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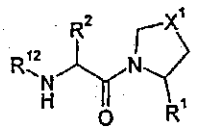
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(54) Title: NOVEL DIPEPTIDYL PEPTIDASE IV (DP-IV) INHIBITORS AS ANTI-DIABETIC AGENTS



(1)

(57) Abstract: The present invention relates to a series of prodrugs of inhibitors of DP-IV with improved properties. The compounds can be used for the treatment of a number of human diseases, including impaired glucose tolerance and type II diabetes. The compounds of the invention are described by general formula (1); wherein R^1 is H or CN; R^2 is selected from CH_2R^5, $CH_2CH_2R^5$ and $C(R^3)(R^4)$-X^2-$(CH_2)_aR^5$; R^3 and R^4 are each independently selected from H and Me; R^5 is selected from $CON(R^6)(R^7)$, $N(R^8)C(=O)R^9$, $N(R^8)C(=S)R^9$, $N(R^8)SO_2R^{10}$ and $N(R^8)R^{10}$; R^6 and R^7 are each independently $R^{11}(CH_2)_b$ or together they are $-(CH_2)_2$-Z-$(CH_2)_2$- or CH_2-o-C_6H_4-Z-CH_2-; R^8 is H or Me; R^9 is selected from $R^{11}(CH_2)_b$, $R^{11}(CH_2)_bO$ and $N(R^6)(R^7)$; R^{10} is $R^{11}(CH_2)_b$; R^{11} is selected from H, alkyl, optionally substituted aryl, optionally substituted aroyl, optionally substituted arylsulphonyl and optionally substituted heteroaryl; R^{12} is selected from $H_2NCH(R^{13})CO$, $H_2NCH(R^{14})CONHCH(R^{15})CO$, $C(R^{16})=C(R^{17})COR^{18}$ and $R^{19}OCO$; R^{13}, R^{14} and R^{15} are selected from the side chains of the proteinaceous amino acids; R^{16} is selected from H, lower alkyl (C_1-C_6) and phenyl; R^{17} is selected from H and lower alkyl (C_1-C_6); R^{18} is selected from H, lower alkyl (C_1-C_6), OH, O-(lower alkyl (C_1-C_6)) and phenyl; R^{19} is selected from lower alkyl (C_1-C_6), optionally substituted phenyl and $R^{20}C(=O)OC(R^{21})(R^{22})$; R^{20}, R^{21} and R^{22} are each independently selected from H and lower alkyl (C_1-C_6); Z is selected from a covalent bond, $-(CH_2)_c$-, -O-, $-SO_d$- and $-N(R^{10})$-; X^1 is S or CH_2; X^2 is O, S or CH_2; a is 1, 2 or 3; b is 0-3; c is 1 or 2; and d is 0, 1 or 2.

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NOVEL DIPEPTIDYL PEPTIDASE IV (DP-IV) INHIBITORS AS ANTI-DIABETIC AGENTS

The present invention relates to novel compounds that are prodrugs of inhibitors of dipeptidyl peptidase IV. The compounds are useful in the treatment of, *inter alia*, type 2 diabetes and impaired glucose tolerance.

BACKGROUND

The enzyme dipeptidyl peptidase IV, herein abbreviated DP-IV (and elsewhere as DAP-IV or DPP-IV) and also known by the classification EC.3.4.14.5, is a serine protease that cleaves the N-terminal dipeptide from peptides that begin with the sequence H-Xaa-Pro (where Xaa is any amino acid, although preferably a lipophilic one, and Pro is proline). It will also accept as substrates peptides that begin with the sequence H-Xaa-Ala (where Ala is alanine). DP-IV was first identified as a membrane-bound protein. More recently a soluble form has been identified.

Initial interest in DP-IV focussed on its role in the activation of T lymphocytes. DP-IV is identical to the T cell protein CD26. It was proposed that inhibitors of DP-IV would be capable of modulating T cell responsiveness, and so could be developed as novel immunomodulators. It was further suggested that CD26 was a necessary co-receptor for HIV, and thus that DP-IV inhibitors could be useful in the treatment of AIDS.

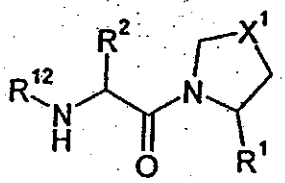
Attention was given to the role of DP-IV outside the immune system. It was recognised that DP-IV has a key role in the degradation of several peptide hormones, including growth hormone releasing hormone (GHRH) and glucagon-like peptide-1 and –2 (GLP-1 and GLP-2). Since GLP-1 is known to have a potentiating effect on the action of insulin in the control of post-prandial blood glucose levels it is clear that DP-IV inhibitors might also be usefully employed in the treatment of type II diabetes and impaired glucose tolerance. At least two DP-IV inhibitors are currently undergoing clinical trials to explore this possibility.

Several groups have disclosed inhibitors of DP-IV. While some leads have been found from random screening programs, the majority of the work in this field has been directed towards the investigation of substrate analogues. Inhibitors of DP-IV that are substrate analogues are disclosed in, for example, US 5,462,928, US 5,543,396,

WO95/15309 (equivalent to US 5,939,560 and EP 0731789), WO98/19998 (equivalent to US 6,011,155), WO99/46272 and WO99/61431. The most potent inhibitors are aminoacyl pyrrolidine boronic acids, but these are unstable and tend to cyclise, while the more stable pyrrolidine and thiazolidine derivatives have a lower affinity for the enzyme and so would require large doses in a clinical situation. Pyrrolidine nitriles appear to offer a good compromise since they have both a high affinity for the enzyme and a reasonably long half-life in solution as the free base. There remains, however, a need for inhibitors of DP-IV with improved properties.

SUMMARY OF THE INVENTION

The present invention relates to a series of prodrugs of inhibitors of DP-IV with improved properties. The compounds can be used for the treatment of a number of human diseases, including impaired glucose tolerance and type II diabetes. Accordingly, the invention further relates to the use of the compounds in the preparation of pharmaceutical compositions, to such compositions *per se*, and to the use of such compositions in human therapy. In a first aspect of the present invention, there is provided, a compound according to general formula 1.



1

or a pharmaceutically acceptable salt thereof, wherein: R^1 is H or CN; R^2 is selected from CH_2R^5, $CH_2CH_2R^5$ and

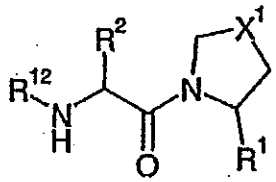
$C(R^3)(R^4)\text{-}X^2\text{-}(CH_2)_aR^5$; R^3 and R^4 are each independently selected from H and Me; R^5 is selected from $CON(R^6)(R^7)$, $N(R^8)C(=O)R^9$, $N(R^8)C(=S)R^9$, $N(R^8)SO_2R^{10}$ and $N(R^8)R^{10}$; R^6 and R^7 are each independently $R^{11}(CH_2)_b$ or together they are $-(CH_2)_2\text{-}Z\text{-}(CH_2)_2-$ or $-CH_2\text{-}o\text{-}C_6H_4\text{-}Z\text{-}CH_2-$; R^8 is H or Me; R^9 is selected from $R^{11}(CH_2)_b$, $R^{11}(CH_2)_bO$ and $N(R^6)(R^7)$; R^{10} is $R^{11}(CH_2)_b$; R^{11} is selected from H, alkyl, optionally substituted aryl, optionally substituted aroyl, optionally substituted arylsulphonyl and optionally substituted heteroaryl; R^{12} is selected from $H_2NCH(R^{13})CO$, $H_2NCH(R^{14})CONHCH(R^{15})CO$, $C(R^{16})=C(R^{17})COR^{18}$ and $R^{19}OCO$; R^{13}, R^{14} and R^{15} are selected from the side chains of the proteinaceous amino acids; R^{16} is selected from H, lower alkyl ($C_1 - C_6$) and phenyl; R^{17} is selected from H and lower alkyl ($C_1 - C_6$); R^{18} is selected from H, lower alkyl ($C_1 - C_6$), OH, O-(lower alkyl ($C_1 - C_6$)) and phenyl; R^{19} is

selected from lower alkyl (C_1 – C_6), optionally substituted phenyl and $R^{20}C(=O)OC(R^{21})(R^{22})$; R^{20}, R^{21} and R^{22} are each independently selected from H and lower alkyl (C_1 – C_6); Z is selected from a covalent bond, $-(CH_2)_c-$, -O-, $-SO_d-$ and $-N(R^{10})-$; X^1 is S or CH_2; X^2 is O, S or CH_2; a is 1, 2 or 3; b is 0 – 3; c is 1 or 2; and d is 0, 1 or 2. With the proviso that if R^1 is CN and X^1 is CH_2, R^{12} is selected from $C(R^{16})=C(R^{17})$ COR^{18} and $R^{19}OCO$ only wherein R^{18}, R^{17} and R^{18} are as defined above and R^{19} is lower alkyl (C_1-C_6).

In a second aspect of the invention, there is provided a pharmaceutical composition comprising a compound as defined above.

In a third aspect of the invention, there is provided the use for a compound as defined above, which is as a component in the preparation of a pharmaceutical composition.

In a fourth aspect of the invention, there is provided the use, in the manufacture of a pharmaceutical composition for the treatment of a disease or medical condition in a human or animal, of a compound according to general formula 1



1

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is H or CN,

R^2 is selected from CH_2R^5, $CH_2CH_2R^5$ and $C(R^3)(R^4)$-X^2-$(CH_2)_aR^5$,

R^3 and R^4 are each independently selected from H and Me;

R^5 is selected from $CON(R^6)(R^7)$, $N(R^8)C(=O)R^9$, $N(R^8)C(=S)R^9$, $N(R^8)SO_2R^{10}$ and $N(R^8)R^{10}$;

R^6 and R^7 are each independently $R^{11}(CH_2)_b$ or together they are $-(CH_2)_2$-Z-$(CH_2)_2-$ or $-CH_2$-o-C_6H_4-Z-CH_2-;

R^8 is H or Me;

R^9 is selected from $R^{11}(CH_2)_b$, $R^{11}(CH_2)_bO$ and $N(R^5)(R^7)$;

R^{10} is $R^{11}(CH_2)_b$;

R^{11} is selected from H, alkyl, optionally substituted aryl, optionally substituted aroyl, optionally substituted arylsulphonyl and optionally substituted heteroaryl;

R^{12} is selected from $H_2NCH(R^{13})CO$, $H_2NCH(R^{14})CONHCH(R^{15})CO$, $C(R^{16})=C(R^{17})COR^{18}$ and $R^{19}OCO$;

R^{13}, R^{14} and R^{15} are selected from the side chains of the proteinaceous amino acids;

R^{16} is selected from H, lower alkyl ($C_1 - C_6$) and phenyl;

R^{17} is selected from H and lower alkyl ($C_1 - C_6$);

R^{18} is selected from H, lower alkyl ($C_1 - C_6$), OH, O-(lower alkyl ($C_1 - C_6$)) and phenyl;

R^{19} is selected from lower alkyl ($C_1 - C_6$), optionally substituted phenyl and $R^{20}C(=O)OC(R^{21})(R^{22})$;

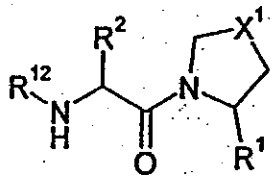
R^{20}, R^{21} and R^{22} are each independently selected from H and lower alkyl ($C_1 - C_6$);

Z is selected from a covalent bond, $-(CH_2)_c-$, -O-, $-SO_d-$ and $-N(R^{10})-$;
X^1 is S or CH_2,
X^2 is O, S or CH_2;
a is 1, 2 or 3
b is 0 – 3;
c is 1 or 2; and
d is 0 – 2; with the proviso that if R^1 is CN and X^1 is CH_2, R^{12} is selected from $C(R^{16})=C(R^{17})COR^{18}$ and $R^{19}OCO$ only wherein R^{16}, R^{17} and R^{18} are as defined above and R^{19} is lower alkyl ($C_1 - C_6$).

