

5

INHIBITORS OF CASPASESTECHNICAL FIELD OF THE INVENTION

The present invention relates to novel classes of compounds which are caspase inhibitors, in particular interleukin-1 β converting enzyme ("ICE") inhibitors. This invention also relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well suited for inhibiting caspase activity and consequently, may be advantageously used as agents against interleukin-1- ("IL-1"), apoptosis-, interferon- γ inducing factor- (IGIF), or interferon- γ - ("IFN- γ ") mediated diseases, including inflammatory diseases, autoimmune diseases, destructive bone disorders, proliferative disorders, infectious diseases, and degenerative diseases. This invention also relates to methods for inhibiting caspase activity and decreasing IGIF production and IFN- γ production and methods for treating interleukin-1, apoptosis-, and interferon- γ - mediated diseases using the compounds and compositions of this invention. This invention also relates to methods of preparing the compounds of this invention.

30

BACKGROUND OF THE INVENTION

Interleukin 1 ("IL-1") is a major pro-inflammatory and immunoregulatory protein that stimulates fibroblast differentiation and proliferation, the production of prostaglandins,

- 2 -

collagenase and phospholipase by synovial cells and chondrocytes, basophil and eosinophil degranulation and neutrophil activation. Oppenheim, J.H. et al, Immunology Today, 7, pp. 45-56 (1986). As such, it is involved in the pathogenesis of chronic and acute inflammatory and autoimmune diseases. For example, in rheumatoid arthritis, IL-1 is both a mediator of inflammatory symptoms and of the destruction of the cartilage proteoglycan in afflicted joints. Wood, D.D. et al., Arthritis Rheum. 26, 975, (1983); Pettipher, E.J. et al., Proc. Natl. Acad. Sci. USA 71, 295 (1986); Arend, W.P. and Dayer, J.M., Arthritis Rheum. 38, 151 (1995). IL-1 is also a highly potent bone resorption agent. Jandiski, J.J., J. Oral Path 17, 145 (1988); Dewhirst, F.E. et al., J. Immunol. 8, 2562 (1985). It is alternately referred to as "osteoclast activating factor" in destructive bone diseases such as osteoarthritis and multiple myeloma. Bataille, R. et al., Int. J. Clin. Lab. Res. 21(4), 283 (1992). In certain proliferative disorders, such as acute myelogenous leukemia and multiple myeloma, IL-1 can promote tumor cell growth and adhesion. Bani, M.R., J. Natl. Cancer Inst. 83, 123 (1991); Vidal-Vanaclocha, F., Cancer Res. 54, 2667 (1994). In these disorders, IL-1 also stimulates production of other cytokines such as IL-6, which can modulate tumor development (Tartour et al., Cancer Res. 54, p. 6243 (1994). IL-1 is predominantly produced by peripheral blood monocytes as part of the inflammatory response and exists in two distinct agonist forms, IL-1 α and IL-1 β . Mosely, B.S. et al., Proc. Nat. Acad. Sci., 84, pp. 4572-4576 (1987); Lonnemann, G. et al., Eur. J. Immunol., 19, pp. 1531-1536 (1989).

IL-1 β is synthesized as a biologically inactive precursor, pIL-1 β . pIL-1 β lacks a

- 3 -

conventional leader sequence and is not processed by a signal peptidase. March, C.J., Nature, 315, pp.641-647 (1985). Instead, pIL-1 β is cleaved by interleukin-1 β converting enzyme ("ICE") between Asp-116 and Ala-117 to produce the biologically active C-terminal fragment found in human serum and synovial fluid. Sleath, P.R., et al., J. Biol. Chem., 265, pp.14526-14528 (1992); A.D. Howard et al., J. Immunol., 147, pp.2964-2969 (1991). ICE is a cysteine protease localized primarily in monocytes. It converts precursor IL-1 β to the mature form. Black, R.A. et al., FEBS Lett., 247, pp.-386-390 (1989); Kostura, M.J. et al., Proc. Natl. Acad. Sci. U.S.A., 86, pp.5227-5231 (1989). Processing by ICE is also necessary for the transport of mature IL-1 β through the cell membrane.

ICE (or caspase-1) is a member of a family of homologous enzymes called caspases. These homologs have sequence similarities in the active site regions of the enzymes. Such homologs (caspases) include TX (or ICE_{rel-II} or ICH-2) (caspase-4) (Faucheu, et al., EMBO J., 14, p. 1914 (1995); Kamens J., et al., J. Biol. Chem., 270, p. 15250 (1995); Nicholson et al., J. Biol. Chem., 270 15870 (1995)), TY (or ICE_{rel-III}) (caspase-5) (Nicholson et al., J. Biol. Chem., 270, p. 15870 (1995); ICH-1 (or Nedd-2) (caspase-2) (Wang, L. et al., Cell, 78, p. 739 (1994)), MCH-2 (caspase-6), (Fernandes-Alnemri, T. et al., Cancer Res., 55, p. 2737 (1995), CPP32 (or YAMA or apopain) (caspase-3) (Fernandes-Alnemri, T. et al., J. Biol. Chem., 269, p. 30761 (1994); Nicholson, D.W. et al., Nature, 376, p. 37 (1995)), CMH-1 (or MCH-3) (caspase-7) (Lippke, et al., J. Biol. Chem., 271(4), p1825-1828 (1996)); Fernandes-Alnemri, T. et al., Cancer Res., (1995)), Mch5 (caspase-8) (Muzio, M. et.al., Cell 85(6), 817-827, (1996)), MCH-6 (caspase-9) (Duan, H. et.al., J.

