10% Palladium on carbon (0.8 g) and cyclohexene (1.6 g) was added to a solution of N^2-tert-butyloxycarbonyl-N^4,N^4-bis(2-[2-(2-methoxyethoxy)ethoxy]-ethyl)-L-asparagine α-benzylester (2.6 g) in methanol (20ml) and the mixture was heated at 55°C for 2hours. The mixture was then cooled, filtered and evaporated. Acetone (20ml) and water (8ml) was added to the residue and concentrated hydrochloric acid (2ml) was added. The mixture was heated at 55°C for 1hour, cooled and adjusted to about pH7 with excess solid sodium bicarbonate. 9-Fluorenylmethyl succinimidyl carbonate (1.36g) was added and the mixture was stirred for 16hours. Volatile material was removed by evaporation and the residue was partitioned between water (25ml) and diethyl ether (50ml) . The aqueous layer was separated and acidified to about pH3 with concentrated hydrochloric acid. The mixture was then extracted with dichloromethane (50ml) . The organic extract was washed with water, dried ($MgSO_4$) and evaporated to give N^2-(9-fluorenylmethyloxycarbonyl)-N^4,N^4-bis(2-[2-(2-methoxyethoxy)ethoxy]ethyl)-L-asparagine [hereinafter referred to as Fmoc-Asp(PE)-OH]

(2g) as a gum; NMR ($CDCl_3$): 2.8 (q, 1H), 3.4 (q, 1H), 3.4 (s, 6H), 3.6 (m, 24H), 4.2-4.6 (m, 4H), 6.3 (d, 1H), 7.4 (m, 4H), 7.6 (m, 2H), 7.8 (d, 2H).

1.2 Preparation of Phv-Ala-Ala-Ala-Il-Val-Ala-Ala-Ala-Pip-NH_2

(Formula II: n = 1; X = carbonyl; $R^1 = R^2 = -CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$)

The peptide was prepared by Fmoc solid phase synthesis starting from Fmoc Rink Amide MBHA Resin (Novabiochem, 0.50 g; 0.25 mmoles) by a mixture of automated synthesis and manual synthesis using a Bond Elut tube (Varian, 15 ml, fitted with a filter in the bottom).

Fmoc-Val-Ala-Ala-Ala-Pip-NH-Resin was first obtained using an ABI 431 automated peptide synthesiser by deprotecting the resin and sequentially coupling and deprotecting with Fmoc-Pip-OH (353 mg, 1 mmol), Fmoc-Ala-OH (311 mg, 1 mmol), Fmoc-Ala-OH (311 mg, 1 mmol), Fmoc-Ala-OH (311 mg, 1 mmol) and Fmoc-Val-OH (339 mg, 1 mmol), following the manufacturer's recommended conditions for single acylations incorporating HBTU/HOBT chemistry, as follows. After deprotection, the resin was washed with DMF (10 x 10-20 ml). The carboxylic acid (1mmol) was activated with HBTU (1 equivalent), HOBT (1 equivalent) and DIPEA (2 equivalents) in DMF for approximately 11 minutes before transfer to the resin. The acylation was carried out for approximately 60 minutes and then the resin was washed with DMF (10 x 10-20 ml). Fmoc deprotection at each stage was carried out using a 20% solution of piperidine in DMF (two treatments with 5 ml for 10 minutes each). After each deprotection, the resin was thoroughly washed with DMF
(5 x 10 ml).

The remaining residues in the sequence were sequentially coupled and deprotected manually. Coupling was carried out by addition to the resin of a solution of the appropriate N-Fmoc-protected amino acid (1 mmol), DMF (1.5 ml), HOBT (165 mg, 1 mmol) and diisopropylcarbodiimide (155 microlitres, 1 mmol). The coupling was left for approximately 30 minutes, washed with DMF (5 x 10 ml) and a small portion of the resin checked for completion of the coupling using the Kaiser test (E. Kaiser, et al, (1970), Anal. Biochem. 34, 595). Deprotection was carried out as described above. In this way Fmoc-Asp(PE)-OH (323 mg, 0.5 mmol), Fmoc-Ala-OH (311 mg, 1 mmol), Fmoc-Ala-OH (311 mg, 1 mmol), Fmoc-

Ala-OH (311 mg, 1 mmol) and 5-phenylvaleric acid (178 mg, 1 mmol) were sequentially coupled to the resin. The phenylvaleric acid required a double couple to obtain a positive result by the Kaiser test.

The peptide was cleaved from the resin using a mixture of trifluoroacetic acid (7.9ml) and triethylsilane (0.395ml). After 2 hours the resin was washed with dichloromethane (approximately 150 ml) and the resulting solution was evaporated to dryness. The resulting solid was partitioned between ether (25ml) and water (25ml) and then the ether was extracted with further portions of water (2 x 25ml). The aqueous phases were combined and freeze dried.

The crude product was purified using preparative RP-HPLC (Vydac 218TP1022 column, 250mm x 22mm), loading the crude material in 10 ml of 20% acetonitrile/water. Elution was with a gradient of acetonitrile-water containing 0.1%TFA at a flow rate of 12ml/minute. The fractions containing product were combined and freeze dried to give Phv-Ala-Ala-Ala-Il-Val-Ala-Ala-Ala-Pip-NH_2 (where II is a residue of the L-amino acid of formula II in which n = 1; X = carbonyl and $R^1 = R^2 = -CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$) as a white solid (83mg).

The product was characterised by HPLC, mass spectroscopy and amino acid analysis, as follows.

RP-HPLC (Vydac C18 column, 218TP54, 4.6x250mm, eluting with acetonitrile and water containing 0.1% TFA, using a 10-50% acetonitrile gradient over 30 minutes, flow rate 1.0 ml/minute), indicated 94% purity, retention time 23.29 minutes.

Mass spectrometry, m/e (ES^+) 1220.7 (MH^+).

Amino acid analysis (acid hydrolysis over 24 hours using a solution of 6N HCl containing 1% phenol at 130°C) gave Ala 5.82, Val 0.98, Asp 1.19.

Fmoc-Pip-OH was obtained by an analogous method to that described in E. Atherton and R. C. Sheppard ("Solid phase peptide synthesis: a practical approach", IRL press, 1989, page 51) for *N*-Fmoc-L-methionine:

Fmoc-Pip-OH: NMR (DMSO-d_6) 1.3 (m, 2H), 1.7 (m, 2H), 2.5 (m, 2H), 2.9 (t, 2H), 3.7 (m, 1H), 4.2 (t, 1H), 4.4 (d, 2H), 7.4 (m, 4H), 7.7 (d, 2H), 7.9 (d, 2H); mass spectrometry m/e (ES^+) 352.2 (MH^+)

Example 2

Preparation of Phv-II-Ala-Ala-Lys-Val-Ala-Ala-Ala-Pip-NH_2 (SEQ ID NO : 2)

(Formula II: n = 1; X = carbonyl; $R^1 = R^2 = -CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$)

The synthesis was performed using similar methodology to that described for Example 1.2 by sequentially coupling (manually) Fmoc-Lys(Boc)-OH (468 mg, 1 mmol), Fmoc-Ala-OH (311 mg, 1 mmol), Fmoc-Ala-OH (311 mg, 1 mmol), Fmoc-Asp(PE)-OH (323 mg, 0.5 mmol) and 5-phenylvaleric acid (178 mg, 1 mmol) to Fmoc-Val-Ala-Ala-Ala-Pip-NH-Resin (obtained using manual coupling). The peptide was cleaved from the resin and the crude product purified using similar conditions to those described in Example 1. The product was characterised by HPLC, mass spectroscopy and amino acid analysis, as follows.

RP-HPLC (20-50% acetonitrile gradient over 30 minutes, flow rate 1.0ml/minute) retention time = 16.4 minutes.

Mass spectrometry, m/e (ES^+) 1277.8 (MH^+).

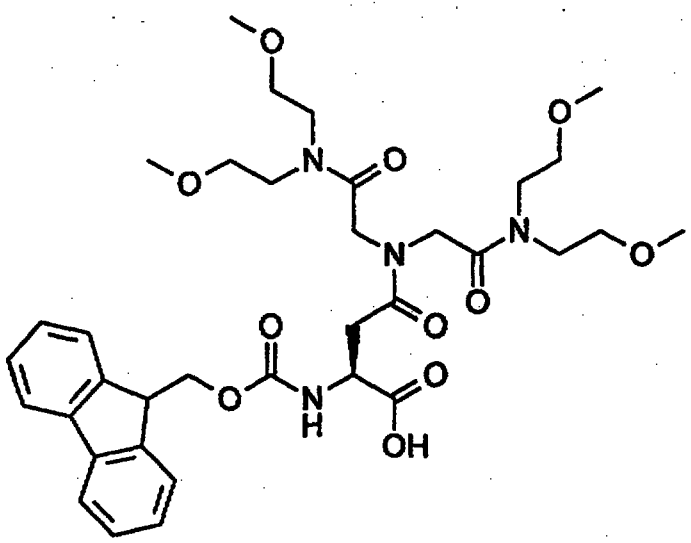
Amino acid analysis gave Asp 1.07, Lys 1.05, Ala 4.9, Val 0.92.

Example 3

Preparation of Phv-Ala-Arg-Ala-II-Thr-IIIa-Ala-Papa-NH_2 (SEQ ID NO : 3)

(Formula II: n = 1; X = carbonyl; $R^1 = R^2 = -CH_2CON(CH_2CH_2OCH_3)_2$)

3.1 Preparation of N^2-(9-fluorenylmethyloxycarbonyl)-N^4,N^4-bis[N,N-bis(2-methoxyethyl)carbamoylmethyl]-L-asparagine



(a) N-methylmorpholine (2 g), hydroxybenzotriazole (4 g), bis(2-methoxyethyl)amine (3.5 g) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.8 g) was added to N-(benzyloxycarbonyl)iminodiacetic acid (2.7 g) in dimethylformamide (15ml) and the mixture was stirred for 16 hours. Volatile material was removed by evaporation and the residue was partitioned between water and dichloromethane. The organic extract was washed with water, dried ($MgSO_4$) and evaporated. The residue was purified by chromatography on silica using a gradient of chloroform increasing to 10% methanol/chloroform to give N-(benzyloxycarbonyl)iminodi-[N,N-bis(2-methoxyethyl)]acetamide as a gum (2.3g); NMR (d_6-DMSO): 3.1-3.4 (4 singlets due to rotamers, 12H), 3.5 (m, 16H), 4.2 (s, 4H), 5.05 (s, 2H), 7.3 (m, 5H).