In a fifth aspect of the invention, there is provided a compound or pharmaceutical composition as defined above or a use as defined above, with the proviso that R^{19} is not t-butyl.

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect, the present invention relates to a series of novel compounds that are prodrugs of inhibitors of DP-IV with improved properties. The compounds of the invention are described by general formula **1**.



1

In this general formula R^1 is either a hydrogen atom (H) or a nitrile group (-CN) and X^1 is either a sulphur atom (S) or CH_2. In one preferred embodiment of the invention, R^1 is H. In another preferred embodiment, R^1 is CN.

R^2 is selected from a group according to CH_2R^5, a group according to $CH_2CH_2R^5$ and a group according to $C(R^3)(R^4)\text{-}X^2\text{-}(CH_2)_aR^5$, where R^3 and R^4 are each independently selected from H and a methyl group (Me), X^2 is O, S or CH_2 and a is 1, 2 or 3. Preferably R^2 is selected from a group according to $CH_2CH_2R^5$ and a group according to $C(R^3)(R^4)\text{-}X^2\text{-}(CH_2)_aR^5$. More preferably R^2 is selected from a group according to $CH_2CH_2R^5$ and a group according to $C(R^3)(R^4)\text{-}X^2\text{-}(CH_2)_aR^5$ where R^3 and R^4 are both H, X^2 is CH_2 and a is 1 or 2. Most preferably R^2 is selected from a group according to $CH_2CH_2CH_2R^5$ and a group according to $CH_2CH_2CH_2CH_2R^5$.

R^5 is selected from a group according to $CON(R^6)(R^7)$, a group according to $N(R^8)C(=O)R^9$, a group according to $N(R^8)C(=S)R^9$, a group according to $N(R^8)SO_2R^{10}$ and a group according to $N(R^8)R^{10}$. In one preferred embodiment of the invention, R^5 is a group according to $CON(R^6)(R^7)$. In another preferred embodiment, R^5 is selected from a group according to $N(R^8)C(=O)R^9$, a group according to $N(R^8)C(=S)R^9$, a group according to $N(R^8)SO_2R^{10}$ and a group according to $N(R^8)R^{10}$.

R^6 and R^7 may each independently a group according to $R^{11}(CH_2)_b$, where b is 0 – 3. Alternatively they may together be a chain $-(CH_2)_2-Z-(CH_2)_2-$ or $-CH_2-o-C_6H_4-Z-CH_2-$, where Z is selected from a covalent bond, $-(CH_2)_c-$, -O-, $-SO_d-$ and $-N(R^{10})-$, c is 1 or 2; and d is 0, 1 or 2, such that, together with the nitrogen atom to which they are attached, they form a five-, six- or seven-membered ring.

R^8 is H or Me.

R^9 is selected from a group according to $R^{11}(CH_2)_b$, a group according to $R^{11}(CH_2)_bO$ and a group according to $N(R^6)(R^7)$.

R^{10} is a group according to $R^{11}(CH_2)_b$.

R^{11} is selected from H, alkyl, optionally substituted aryl, optionally substituted aroyl, optionally substituted arylsulphonyl and optionally substituted heteroaryl.

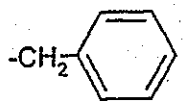
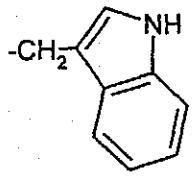
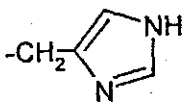
The term alkyl, as used herein, denotes saturated hydrocarbon groups with between 1 and 10 carbon atoms, including straight-chain, branched and mono- and polycycloalkyl groups, such as methyl, ethyl, propyl, isopropyl, *n*-butyl, *tert*-butyl, cyclopentyl, cyclohexyl, cyclohexylmethyl, 2-cyclohexyl-2-propyl, bicyclo[2.2.2]octyl and the like.

The term aryl, as used herein, denotes monocyclic and fused bicyclic aromatic groups, including carbocyclic groups, such as phenyl and naphthyl, and heteroaryl groups with up to three heteroatoms selected from nitrogen, oxygen and sulphur, such as pyrrolyl, furyl, thienyl, pyrazolyl, imidazolyl, oxazolyl, isothiazolyl, pyridyl, pyrimidinyl, indolyl, quinolinyl and the like. Unless otherwise specified, an aryl, aroyl, arylsulphonyl or heteroaryl group may optionally be substituted with up to three groups independently selected from alkyl, OH, alkoxy, O-alkyl, Cl, F, Br, NH_2, amino (including alkylamino NH-alkyl and dialkylamino $N(alkyl)_2$), CO_2H, CO_2-alkyl, $CONH_2$, CONH-alkyl, $CON(alkyl)_2$, acyl, carboxy, carboxyalkyl, carboxamido, NO_2 and CN.

R^{12} is selected from a group according to $H_2NCH(R^{13})CO$, a group according to $H_2NCH(R^{14})CONHCH(R^{15})CO$, a group according to $C(R^{16})=C(R^{17})COR^{18}$ and a group according to $R^{19}OCO$. In one preferred embodiment of the invention R^{12} is selected

from a group according to $H_2NCH(R^{13})CO$ and a group according to $H_2NCH(R^{14})CONHCH(R^{15})CO$. In another preferred embodiment of the invention R^{12} is a group according to $R^{19}OCO$.

R^{13}, R^{14} and R^{15} are selected from the side chains of the proteinaceous amino acids, as listed in the following Table.

Amino acid	Side chain	Amino acid	Side chain
Alanine	$-CH_3$	Leucine	$-CH_2CH(CH_3)_2$
Arginine	$-(CH_2)_3NHC(:NH)NH_2$	Lysine	$-(CH_2)_4NH_2$
Asparagine	$-CH_2CONH_2$	Methionine	$-CH_2CH_2SCH_3$
Aspartic acid	$-CH_2CO_2H$	Phenylalanine	
Cysteine	$-CH_2SH$	Serine	$-CH_2OH$
Glutamic acid	$-CH_2CH_2CO_2H$	Threonine	$-CH_2CH(OH)CH_3$
Glutamine	$-CH_2CH_2CONH_2$	Tryptophan	
Glycine	$-H$	Tyrosine	
Histidine		Valine	$-CH(CH_3)_2$
Isoleucine	$-CH(CH_3)CH_2CH_3$		

R^{16} is selected from H, lower alkyl ($C_1 - C_6$ alkyl) and phenyl.

R^{17} is selected from H and lower alkyl ($C_1 - C_6$).

R^{18} is selected from H, lower alkyl ($C_1 - C_6$), OH, O-(lower alkyl ($C_1 - C_6$)) and phenyl.

R^{19} is selected from lower alkyl (C_1 – C_6), optionally substituted phenyl and $R^{20}C(=O)OC(R^{21})(R^{22})$.

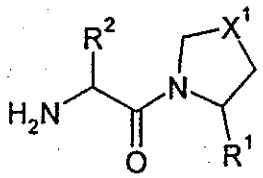
R^{20}, R^{21} and R^{22} are each independently selected from H and lower alkyl (C_1 – C_6).

In a preferred embodiment, X^1 is CH_2 and R^1 is CN. For this embodiment, preferred R^5 groups are $CON(R^6)(R^7)$, $N(R^8)C(=O)R^9$, $N(R^8)C(=S)R^9$ and $N(R^8)R^{10}$. In another preferred embodiment, X^1 is CH_2 and R^1 is H. In another preferred embodiment X^1 is S. In a further preferred embodiment X^1 is S and R^1 is H.

Preferred compositions according to the invention may have improved activity and/or improved pharmacological profile. Preferred compositions may have in vivo stability characteristics which make them particularly suitable for use as pro-drugs.

Certain of the compounds of the present invention have acidic or basic properties and so can exist as salts. Insofar as such salts are non-toxic and otherwise pharmaceutically acceptable, they are included within the scope of the invention. Examples of such salts include, but are not limited to, the acetate, hydrochloride, sulphate, phosphate and benzoate salts of basic compounds, and the sodium, potassium and tetra-alkyl ammonium salts of acidic compounds.

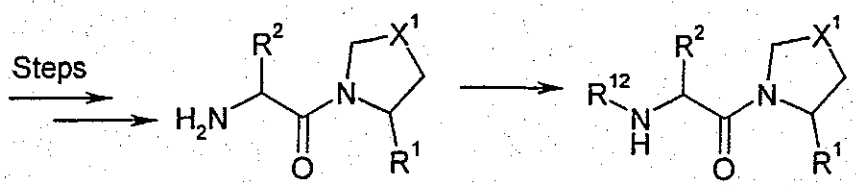
Following administration, the compounds of the present invention are transformed into compounds according to general formula 2. These compounds are potent inhibitors of dipeptidyl peptidase IV.



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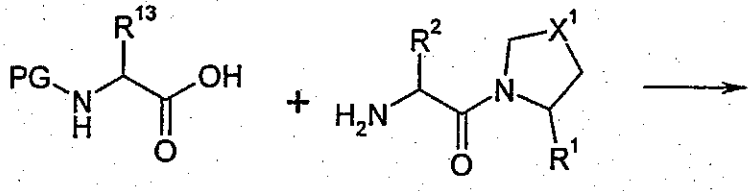
Accordingly, the compounds of the invention can be used for the treatment of a number of human diseases, including impaired glucose tolerance and type II diabetes. Further aspects of the invention therefore relate to the use of the compounds in the preparation of pharmaceutical compositions, to such compositions *per se*, and to the use of such compositions in human therapy.

The compounds of the present invention may be prepared according to methods that are well known in the field of organic chemistry, and particularly peptide chemistry. One strategy is to prepare the corresponding primary amine according to general formula **2** and then derivatise this.

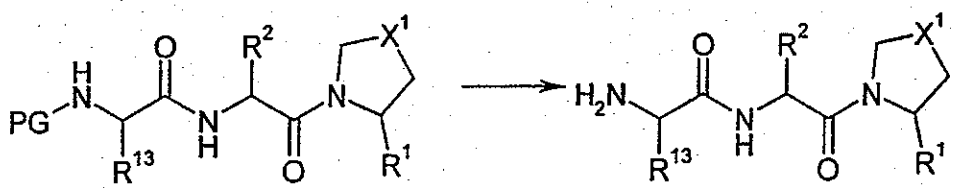


2 **1**

When R^{12} is $H_2NCH(R^{13})CO$ then the final transformation may be accomplished in two steps by the reaction of **2** with a protected amino acid derivative followed by a deprotection step.