- 4 -

Biol. Chem., 271(34), p. 16720-16724 (1996)), Mch4 (caspase-10) (Vincenz, C. et.al., J. Biol. Chem., 272, p. 6578-6583 (1997); Fernandes-Alnemri, T. et.al., Proc. Natl. Acad. Sci. 93, p. 7464-7469 (1996)), Ich-3 (caspase-11) (Wang, S. et.al., J. Biol. Chem., 271, p. 20580-20587 (1996)), mCASP-12 (caspase-12), (Van de Craen, M. et.al., FEBS Lett. 403, p. 61-69 (1997); Yuan, Y. and Miura, M. PCT Publication WO95/00160 (1995)), ERICE (caspase-13), (Humke, E.W., et.al., J. Biol. Chem., 273(25) p. 15702-15707 (1998)), and MICE (caspase-14) (Hu, S. et.al., J. Biol. Chem., 273(45) p. 29648-29653 (1998)).

Each of these ICE homologs, as well as ICE itself, is capable of inducing apoptosis when overexpressed in transfected cell lines. Inhibition of one or more of these homologs with the peptidyl ICE inhibitor Tyr-Val-Ala-Asp-chloromethylketone results in inhibition of apoptosis in primary cells or cell lines. Lazebnik et al., Nature, 371, p. 346. (1994).

Caspases also appear to be involved in the regulation of programmed cell death or apoptosis. Yuan, J. et al., Cell, 75, pp.641-652 (1993); Miura, M. et al., Cell, 75, pp. 653-660 (1993); Nett-Fiordalisi, M.A. et al., J. Cell Biochem., 17B, p. 117 (1993). In particular, ICE or ICE homologs are thought to be associated with the regulation of apoptosis in neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. Marx, J. and M. Baringa, Science, 259, pp. 760-762 (1993); Gagliardini, V. et al., Science, 263, pp. 826-828 (1994). Therapeutic applications for inhibition of apoptosis may include treatment of Alzheimer's disease, Parkinson's disease, stroke, myocardial infarction, spinal atrophy, and aging.

- 5 -

ICE has been demonstrated to mediate apoptosis (programmed cell death) in certain tissue types. Steller, H., Science, 267, p. 1445 (1995); Whyte, M. and Evan, G., Nature, 376, p. 17 (1995);
5 Martin, S.J. and Green, D.R., Cell, 82, p. 349 (1995); Alnemri, E.S., et al., J. Biol. Chem., 270, p. 4312 (1995); Yuan, J. Curr. Opin. Cell Biol., 7, p. 211 (1995). A transgenic mouse with a disruption of the ICE gene is deficient in Fas-mediated apoptosis (Kuida,
10 K. et al., Science 267, 2000 (1995)). This activity of ICE is distinct from its role as the processing enzyme for pro-IL-1 β . It is conceivable that in certain tissue types, inhibition of ICE may not affect secretion of mature IL-1 β , but may inhibit apoptosis.

15 Enzymatically active ICE has been previously described as a heterodimer composed of two subunits, p20 and p10 (20kDa and 10kDa molecular weight, respectively). These subunits are derived from a 45kDa proenzyme (p45) by way of a p30 form, through an
20 activation mechanism that is autocatalytic. Thornberry, N.A. et al., Nature, 356, pp.768-774 (1992). The ICE proenzyme has been divided into several functional domains: a prodomain (p14), a p22/20 subunit, a polypeptide linker and a p10 subunit.
25 Thornberry et al., supra; Casano et al., Genomics, 20, pp. 474-481 (1994).

Full length p45 has been characterized by its cDNA and amino acid sequences. PCT patent applications WO 91/15577 and WO 94/00154. The p20 and p10 cDNA and
30 amino acid sequences are also known. Thornberry et al., supra. Murine and rat ICE have also been sequenced and cloned. They have high amino acid and nucleic acid sequence homology to human ICE. Miller, D.K. et al., Ann. N.Y. Acad. Sci., 696, pp. 133-148 (1993);
35 Molineaux, S.M. et al., Proc. Nat. Acad. Sci., 90, pp.

- 6 -

1809-1813 (1993). The three-dimensional structure of ICE has been determined at atomic resolution by X-ray crystallography. Wilson, K.P., et al., Nature, 370, pp. 270-275 (1994). The active enzyme exists as a tetramer of two p20 and two p10 subunits.