(b) 10% Palladium on carbon (0.2 g) and cyclohexene (2 ml) was added to N-(benzyloxycarbonyl)iminodi-[N,N-bis(2-methoxyethyl)]acetamide (3.5 g) in ethanol (20 ml) and the mixture was stirred at 55°C under nitrogen for 4 hours. The reaction mixture was then cooled, filtered and evaporated. N-methylmorpholine (1.4 g), hydroxybenzotriazole (1.85 g), N-tert-butyloxycarbonyl-L-aspartic acid α-benzyl ester (2.4 g) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.6 g) was added to a solution of the residue in dimethylformamide (15 ml) with stirring. The mixture was stirred for 16 hours and then added to 2% acetic acid in water (50 ml) and extracted with diethyl ether (2 x 50ml). The combined organic extract was washed with sodium bicarbonate solution , dried ($MgSO_4$) and evaporated to give N^2-tert-butyloxycarbonyl-N^4,N^4-bis[N,N-bis(2-

methoxyethyl)carbamoylmethyl]-L- asparagine α-benzylester (4.1)g as a gum; NMR (d_6-DMSO): 1.4 (s, 9H), 2.6 (m, 2H), 3.2 (overlapping singlets due to rotamers, 12H), 3.4 (m, 16H), 4.0-4.4 (m, 5H), 5.1 (s, 2H), 6.8 (d, 1H), 7.4 (s, 5H).

(c) Using an analogous procedure to that described in Example 1, part (c), but using N^2-tert-butyloxycarbonyl-N^4,N^4-bis[N,N-bis(2-methoxyethyl)-carbamoylmethyl]-L- asparagine α-benzylester (4 g) as starting material, there was thus obtained N^2-(9-fluorenylmethyloxycarbonyl)-N^4,N^4-bis[N,N-bis(2-methoxyethyl)carbamoylmethyl]-L-asparagine (hereinafter referred to as FMoc-Asp(TE)-OH) (3 g) as a gum; NMR ($CDCl_3$): 2.8 (m, 1H), 3.1 (m, 1H), 3.3 (overlapping singlets due to rotamers), 12H), 3.6 (m, 16H), 4.2-4.8 (m, 8H), 6.4 (d, 1H), 7.4 (m, 4H), 7.6 (d, 2H), 7.8 (d, 2H).

3.2 Preparation of Phv-Ala-Arg-Ala-II-Thr-IIIa-Ala-Papa-NH_2

(Formula II: n = 1; X = carbonyl; $R^1 = R^2 = -CH_2CON(CH_2CH_2OCH_3)_2$)

The synthesis was performed using similar methodology to that described for Example 1.2 by deprotecting the resin and sequentially coupling and deprotecting with Fmoc-Papa-OH, Fmoc-Ala-OH, (2S)-[(3R)-3-(N-9-fluorenylmethyloxycarbonylamino)-2-oxo-pyrrolidin-1-yl]propionic acid (Fmoc-IIIa-OH), Fmoc-Thr(OtBu)-OH, Fmoc-Asp(TE)-OH, Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ala-OH and 5-phenylvaleric acid at the appropriate coupling stages, which steps were all carried out manually. The product was characterised by HPLC, mass spectroscopy and amino acid analysis, as follows.

RP-HPLC (10-50% acetonitrile gradient over 30 minutes, flow rate 1.0ml/minute) retention time = 21.88 minutes.

Mass spectrometry, m/e (ES^+) 1395.8 (MH^+).

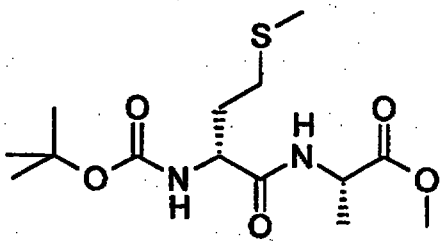
Amino acid analysis gave Asp 1.04, Thr 0.93, Ala 3.12, Arg 0.92.

Fmoc-Papa-OH was obtained by an analogous method to that described in E. Atherton and R. C. Sheppard ("Solid phase peptide synthesis: a practical approach", IRL press, 1989, page 51) for *N*-Fmoc-L-methionine:

Fmoc-Papa-OH: NMR (DMSO-d_6) 3.5 (s, 2H), 4.25 (t, 1H), 4.5 (d, 2H), 7.1 (d, 2H), 7.4 (m, 6H), 7.75 (d, 2H), 7.9 (d, 2H), 9.6 (s, 1H); mass spectrometry m/e (ES^-) 372.1 $(M-H)^-$

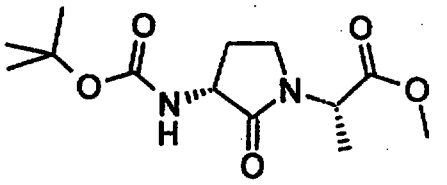
(2S)-2-[(3R)-3-(N-[9-fluorenylmethyloxycarbonyl]amino)-2-oxo-pyrrolidin-1-yl]propionic acid (Fmoc-IIIa-OH) was obtained as follows:

(i) Synthesis of Boc-(D)-Met-(L)-Ala-OMe



N-methylmorpholine (5.6g), L-alanine methyl ester hydrochloride (3.9g), HOBt (4.6g) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (5.3g) was added to a solution of Boc-(D)-Methionine (7g, 0.028mol) in dry DMF (50ml). The mixture was stirred overnight. Solvent was removed by evaporation and the residue was partitioned between dichloromethane (100ml) and 5% aqueous acetic acid (50ml). On standing HOBt crystallised and was removed by filtration, and the organic layer was separated and washed with aqueous sodium bicarbonate, dried ($MgSO_4$) and evaporated. The residue (8.5g) was purified by flash chromatography in a sinter funnel eluting with a mixture of dichloromethane and ether (0% to 100% ether). The fractions containing product were combined and evaporated to give Boc-(D)-Met-(L)-Ala-OMe (7.2 g) as a gum which crystallised on standing; NMR ($CDCl_3$): 1.4 (d, 3H), 1.45 (s, 9H), 1.95 (m, 1H), 2.1 (s, 3H), 2.1 (m, 1H), 2.6 (m, 2H), 3.75 (s, 3H), 4.3 (bs, 1H), 4.6 (m, 1H), 5.3 (m, 1H), 6.9 (bs, 1H).

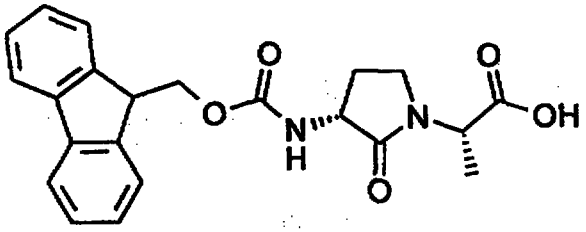
(ii) Synthesis of methyl (2S)- 2-[(3R)-3-(N-[tert-butyloxycarbonyl]amino)-2-oxo-pyrrolidin-1-yl]propionate



Note : This sequence must be conducted under dry conditions with dry solvents otherwise epimerisation will occur. Methyl iodide (10ml) was added to Boc-(D)-Met-(L)-Ala-OMe (8g) in a mixture of DMF (20ml) and dichloromethane (20ml) and the mixture was allowed to

stand for 16 hours and then evaporated to dryness. Further dichloromethane (2x50ml) was added and evaporated to remove residual methyl iodide and the residue was dissolved in a mixture of DMF (300ml) and dichloromethane (300 ml). The mixture was cooled to ~5°C and sodium hydride (0.76g of an 80% dispersion in mineral oil) was added in one portion and the mixture was stirred at this temperature for 2 hours. Saturated aqueous ammonium chloride (50 ml) was added and the mixture was evaporated to dryness and then partitioned between water and ether. The ether extract was washed with brine and dried and evaporated to give a gum which was purified by flash chromatography on a sinter funnel (25% ethyl acetate:hexane to 100% ethylacetate) to give methyl (2S)-2-[(3R)-3-(N-[tert-butyloxycarbonyl]amino)-2-oxo-pyrrolidin-1-yl]propionate as a gum (4.2g) which crystallised on standing: NMR ($CDCl_3$): 1.4 (s, 9H), 1.4 (d, 3H), 1.8 (m, 1H), 2.6 (m, 1H), 3.4 (m, 2H), 3.7 (m, 3H), 4.2 (m, 1H), 4.9 (q, 1H), 5.2 (bs, 1H).

(iii) Synthesis of (2S)-2-[(3R)-3-(N-[9-fluorenylmethyloxycarbonyl]amino)-2-oxo-pyrrolidin-1-yl]propionic acid (Fmoc-IIIa-OH)



Methyl (2S)-2-[(3R)-3-(N-[tert-butyloxycarbonyl]amino)-2-oxo-pyrrolidin-1-yl]propionate (4g) was refluxed in a mixture of acetone (60ml), water (40ml) and concentrated hydrochloric acid (24ml) for 3 hours and then the mixture was evaporated to dryness. Water was added and the evaporation repeated. The residue was dissolved in water (15ml) and excess solid sodium bicarbonate was added. 9-Fluorenylmethyl succinimidyl carbonate (5.2g) in acetone (30ml) was added. The mixture was stirred for 16 hours and then the solvent was removed by evaporation and the residue was partitioned between water and ether. The aqueous layer was separated, its pH adjusted to ~3 with hydrochloric acid, and extracted with dichloromethane. The organic layer was washed with water, dried ($MgSO_4$) and evaporated to give a white foam which crystallised on trituration with ether to give (2S)-2-[(3R)-3-(N-[9-fluorenylmethyloxycarbonyl]amino)-2-oxo-pyrrolidin-1-yl]propionic acid (4.2g) as a white

solid, mp. 191-3°C (dec); NMR ($CDCl_3$): 1.4 (d, 3H), 2.0 (m, 1H), 2.6 (m, 1H), 3.4 (m, 2H), 4.2 (t, 1H), 4.4 (m, 3H), 4.9 (m, 1H), 5.8 (bs, 1H), 7.4 (m, 4H), 7.6 (d, 2H), 7.7 (d, 2H).

Example 4

Preparation of Phv-Ala-Ala-Ala-II-Val-Ala-Ala-Ala-Pip-NH_2 (SEQ ID NO : 4)

(Formula II: n = 1; X = carbonyl; $R^1 = R^2 = -CH_2CON(CH_2CH_2OCH_3)_2$)

The synthesis was performed using similar methodology to that described for Example 1.2, using Fmoc-Asp(TE)-OH instead of Fmoc-Asp(PE)-OH at the appropriate coupling stage. The product was characterised by HPLC, mass spectroscopy and amino acid analysis, as follows.