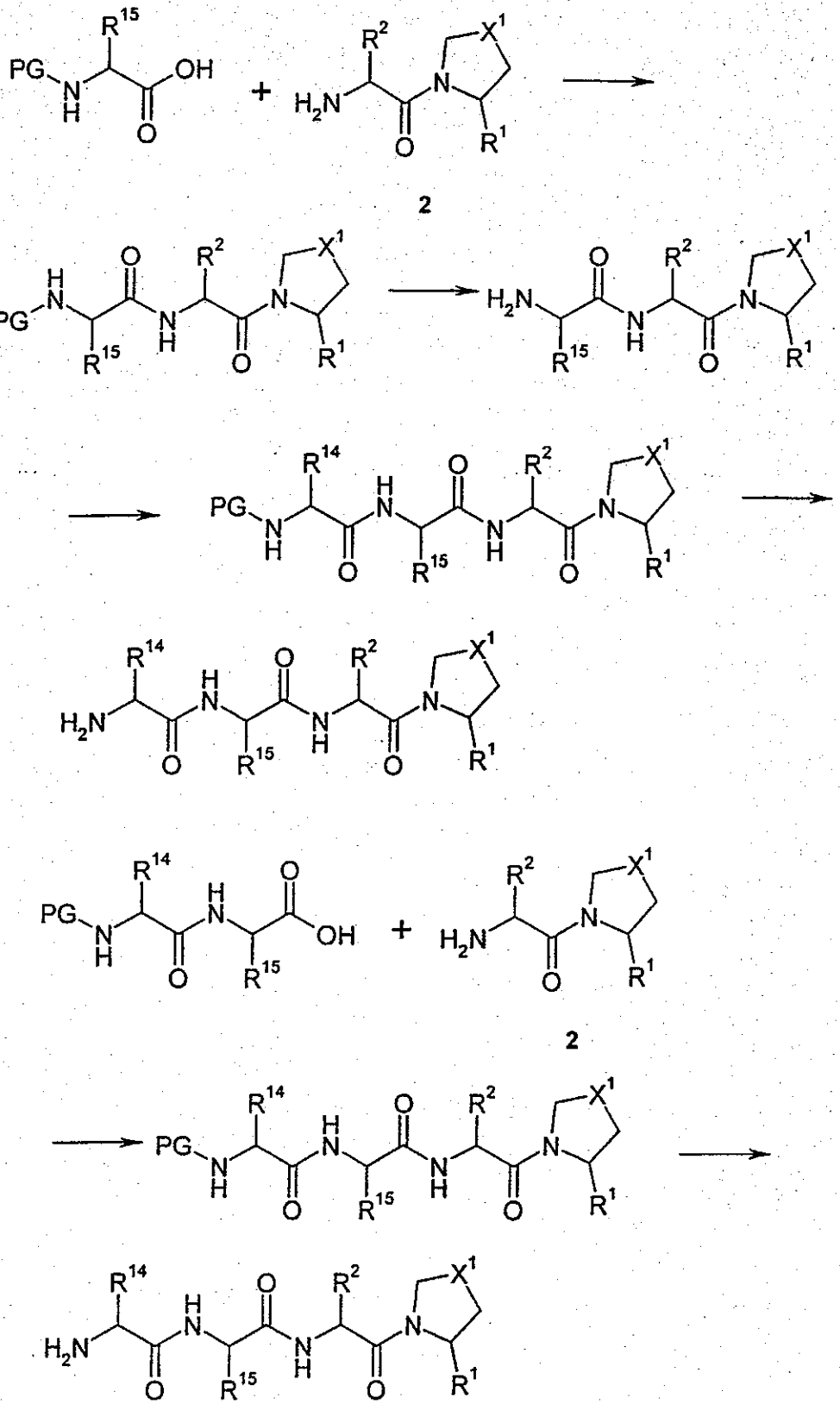


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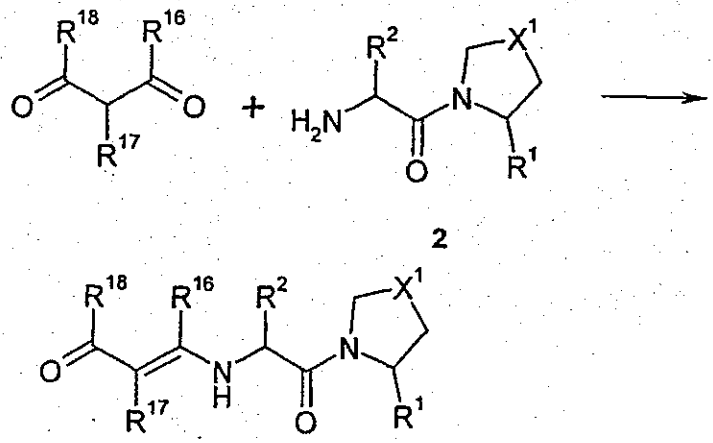
In the above scheme, PG is a protecting group such as tert-butyloxycarbonyl (BOC), 9-fluorenylmethyloxycarbonyl (FMOC) or benzyloxycarbonyl.

When R^{12} is $H_2NCH(R^{14})CONHCH(R^{15})CO$ then the final transformation may be accomplished analogously by the reaction of **2** with a protected dipeptide derivative followed by a deprotection step, or in a slightly longer way with two cycles of coupling and deprotection.



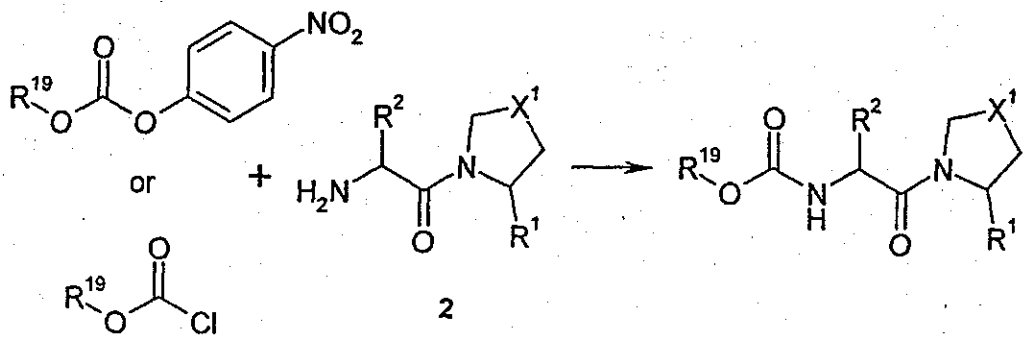
or

When R^{12} is $C(R^{16})=C(R^{17})COR^{18}$ then the final transformation may be accomplished by the reaction of **2** with a suitable 1,3-dicarbonyl compound.



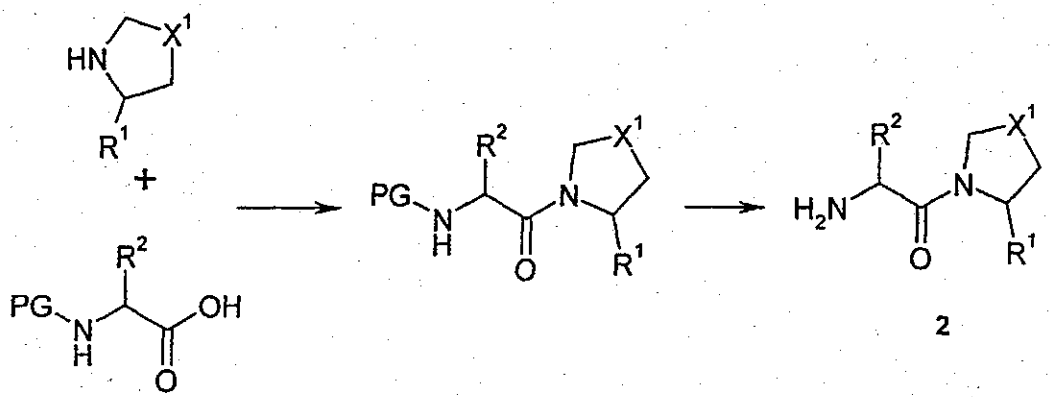
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When R^{12} is $R^{19}OCO$ then the final transformation may be accomplished by the reaction of **2** with a suitable active carbonic acid half ester derivative, such as a chloroformate or a para-nitrophenyl carbonate.



2

The intermediate **2** may be prepared by the coupling of a protected amino acid with a pyrrolidine or thiazolidine derivative, followed by a deprotection step.



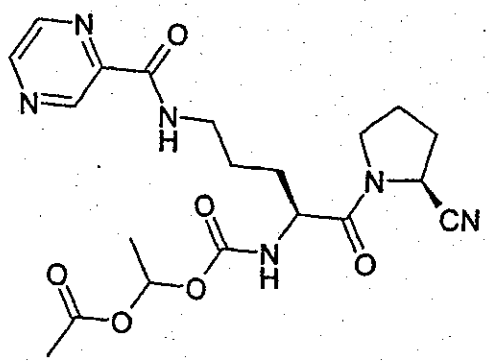
2

Alternatively, it may be more convenient to elaborate the functionality of R^2 after the assembly of the backbone of **2**.

These general methods are further illustrated in the following non-limiting Examples.

EXAMPLE 1

(2*S*)-1-[*N*$^{\alpha}$-(1'-Acetoxyethoxycarbonyl)-*N*$^{\omega}$-(pyrazinyl-2-carbonyl)-L-ornithinyl]-pyrrolidine-2-carbonitrile



1A. *N*-(2-Nitrobenzenesulphenyl)-L-proline

L-Proline (25g, 217mmol) was dissolved in 2M NaOH (110ml, 220mmol) and dioxan (120ml). A solution of 2-nitrobenzenesulphenyl chloride (42g, 222mmol) in dioxan (60ml) was slowly added at the same time as 2M NaOH (110ml, 220mmol). The mixture was stirred for 2 hours at room temperature then poured into water (500ml). The solid was removed by filtration. The pH of the filtrate was adjusted to pH3 with 2M HCl and the solution was extracted with ethyl acetate (3 x 500ml). The combined organic extracts were washed with water (4 x 200ml) and brine (1 x 200ml), dried (Na_2SO_4) and evaporated *in vacuo* to give an orange solid identified as *N*-(2-nitrobenzenesulphenyl)-L-proline (58.1g, 217mmol, 100%).

1B. *N*-(2-Nitrobenzenesulphenyl)-L-proline succinimidyl ester

N-(2-Nitrobenzenesulphenyl)-L-proline (57.9g, 216mmol) was dissolved in CH_2Cl_2/DMF (9:1, 500ml). *N*-Hydroxysuccinimide (37.3g, 324mmol) and water-soluble carbodiimide (51.8g, 260mmol) were added. The mixture was stirred for 18 hours at room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (1000ml). The solution was washed with water (4 x 200ml) and brine (1 x 200ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow solid identified as *N*-(2-nitrobenzenesulphenyl)-L-proline succinimidyl ester (78.9g, 216mmol, 100%).

1C. *N*-(2-Nitrobenzenesulphenyl)-L-prolinamide

N-(2-Nitrobenzenesulphenyl)-L-proline succinimidyl ester (78.5g, 215mmol) was dissolved in dioxan (500ml). Ammonia (35%, 100ml) was added. The mixture was stirred at room temperature for 2 hours then poured into water (700ml). The precipitate was collected, washed with water (200ml), dried over P_2O_5 and recrystallised from ethyl acetate/pet ether 60-80 to give a yellow solid identified as *N*-(2-nitrobenzenesulphenyl)-L-prolinamide (49.6g, 185mmol, 86%).

1D. (2*S*)-1-(2-Nitrobenzenesulphenyl)pyrrolidine-2-carbonitrile

N-(2-Nitrobenzenesulphenyl)-L-prolinamide (49g, 183mmol) was dissolved in dry THF (300ml). The solution was cooled to 0°C, triethylamine (36.7g, 367mmol) was added followed by the slow addition of trifluoroacetic anhydride (77g, 367mmol). The pH was adjusted to pH9 with triethylamine. The mixture was stirred for 30min then diluted with ethyl acetate (500ml), washed with water (1 x 200ml) and brine (1 x 200ml), dried (Na_2SO_4) and evaporated *in vacuo* to give an orange oil which was purified by flash chromatography on silica gel (eluant: 80% pet ether 60-80, 20% ethyl acetate) to give a yellow solid identified as (2*S*)-1-(2-nitrobenzenesulphenyl)pyrrolidine-2-carbonitrile (38.9g, 150mmol, 82%).

1E. (2*S*)-Pyrrolidine-2-carbonitrile hydrochloride

(2*S*)-1-(2-nitrobenzenesulphenyl)pyrrolidine-2-carbonitrile (38.5g, 149mmol) was dissolved in diethyl ether (200ml). 4M HCl/Dioxan (150ml, 600mmol) was slowly added. The mixture was stirred for 2h at room temperature then poured into diethyl ether (1000ml). The solid was collected, washed with diethyl ether (500ml) and recrystallised from methanol/diethyl ether to give a white solid identified as (2*S*)-pyrrolidine-2-carbonitrile hydrochloride (18.9g, 142.5mmol, 96%).

1F. (2*S*)-1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-pyrrolidine-2-carbonitrile.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithine (2.5g, 7.4mmol) was dissolved in CH_2Cl_2 (50ml). This solution was cooled to 0°C, (2*S*)-pyrrolidine-2-carbonitrile hydrochloride (1.2g, 9.1mmol) and PyBOP (4.3g, 8.23mmol) were added, and the pH adjusted to pH9 with triethylamine. The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken

up in ethyl acetate (200ml). The solution was washed with 0.3M $KHSO_4$ (2 x 50ml), sat. $NaHCO_3$ (2 x 50ml), water (2 x 50ml) and brine (1 x 50ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil which was purified by flash chromatography on silica gel (eluant: 80% ethyl acetate, 20% pet. ether, 60-80) to give a colourless oil identified as (2*S*)-1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-pyrrolidine-2-carbonitrile (2.98g, 7.16mmol, 97%).