Recently, ICE and other members of the ICE/CED-3 family have been linked to the conversion of pro-IGIF to IGIF or to the production of IFN- γ *in vivo* (PCT application PCT/US96/20843, publication no. WO 97/22619, which is incorporated herein by reference). IGIF is synthesized *in vivo* as the precursor protein "pro-IGIF".

Interferon-gamma inducing factor (IGIF) is an approximately 18-kDa polypeptide that stimulates T-cell production of interferon-gamma (IFN- γ). IGIF is produced by activated Kupffer cells and macrophages *in vivo* and is exported out of such cells upon endotoxin stimulation. Thus, a compound that decreases IGIF production would be useful as an inhibitor of such T-cell stimulation which in turn would reduce the levels of IFN- γ production by those cells.

IFN- γ is a cytokine with immunomodulatory effects on a variety of immune cells. In particular, IFN- γ is involved in macrophage activation and Th1 cell selection (F. Belardelli, APMIS, 103, p. 161 (1995)). IFN- γ exerts its effects in part by modulating the expression of genes through the STAT and IRF pathways (C. Schindler and J.E. Darnell, Ann. Rev. Biochem., 64, p. 621 (1995); T. Taniguchi, J. Cancer Res. Clin. Oncol., 121, p. 516 (1995)).

Mice lacking IFN- γ or its receptor have multiple defects in immune cell function and are resistant to endotoxic shock (S. Huang et al., Science, 259, p.1742 (1993); D. Dalton et al., Science, 259, p. 1739 (1993); B.D. Car et al., J. Exp. Med., 179, p.1437

- 7 -

(1994)). Along with IL-12, IGIF appears to be a potent inducer of IFN- γ production by T cells (H. Okamura et al., Infection and Immunity, 63, p.3966 (1995); H. Okamura et al., Nature, 378, p.88 (1995); S. Ushio et al., J.Immunol., 156, p.4274 (1996)).

IFN- γ has been shown to contribute to the pathology associated with a variety of inflammatory, infectious and autoimmune disorders and diseases. Thus, compounds capable of decreasing IFN- γ production would be useful to ameliorate the effects of IFN- γ related pathologies.

Accordingly, compositions and methods capable of regulating the conversion of pro-IGIF to IGIF would be useful for decreasing IGIF and IFN- γ production in vivo, and thus for ameliorating the detrimental effects of these proteins which contribute to human disorders and diseases.

Caspase inhibitors represent a class of compounds useful for the control of inflammation or apoptosis or both. Peptide and peptidyl inhibitors of ICE have been described (PCT patent applications WO 91/15577, WO 93/05071, WO 93/09135, WO 93/12076, WO 93/14777, WO 93/16710, WO 95/35308, WO 96/30395, WO 96/33209 and WO 98/01133; European patent applications 503 561, 547 699, 618 223, 623 592, and 623 606; and US patent nos. 5,434,248, 5,710,153, 5,716,929, and 5,744,451). Such peptidyl inhibitors of ICE have been observed to block the production of mature IL-1 β in a mouse model of inflammation (vide infra) and to suppress growth of leukemia cells in vitro (Estrov et al., Blood, 84, 380a (1994)). However, due to their peptidic nature, such inhibitors are typically characterized by undesirable pharmacologic properties, such as poor cellular penetration and cellular activity, poor oral

- 8 -

absorption, instability and rapid metabolism.

Plattner, J.J. and D.W. Norbeck, in Drug Discovery Technologies, C.R. Clark and W.H. Moos, Eds. (Ellis Horwood, Chichester, England, 1990), pp.92-126. These
5 properties has hampered their development into effective drugs.

Non-peptidyl compounds have also been reported to inhibit ICE *in vitro*. PCT patent application WO 95/26958; US Patent 5,552,400; Dolle et
10 al., J. Med. Chem., 39, pp. 2438-2440 (1996).

It is not clear however whether these compounds have the appropriate pharmacological profiles to be therapeutically useful.

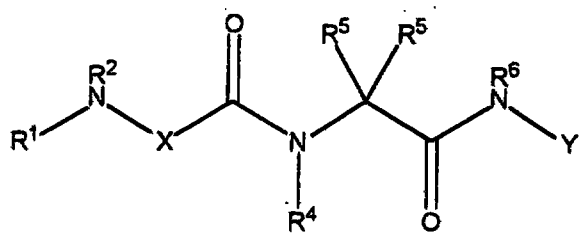
Accordingly, the need exists for compounds
15 that can effectively inhibit caspases, and that have favorable *in vivo* activity, for use as agents for preventing and treating chronic and acute forms of IL-1-, apoptosis-, IGIF-, or IFN- γ -mediated diseases, as well as inflammatory, autoimmune, destructive bone,
20 proliferative, infectious, or degenerative diseases.