RP-HPLC (10-50% acetonitrile gradient over 30 minutes, flow rate 1.0ml/minute) retention time = 23.9 minutes.

Mass spectrometry, m/e (ES+) 1274.7 (MH^+).

Amino acid analysis gave Asp 1.03, Ala 5.94, Val 0.98.

Example 5

Preparation of Phv-Arg-Ala-Ala-IIIa-Ala-II-Ala-Papa-NH_2 (SEQ ID NO : 5)
(Formula II: n = 1; X = carbonyl; $R^1 = R^2 = -CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$)

The synthesis was performed using similar methodology to that described for Example 1.2, by sequentially coupling and deprotecting (manually) Fmoc-Papa-OH, Fmoc-Ala-OH, Fmoc-Asp(PE)-OH, Fmoc-Ala-OH, Fmoc-IIIa-OH, Fmoc-Ala-OH (twice), Fmoc-Arg(Pbf)-OH and 5-phenylvaleric acid, followed by cleavage from the resin and purification by preparative RP-HPLC. The product was characterised by HPLC, mass spectroscopy and amino acid analysis, as follows.

RP-HPLC (10-50% acetonitrile gradient over 30 minutes, flow rate 1.0ml/minute) retention time = 20.45 minutes.

Mass spectrometry, m/e (ES+) 656.4 ($M+2H^{++}$).

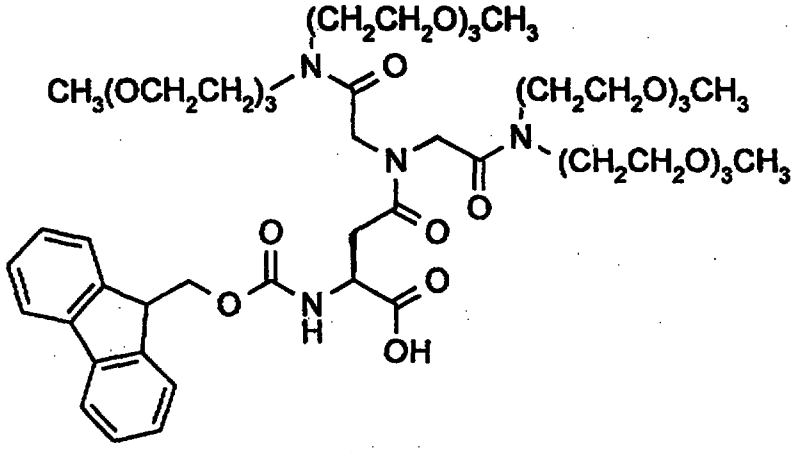
Amino acid analysis gave Arg 1.06, (Ala + IIIa) 4.85, Asp 1.09

Example 6

Preparation of Phv-Arg-Ala-Ala-II-Thr-IIIa-Ala-Papa-NH_2 (SEQ ID NO : 6)

(Formula II: n = 1; X = carbonyl; $R^1 = R^2 = CH_2CON[(CH_2CH_2O)_3CH_3]_2$

6.1 Preparation of N^2-(9-fluorenylmethyloxycarbonyl)-N^4,N^4-bis[N,N-bis(2-[2-(2-methoxyethoxy)ethoxy]ethyl)carbamoylmethyl]-L-asparagine



Using an analogous procedure to that described in Example 3.1 for the preparation of FMoc-Asp(TE)-OH, but using a proportionate amount of bis(2-2[-(2-methoxyethoxy)ethoxy]ethyl)amine in part (a) instead of bis(2-methoxyethyl)amine, there was thus obtained N^2-(9-Fluorenylmethyloxycarbonyl)-N^4,N^4-bis[N,N-bis(2-[2-(2-methoxyethoxy)ethoxy]ethyl)carbamoylmethyl]-L-asparagine (hereinafter referred to as FMoc-Asp(TPE)-OH) as an oil; NMR ($CDCl_3$): 2.9 (m, 1H), 3.1 (m, 1H), 3.4 (m, 12H), 3.6 (m, 48H), 4.2-4.6 (m, 8H), 7.4 (m, 4H), 7.6 (m, 2H), 7.8 (d, 2H).

The following intermediates were obtained on carrying out steps (a) and(b) respectively: N-(benzyloxycarbonyl)iminodi-[N,N-bis(2-[2-(2-methoxyethoxy)ethoxy]ethyl)]-acetamide; NMR ($CDCl_3$): 3.4 (m, 12H), 3.6 (m, 48H), 4.3 (s, 2H), 4.4 (s, 2H), 5.05 (s, 2H), 7.3 (s, 5H). N^2-tert-butyloxycarbonyl-N^4,N^4-bis[N,N-bis(2-[2-(2-methoxyethoxy)ethoxy]ethyl)carbamoylmethyl]-L-asparagine α-benzylester; NMR ($CDCl_3$): 1.9 (s, 9H), 2.7 (m, 1H), 3.05 (m, 1H), 3.4 (m, 12H), 3.6 (m, 48H), 4.4 (m, 5H), 5.2 (s, 2H), 5.9 (bd, 1H), 7.4 (m, 5H).

6.2 Preparation of Phv-Arg-Ala-Ala-II-Thr-IIIa-Ala-Papa-NH_2

(Formula II: n = 1; X = carbonyl; $R^1 = R^2 = -CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$)

The synthesis was performed using similar methodology to that described for Example 1.2, by sequentially coupling and deprotecting (manually) Fmoc-Papa-OH, Fmoc-Ala-OH, Fmoc-IIIa-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Asp(TPE)-OH, Fmoc-Ala-OH (twice), Fmoc-Arg(Pbf)-OH and 5-phenylvaleric acid, followed by cleavage from the resin and purification by preparative RP-HPLC. The product was characterised by HPLC, mass spectroscopy and amino acid analysis, as follows.

RP-HPLC (10-50% acetonitrile gradient over 30 minutes, flow rate 1.0ml/minute) retention time = 23.61 minutes.

Mass spectrometry, m/e (ES+) 1748.0 (MH^+).

Amino acid analysis gave Arg 1.01, Ala 3.18, IIIa 1.05, Asp 0.97, Thr 0.78

Example 7

Preparation of Phv-Ala-Ala-Ala-II-Thr-Pro-Arg-Gly-Papa-NH_2 (SEQ ID NO : 7)

(Formula II: n = 1; X = carbonyl; $R^1 = R^2 = -CH_2CON(CH_2CH_2OCH_3)_2$)

The synthesis was performed using similar methodology to that described for Example 1.2, by sequentially coupling and deprotecting (manually) Fmoc-Papa-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Asp(TE)-OH, Fmoc-Ala-OH (three times) and 5-phenylvaleric acid, followed by cleavage from the resin and purification by preparative RP-HPLC. The product was characterised by HPLC, mass spectroscopy and amino acid analysis, as follows.

RP-HPLC (20-50% acetonitrile gradient over 30 minutes, flow rate 1.0ml/minute) retention time = 16.07 minutes.

Mass spectrometry, m/e (ES+) 1395.7 (MH^+).

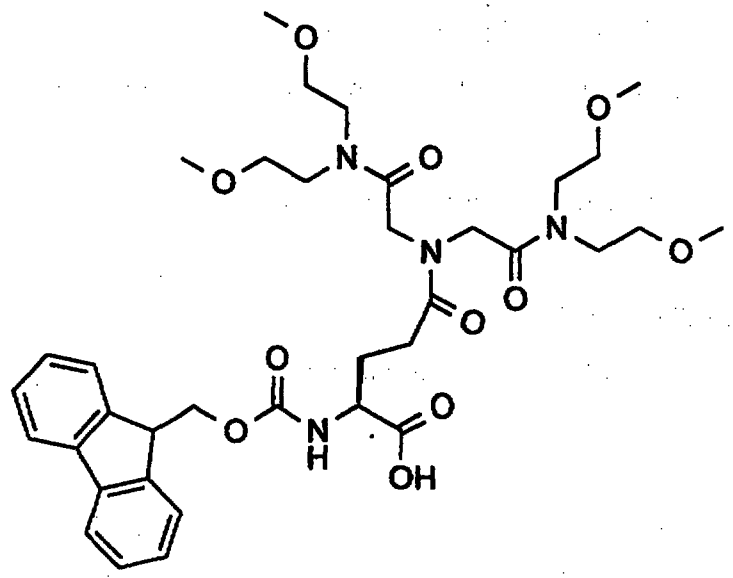
Amino acid analysis gave Arg 1.01, Ala 3.06, Asp 1.02, Thr 0.92, Pro 0.92, Gly 1.05

Example 8

Preparation of Phv-II-Arg-Ala-His-Val-IIIa-Ala-Papa-NH_2 (SEQ ID NO : 8)

(Formula II: n = 2; X = carbonyl; $R^1 = R^2 = -CH_2CON(CH_2CH_2OCH_3)_2$)

8.1 Preparation of N^2-(9-fluorenylmethyloxycarbonyl)-N^4,N^4-bis[N,N-bis(2-methoxyethyl)carbamoylmethyl]-L-glutamine



Using an analogous procedure to that described in Example 3.1 for the preparation of FMoc-Asp(TE)-OH, but using a proportionate amount of N-tert-butyloxycarbonyl-L-glutamic acid α-benzylester in part (b) instead of N-tert-butyloxycarbonyl-L-aspartic acid α-benzylester, there was thus obtained N^2-(9-Fluorenylmethyloxycarbonyl)-N^4,N^4-bis[N,N-bis(2-methoxyethyl)carbamoylmethyl]-L-glutamine (hereinafter referred to as FMoc-Glu(TE)-OH) as an oil; NMR ($CDCl_3$): 2.0-2.4 (m, 4H), 3.3 (m, 12H), 3.5 (m, 16H), 4.2-4.6 (m, 8h), 7.4 (m, 4H), 7.6 (m, 2H), 7.8 (d, 2H).

The following intermediate was obtained on carrying out step (b):
N^2-tert-butyloxycarbonyl-N^4,N^4-bis[N,N-bis(2-methoxyethyl)carbamoylmethyl]-L-glutamine α-benzylester as a gum; NMR ($CDCl_3$): 1.4 (s, 9H), 2.0 (m, 1H), 2.2 (m, 1H), 2.4 (m, 2H), 3.3 (s, 12H), 3.5 (m, 16H), 4.1-4.5 (m, 5H), 5.2 (s, 2H), 7.3 (s, 5H).