1G. (2*S*)-1-[N^{ω}-(Pyrazinyl-2-carbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile trifluoroacetate

(2*S*)-1-[N^{α}-*tert*-Butyloxycarbonyl-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile (2.8g, 6.7mmol) was dissolved in trifluoroacetic acid (5ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* to give a colourless oil identified as (2*S*)-1-[N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile trifluoroacetate (1.5g, 3.48mmol, 52%).

1H. (2*S*)-1-[N^{α}-(1'-Acetoxyethoxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]-pyrrolidine-2-carbonitrile

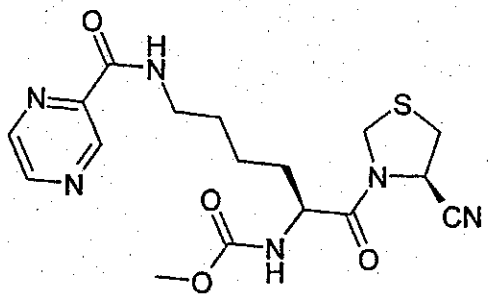
A solution of (2*S*)-1-[N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile trifluoroacetate (200mg, 0.47mmol), α-acetoxyethyl *p*-nitrophenyl carbonate (140mg, 0.52 mmol; prepared according to Alexander *et al.*, J. Med. Chem. 31, 318, 1988) and triethylamine (60mg, 0.6mmol) in dichloromethane (25ml) was stirred at room temperature for 18 hours then evaporated *in vacuo*. The residue was taken up in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (eluant: 98% chloroform, 2%methanol) to give a white solid identified as (2*S*)-1-[N^{α}-(1'-acetoxyethoxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile (30mg, 0.07mmol,14%).

$[M+H]^+$ = 447.2

^1H NMR ($CDCl_3$): δ 1.41-1.48 (3H,m), 1.72-1.86 (4H,m), 2.02 (3H,d,J=7.7Hz), 2.11-2.28 (4H,m), 3.51-3.57 (2H,m), 3.68-3.69 (2H,m), 4.47-4.48 (1H,m), 4.74-4.76 (1H,m), 5.55-5.59 (1H,m), 6.75-6.78 (1H,m), 7.89-7.91 (1H,m), 8.52 (1H,d,J=1.9Hz), 8.76 (1H,d,J=2.5Hz), 9.3 (1H,d,J=1.5Hz) ppm.

EXAMPLE 2

(4*R*)-3-[N^{α}-Methoxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-lysinyl]thiazolidine-4-carbonitrile



2A. (4*R*)-3-(*tert*-Butyloxycarbonyl)thiazolidine-4-carboxamide

(4*R*)-3-(*tert*-Butyloxycarbonyl)thiazolidine-4-carboxylic acid (12.5g, 54.1mmol) was dissolved in CH_2Cl_2/DMF (9:1, 150ml). To this solution at 0°C was added 1-hydroxybenzotriazole hydrate (14.6g, 108mmol) and water-soluble carbodiimide (13.0g, 65mmol). The mixture was stirred for 1 hour at 0°C then ammonia (35%, 50ml) was added. The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (500ml). The solution was washed with 0.3M $KHSO_4$ (2 x 100ml), sat. $NaHCO_3$ (2 x 100ml), water (2 x 100ml) and brine (1 x 100ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil. The residue was purified by flash chromatography on silica gel (eluant: 2% methanol, 98% chloroform) to give a colourless oil identified as (4*R*)-3-(*tert*-butyloxycarbonyl)thiazolidine-4-carboxamide (8.9g, 38.4mmol,71%).

2B. (4*R*)-Thiazolidine-4-carboxamide hydrochloride

(4*S*)-3-(*tert*-Butyloxycarbonyl)thiazolidine-4-carboxamide (8.6g, 37.1mmol) was dissolved in 4M HCl/dioxan (50ml). The mixture was stirred for 1 hour at room temperature then the solvent was evaporated *in vacuo* to give a white solid identified as (4*R*)-thiazolidine-4-carboxamide hydrochloride (6.2g, 36.8mmol, 99%).

2C. (4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-lysinyl]thiazolidine-4-carboxamide

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-lysine (5g, 10.7mmol) was dissolved in CH_2Cl_2 (100ml). This solution was cooled to 0°C, (4*R*)-thiazolidine-4-carboxamide hydrochloride (1.78g, 11.7mmol) and PyBOP (6.7g, 12.8mmol) were

added, and the pH was adjusted to pH9 with triethylamine. The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (200ml). The solution was washed with 0.3M $KHSO_4$ (2 x 50ml), sat. $NaHCO_3$ (2 x 50ml), water (2 x 50ml) and brine (1 x 50ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil. The residue was purified by flash chromatography on silica gel (eluant: 2% methanol, 98% chloroform) to give a colourless oil identified as (4*R*)-3-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxy-carbonyl)-L-lysinyl]thiazolidine-4-carboxamide (2.81g, 4.8mmol, 44%).

2D. (4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-lysinyl]thiazolidine-4-carbonitrile

(4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-lysinyl]-thiazolidine-4-carboxamide (2.7g, 4.7mmol) was dissolved in dry THF (100ml). The solution was cooled to 0°C and triethylamine (1.0g, 10mmol) was added followed by the slow addition of trifluoroacetic anhydride (2.0g, 9.5mmol). The pH was adjusted to pH9 with triethylamine. The mixture was stirred for 30min then diluted with ethyl acetate (100ml), washed with water (1 x 50ml) and brine (1 x 50ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 60% pet ether 60-80, 40% ethyl acetate) to give a colourless oil identified as (4*R*)-3-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-lysinyl]-thiazolidine-4-carbonitrile (2.14g, 3.81mmol, 82%).

2E. (4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-L-lysinyl]thiazolidine-4-carbonitrile

(4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-lysinyl]-thiazolidine-4-carbonitrile (1.9g, 3.4mmol) was dissolved in THF (40ml). Diethylamine (10ml) was added. The mixture was stirred for 2h at room temperature then the solvent was removed *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 90% chloroform, 7% methanol, 3% triethylamine) to give a colourless oil identified as (4*R*)-3-[N^{α}-(*tert*-butyloxycarbonyl)-L-lysinyl]thiazolidine-4-carbonitrile (863mg, 2.5mmol, 75%).

2F. (4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-lysinyl]-thiazolidine-4-carbonitrile

(4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-L-lysinyl]thiazolidine-4-carbonitrile (100mg, 0.29mmol) was dissolved in CH_2Cl_2 (20ml). To this solution at 0°C were added 2-

pyrazinecarboxylic acid (43mg, 0.35mmol) and PyBOP (170mg, 0.33mmol) and the pH was adjusted to pH9 with triethylamine. The mixture was stirred for 18 h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$ (2 x 20ml), sat. $NaHCO_3$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 2% methanol, 98% chloroform) to give a colourless oil identified as (4*R*)-3-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-lysinyl]thiazolidine-4-carbonitrile (112mg, 0.25mmol, 86%).

2G. (4*R*)-3-[(N^{α}-Methoxycarbonyl)- N^{ω}-(pyrazinyl-2-carbonyl)-L-lysinyl]-thiazolidine-4-carbonitrile

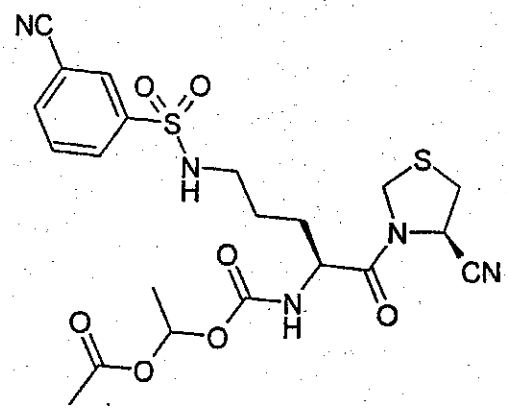
(4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-lysinyl]thiazolidine-4-carbonitrile (160mg, 0.36mmol) was dissolved in 4M HCl/dioxan (30ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (25 ml). Methyl chloroformate (50mg, 0.53mmol) and triethylamine (60mg, 0.6 mmol) were added and the solution was stirred at room temperature for 18 hours then solution was evaporated *in vacuo*. The residue was taken up in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (eluant: 90% ethyl acetate: 10% pet. ether 60-80) to give a white solid identified as (4*R*)-3-[(N^{α}-methoxycarbonyl)-N^{ω}-(pyrazinyl-2-carbonyl)-L-lysinyl]thiazolidine-4-carbonitrile (52mg, 0.13mmol, 35%).

$[M+H]^+$ = 407.1

^1H NMR ($CDCl_3$): δ 1.33-1.48 (4H,m), 1.63-1.82 (2H,m), 3.21-3.27 (2H,m), 3.45-3.60 (2H,m), 3.63 (3H,s), 4.44-4.46 (1H,m), 4.63 (1H,d,J=8.4Hz), 4.86 (1H,d,J=8.5Hz), 5.23-5.27 (1H,m), 5.53 (1H,d,J=8.2Hz), 7.85-7.87 (1H,m), 8.50-8.51 (1H,m), 8.73 (1H,d,J=2.5Hz), 9.38 (1H,d,J = 1,3Hz) ppm.

EXAMPLE 3

(4*R*)-3-[N$^{\alpha}$-(1'-Acetoxyethoxycarbonyl)-*N*$^{\omega}$-(3-cyanobenzenesulphonyl)-L-ornithinyl]thiazolidine-4-carbonitrile



3A. (4*R*)-3-[*N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\omega}$-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]thiazolidine-4-carboxamide

N$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\omega}$-(9-fluorenylmethyloxycarbonyl)-L-ornithine (2.8g, 6.2mmol) was dissolved in CH_2Cl_2 /DMF (9:1, 100ml). This solution was cooled to 0°C, (4*R*)-thiazolidine-4-carboxamide hydrochloride (1.78g, 11.7mmol), 1-hydroxybenzotriazole hydrate (1.1g, 8.1mmol) and water-soluble carbodiimide (1.5g, 7.5mmol) were added, and the pH was adjusted to pH8 with N-methylmorpholine. The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (200ml). The solution was washed with 0.3M $KHSO_4$ (2 x 50ml), sat. $NaHCO_3$ (2 x 50ml), water (2 x 50ml) and brine (1 x 50ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil. The residue was purified by flash chromatography on silica gel (eluant: 85% ethyl acetate, 15% pet. ether 60-80) to give a colourless oil identified as (4*R*)-3-[*N*$^{\alpha}$-(*tert*-butyloxycarbonyl)-*N*$^{\omega}$-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]thiazolidine-4-carboxamide (2.26g, 3.9mmol, 66%).

3B. (4*R*)-3-[*N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\omega}$-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]thiazolidine-4-carbonitrile

(4*R*)-3-[*N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\omega}$-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]-thiazolidine-4-carboxamide (2.1g, 3.7mmol) was dissolved in dry THF (100ml). The solution was cooled to 0°C, triethylamine (740mg, 7.4mmol) was added followed by the slow addition of trifluoroacetic anhydride (1.65g, 7.9mmol). The pH was adjusted to pH9 with triethylamine. The mixture was stirred for 30min then diluted with ethyl

acetate (100ml), washed with water (1 x 50ml) and brine (1 x 50ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 45% pet ether 60-80, 55% ethyl acetate) to give a colourless oil identified as (4*R*)-3-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]-thiazolidine-4-carbonitrile (1.73g, 3.14mmol, 85%).