SUMMARY OF THE INVENTION

The present invention provides novel classes of compounds, and pharmaceutically acceptable
25 derivatives thereof, that are useful as caspase inhibitors, in particular, as ICE inhibitors. These compounds can be used alone or in combination with other therapeutic or prophylactic agents, such as antibiotics, immunomodulators or other anti-
30 inflammatory agents, for the treatment or prophylaxis of diseases mediated by IL-1, apoptosis, IGIF, or IFN- γ . According to a preferred embodiment, the compounds of this invention are capable of binding to the active site of a caspase and inhibiting the activity of that
35 enzyme.

- 9 -

It is a principal object of this invention to provide novel classes of compounds represented by formula I, which have favorable in vivo profiles:



5

wherein the various substituents are described herein.

It is a further objective of this invention to provide pharmaceutical compositions, including multi-component compositions. This invention also provides methods for using and preparing the compounds of this invention and related compounds.

10

DETAILED DESCRIPTION OF THE INVENTION

15

In order that the invention described herein may be more fully understood, the following detailed description is set forth.

The following abbreviations and definitions are used throughout the application.

20

Abbreviations

Ac ₂ O	acetic anhydride
MeCN	acetonitrile
AMC	aminomethyl coumarin
n-Bu	normal-butyl
DMF	dimethylformamide
DIEA	<i>N,N</i> -diisopropylethylamine
DMA	<i>N,N</i> -dimethylacetamide
EDC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
Et ₂ O	diethyl ether

- 10 -

EtOAc	ethyl acetate
Fmoc	9-fluorenylmethoxycarbonyl
HBTU	O-benzotriazol-1-yl-N,N,N,N'- tetramethyluronium hexafluorophosphate
HOBT	1-hydroxybenzotriazole hydrate
MeOH	methanol
NMP	N-methylpyrrolidinone
TFA	trifluoroacetic acid
pNA	p-nitroaniline

The term "caspase" refers to an enzyme that is a member of the family of enzymes that includes ICE (see H. Hara, Natl. Acad. Sci., 94, pp. 2007-2012 (1997)).

The terms "HBV", "HCV" and "HGV" refer to hepatitis-B virus, hepatitis-C virus and hepatitis-G virus, respectively.

The term " K_i " refers to a numerical measure of the effectiveness of a compound in inhibiting the activity of a target enzyme such as ICE. Lower values of K_i reflect higher effectiveness. The K_i value is derived by fitting experimentally determined rate data to standard enzyme kinetic equations (see I.H. Segel, Enzyme Kinetics, Wiley-Interscience, 1975).

The term "interferon gamma inducing factor" or "IGIF" refers to a factor which is capable of stimulating the endogenous production of IFN- γ .

The term "caspase inhibitor" refer to a compound which is capable of demonstrating detectable inhibition of one or more caspases. The term "ICE inhibitor" refers to a compound which is capable of demonstrating detectable inhibition of ICE and optionally one or more additional caspases. Inhibition of these enzymes may be determined using the methods described and incorporated by reference herein.

The skilled practitioner realizes that an *in vivo* enzyme inhibitor is not necessarily an *in vitro*

- 11 -

enzyme inhibitor. For example, a prodrug form of a compound typically demonstrates little or no activity in *in vitro* assays. Such prodrug forms may be altered by metabolic or other biochemical processes in the patient to provide an *in vivo* ICE inhibitor.

The term "cytokine" refers to a molecule which mediates interactions between cells.

The term "condition" refers to any disease, disorder or effect that produces deleterious biological consequences in a subject.

The term "subject" refers to an animal, or to one or more cells derived from an animal. Preferably, the animal is a mammal, most preferably a human. Cells may be in any form, including but not limited to cells retained in tissue, cell clusters, immortalized cells, transfected or transformed cells, and cells derived from an animal that have been physically or phenotypically altered.

The term "patient" as used in this application refers to any mammal, preferably humans.

The term "alkyl" refers to a straight-chained or branched, saturated aliphatic hydrocarbon containing 1 to 6 carbon atoms.

The term "alkenyl" refers to a straight-chained or branched unsaturated hydrocarbon containing 2 to 6 carbon atoms and at least one double bond.

The term "alkynyl" refers to a straight-chained or branched unsaturated hydrocarbon containing 2 to 6 carbon atoms and at least one triple bond.

The term "cycloalkyl" refers to a mono- or polycyclic, non-aromatic, hydrocarbon ring system which may optionally contain unsaturated bonds in the ring system. Examples include cyclohexyl, adamantyl, norbornyl, and spirocyclopentyl.

- 12 -

The term "aryl" refers to a mono- or polycyclic ring system which contains 6, 10, 12 or 14 carbons in which at least one ring of the ring system is aromatic. The aryl groups of this invention are optionally singly or multiply substituted with R¹¹.
5 Examples of aryl ring systems include, phenyl, naphthyl, and tetrahydronaphthyl.