8.2 Preparation of Phv-II-Arg-Ala-His-Val-IIIa-Ala-Papa-NH_2
(Formula II: n = 2; X = carbonyl; $R^1 = R^2 = -CH_2CON(CH_2CH_2OCH_3)_2$)

The synthesis was performed using similar methodology to that described for Example 1.2, by sequentially coupling and deprotecting (manually) Fmoc-Papa-OH, Fmoc-Ala-OH, Fmoc-

IIIa-OH, Fmoc-Val-OH, Fmoc-His(Trt)-OH, Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(TE)-OH and 5-phenylvaleric acid, followed by cleavage from the resin and purification by preparative RP-HPLC. The product was characterised by HPLC, mass spectroscopy and amino acid analysis, as follows.

RP-HPLC (10-50% acetonitrile gradient over 30 minutes, flow rate 1.0ml/minute) retention time = 22.23 minutes.

Mass spectrometry, m/e (ES+) 1472.2 (MH^+).

Amino acid analysis gave Arg 0.95, Ala 2.17, His 0.98, Glu 0.99, Val 0.92, IIIa 0.96

Example 9

The compounds of the invention may be administered for therapeutic or prophylactic use to warm-blooded animals such as man in the form of conventional pharmaceutical compositions, a typical example of which includes the following:-

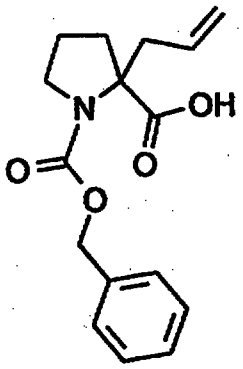
Injectable Solution

0.01 to 100 mg of active ingredient is dissolved in up to 2 ml of an aqueous injection vehicle to give a concentration of active ingredient between 0.01 to 100 mg/ml. The aqueous injection vehicle is buffered to a pH between 5 and 8 using a pharmaceutically acceptable buffer (for example, phosphate, or acetate) and contains a pharmaceutically acceptable tonicity adjustment agent (for example, sodium chloride or dextrose) added to achieve isotonicity. The vehicle may optionally also contain other pharmaceutically acceptable excipients such as solubilising agents (for example, DMSO, ethanol, propylene glycol or polyethylene glycol) preservatives and antioxidants. The active ingredient may typically be an Example described hereinbefore and may conveniently be present as a pharmaceutically acceptable salt.

Note:

(1) For peptides containing a group of the formula IV, (S)-2-[1-(9-fluorenylmethyloxycarbonyl)-6-oxo-1,7-diazaspiro[4.4]non-7-yl]propionic acid (Fmoc-IV-OH) may be obtained as follows:

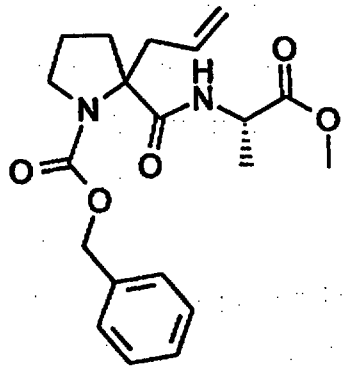
(i) Synthesis of (RS)-2-allyl-N-(benzyloxycarbonyl)proline



N-benzyloxycarbonylproline methyl ester (13g) in THF (20ml) was added dropwise to lithium diisopropylamide (27.5 ml, 2M in hexane/THF) in THF (100ml) at -78°C under nitrogen. The mixture was stirred for 30 minutes and then allyl iodide (5.5ml) was added dropwise and the mixture stirred for a further 30 minutes and then allowed to warm to ambient temperature. The mixture was then added to aqueous ammonium chloride (200ml) and extracted with ether (2x200ml). The ether layer was evaporated and the residue was purified by chromatography on silica using a gradient of hexane increasing to 20% ethyl acetate:hexane. The appropriate fractions, evaporated to dryness, gave methyl (RS)-2-allyl-N-(benzyloxycarbonyl)prolinate (9g) as an oil.

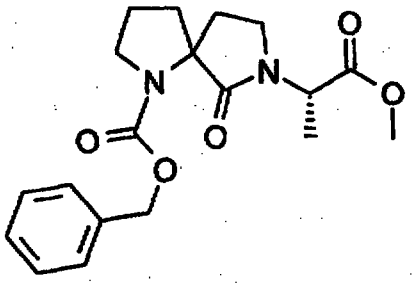
8.5 g of this material was dissolved in methanol (40ml) and sodium hydroxide (4.5g) added in water (20ml) and the mixture refluxed for 60 minutes. The pH of the mixture was then adjusted to 7 with concentrated hydrochloric acid and the methanol was removed by evaporation. The pH of the mixture was adjusted to 3 and the mixture was extracted with ether (2x50 ml) . The combined ether extracts were evaporated to give (RS)-2-allyl-N-(benzyloxycarbonyl)proline as a gum; NMR (d_6-DMSO (373K)): 1.9 (m, 2H), 2.1 (m, 2H), 2.6 (q, 1H), 2.9 (q , 1H), 3.4 (m, 1H), 3.6 (m, 1H), 5.0 (m, 4H), 5.75 (m, 1H), 7.3 (m, 5H).

(ii) Synthesis of [(RS)-2-allyl-N-(benzyloxycarbonyl)]prolyl-(S)-alanine methyl ester



HOBt (7.7g), N-methylmorpholine (6.6g), L-alanine methyl ester hydrochloride (4.5g) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide (5.7g) was added to (RS)-2-allyl-N-(benzyloxycarbonyl)proline (6.5g) in DMF (30ml) and the mixture was stirred for 18 hours and then evaporated. The residue was partitioned between ether and water, filtered to remove HOBt and the organic layer separated. The organic layer was evaporated and the residue was purified by chromatography on silica using a gradient of 20% ethyl acetate in hexane increasing to 50% ethyl acetate in hexane. The appropriate fractions were combined and evaporated to dryness to give [(RS)-2-allyl-N-(benzyloxycarbonyl)]prolyl-(S)-alanine methyl ester (7g); NMR (d_6-DMSO (373K)): some doubling of peaks due to the mixture of diastereoisomers, 1.25 and 1.3 (2d, 3H), 1.75 (m, 2H), 2.2 (m, 2H), 2.65 (m, 1H), 2.9 (m, 1H), 3.4 (m, 1H), 3.65 (2s, 3H), 3.7 (m, 1H), 4.3 (2q, 1H), 5.0 (m, 4H), 5.7 (m, 1H), 7.3 (m, 5H), 7.4 and 7.5 (bs, 1H).

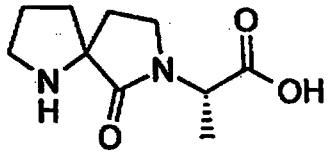
(iii) Synthesis of methyl (S)-2-(1-benzyloxycarbonyl-6-oxa-1,7-diazaspiro[4.4]non-7-yl)propionate. (CbZ-IV-OMe)



Osmium tetroxide (1.5ml of 4% aqueous solution) was added to [(RS)-2-allyl-N-(benzyloxycarbonyl)]prolyl-(S)-alanine methyl ester (1.45g) in a mixture of methanol (30ml) and water (20ml). The mixture was stirred under argon for 10 minutes and then sodium periodate (2.45g) was added in portions. The mixture was stirred for 2 hours and then water (100ml) was added and the mixture was extracted with ethyl acetate (2x70ml). The combined extracts were dried and evaporated to give 1.4g of a gum. The gum was dissolved in dichloromethane (30ml) and triethylsilane (0.65g) and then trifluoroacetic acid (4g) were added dropwise. The mixture was stirred for 3 hours, evaporated and the residue partitioned between aqueous sodium bicarbonate and ether. The ether extract was separated and evaporated to dryness. The residue was purified by chromatography on silica using a gradient of 25% ethyl acetate in hexane increasing to 100% ethyl acetate. The appropriate fractions were combined and evaporated to dryness to give methyl (S)-2-(1-benzyloxycarbonyl-6-oxo-

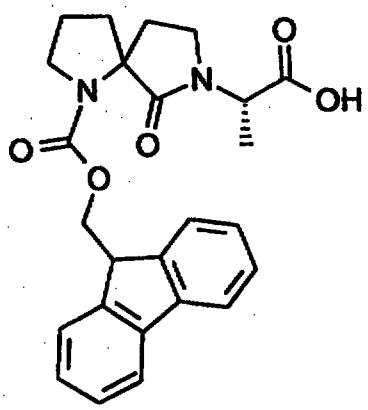
1,7-diazaspiro[4.4]non-7-yl)propionate (0.8g); NMR (d_6-DMSO (373K)): some doubling of peaks due to the mixture of diastereoisomers, 1.25 and 1.35 (2d, 3H), 1.95 (m, 6H), 3.1-3.5 (m, 4H), 3.6 and 3.65 (2s, 3H), 4.5 and 4.65 (2q, 1H), 5.05 (m, 2H), 7.25 (m, 5H).

(iv) Synthesis of (S)-2-(6-oxo-1,7-diazaspiro-[4.4]non-7-yl)propionic acid (H-IV-OH)



Potassium carbonate (2.5 g) was added to methyl (S)-2-(1-benzyloxycarbonyl-6-oxo-1,7-diazaspiro[4.4]non-7-yl)propionate (3.3g) in a mixture of methanol (40ml) and water (40ml) and the mixture was stirred at ambient temperature for 10 hours. The pH was adjusted to ~5 with concentrated hydrochloric acid and the mixture was evaporated to dryness. The residue was dissolved in water (40ml) and the pH adjusted to 3 with concentrated hydrochloric acid. The mixture was then extracted with dichloromethane (2x50ml). The combined extracts were dried ($MgSO_4$) and evaporated to yield a foam (2.8g). The foam was dissolved in methanol (20ml) and cyclohexene (0.7g) was added followed by 10% Pd/C (0.5g). The mixture was refluxed for 2 hours, cooled, filtered and the filtrate was evaporated to give (S)-2-(6-oxo-1,7-diazaspiro[4.4]non-7-yl)propionic acid as a foam (1.9g); NMR (d_6-DMSO): some doubling of peaks due to the mixture of diastereoisomers, 1.25 and 1.3 (2s, 3H), 1.8 (m, 4H), 2.0 (m, 2H), 3.0 (m, 2H), 3.3 (m, 2H), 4.5 (m, 1H).