3C. (4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithinyl]thiazolidine-4-carbonitrile

(4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]-thiazolidine-4-carbonitrile (1.6g, 2.9mmol) was dissolved in THF (40ml). Diethylamine (10ml) was added. The mixture was stirred for 2h at room temperature then the solvent was removed *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 90% chloroform, 7% methanol, 3% triethylamine) to give a colourless oil identified as (4*R*)-3-[N^{α}-(*tert*-butyloxycarbonyl)-L-ornithinyl]thiazolidine-4-carbonitrile (902mg, 2.75mmol, 95%).

3D. (4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(3-cyanobenzenesulphonyl)-L-ornithinyl]thiazolidine-4-carbonitrile

(4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithinyl]thiazolidine-4-carbonitrile (207mg, 0.63mmol) was dissolved in CH_2Cl_2 (25ml). To this solution at 0°C was added 3-cyanobenzenesulphonyl chloride (135mg, 0.67mmol) and the pH was adjusted to pH9 with triethylamine. The mixture was stirred for 18 h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$ (2 x 20ml), sat. $NaHCO_3$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 45% ethyl acetate: 55% pet. ether 60-80°C) to give a colourless oil identified as (4*R*)-3-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(3-cyanobenzenesulphonyl)-L-ornithinyl]thiazolidine-4-carbonitrile (162mg, 0.33mmol, 52%).

3E. (4*R*)-3-[N^{α}-(1'-Acetoxyethoxycarbonyl)-N^{ω}-(3-cyanobenzenesulphonyl)-L-ornithinyl]thiazolidine-4-carbonitrile

(4*R*)-3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(3-cyanobenzenesulphonyl)-L-ornithinyl]-thiazolidine-4-carbonitrile (142mg, 0.29mmol) was dissolved in trifluoroacetic acid (5ml). The mixture was stirred for 1 hour at room temperature then the solvent was

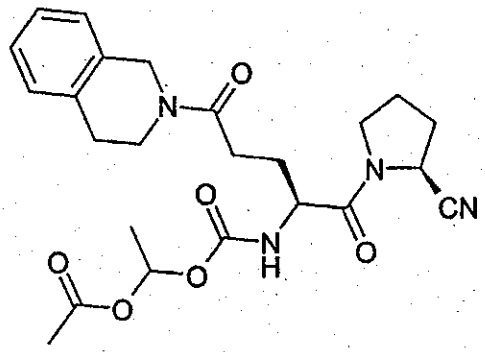
removed *in vacuo*. The residue was dissolved in dichloromethane (25ml) and α-acetoxyethyl *p*-nitrophenyl carbonate (108mg, 0.40 mmol; prepared according to Alexander *et al.*, J. Med. Chem. 31, 318, 1988) and triethylamine (60mg, 0.6mmol) were added. The reaction was stirred at room temperature for 18 hours, then evaporated *in vacuo*. The residue taken up in ethyl acetate (70ml) and the solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (eluant: 70% ethyl acetate: 30% pet. ether 60-80) to give a white solid identified as (4*R*)-3-[N^{α}-(1'-acetoxyethoxycarbonyl)-N^{ω}-(3-cyanobenzenesulphonyl)-L-ornithinyl]thiazolidine-4-carbonitrile (32mg, 0.06mmol, 21%).

$[M+H]^+ = 524.0$

^1H NMR ($CDCl_3$): δ 1.20-1.22 (2H,m), 1.43-1.46 (3H,m), 1.59-1.78 (4H,m), 2.03-2.06 (3H,m), 3.03 (2H,d,J=4.2Hz), 3.29-3.33 (2H,m), 4.61-4.66 (1H,m), 4.79-4.84 (1H,m), 5.16-5.20 (1H,m), 5.73-5.82 (1H,m), 6.74-6.76 (1H,m), 7.63-7.69 (1H,m), 7.83-7.86 (1H,m), 8.10-8.16 (2H,m) ppm.

EXAMPLE 4

(2*S*,2'*S*)-1-[2'-(1''-Acetoxyethoxycarbonylamino)-5'-oxo-5'-(tetrahydroisoquinolin-2-yl)pentanoyl]pyrrolidine-2-carbonitrile



4A. (2*S*)-1-[*N*-(*tert*-Butyloxycarbonyl)-O^{ω}-methyl-L-glutamyl]pyrrolidine-2-carbonitrile

N-(*tert*-Butyloxycarbonyl)-O^{ω}-methyl-L-glutamic acid (1.0g, 3.83mmol) was dissolved in CH_2Cl_2 /DMF (9:1, 20ml). To this solution at 0°C were added 1-hydroxybenzotriazole hydrate (788mg, 5.84mmol), water-soluble carbodiimide (877mg, 4.38mmol), (2*S*)-pyrrolidine-2-carbonitrile hydrochloride (609mg, 4.6mmol) and triethylamine (65mg,

0.65mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$ (2 x 20ml), sat. $NaHCO_3$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 50% ethyl acetate, 50% pet. ether 60-80) to give a brown oil identified as (2*S*)-1-[*N*-(*tert*-butyloxycarbonyl)-O^{ω}-methyl-L-glutamyl]pyrrolidine-2-carbonitrile (290mg, 0.86mmol, 22%).

4B. (2*S*)-1-[*N*-(*tert*-Butyloxycarbonyl)-L-glutamyl]pyrrolidine-2-carbonitrile

(2*S*)-1-[*N*-(*tert*-Butyloxycarbonyl)-O^{ω}-methyl-L-glutamyl]pyrrolidine-2-carbonitrile (250mg, 0.74mmol) was dissolved in dioxan (5ml). 1M Lithium hydroxide (1.1ml, 1.1mmol) was added. The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 1M $KHSO_4$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a colourless oil identified as (2*S*)-1-[*N*-(*tert*-butyloxycarbonyl)-L-glutamyl]pyrrolidine-2-carbonitrile (200mg, 0.61mmol, 83%).

4C. (2*S*,2'*S*)-1-[2'-(*tert*-Butyloxycarbonylamino)-5'-oxo-5'-(tetrahydroisoquinolin-2-yl)pentanoyl]pyrrolidine-2-carbonitrile

(2*S*)-1-[*N*-(*tert*-Butyloxycarbonyl)-L-glutamyl]pyrrolidine-2-carbonitrile (200mg, 0.61mmol) was dissolved in CH_2Cl_2/DMF (9:1, 20ml). To this solution at 0°C were added 1-hydroxybenzotriazole hydrate (98mg, 0.73mmol), water-soluble carbodiimide (140mg, 0.73mmol), tetrahydroisoquinoline (109mg, 0.82mmol) and triethylamine(150mg, 1.5mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$ (2 x 20ml), sat. $NaHCO_3$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil. The residue was purified by flash chromatography on silica gel (eluant: 5% methanol, 97% chloroform) to give a colourless oil identified as .(2*S*,2'*S*)-1-[2'-(*tert*-butyloxycarbonylamino)-5'-oxo-5'-(tetrahydroisoquinolin-2-yl)pentanoyl]pyrrolidine-2-carbonitrile (149mg, 0.34mmol, 56%).

4D. (2*S*,2′*S*)-1-[2′-(1″-Acetoxyethoxycarbonylamino)-5′-oxo-5′-(tetrahydro-isoquinolin-2-yl)pentanoyl]pyrrolidine-2-carbonitrile

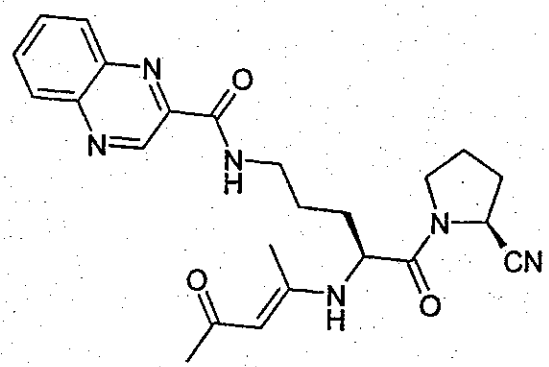
(2*S*,2′*S*)-1-[2′-(*tert*-butyloxycarbonylamino)-5′-oxo-5′-(tetrahydroisoquinolin-2-yl)-pentanoyl]pyrrolidine-2-carbonitrile (149mg, 0.34mmol) was dissolved in trifluoroacetic acid (20ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (25ml) and α-acetoxyethyl *p*-nitrophenyl carbonate (100mg, 0.37 mmol; prepared according to Alexander *et al.*, J. Med. Chem. 31, 318, 1988) and triethylamine (40mg, 0.4mmol) were added. The reaction was stirred at room temperature for 18 hours then evaporated *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (eluant: 90% ethyl acetate, 10% pet. ether 60-80°C) to give a white solid identified as (2*S*,2′*S*)-1-[2′-(1″-Acetoxyethoxycarbonylamino)-5′-oxo-5′-(tetrahydroisoquinolin-2-yl)pentanoyl]pyrrolidine-2-carbonitrile (58mg, 0.12mmol, 36%).

$[M+H]^+$ = 471.2

^{1}H NMR ($CDCl_3$): δ 1.40-1.44 (3H,m), 2.00-2.07 (3H,m), 2.13-2.40 (9H,m), 2.82-2.91 (2H,m), 3.63-3.70 (2H,m), 3.96-4.18 (1H,m), 4.57-4.61 (2H,m), 4.72-4.75 (2H,m), 5.78-5.80 (1H,m), 6.69-6.75 (1H,m), 7.10-7.25 (4H,m) ppm.

EXAMPLE 5

(2*S*)-1-[N^{α}-(4′-Oxopent-2′-en-2′-yl)-N^{ω}-(quinoxalinyl-2-carbonyl)-L-ornithinyl]-pyrrolidine-2-carbonitrile



5A. 1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]-L-prolineamide

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-ornithine (5g, 11.0mmol) was dissolved in CH_2Cl_2 (40ml). This solution was cooled to 0°C, L-prolineamide (1.4g, 12.2 mmol) and PyBOP (6.3g, 12.1mmol) were added, and the pH was adjusted to pH9 with triethylamine. The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in chloroform (200ml). The solution was washed with 0.3M $KHSO_4$ (2 x 50ml), sat. $NaHCO_3$ (2 x 50ml), water (2 x 50ml) and brine (1 x 50ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil. The residue was purified by flash chromatography on silica gel (eluant: 98% chloroform, 2% methanol) to give a colourless oil identified as 3-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]-L-prolineamide (4.2g, 7.6mmol, 69%).

5B. (2*S*)-1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile

N-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]-L-proline-amide (4.1g, 7.4mmol) was dissolved in dry THF (100ml). The solution was cooled to 0°C and triethylamine (820mg, 8.2mmol) was added followed by the slow addition of trifluoroacetic anhydride (1.7g, 8.1mmol). The pH was adjusted to pH9 with triethylamine. The mixture was stirred for 30min then diluted with ethyl acetate (100ml), washed with water (1 x 50ml) and brine (1 x 50ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 20% ethyl acetate, 80% pet. ether 60-80) to give a colourless oil identified as (2*S*)-1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile (3.5g, 6.5mmol, 87%).

5C. (2*S*)-1-[N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile

(2*S*)-1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]-pyrrolidine-2-carbonitrile (3.4g, 6.4mmol) was dissolved in THF (40ml). Diethylamine (10ml) was added. The mixture was stirred for 2h at room temperature then the solvent was removed *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 90% chloroform, 7% methanol, 3% triethylamine) to give a colourless oil identified as (2*S*)-1-[N^{α}-(*tert*-butyloxycarbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile (1.48g, 4.77mmol, 75%).

5D. (2*S*)-1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(quinoxalinyl-2-carbonyl)-L-ornithinyl]-pyrrolidine-2-carbonitrile

(2*S*)-1-[N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile (300mg, 0.97mmol) was dissolved in CH_2Cl_2 (25ml). To this solution at 0°C was added 2-quinoxaloyl chloride (200mg, 1.04mmol) and the pH was adjusted to pH9 with triethylamine. The mixture was stirred for 18 h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$ (2 x 20ml), sat. $NaHCO_3$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 60% ethyl acetate, 40% pet. ether 60-80) to give a colourless oil identified as (2*S*)-1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(quinoxalinyl-2-carbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile (310mg, 0.67mmol, 69%).