The term "heteroaryl" refers to a mono- or polycyclic ring system which contains 1 to 15 carbon atoms and 1 to 4 heteroatoms, and in which at least one ring of the ring system is aromatic. Heteroatoms are sulfur, nitrogen or oxygen. The heteroaryl groups of this invention are optionally singly or multiply substituted with R¹¹.
10

The term "heterocyclic" refers to a mono- or polycyclic ring system which contains 1 to 15 carbon atoms and 1 to 4 heteroatoms, in which the mono- or polycyclic ring system may optionally contain unsaturated bonds but is not aromatic. Heteroatoms are independently sulfur, nitrogen, or oxygen.
15
20

The term "alkylaryl" refers to an alkyl group, wherein a hydrogen atom of the alkyl group is replaced by an aryl radical.

The term "alkylheteroaryl" refers to an alkyl group, wherein a hydrogen atom of the alkyl group is replaced by a heteroaryl radical.
25

The term "amino acid side chain" refers to any group attached to the α carbon of a naturally or non-naturally occurring amino acid.

The term "substitute" refers to the replacement of a hydrogen atom in a compound with a substituent group.
30

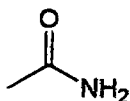
The term "straight chain" refers to a contiguous unbranching string of covalently bound atoms. The straight chain may be substituted, but
35

- 13 -

these substituents are not a part of the straight chain.

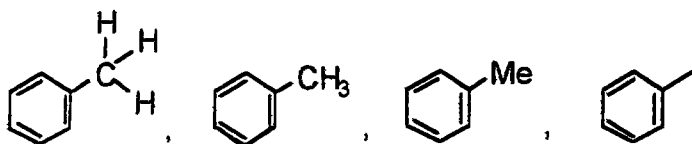
In chemical formulas, parenthesis are used herein to denote connectivity in molecules or groups.

5 In particular, parentheses are used to indicate: 1) that more than one atom or group is bonded to a particular atom; or 2) a branching point (i.e., the atom immediately before the open parenthesis is bonded both to the atom or group in the parentheses and the
10 atom or group immediately after the closed parenthesis). An example of the first use is "-N(alkyl)₂", indicating two alkyl groups bond to an N atom. An example of the second use is "-C(O)NH₂", indicating a carbonyl group and an amino ("NH₂") group
15 both bonded to the indicated carbon atom. A "-C(O)NH₂" group may be represented in other ways, including the following structure:



20

Substituents may be represented in various forms. These various forms are known to the skilled practitioner and may be used interchangeably. For example, a methyl substituent on a phenyl ring may be
25 represented in any of the following forms:



Various forms of substituents such as methyl are used herein interchangeably.

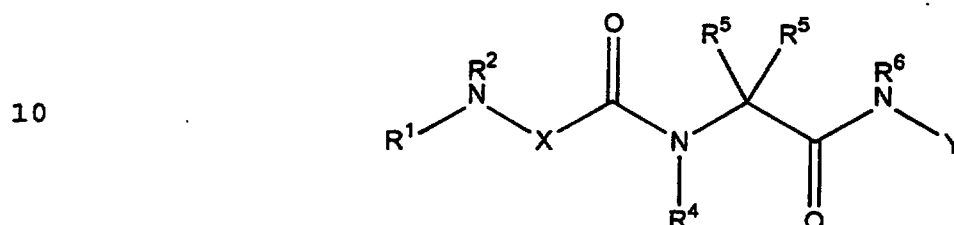
30

Other definitions are set forth in the specification where necessary.

-14-

Compounds of this Invention

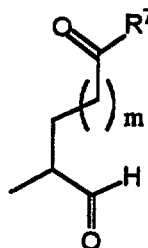
The compounds of one embodiment A of this invention are those of formula I:



wherein:

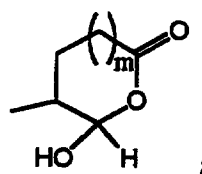
Y is:

(a)



provided that when R⁷ is -OH then Y can also be:

(b)



X is -C(R³)₂- or -N(R³)-;

m is 0 or 1;

30 R¹ is H, -C(O)R⁸, -C(O)C(O)R⁸, -S(O)₂R⁸,
 -S(O)R⁸, -C(O)OR⁸, -C(O)N(H)R⁸, -S(O)₂N(H)-R⁸,
 -S(O)N(H)-R⁸, -C(O)C(O)N(H)R⁸, -C(O)CH=CHR⁸,
 -C(O)CH₂OR⁸, -C(O)CH₂N(H)R⁸, -C(O)N(R⁸)₂, -S(O)₂N(R⁸)₂,

- 15 -

-S(O)N(R⁸)₂, -C(O)C(O)N(R⁸)₂, -C(O)CH₂N(R⁸)₂, -CH₂R⁸,
-CH₂-alkenyl-R⁸, or -CH₂-alkynyl-R⁸;