(v) Synthesis of (S)-2-[1-(9-fluorenylmethyloxycarbonyl)-6-oxo-1,7-diazaspiro-[4.4]non-7-yl]propionic acid (Fmoc-IV-OH)



Excess solid sodium bicarbonate was added to (S)-2-(6-oxo-1,7-diazaspiro[4.4]non-7-yl)propionic acid (0.42g) in water (2ml) and then 9-fluorenylmethyl succinimidyl carbonate

(0.7g) in acetone (3ml) was added. The mixture was stirred for 18 hours. The mixture was then added to water (10ml), extracted with ether (10ml) and the aqueous layer separated. (The ether extracts were discarded). The pH of the aqueous layer was adjusted to ~3 with concentrated hydrochloric acid and then it was extracted with dichloromethane (2x10ml). The combined extracts were separated, dried ($MgSO_4$) and evaporated to give (S)-2-[1-(9-fluorenylmethyloxycarbonyl)-6-oxo-1,7-diazaspiro[4.4]non-7-yl]propionic acid (0.62g) as a white foam; NMR (d_6-DMSO (373K)): some doubling of peaks due to the mixture of diastereoisomers, 1.3 (2d, 3H), 1.6-2.0 (m, 6H), 3.05 (m, 1H), 3.2-3.45 (m, 3H), 4.2-4.4 (m, 1H), 4.5 (m, 1H), 6.2 (s, 2H), 7.35 (m, 4H), 7.8 (m, 4H).

(2) For compounds containing a group of the formula Va, (3S)-3-(9-fluorenylmethoxycarbonylamino)-2-oxoperhydroazepine-1-acetic acid (Fmoc-Va-OH) may be obtained as follows:

(i) (3S)-3-Amino-ε-caprolactam (25g) and triethylamine (19.7g) were dissolved in THF (200ml) and cooled to 5°C. Benzyl chloroformate (33g) in THF (50ml) was added dropwise over 30 minutes. The reaction mixture was stirred at ambient temperature for 18 hours and then poured into water (500ml). The reaction mixture was extracted with ethyl acetate (3x100ml). The combined extracts were dried ($MgSO_4$) and evaporated. The residue was triturated with ether (40ml) and filtered to give 20g of (S)-3-benzyloxycarbonylamino-ε-caprolactam as a white solid; NMR (d_6-DMSO): 1.1-2(m, 6H), 2.9-3.2(m, 2H), 4.1-4.25 (m, 1H), 5.0 (s, 2H), 7.25-7.4 (m, 5H), 7.75 (t, 1H).

(ii) A mixture of sodium hydride (2g) in DMF (100ml) was cooled under a stream of argon to 0°C. (3S)-3-Benzyloxycarbonylamino-ε-caprolactam (10g) was added portionwise over 20 minutes at such a rate to keep the reaction temperature below 5°C. Stirring was continued at 0°C for 40 minutes and tert-butyl bromo acetate (8.2g) was then added dropwise over 10 minutes. The reaction mixture was stirred at 0°C for 1 hour and for a further 18 hours at ambient temperature. The reaction mixture was poured into water (600ml) and extracted with ethyl acetate (6x75ml). The combined extracts were washed with water (3x100ml), dried ($MgSO_4$) and evaporated. The residue was purified by MPLC on silica eluting with 20% ethyl acetate/dichloromethane to give tert-butyl (3S)-3-benzyloxycarbonylamino-2-oxoperhydroazepine-1-acetate (15g) as a clear oil; NMR (d_6-DMSO): 1.4 (s, 9H), 1.5-1.9 (m,

6H), 3.5-3.65 (m, 1H), 3.9-4.15 (m, 2H), 4.4 (m, 1H), 5.0 (s, 2H), 7.1 (d, 1H), 7.35 (m, 5H); mass spectroscopy, m/e (ES+) 377 (MH^+).

(iii) tert-Butyl (3S)-3-benzyloxycarbonylamino-2-oxoperhydroazepine-1-acetate (15g) was disolved in ethanol (150ml) and purged with argon. 10% palladium or carbon (1.5g) was added and the flask evacuated and filled with hydrogen from a balloon. The reaction mixture was stirred at ambient temperature for 4 hours. The reaction mixture was then purged with argon and filtered through diatomaceous earth. The filtrate was evaporated to give tert-butyl (3S)-3-amino-2-oxoperhydroazepine-1-acetate (7.7g) as a viscous oil; NMR ($CDCl_3$): 1.45 (s, 9H), 1.55-2.05 (m, 6H), 3.2-3.3 (d, 1H), 3.55-3.75 (two overlapping doublets, 2H), 3.95-4.25 (q, 2H); mass spectroscopy, m/e (ES+) 243.2 (MH^+).

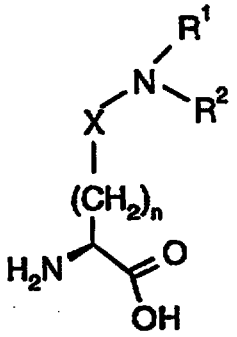
(iv) A solution of tert-butyl (3S)-3-amino-2-oxoperhydroazepine-1-acetate (7g) in THF (50me) was added to a solution of sodium carbonate (3g) in water (30ml). A solution of N-(9-fluorenylmethoxycarbonyloxy)succimimide (9.7g) in THF (100ml) was then added dropwise over 30 minutes with stirring. The reaction mixture was stired at ambient temperature for a further 30 minutes. Water (200ml) was added and the reaction mixture extracted with ethyl acetate (3x100ml). The combined extracts were washed with brine (100ml), dried ($MgSO_4$) and evaporated. The residue was purified by MPLC on silica, eluting initially with dichloromethane and gradually increasing to 15% ethyl acetate/dichloromethane to give tert-butyl (3S)-3-(9-fluorenylmethoxycarbonylamino)-2-oxoperhydroazepine-1-acetate (10.9g) as a clear oil; NMR (d_6-DMSO): 1.45 (s, 9H), 1.5-2.15 (m, 6H), 3.1-3.25 (m, 1H), 3.6-3.75 (m, 1H), 4.0-4.5 (m, 5H), 6.25 (d, 1H), 7.25-7.45 (m, 4H), 7.6 (d, 2H), 7.75 (d, 2H); mass spectroscopy, m/e (ES+) 465.2 (MH^+).

v) tert-Butyl (3S)-3-(9-fluorenylmethoxycarbonylamino)-2-oxoperhydroazepine-1-acetate (10.5g) was dissolved in dichloromethane (30ml) and trifluoroacetic acid (20ml) was added. The reaction mixture was stirred at ambient temperature for 18 hours. Dichloromethane (100ml) was added and the reaction mixture was washed with water (4x100ml), dried ($MgSO_4$) and evaporated. The residue was purified by MPLC on silica eluting with 25% ethylacetate/dichloromethane, followed by ethyl acetate, to give an oil. Trituration with isohexane gave a white foam which was filtered and dred under vacuum at

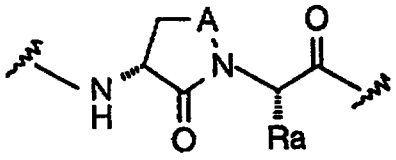
60°C to give (3S)-3-(9-fluorenylmethoxycarbonylamino)-2-oxoperhydroazepine-1-acetic acid (5.5g); NMR (d_6-DMSO): 1.4-1.95 (m, 1H), 3.15-3.4 (m, 1H), 3.55-3.7 (m, 1H), 3.9-4.2 (m, 2H), 4.25-4.45 (m, 2H), 7.15 (d, 1H), 7.25-7.45(m, 4H), 7.75 (m, 2H), 7.85 (d, 2H); mass spectrometry, m/e (ES-) 407.1 $(M-H)^-$.

Chemical Formulae

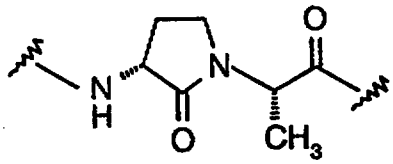
$$P—AA^1—AA^2—AA^3—AA^4—AA^5—AA^6—AA^7—AA^8—Q \qquad I$$



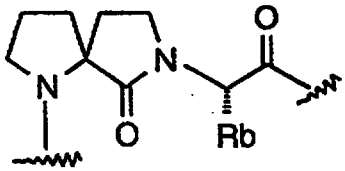
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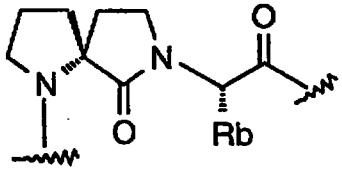
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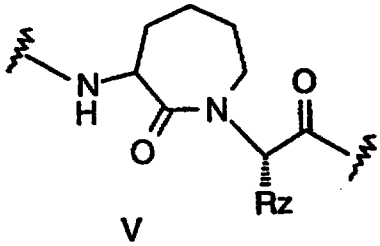
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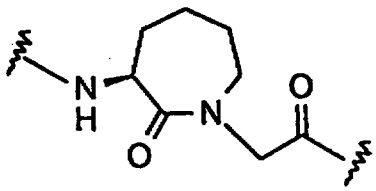
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IVa