5E. (2*S*)-1-[N^{α}-(4'-Oxopent-2'-en-2'-yl)-N^{ω}-(quinoxalinyl-2-carbonyl)-L-ornithinyl]-pyrrolidine-2-carbonitrile

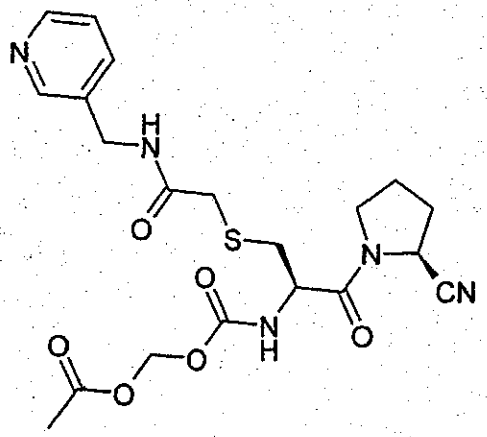
(2*S*)-1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(quinoxalinyl-2-carbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile (160mg, 0.34mmol) was dissolved in trifluoroacetic acid (20 ml). The mixture was stirred for one hour at room temperature then the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (25ml) and 2,4-pentanedione (48mg, 0.48mmol) and triethylamine (100mg, 1.0mmol) were added. The reaction was stirred at room temperature for 18 hours then evaporated *in vacuo*. The residue taken up in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (eluant: 98% chloroform, 2% methanol) to give a white solid identified as (2*S*)-1-[N^{α}-(4'-oxopent-2'-en-2'-yl)-N^{ω}-(quinoxalinyl-2-carbonyl)-L-ornithinyl]pyrrolidine-2-carbonitrile (87mg, 0.19mmol, 57%).

$[M+H]^+ = 449.2$

^{1}H NMR ($CDCl_3$): δ 1.89-2.02 (10H,2 x s+m), 2.13-2.24 (4H,m), 3.57-3.62 (5H,m), 4.3-4.6 (1H,m), 4.70-4.81 (1H,m), 5.02 (1H,s), 7.83-7.88 (2H,m), 8.10-8.19 (3H,m), 9.62 (1H,s), 11.0-11.2 (1H,m) ppm.

EXAMPLE 6

(2*S*)-1-[*N*-Acetoxymethoxycarbonyl-*S*-(3-picolylcarbamoylmethyl)-L-cysteinyl]-pyrrolidine-2-carbonitrile



6A. *S*-(Benzyloxycarbonylmethyl)-*N*-(*tert*-butyloxycarbonyl)-L-cysteine

N-(*tert*-Butyloxycarbonyl)-L-cysteine (3.5g, 15.8mmol), benzyl 2-bromoacetate (3.7g,16.1mmol) and triethylamine (1.8g, 18.0mmol) were dissolved in THF (100ml). The mixture was stirred for 18 hours at room temperature then diluted with ethyl acetate (100ml), washed with 0.3M $KHSO_4$, sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 95% chloroform, 4% methanol, 1% acetic acid) to give a colourless oil identified as *S*-(benzyloxycarbonylmethyl)-*N*-(*tert*-butyloxycarbonyl)–L-cysteine (5.2g, 14.1mmol, 89%).

6B. (2*S*)-1-[*S*-(Benzyloxycarbonylmethyl)-*N*-(*tert*-butyloxycarbonyl)–L-cysteinyl]-pyrrolidine-2-carbonitrile

S-(Benzyloxycarbonylmethyl)-*N*-(*tert*-butyloxycarbonyl)–L-cysteine (5.10g, 13.8mmol) was dissolved in CH_2Cl_2 (200ml). This solution was cooled to 0°C, (2*S*)-pyrrolidine-2-carbonitrile hydrochloride (2.1g, 15.8mmol) and PyBOP (8.0g, 15.3mmol) were added, and the pH adjusted to pH9 with triethylamine. The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (150ml). The solution was washed with 0.3M $KHSO_4$ (1 x 50ml), sat. $NaHCO_3$ (1 x 50ml), water (1 x 50ml) and brine (1 x 50ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil which was purified by flash chromatography on silica gel (eluant: 40% ethyl acetate, 60% pet. ether 60-80) to give a colourless oil

identified as (2*S*)-1-[*S*-(benzyloxycarbonylmethyl)-*N*-(*tert*-butyloxycarbonyl)-L-cysteinyl]pyrrolidine-2-carbonitrile (5.82g, 13.0mmol, 94%).

6C. (2*S*)-1-[*N*-(*tert*-Butyloxycarbonyl)-*S*-(carboxymethyl)-L-cysteinyl]pyrrolidine-2-carbonitrile

(2S)-1-[*S*-(Benzyloxycarbonylmethyl)-*N*-(*tert*-butyloxycarbonyl)-L-cysteinyl]pyrrolidine-2-carbonitrile (1.31g, 2.9mmol) was dissolved in THF(100ml). 1M Lithium hydroxide (3.5ml, 3.5mmol) was added. The mixture was stirred for 3 hours at room temperature then with ethyl acetate (100ml), washed with 1M citric acid, water and brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a colourless oil which was purified by flash chromatography on silica gel (eluant: 97% chloroform, 2% methanol, 1% acetic acid) to give a colourless oil identified as (2*S*)-1-[*N*-(*tert*-butyloxycarbonyl)-*S*-(carboxymethyl)-L-cysteinyl]pyrrolidine-2-carbonitrile (860mg, 2.4mmol, 82%).

6D. (2*S*)-1-[*N*-(*tert*-Butyloxycarbonyl)-*S*-(3-picolylcarbamoylmethyl))-L-cysteinyl]-pyrrolidine-2-carbonitrile

(2S)-1-[*N*-(*tert*-Butyloxycarbonyl)-*S*-(carboxymethyl)-L-cysteinyl]pyrrolidine-2-carbonitrile (150mg, 0.42mmol) was dissolved in CH_2Cl_2 (20ml). The solution was cooled to 0°C, 3-(aminomethyl)pyridine (53mg, 0.5mmol) and PyBOP (270mg, 0.52mmol) were added, and the pH was adjusted to pH9 with triethylamine. The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$ (1 x 20ml), sat. $NaHCO_3$ (1 x 20ml), water (1 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil which was purified by flash chromatography on silica gel (eluant: 96% chloroform, 4% methanol) to give a colourless oil identified as (2*S*)-1-[*N*-(*tert*-butyloxycarbonyl)-*S*-(3-picolylcarbamoylmethyl))-L-cysteinyl]pyrrolidine-2-carbonitrile (170mg, 0.38mmol, 91%).

6E. (2*S*)-1-[N^{α}-Acetoxymethoxycarbonyl-*S*-(3-picolylcarbamoylmethyl)-L-cysteinyl]pyrrolidine-2-carbonitrile

(2*S*)-1-[*N*-(*tert*-Butyloxycarbonyl)-*S*-(3-picolylcarbamoylmethyl)-L-cysteinyl]pyrrolidine-2-carbonitrile (130mg, 0.29mmol) was dissolved in trifluoroacetic acid (20ml). The solution was stirred for 1 hour at room temperature then the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (25ml) and acetoxymethyl *p*-

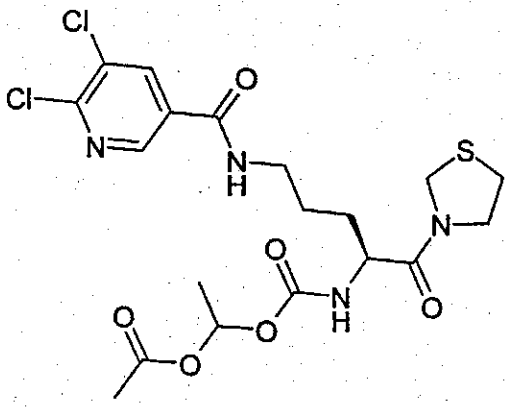
nitrophenyl carbonate (80mg, 0.31mmol; prepared according to Alexander *et al.*, J. Med. Chem. 31, 318, 1988) and triethylamine (40mg, 0.4mmol) were added. The solution was stirred at room temperature for 18 hours then evaporated *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (eluant: 90% ethyl acetate, 10% pet. ether 60-80) to give a white solid identified as (2*S*)-1-[*N*$^{\alpha}$-acetoxymethoxycarbonyl-*S*-(3-picolylcarbamoylmethyl)-L-cysteinyl]pyrrolidine-2-carbonitrile (33mg, 0.071mmol, 24%).

$[M+H]^+$ = 464.0

^{1}H NMR ($CDCl_3$): δ 2.08 (3H,s), 2.13-2.29 (4H,m), 2.89 (2H,d,J=6.9Hz), 3.20-3.29 (2H,m), 3.61-3.74 (2H,m), 4.46 (2H,d,J=5.9Hz), 4.60-4.71 (2H,m), 5.68 (2H,s), 6.12 (1H,d,J=8.6Hz), 7.16-7.27 (2H,m), 7.66 (1H,d,J=8.1Hz), 8.50 (1H,d,J=4.7Hz), 8.56 (1H,s) ppm.

EXAMPLE 7

3-[*N*$^{\alpha}$-(1′- Acetoxyethoxycarbonyl)-*N*$^{\omega}$-(5,6-dichloronicotinoyl)-L-ornithinyl]-thiazolidine



7A. 3-[*N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\omega}$-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]thiazolidine

N$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\omega}$-(9-fluorenylmethyloxycarbonyl)-L-ornithine (2.73g, 6mmol) was dissolved in CH_2Cl_2/DMF (9:1, 100ml). To this solution at 0°C were added 1-hydroxybenzotriazole hydrate (1.53g, 10mmol), water-soluble carbodiimide (1.34g, 7mmol), thiazolidine (1.28g, 18mmol) and triethylamine (80mg, 8mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed

in vacuo and the residue was taken up in ethyl acetate (100ml). The solution was washed with 0.3M $KHSO_4$ (2 x 25ml), sat. $NaHCO_3$ (2 x 25ml), water (2 x 25ml) and brine (1 x 25ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 75% ethyl acetate, 25% pet. ether 60-80) to give a white solid identified as 3-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]thiazolidine (2.55g, 4.85mmol, 81%).

7B. 3-[N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithinyl]thiazolidine

3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(9-fluorenylmethyloxycarbonyl)-L-ornithinyl]thiazolidine (1.15g, 2.13mmol) was dissolved in acetonitrile (20ml). Diethylamine (5ml) was added. The mixture was stirred for 90min at room temperature then the solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel (eluant: 90% chloroform, 7% methanol, 3% triethylamine) to give a pale yellow oil identified as 3-[N^{α}-(*tert*-butyloxycarbonyl)-L-ornithinyl]thiazolidine (530mg, 1.67mmol, 78%).

7C. 3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]-thiazolidine

3-[N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithinyl]thiazolidine (600mg, 1.96mmol) was dissolved in CH_2Cl_2/DMF (9:1, 50ml). To this solution at 0°C were added 1-hydroxybenzotriazole hydrate (360mg, 2.36mmol), water-soluble carbodiimide (472mg, 2.36mmol), 5,6-dichloronicotinic acid (416mg, 2.16mmol) and triethylamine (360mg, 3.6mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$ (2 x 20ml), sat. $NaHCO_3$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 2% methanol, 98% chloroform) to give a sticky white solid identified as 3-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]thiazolidine (512mg, 1.08mmol, 56%).

7D. 3-[N^{α}-(1'-Acetoxyethoxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]-thiazolidine

3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]thiazolidine (128mg, 0.27mmol) was dissolved in 4M HCl/dioxan (20ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo*. The residue

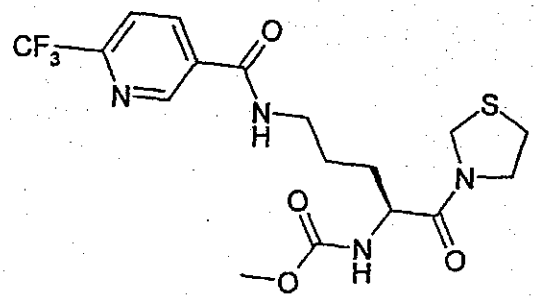
was dissolved in dichloromethane (25ml) and α-acetoxyethyl *p*-nitrophenyl carbonate (83mg, 0.3mmol; prepared according to Alexander *et al.*, J. Med. Chem. 31, 318, 1988) and triethylamine (40mg, 0.4mmol) were added. The solution was stirred at room temperature for 18 hours then evaporated *in vacuo*. The residue was taken up in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (eluant: 75% ethyl acetate, 25% pet. ether 60-80) to give a white solid identified as 3-[N^{α}-(1'-acetoxyethoxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]thiazolidine (67mg, 0.13mmol, 47%).