5 R² is -H and each R³ is independently -H, an
amino acid side chain, -R⁸, alkenyl-R⁹, or alkynyl-R⁹,
or R² and one R³ together with the atoms to which they
are bound, form a 3 to 7 membered cyclic or
heterocyclic ring system, wherein a hydrogen atom bound
10 to any -alkyl or -cycloalkyl carbon atom is optionally
replaced by -R¹⁰, a hydrogen atom bound to any -aryl or
-heteroaryl carbon atom is optionally replaced by -R¹¹,
a hydrogen atom bound to any nitrogen atom of the ring
system is optionally replaced by -R¹;

15 R⁴ is -H and each R⁵ is independently -H, an
amino acid side chain, -R⁸, -alkenyl-R⁹, or -alkynyl-
R⁹, or R⁴ and one R⁵ together with the atoms to which
they are bound form a 3 to 7 membered cyclic or
heterocyclic ring system, wherein a hydrogen atom bound
20 to any -alkyl or -cycloalkyl carbon atom is optionally
replaced by R¹⁰, a hydrogen atom bound to any -aryl or
-heteroaryl carbon atom is optionally replaced by R¹¹,
and a hydrogen atom bound to any nitrogen atom of the
ring system is optionally replaced with R¹;

25

R⁶ is -H;

R⁷ is -OH, -OR⁸, or -N(H)OH;

30 each R⁸ is independently -alkyl, -cycloalkyl,
-aryl, -heteroaryl, -heterocyclyl, -alkylcycloalkyl
-alkylaryl, -alkylheteroaryl, or -alkylheterocyclyl,

- 16 -

wherein a hydrogen atom bound to any -alkyl or
-cycloalkyl carbon atom is optionally replaced by R^{10} ,
a hydrogen atom bound to any -aryl or -heteroaryl
carbon atom is optionally replaced by R^{11} , and a
5 hydrogen atom bound to any nitrogen atom is optionally
replaced by R^1 ;

each R^9 is independently -aryl, -heteroaryl,
cycloalkyl, or -heterocyclyl, wherein a hydrogen atom
10 bound to any -alkyl or -cycloalkyl carbon atom is
optionally replaced by R^{10} , a hydrogen atom bound to
any -aryl or -heteroaryl carbon atom is optionally
replaced by R^{11} , and a hydrogen atom bound to any
nitrogen atom is optionally replaced by R^1 ;

15 each R^{10} is independently -OH, -SH, -F, -Cl,
-Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H,
-N(H)C(O)NH₂, -perfluoroalkyl, -O-alkyl, -O-aryl,
-O-alkylaryl, -N(H)alkyl, -N(H)aryl, -N(H)-alkylaryl,
20 -N(alkyl)₂, -C(O)N(H)alkyl, -C(O)N(alkyl)₂,
-N(H)C(O)alkyl, -N(H)C(O)N(H)alkyl, -N(H)C(O)N(alkyl)₂,
-S-alkyl, -S-aryl, -S-alkylaryl, -S(O)₂alkyl,
-S(O)alkyl, -C(O)alkyl, -CH₂NH₂, -CH₂N(H)alkyl, or
-CH₂N(alkyl)₂, -alkyl, -cycloalkyl, -aryl, -heteroaryl,
25 -heterocyclyl, -alkylcycloalkyl -alkylaryl,
-alkylheteroaryl, or -alkylheterocyclyl, wherein a
hydrogen atom bound to any -aryl or -heteroaryl carbon
atom is optionally replaced by R^{11} and a hydrogen atom
bound to any nitrogen atom is optionally replaced by
30 R^1 ; and

each R^{11} is independently -OH, -SH, -F, -Cl,
-Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H,
-N(H)C(O)NH₂, -alkyl, -cycloalkyl, -perfluoroalkyl, -O-
35 alkyl, -O-aryl, -O-alkylaryl, -N(H)alkyl, -N(H)aryl,

- 17 -

-N(H)-alkylaryl, -N(alkyl)₂, -C(O)N(H)alkyl,
 -C(O)N(alkyl)₂, -N(H)C(O)alkyl, -N(H)C(O)N(H)alkyl,
 -N(H)C(O)N(alkyl)₂, -S-alkyl, -S-aryl, -S-alkylaryl,
 -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl, -CH₂NH₂,
 5 -CH₂N(H)alkyl, or -CH₂N(alkyl)₂.

In an alternative form of embodiment A:

R¹ is H, -R⁸, -C(O)R⁸, -C(O)C(O)R⁸, -S(O)₂R⁸,
 10 -S(O)R⁸, -C(O)OR⁸, -C(O)N(H)R⁸, -S(O)₂N(H)-R⁸,
 -S(O)N(H)-R⁸, -C(O)C(O)N(H)R⁸, -C(O)CH=CHR⁸,
 -C(O)CH₂OR⁸, -C(O)CH₂N(H)R⁸, -C(O)N(R⁸)₂, -S(O)₂N(R⁸)₂,
 -S(O)N(R⁸)₂, -C(O)C(O)N(R⁸)₂, -C(O)CH₂N(R⁸)₂, -CH₂R⁸,
 -CH₂-alkenyl-R⁸, or -CH₂-alkynyl-R⁸;