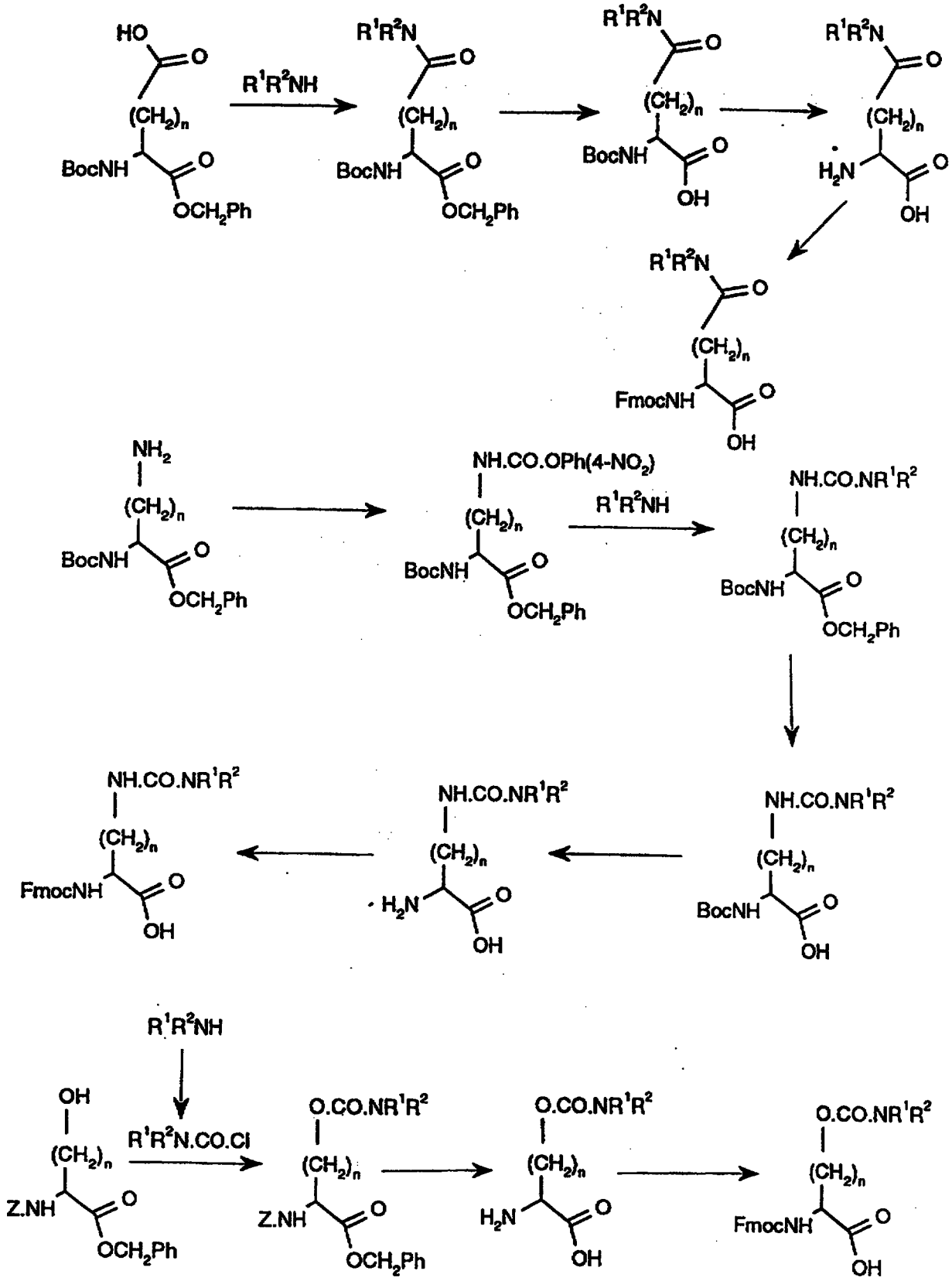


V

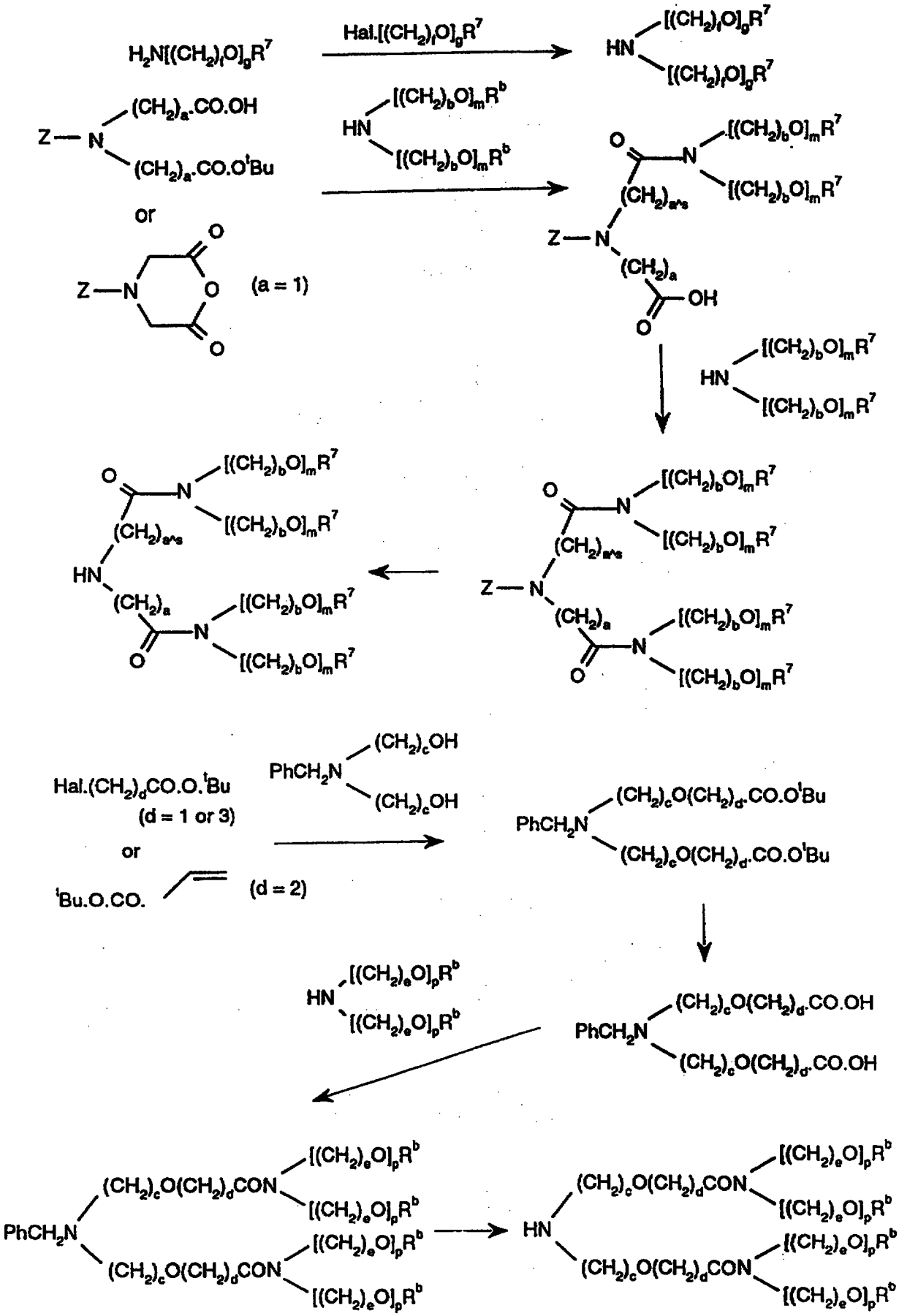


Va

Scheme 1


HO
O
(CH2)n
BocNH
O
OCH2Ph
R1R2NH
R1R2N
OH
H2N
FmocNH
NH2
NH.CO.OPh(4-NO2)
NH.CO.NR1R2
R1R2N.CO.Cl
O.CO.NR1R2
Z.NH

Scheme 2


Hal.[(CH2)fO]gR7
H2N[(CH2)fO]gR7
HN
[(CH2)fO]gR7
[(CH2)fO]gR7
Z—N
(CH2)a.CO.OH
(CH2)a.CO.OtBu
[(CH2)bO]mRb
HN
[(CH2)bO]mRb
[(CH2)bO]mRb
or
O
O
Z—N
O
O
(a = 1)
O
N
[(CH2)bO]mR7
[(CH2)bO]mR7
(CH2)a-1
Z—N
(CH2)a
OH
O
HN
[(CH2)bO]mR7
[(CH2)bO]mR7
Hal.(CH2)dCO.O.tBu
(d = 1 or 3)
or
tBu.O.CO.
(d = 2)
PhCH2N
(CH2)cOH
(CH2)cOH
(CH2)cO(CH2)d.CO.OtBu
(CH2)cO(CH2)d.CO.OtBu
HN
[(CH2)eO]pRb
[(CH2)eO]pRb
PhCH2N
(CH2)cO(CH2)d.CO.OH
(CH2)cO(CH2)d.CO.OH
(CH2)cO(CH2)dCON
[(CH2)eO]pRb
[(CH2)eO]pRb

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: ZENECA LIMITED
(B) STREET: 15 STANHOPE GATE
(C) CITY: LONDON
(E) COUNTRY: UNITED KINGDOM
(F) POSTAL CODE (ZIP): W1Y 6LN
(G) TELEPHONE: 0171 304 5000
(H) TELEFAX: 0171 304 5151
(I) TELEX: 0171 834 2042

(ii) TITLE OF INVENTION: PEPTIDE DERIVATIVES

(iii) NUMBER OF SEQUENCES: 8

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9611881.5
(B) FILING DATE: 07-JUN-1996

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9622890.3
(B) FILING DATE: 02-NOV-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "OTHER"
/note= "5-Phenylpentanoyl-Ala"

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:4
(D) OTHER INFORMATION:/product= "OTHER"
/note= "[N4,N4-bis(2-[2-(2-methoxyethoxy)ethoxy]ethyl)]-Asn"

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:8
(D) OTHER INFORMATION:/product= "OTHER"
/note= "Ala-piperidine-4-carboxamide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "OTHER"
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"5-Phenylpentanoyl-[N4,N4-bis(2-[2-(2-methoxyethoxy)ethoxy]ethyl)]-Asn"

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:8
(D) OTHER INFORMATION:/product= "OTHER"
/note= "Ala-piperidine-4-carboxamide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Xaa Ala Ala Lys Val Ala Ala Xaa
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
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(B) LOCATION:1
(D) OTHER INFORMATION:/product= "OTHER"
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(ix) FEATURE:
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(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:6
(D) OTHER INFORMATION:/product= "OTHER"
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"[(S)-2-((R)-3-amino-2-oxopyrrolidin-1-yl)propanoyl]-Ala-4-aminophenylacetamide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Xaa Arg Ala Xaa Thr Xaa
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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "OTHER"
/note= "5-Phenylpentanoyl-Ala"

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:4
(D) OTHER INFORMATION:/product= "OTHER"
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(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:8
(D) OTHER INFORMATION:/product= "OTHER"
/note= "Ala-piperidine-4-carboxamide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Xaa Ala Ala Xaa Val Ala Ala Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "OTHER"
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(A) NAME/KEY: Peptide
(B) LOCATION:4
(D) OTHER INFORMATION:/product= "OTHER"
/note= "[(S)-2-((R)-3-amino-2-oxopyrrolidin-1-yl)propanoyl]-Ala"