$[M+H]^+ = 509.0$

^1H NMR ($CDCl_3$): δ 1.44-1.46 (3H, m), 1.63-1.99 (3H,br m), 1.99-2.04 (4H,m), 2.98-3.06 (2H,m), 3.46-3.48 (2H,m), 3.50-3.80 (2H,m), 4.47-4.56 (3H,m), 5.81-5.91 (1H,m), 6.74-6.75 (1H,m), 7.24-7.33 (1H,m), 8.24-8.25 (1H,m), 8.69-8.71 (1H,m) ppm.

EXAMPLE 8

3-[N^{α}-Methoxycarbonyl-N^{ω}-(6-trifluoromethylnicotinoyl)-L-ornithinyl]thiazolidine



8A. 3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(6-trifluoromethylnicotinoyl)-L-ornithinyl]-thiazolidine

3-[N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithinyl]thiazolidine (150mg, 0.49mmol) was dissolved in CH_2Cl_2/DMF (9:1, 20ml). To this solution at 0°C were added 1-hydroxybenzotriazole hydrate (100mg, 0.74mmol), water-soluble carbodiimide (118mg, 0.59mmol), 6-trifluoromethylnicotinic acid (104mg, 0.54mmol) and triethylamine (100mg, 1.0mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$ (2 x 20ml), sat. $NaHCO_3$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was

purified by flash chromatography on silica gel (eluant: 6% methanol, 94% chloroform) to give a sticky white solid identified as 3-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(6-trifluoromethylnicotinoyl)-L-ornithinyl]thiazolidine (76mg, 0.16mmol, 32%).

8B. 3-[N^{α}-Methoxycarbonyl-N^{ω}-(6-trifluoromethylnicotinoyl)-L-ornithinyl]-thiazolidine

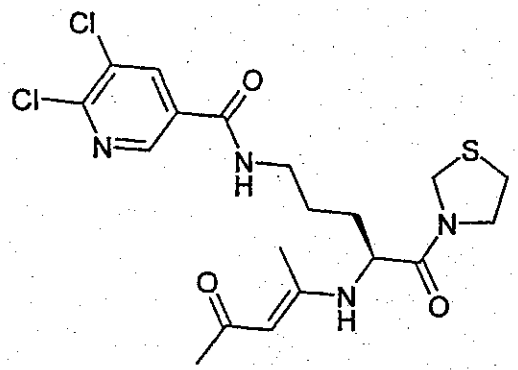
3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(6-trifluoromethylnicotinoyl)-L-ornithinyl]thiazolidine (76mg, 0.16mmol) was dissolved in 4M HCl/dioxan (20ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (25ml) and methyl chloroformate (17mg, 0.18 mmol) and triethylamine (20mg, 0.2mmol) were added. The solution was stirred at room temperature for 18 hours then evaporated *in vacuo*. The residue was taken up in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (eluant: 3% methanol, 97% chloroform) to give a white solid identified as 3-[N^{α}-methoxycarbonyl-N^{ω}-(6-trifluoromethylnicotinoyl)-L-ornithinyl]thiazolidine (66mg, 0.15mmol, 96%).

$[M+H]^+ = 435.1$

^1H NMR ($CDCl_3$): δ 1.36-1.79 (4H,m), 2.98-3.11 (2H,m), 3.48-3.60 (2H,m), 3.64 (3H,s), 3.72-4.10 (3H,m), 4.57-4.60 (2H,m), 5.63-5.76 (1H,m), 6.55 (1H,br m), 7.54-7.55(1H,m), 8.77-8.79 (2H,m) ppm.

EXAMPLE 9

3-[N^{ω}-(5,6-Dichloronicotinoyl)-N^{α}-(4'-oxopent-2'-en-2'-yl)-L-ornithinyl]thiazolidine



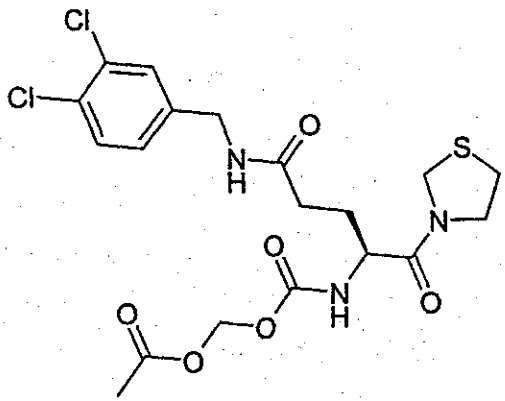
3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(5,6-dichloronicotinoyl)-L-ornithinyl]thiazolidine (162mg, 0.34mmol) was dissolved in 4M HCl/dioxan (20ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (25ml) and 2,4-pentanedione (100mg, 0.37 mmol) and triethylamine (40mg, 0.4mmol) were added. The solution was stirred at room temperature for 18 hours then evaporated *in vacuo*. The residue was taken up in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (eluant: 90% ethyl acetate, 10% pet. ether 60-80) to give a white solid identified as 3-[N^{ω}-(5,6-dichloronicotinoyl)-N^{α}-4'-oxopent-2'-en-2'-yl)-L-ornithinyl]thiazolidine (63mg, 0.14mmol, 40%).

$[M+H]^+ = 461.3$

^1H NMR ($CDCl_3$): δ 1.74-1.86 (6H,m), 1.87 (3H,s), 1.97 (3H,s), 2.94-3.11 (2H,m), 3.46-3.51 (2H,m), 3.75-3.79 (1H,m), 4.48-4.57 (2H,m), 5.01 (1H,s), 7.60-7.90 (1H,m), 8.34 (1H,d,J=2.0Hz), 8.78 (1H,d,J=2.3Hz), 11.01 (1H,d,J=8.2Hz) ppm.

EXAMPLE 10

3-[N^{α}-(Acetoxymethoxycarbonyl)-N^{ω}-(3,4-dichlorobenzyl)-L-glutaminyl]thiazolidine



10A. 3-[*N*-(*tert*-Butyloxycarbonyl)-O^{ω}-methyl-L-glutamyl]thiazolidine

N-(*tert*-Butyloxycarbonyl)-O^{ω}-methyl-L-glutamic acid (6.28g, 24mmol) was dissolved in CH_2Cl_2/DMF (9:1, 100ml). To this solution at 0°C were added 1-hydroxybenzotriazole hydrate (5.5g, 36mmol), water-soluble carbodiimide (5.38g, 28mmol), thiazolidine (2.48g, 28mmol) and triethylamine (3.0g, 30mmol). The mixture was stirred for 18h at

0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (150ml). The solution was washed with 0.3M $KHSO_4$ (2 x 30ml), sat. $NaHCO_3$ (2 x 30ml), water (2 x 30ml) and brine (1 x 30ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 70% ethyl acetate, 30% pet. ether 60-80) to give a brown oil identified as 3-[*N*-(*tert*-butyloxycarbonyl)-O^{ω}-methyl-L-glutamyl]thiazolidine (4.0g, 12mmol, 50%).

10B. 3-[*N*-(*tert*-Butyloxycarbonyl)-L-glutamyl]thiazolidine

3-[*N*-(*tert*-Butyloxycarbonyl)-O^{ω}-methyl-L-glutamyl]thiazolidine (3.23g, 9.73mmol) was dissolved in THF (50ml). 1M Lithium hydroxide (11ml, 11mmol) was added. The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 1M $KHSO_4$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a colourless oil identified as 3-[*N*-(*tert*-butyloxycarbonyl)-L-glutamyl]thiazolidine (3.0g, 9.4mmol, 97%).

10C. 3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(3,4-dichlorobenzyl)-L-glutaminyl]-thiazolidine

3-[*N*-(*tert*-Butyloxycarbonyl)-L-glutamyl]thiazolidine (200mg, 0.63mmol) was dissolved in CH_2Cl_2/DMF (9:1, 10ml). To this solution at 0°C was added 1-hydroxybenzotriazole hydrate (119mg, 0.76mmol), water-soluble carbodiimide (163mg, 0.88mmol), 3,4-dichlorobenzylamine (111mg, 0.83mmol) and triethylamine(126mg, 1.26mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$ (2 x 20ml), sat. $NaHCO_3$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo* to give a yellow oil. The residue was purified by flash chromatography on silica gel (eluant: ethyl acetate) to give a colourless oil identified as 3-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(3,4-dichlorobenzyl)-L-glutaminyl]thiazolidine (295mg, 0.62mmol, 98%).

10D. 3-[N^{α}-(Acetoxymethoxycarbonyl)-N^{ω}-(3,4-dichlorobenzyl)-L-glutaminyl]-thiazolidine

3-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(3,4-dichlorobenzyl)-L-glutaminyl]thiazolidine (150mg, 0.32mmol) was dissolved in 4M HCl/dioxan (20ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo*. The residue was

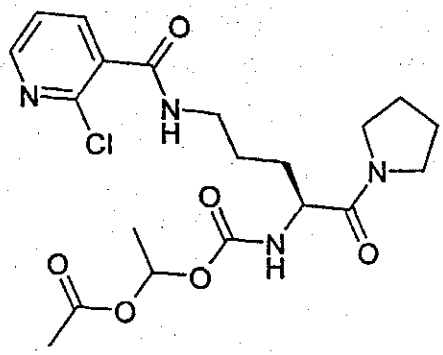
dissolved in dichloromethane (25ml) and acetoxymethyl *p*-nitrophenyl carbonate (95mg, 0.35mmol; prepared according to Alexander *et al.*, J. Med. Chem. 31, 318, 1988) and triethylamine (64mg, 0.64mmol) were added. The solution was stirred at room temperature for 18 hours then evaporated *in vacuo*. The residue taken up in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (eluant: ethyl acetate) to give a white solid identified as 3-[N^{α}-(acetoxymethoxycarbonyl)-N^{ω}-(3,4-dichlorobenzyl)-L-glutaminyl]thiazolidine (88mg, 0.18mmol, 56%).

$[M+H]^+$ = 492.0

^1H NMR ($CDCl_3$): δ 1.44-1.46 (3H,m), 1.63-1.99 (3H,br m), 1.99-2.04 (4H,m), 2.98-3.06 (2H,m), 3.46-3.48 (2H,m), 3.50-3.80 (2H m), 4.47-4.56 (3H,m), 5.81-5.91 (1H,m), 6.74-6.75 (1H,m), 7.24-7.33 (1H,m), 8.24-8.25 (1H,m), 8.69-8.71 (1H,m) ppm.

EXAMPLE 11

1-[N^{α}-(1'-Acetoxyethoxycarbonyl)-N^{ω}-(2-chloronicotinoyl)-L-ornithinyl]pyrrolidine



11A. 1-[N^{ω}-(Benzyloxycarbonyl)-N^{α}-(*tert*-butyloxycarbonyl)-L-ornithinyl]pyrrolidine

N^{ω}-(Benzyloxycarbonyl)-N^{α}-(*tert*-butyloxycarbonyl)-L-ornithine (5.49g, 15mmol) was dissolved in CH_2Cl_2/DMF (9:1, 100ml). To this solution at 0°C were added 1-hydroxybenzotriazole hydrate (3.37g, 22mmol), water-soluble carbodiimide (3.46g, 18mmol), pyrrolidine (1.28g, 18mmol) and triethylamine (200mg, 20mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (200ml). The solution was washed with 0.3M $KHSO_4$ (2 x 50ml), sat. $NaHCO_3$ (2 x 50ml), water (2 x 50ml) and

brine (1 x 50ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 90% ethyl acetate, 10% pet. ether 60-80) to give a colourless oil identified as 1-[N^{ω}-(benzyloxycarbonyl)-N^{α}-(*tert*-butyloxycarbonyl)-L-ornithinyl]pyrrolidine (5.15g, 12.3mmol, 82%).