15 R² is -H and each R³ is independently -H, an
 amino acid side chain, -R⁸, alkenyl-R⁹, or alkynyl-R⁹,
 or each R³, together with the atom to which they are
 bound, form a 3 to 7 membered cyclic or heterocyclic
 20 cyclic ring system, or R² and one R³ together with the
 atoms to which they are bound, form a 3 to 7 membered
 cyclic or heterocyclic ring system, wherein a hydrogen
 atom bound to any -alkyl or -cycloalkyl carbon atom is
 optionally replaced by -R¹⁰, a hydrogen atom bound to
 25 any -aryl or -heteroaryl carbon atom is optionally
 replaced by -R¹¹, a hydrogen atom bound to any nitrogen
 atom of the ring system is optionally replaced by -R¹;

each R¹⁰ is independently -OH, -SH, -F, -Cl,
 30 -Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H,
 -N(H)C(O)NH₂, -perfluoroalkyl, -O-alkyl, -O-aryl,
 -O-alkylaryl, -N(H)alkyl, -N(H)aryl, -N(H)-alkylaryl,
 -N(alkyl)₂, -C(O)N(H)alkyl, -C(O)N(alkyl)₂,
 -N(H)C(O)alkyl, -N(H)C(O)Oalkyl, -N(H)C(O)Oaryl,

- 18 -

-N(H)C(O)Oalkylaryl, -N(H)C(O)Oheteroaryl,
-N(H)C(O)Oalkylheteroaryl, -N(H)C(O)Ocycloalkyl,
-N(H)C(O)N(H)alkyl, -N(H)C(O)N(alkyl)₂,
-N(H)C(O)N(H)aryl, -N(H)C(O)N(H)alkylaryl,
5 -N(H)C(O)N(H)heteroaryl, -N(H)C(O)N(H)alkylheteroaryl,
-N(H)C(O)N(H)cycloalkyl, -S-alkyl, -S-aryl,
-S-alkylaryl, -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl,
-CH₂NH₂, -CH₂N(H)alkyl, or -CH₂N(alkyl)₂, -alkyl,
-cycloalkyl, -aryl, -heteroaryl, -heterocyclyl,
10 -alkylcycloalkyl -alkylaryl, -alkylheteroaryl, or
-alkylheterocyclyl, wherein a hydrogen atom bound to
any -aryl or -heteroaryl carbon atom is optionally
replaced by R¹¹ and a hydrogen atom bound to any
nitrogen atom is optionally replaced by R¹; and
15 the other substituents are as defined above.

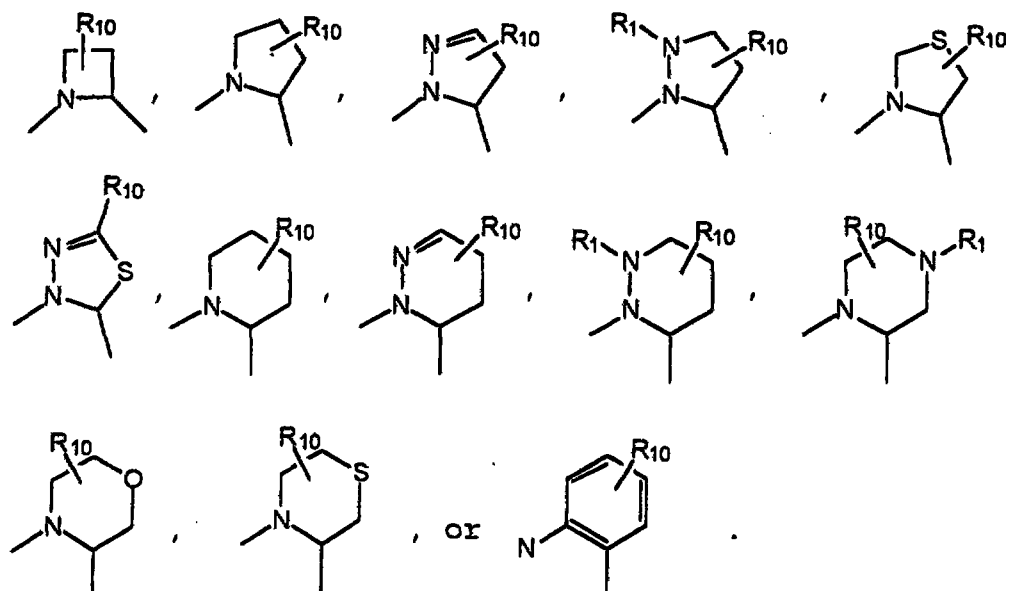
Preferably, in any of the above embodiments:

20 m is 0;