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:5
(D) OTHER INFORMATION:/product= "OTHER"
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(A) NAME/KEY: Peptide
(B) LOCATION:6
(D) OTHER INFORMATION:/product= "OTHER"
/note= "Ala-4-aminophenylacetamide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Xaa Ala Ala Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "OTHER"
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(B) LOCATION:4
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(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:6
(D) OTHER INFORMATION:/product= "OTHER"
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Xaa Ala Ala Xaa Thr Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

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(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
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(D) OTHER INFORMATION:/product= "OTHER"
/note= "5-Phenylpentanoyl-Ala"

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:4
(D) OTHER INFORMATION:/product= "OTHER"
/note= "(N4,N4-bis[N,N-bis(2-methoxyethyl)carbamoylmethyl])-Asn"

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION:8
(D) OTHER INFORMATION:/product= "OTHER"
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Xaa Ala Ala Xaa Thr Pro Arg Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
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(ix) FEATURE:
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"{(S)-2-((R)-3-amino-2-oxopyrrolidin-1-yl)propanoyl}-Ala-4-aminophenylacetamide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Xaa Arg Ala His Val Xaa
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CLAIMS

What we claim is:-

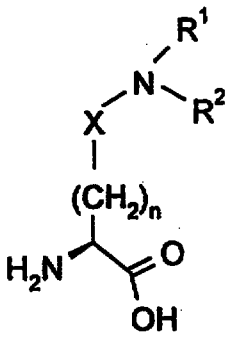
1. A peptide derivative of the formula I,

P-AA^1-AA^2-AA^3-AA^4-AA^5-AA^6-AA^7-AA^8-Q

or a pharmaceutically acceptable salt thereof, wherein

P is a hydrophobic residue;

AA^1, AA^2, AA^3, AA^4, AA^5, AA^6, AA^7 and AA^8 are L-amino acid residues in which 1, 2 or 3 of AA^1, AA^4 and AA^7 are selected from a residue of the L-amino acid of formula II



II

wherein n is an integer 1, 2, 3 or 4;

X is -NH-CO-, -CO- or -O.CO- ;

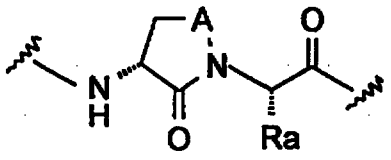
R^1 and R^2 are selected from (A), (B) and (C) wherein

(A) is a group of the formula $-(CH_2)_a$-CO-N(R^3)(R^4) in which a is an integer 1 or 2 and R^3 and R^4 are independently selected from a group $-[(CH_2)_bO]_m$-R^a in which R^a is methyl or ethyl and m is an integer 1, 2, 3, 4 or 5; when m is 1, b is 2 or 3 and when m is 2, 3, 4 or 5, the value of b in each $-(CH_2)_bO$- unit is independently selected from 2 and 3;

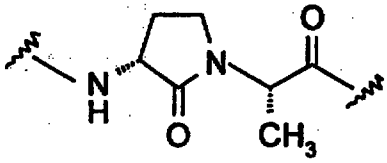
(B) is a group of the formula $-(CH_2)_cO(CH_2)_d$-CO-N(R^5)(R^6) in which c is an integer 2 or 3, d is an integer 1, 2 or 3, and R^5 and R^6 are independently selected from a group $-[(CH_2)_eO]_p$-R^b in which R^b is methyl or ethyl and p is an integer 1, 2, 3, 4 or 5; when p is 1, e is 2 or 3 and when p is 2, 3, 4 or 5, the value for e in each $-(CH_2)_eO$- unit is independently selected from 2 and 3; and

(C) is a group of the formula $-[(CH_2)_fO]_g-R^7$ in which R^7 is methyl or ethyl and g is an integer 1, 2, 3, 4 or 5; when g is 1, f is 2 or 3 and when g is 2, 3, 4 or 5, the value for f in each $-(CH_2)_fO-$ unit is independently selected from 2 and 3;

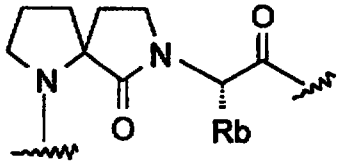
or AA^1, AA^2, AA^3, AA^6, AA^7, and AA^8 are L-amino acid residues in which one or both of AA^1 and AA^7 are selected from a residue of the L-amino acid of formula II as defined above and AA^4 together with AA^5 form a group of the formula III, IIIa, IV, IVa, V or Va;



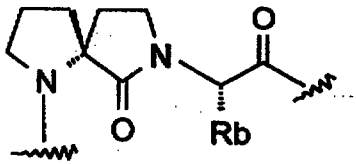
III



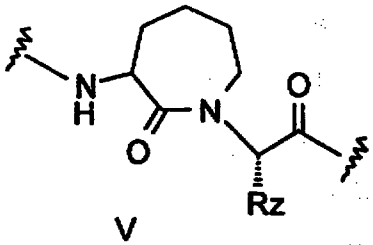
IIIa



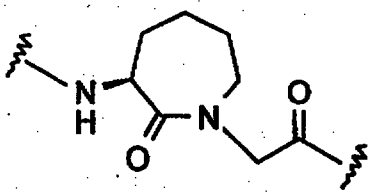
IV



IVa



V



Va

in which Ra, Rb and Rz are independently selected from hydrogen and (1-4C)alkyl, and A is oxygen or methylene;

or AA^1, AA^2, AA^3, AA^4, AA^5 and AA^8 are L-amino acid residues in which one or both of AA^1 and AA^4 are selected from a residue of the L-amino acid of formula II as defined above and AA^6 together with AA^7 form a group of the formula III, IIIa, IV, IVa, V or Va as defined above;

and Q is OH, NH_2, NRcRd wherein Rc is selected from (1-4C)alkyl, 2-carbamoylcyclopentyl, 2-pyridylmethyl, 4-carbamoylcyclohexyl, 4-carbamoylcyclohexylmethyl, 3-carbamoylphenyl, 4-carbamoylphenyl, 4-(carbamoylmethyl)phenyl, 4-(carboxymethyl)phenyl, 2-morpholinoethyl and a group of the formula $-A^1-G^1$ in which A^1 is (3-7C)alkylene or

A^1 is selected from

(1) a group of the formula $-A^2-B^2-$ in which A^2 is p-phenylene or 1,4-cyclohexylene and B^2 is (1-4C)alkylene or A^2 is methylene and B^2 is p-phenylene or 1,4-cyclohexylene; and

(2) a group of the formula $-A^3-B^3-C^3-$ in which A^3 is methylene, B^3 is p-phenylene or 1,4-cyclohexylene and C^3 is (1-3C)alkylene; and

G^1 is a group of the formula $-N=C[N(Rp)_2]_2$ in which each Rp is independently selected from hydrogen, methyl, ethyl and propyl; and Rd is hydrogen or (1-4C)alkyl; or Q is 1-piperazinyl, 4-methyl-1-piperazinyl, 4-(2-(2-hydroxyethoxy)ethyl)-1-piperazinyl, 4-amidino-1-piperazinyl, 1-piperidyl or 4-substituted-1-piperidyl wherein the 4-substituent is selected from carboxy, carbamoyl, N-(2-aminoethyl)carbamoyl and N-(4-aminobutyl)carbamoyl; or Q is a sequence of 1 to 6 amino acid residues or an amide thereof.

2. A peptide derivative, or a pharmaceutically acceptable salt thereof, as claimed in claim 1 which is a peptide derivative of the formula $P-AA^1-AA^2-AA^3-II-AA^5-AA^6-AA^7-AA^8-Q$ wherein AA^1, AA^2, AA^3, AA^5, AA^6, AA^7 and AA^8 are L-amino acid residues, P and Q have any of the meanings defined in claim 1, and II is a residue of an L-amino acid of formula II as defined in claim 1.

3. A peptide derivative, or a pharmaceutically acceptable salt thereof, as claimed in claim 1 which is a peptide derivative of the formula $P-AA^1-AA^2-AA^3-IIIa-AA^5-II-AA^8-Q$ wherein AA^1, AA^2, AA^3, AA^5 and AA^8 are L-amino acid residues, P and Q have any of the meanings defined in claim 1, II is a residue of an L-amino acid of formula II as defined in claim 1, and IIIa is a group of the formula IIIa as defined in claim 1.

4. A peptide derivative, or a pharmaceutically acceptable salt thereof, as claimed in claim 1 which is a peptide derivative of the formula $P-AA^1-AA^2-AA^3-II-AA^5-IIIa-AA^8-Q$ wherein AA^1, AA^2, AA^3, AA^5 and AA^8 are L-amino acid residues, P and Q have any of the meanings

defined in claim 1, II is a residue of an L-amino acid of formula II as defined in claim 1, and IIIa is a group of the formula IIIa as defined in claim 1.

5. A peptide derivative, or a pharmaceutically acceptable salt thereof, a claimed in claim 1 which is a peptide derivative of the formula formula P-II-AA^2-AA^3-AA^4- AA^5-IIIa-AA^8-Q wherein AA^2, AA^3, AA^4, AA^5 and AA^8 are L-amino acid residues, P and Q have any of the meanings defined in claim 1, II is a residue of an L-amino acid of formula II as defined in claim 1, and IIIa is a group of the formula IIIa as defined in claim 1.

6. A peptide derivative, or pharmaceutically acceptable salt thereof, as claimed in any of claims 1 to 5 in which P is an aliphatic, aromatic or mixed aliphatic/aromatic organic group of from 5 to 20 carbon atoms, or a heteroaromatic or mixed aliphatic/heteroaromatic organic group of from 5 to 20 carbon atoms and 1, 2 or 3 heteroatoms selected from oxygen, sulphur and nitrogen.

7. A peptide derivative, or a phamaceutically acceptable salt thereof, as claimed in any of claims 1 to 6 wherein, in the residue of the L-amino acid of formula II, n = 1 or 2, X = carbonyl and R^1 and R^2 are both identical groups of the formula (A) or are both identical groups of the formula (C) as defined in claim 1.

8. A peptide derivative, or a pharmaceutically acceptable salt thereof, as claimed in claim 7 wherein R^1 and R^2 are both. $-CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$ or are both $-CH_2CON(CH_2CH_2OCH_3)_2$ or are both $-CH_2CON[(CH_2CH_2O)_3CH_3]_2$.