11B. 1-[N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithinyl]pyrrolidine

To a solution of 1-[N^{ω}-(benzyloxycarbonyl)-N^{α}-(*tert*-butyloxycarbonyl)-L-ornithinyl]-pyrrolidine (2.15g, 5.13mmol) in methanol (80ml) was added 10% Pd/C (400mg). The mixture was stirred under a hydrogen atmosphere for 2 hours then the catalyst was filtered off and washed with methanol (50ml). The combined filtrates were evaporated *in vacuo* to give an off white solid identified as 1-[N^{α}-(*tert*-butyloxycarbonyl)-L-ornithinyl]pyrrolidine (1.35g, 4.74mmol, 94%).

11C. 1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(2-chloronicotinoyl)-L-ornithinyl]pyrrolidine

1-[N^{α}-(*tert*-Butyloxycarbonyl)-L-ornithinyl]pyrrolidine (204mg, 0.72mmol) was dissolved in CH_2Cl_2 (20ml). To this solution was added 2-chloronicotinoyl chloride (130mg, 0.74mmol) and triethylamine (200mg, 2.0mmol). The mixture was stirred for 18h at 0°C to room temperature then the solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (70ml). The solution was washed with 0.3M $KHSO_4$ (2 x 20ml), sat. $NaHCO_3$ (2 x 20ml), water (2 x 20ml) and brine (1 x 20ml), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluant: 10% methanol, 90% chloroform) to give a sticky white solid identified as 1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-(2-chloronicotinoyl)-L-ornithinyl]pyrrolidine (236mg, 0.56mmol, 78%).

11D. 1-[N^{α}-(1'-Acetoxyethoxycarbonyl)-N^{ω}-(2-chloronicotinoyl)-L-ornithinyl]-pyrrolidine

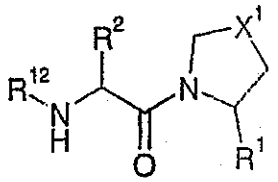
1-[N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-(2-chloronicotinoyl)-L-ornithinyl]pyrrolidine (206mg, 0.49mmol) was dissolved in 4M HCl/dioxan (20ml). The mixture was stirred for 1 hour at room temperature then the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (25ml) and α-acetoxyethyl *p*-nitrophenyl carbonate (140mg, 0.52 mmol; prepared according to Alexander *et al.*, J. Med. Chem. 31, 318, 1988) and triethylamine (40mg, 0.4mmol) were added. The solution was stirred at room temperature for 18 hours then evaporated *in vacuo*. The residue was taken up

in ethyl acetate (70ml). The solution was washed with sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel (eluant: 92% chloroform, 8% methanol) to give a white solid identified as 1-[N^{α}-(1'-acetoxyethoxycarbonyl)-N^{ω}-(2-chloronicotinoyl)-L-ornithinyl]pyrrolidine (127mg, 0.28mmol, 57%).

$[M+H]^+$ = 455.1

^1H NMR ($CDCl_3$): δ 1.42-1.49 (3H,m), 1.83-1.95 (8H,m), 2.02 (3H,d,J=1.5Hz), 3.32-3.71 (6H,m), 4.45-4.47 (1H,m), 5.75-5.85 (1H,m), 6.72-6.77 (2H.m), 7.27-7.33 (1H,m), 7.97-8.06 (1H,m), 8.40-8.43 (1H,m) ppm.

1 A compound according to general formula 1



1

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is H or CN,

R^2 is selected from CH_2R^5, $CH_2CH_2R^5$ and $C(R^3)(R^4)$-X^2-$(CH_2)_aR^5$,

R^3 and R^4 are each independently selected from H and Me;

R^5 is selected from $CON(R^6)(R^7)$, $N(R^8)C(=O)R^9$, $N(R^8)C(=S)R^9$, $N(R^8)SO_2R^{10}$ and $N(R^8)R^{10}$;

R^6 and R^7 are each independently $R^{11}(CH_2)_b$ or together they are $-(CH_2)_2$-Z-$(CH_2)_2$- or $-CH_2$-o-C_6H_4-Z-CH_2-;

R^8 is H or Me;

R^9 is selected from $R^{11}(CH_2)_b$, $R^{11}(CH_2)_bO$ and $N(R^6)(R^7)$;

R^{10} is $R^{11}(CH_2)_b$;

R^{11} is selected from H, alkyl, optionally substituted aryl, optionally substituted aroyl, optionally substituted arylsulphonyl and optionally substituted heteroaryl;

R^{12} is selected from $H_2NCH(R^{13})CO$, $H_2NCH(R^{14})CONHCH(R^{15})CO$, $C(R^{16})=C(R^{17})COR^{18}$ and $R^{19}OCO$;

R^{13}, R^{14} and R^{15} are selected from the side chains of the proteinaceous amino acids;

R^{16} is selected from H, lower alkyl ($C_1 - C_6$) and phenyl;

R^{17} is selected from H and lower alkyl ($C_1 - C_6$);

R^{18} is selected from H, lower alkyl ($C_1 - C_6$), OH, O-(lower alkyl ($C_1 - C_6$)) and phenyl;

R^{19} is selected from lower alkyl ($C_1 - C_6$), optionally substituted phenyl and $R^{20}C(=O)OC(R^{21})(R^{22})$;

R^{20}, R^{21} and R^{22} are each independently selected from H and lower alkyl ($C_1 - C_6$);

Z is selected from a covalent bond, $-(CH_2)_c-$, -O-, $-SO_d-$ and $-N(R^{10})-$;
X^1 is S or CH_2,
X^2 is O, S or CH_2;
a is 1, 2 or 3
b is 0 – 3;
c is 1 or 2; and
d is 0 – 2;
with the proviso that if R^1 is CN and X^1 is CH_2, R^{12} is selected from $C(R^{16})=C(R^{17})COR^{18}$ and $R^{19}OCO$ only wherein R^{18}, R^{17} and R^{18} are as defined above and R^{19} is lower alkyl ($C_1 - C_6$).

2 A compound according to Claim 1 wherein R^1 is H.

3 A compound according to Claim 1 wherein R^1 is CN.

4 A compound according to any of Claims 1 to 3 wherein R^2 is selected from $CH_2CH_2R^5$ and $C(R^3)(R^4)-X^2-(CH_2)_aR^5$.

5 A compound according to claim 2 or claim 3 wherein X^1 is CH_2.

6. A compound according to claim 1 or claim 2 wherein X^1 is S.

7 A compound according to Claim 4 wherein R^3 and R^4 are both H, X^2 is CH_2 and a is 1 or 2.

8 A compound according to Claim 7 wherein R^2 is selected from $CH_2CH_2CH_2R^5$ and $CH_2CH_2CH_2CH_2R^5$.

9 A compound according to any of Claims 1 to 8 wherein R^5 is $CON(R^6)(R^7)$.

10 A compound according to any of Claims 1 to 4 and 6 to 8 wherein R^5 is selected from $N(R^8)C(=O)R^9$, $N(R^8)C(=S)R^9$, $N(R^8)SO_2R^{10}$ and $N(R^8)R^{10}$.

11 A compound according to claim 5 wherein R^5 is selected from $CON(R^6)(R^7)$, $N(R^8)C(=O)R^9$, $N(R^8)C(=S)R^9$ and $N(R^8)R^{10}$.

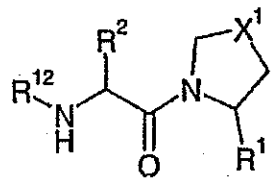
12 A compound according to any of Claims 1 to 11 wherein R^{12} is selected from $H_2NCH(R^{13})CO$ and $H_2NCH(R^{14})CONHCH(R^{15})CO$.

13 A compound according to any of Claims 1 to 11 wherein R^{12} is $R^{19}OCO$.

14 A pharmaceutical composition comprising a compound according to any of Claims 1 to 13.

15 The use for a compound according to any of Claims 1 to 13, which is as a component in the preparation of a pharmaceutical composition.

16 The use, in the manufacture of a pharmaceutical composition for the treatment of a disease or medical condition in a human or animal, of a compound according to general formula 1



1

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is H or CN,

R^2 is selected from CH_2R^5, $CH_2CH_2R^5$ and $C(R^3)(R^4)$-X^2-$(CH_2)_aR^5$,

R^3 and R^4 are each independently selected from H and Me;

R^5 is selected from $CON(R^6)(R^7)$, $N(R^8)C(=O)R^9$, $N(R^8)C(=S)R^9$, $N(R^8)SO_2R^{10}$ and $N(R^8)R^{10}$;

R^6 and R^7 are each independently $R^{11}(CH_2)_b$ or together they are $-(CH_2)_2$-Z-$(CH_2)_2$- or $-CH_2$-o-C_6H_4-Z-CH_2-;

R^8 is H or Me;

R^9 is selected from $R^{11}(CH_2)_b$, $R^{11}(CH_2)_bO$ and $N(R^8)(R^7)$;

R^{10} is $R^{11}(CH_2)_b$;

R^{11} is selected from H, alkyl, optionally substituted aryl, optionally substituted aroyl, optionally substituted arylsulphonyl and optionally substituted heteroaryl;

R^{12} is selected from $H_2NCH(R^{13})CO$, $H_2NCH(R^{14})CONHCH(R^{15})CO$, $C(R^{16})=C(R^{17})COR^{18}$ and $R^{19}OCO$;

R^{13}, R^{14} and R^{15} are selected from the side chains of the proteinaceous amino acids;

R^{16} is selected from H, lower alkyl ($C_1 - C_6$) and phenyl;

R^{17} is selected from H and lower alkyl ($C_1 - C_6$);

R^{18} is selected from H, lower alkyl ($C_1 - C_6$), OH, O-(lower alkyl ($C_1 - C_6$)) and phenyl;

R^{19} is selected from lower alkyl ($C_1 - C_6$), optionally substituted phenyl and $R^{20}C(=O)OC(R^{21})(R^{22})$;

R^{20}, R^{21} and R^{22} are each independently selected from H and lower alkyl (C_1 – C_6);

Z is selected from a covalent bond, -$(CH_2)_c$-, -O-, -SO_d- and –$N(R^{10})$-;
X^1 is S or CH_2,
X^2 is O, S or CH_2;
a is 1, 2 or 3
b is 0 – 3;
c is 1 or 2; and
d is 0 – 2; with the proviso that if R^1 is CN and X^1 is CH_2, R^{12} is selected from $C(R^{16})=C(R^{17})COR^{18}$ and $R^{19}OCO$ only wherein R^{16}, R^{17} and R^{18} are as defined above and R^{19} is lower alkyl (C_1 – C_6).

17 The use according to claim 15 or 16 wherein the pharmaceutical composition is for the treatment of impaired glucose tolerance, type II diabetes or hyperglycaemia.

18 The use according to claim 15 or 16 wherein the pharmaceutical composition is for the treatment of a disease or medical condition which is due to a DP-IV mediated process.

19 The use of a compound according to any of Claims 1 to 13, or a prodrug thereof, in the manufacture of a medicament for the treatment of a disease or medical condition which is due to a DP-IV mediated process.

20 A compound or pharmaceutical composition according to any of Claims 1 to 14, or a use according to any of claims 15 to 19, with the proviso that R^{19} is not t-butyl.

21 A compound according to claim 1 substantially as herein described with reference to any one of the examples thereof.

22 A compound according to any one of claims 1 to 13 or claim 20 substantially as herein described.

23 A pharmaceutical composition according to claim 14 substantially as herein described with reference to any one of the examples thereof.

24 A pharmaceutical composition according to claim 14 or claim 20 substantially as herein described.

25 The use according to claim 15 or claim 16 substantially as herein described with reference to any one of the examples thereof.

26 The use according to any one of claims 15 to 20 substantially as herein described.