9. A peptide derivative, or a pharmaceutically acceptable salt thereof, as claimed in any preceding claim, wherein AA^1 to AA^8 when present are selected from residues of the following amino acids:

AA^1 selected from Ala, Ile, Tyr, Val, Glu, Lys, Arg, Gly, Gap, $GapMe_4$ and 3,3,3-trifluoroalanine;

AA^2 selected from Ala, Lys, Glu, Sar, Val, Arg, Gly, Pro, Ile, Tic, 3,3,3-trifluoroalanine and N^6-diethylLys;

AA^3 selected from Ala, His, Gln, Val, Thr, Glu, Gly, Asp, Asn and N^3-diethylDap;

AA^4 selected from Ala, Lys, Asn, Arg, Thr, Glu, Sar, Gly, Pro, His and N^6-diethylLys;

AA^5 selected from Thr, Val, Ala, Gly, Dap, Dab, Pro, Hyp, Asn, Ser, and N^3-diethylDap;

AA^6 selected from Gly, Leu, Lys, Ala, Pro, Glu, Sar, His and Dap;

AA^7 selected from Pro, Ala, Lys, Arg, Glu, Sar, Gly, Oic and Dic;

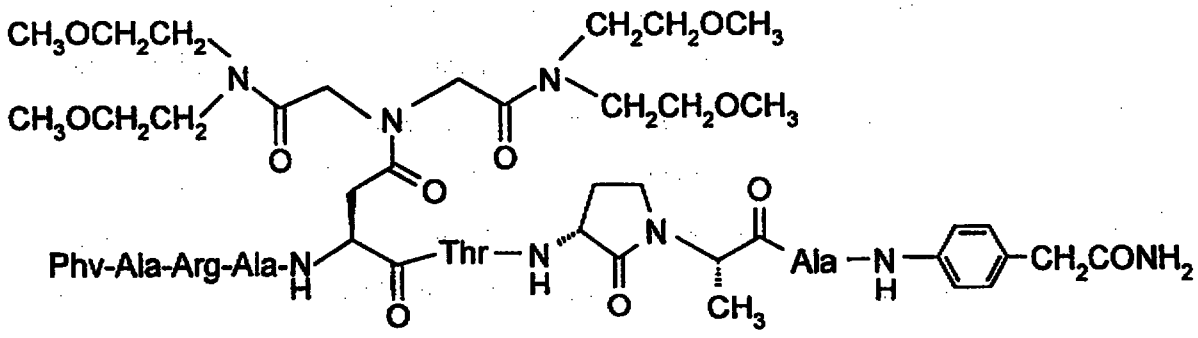
and

AA^8 selected from Ala, Gly, Dap, azaalanine and azaglycine.

10. A peptide derivative, or a pharmaceutically acceptable salt thereof, as claimed in any preceding claim, wherein Q is selected from 4-carbamoyl-1-piperidyl and 4-(carbamoylmethyl)anilino.

11. A peptide derivative, or a pharmaceutically acceptable salt thereof, as claimed in any preceding claim wherein hydrophobic group P is 5-phenylvaleryl.

12. A peptide derivative as claimed in claim 1, or a pharmaceutically acceptable salt thereof, which is the compound.

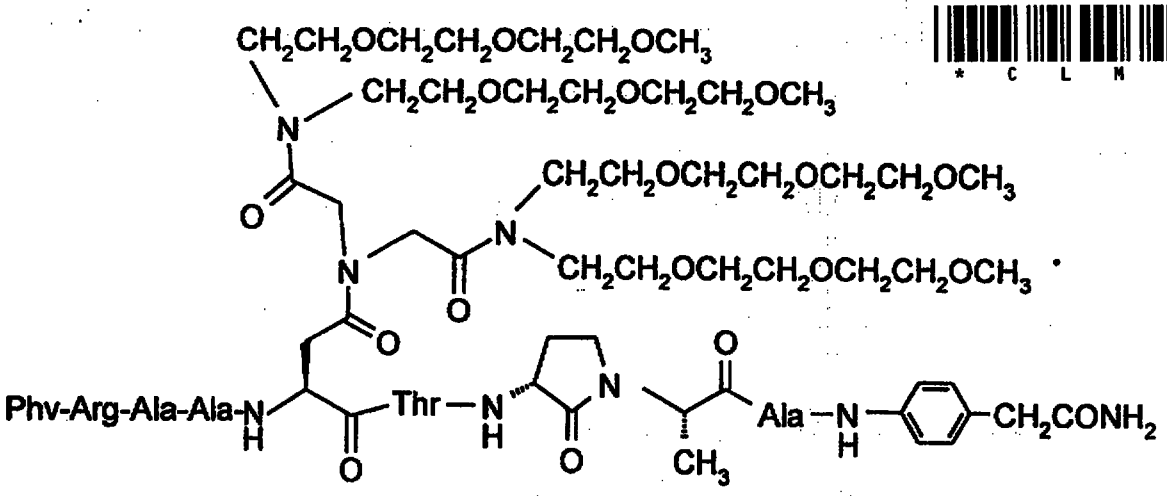


or a pharmceutically acceptable salt thereof, in which Phv represents a 5-phenylvaleryl group.

13. A peptide derivative as claimed in claim 1, or a pharmaceutically acceptable salt therof, which is the compound

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or a pharmaceutically acceptable salt thereof, in which Phv represent a 5-phenylvaleryl group.

14. A pharmaceutical composition which comprises a peptide derivative of the formula I or a pharmaceutically acceptable salt thereof, as claimed in any one of claims 1 to 13, in association with a pharmaceutically acceptable diluent or carrier.

15. A process for the manufacture of a peptide derivative, or a pharmaceutically acceptable salt thereof, as claimed in claim 1, comprising sequentially coupling in the appropriate order suitably protected amino acids or sequences of two or more suitably protected amino acids, a suitably protected group of the formula H-II-OH, H-III-OH, H-IIIa-OH, H-IV-OH, H-IVa-OH, H-V-OH or H-Va-OH and optionally a suitably protected group of the formula H-Q, followed by optional functional group modification of the N-terminal amino group, to introduce a hydrophobic group P, and removal of any remaining protecting groups and any solid support.

16. A method for treating a MHC class II dependent T-cell mediated autoimmune or inflammatory disease which comprises administering to a warm-blooded mammal in need of such treatment an effective amount of a peptide derivative of formula I, or a pharmaceutically acceptable salt thereof, as claimed in claim 1.

17. A method as claimed in claim 16 for treating rheumatoid arthritis or cystic fibrosis.

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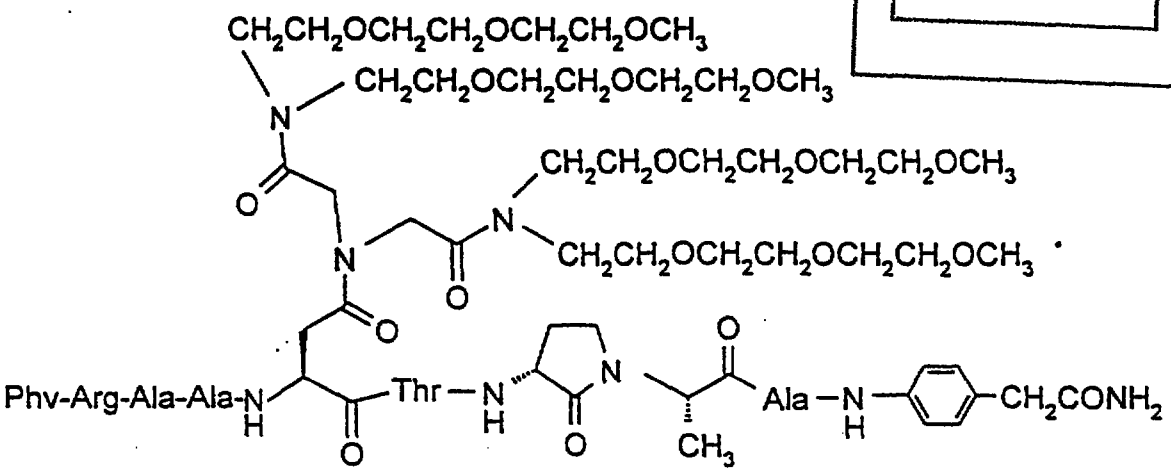
18. The use of a peptide derivative of formula I, or a pharmaceutically acceptable salt thereof, as claimed in claim 1, in the production of a novel medicament for use in the treatment of a MHC class II dependent T-cell mediated autoimmune or inflammatory disease.

19. A protected or unprotected amino acid of the formula II, or a salt thereof, wherein n, X, R^1 and R^2 have any of the meanings defined in claim 1.

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or a pharmaceutically acceptable salt thereof, in which Phv represent a 5-phenylvaleryl group.

14. A pharmaceutical composition which comprises a peptide derivative of the formula I or a pharmaceutically acceptable salt thereof, as claimed in any one of claims 1 to 13, in association with a pharmaceutically acceptable diluent or carrier.

15. A process for the manufacture of a peptide derivative, or a pharmaceutically acceptable salt thereof, as claimed in claim 1, comprising sequentially coupling in the appropriate order suitably protected amino acids or sequences of two or more suitably protected amino acids, a suitably protected group of the formula H-II-OH, H-III-OH, H-IIIa-OH, H-IV-OH, H-IVa-OH, H-V-OH or H-Va-OH and optionally a suitably protected group of the formula H-Q, followed by optional functional group modification of the N-terminal amino group, to introduce a hydrophobic group P, and removal of any remaining protecting groups and any solid support.

16. The use of a peptide derivative of formula I, or a pharmaceutically acceptable salt thereof, as claimed in claim 1, in the production of a novel medicament for use in the treatment of a MHC class II dependent T-cell mediated autoimmune or inflammatory disease.

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17. The use of a peptide derivative of fomula I, or a pharmaceutically acceptable salt thereof, as claimed in claim 1, in the production of a novel medicament for use in the treatment of rheumatoid arthritis or cystic fibrosis.

18. A protected or unprotected amino acid of the formula II, or a salt thereof, wherein n, X, R^1 and R^2 have any of the meanings defined in claim 1.

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(57) Abstract

The invention concerns pharmaceutically useful peptide derivatives of the formula (I), P-AA^1-AA^2-AA^3-AA^4-AA^5-AA^6-AA^7-AA^8-Q in which P, AA^1, AA^2, AA^3, AA^4, AA^5, AA^6, AA^7, AA^8, and Q have the various meanings defined herein and their pharmaceutically acceptable salts, and pharmaceutical compositions containing them. The novel peptide derivatives are of value in treating MHC class I dependent T-cell mediated autoimmune or inflammatory diseases, such as rheumatoid arthritis. The invention further concerns processes for the manufacture of the novel peptide derivatives and the use of the compounds in medical treatment.