R² is -H;

one R³ is -H and the other R³ is -R⁸,
25 -alkenyl-R⁹, or -alkynyl-R⁹; or

R⁴ and one R⁵ together with the atoms to
which they are bound form a 3 to 7 membered cyclic or
heterocyclic ring system, wherein a hydrogen atom bound
30 to any -alkyl or -cycloalkyl carbon atom is optionally
replaced by R¹⁰, a hydrogen atom bound to any -aryl or
-heteroaryl carbon atom is optionally replaced by R¹¹,
and a hydrogen atom bound to any nitrogen atom of the
ring system is optionally replaced with R¹, wherein the
35 ring system is:

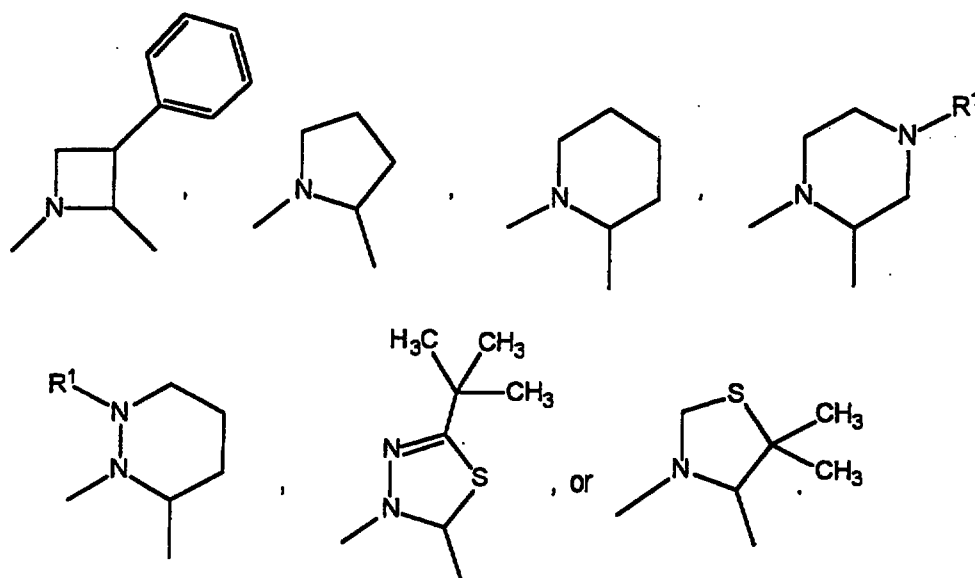


In an alternative preferred embodiment, X is
 5 $C(R^3)_2$ or one R^3 is an amino acid side chain, $-R^8$,
 alkenyl- R^9 , or alkynyl- R^9 .

More preferably, one R^3 is $-H$ and the other
 R^3 is $-alkyl$; or

10 R^4 and one R^5 together with the atoms to
 which they are bound form a 3 to 7 membered cyclic or
 heterocyclic ring system, wherein any hydrogen atom
 bound to a carbon atom of the ring system is optionally
 replaced by R^{10} and any hydrogen atom bound to a
 15 nitrogen atom of the ring system is optionally replaced
 by R^1 , selected from:

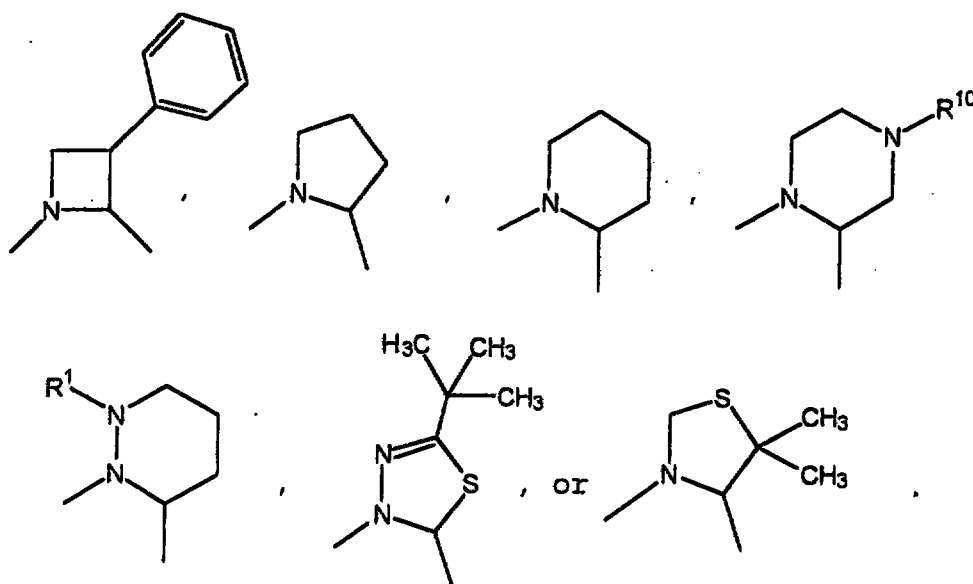
-20-



5 Most preferably, one R³ is -H and the other R³ is -C(H)(CH₃)₂ or -C(CH₃)₃; and

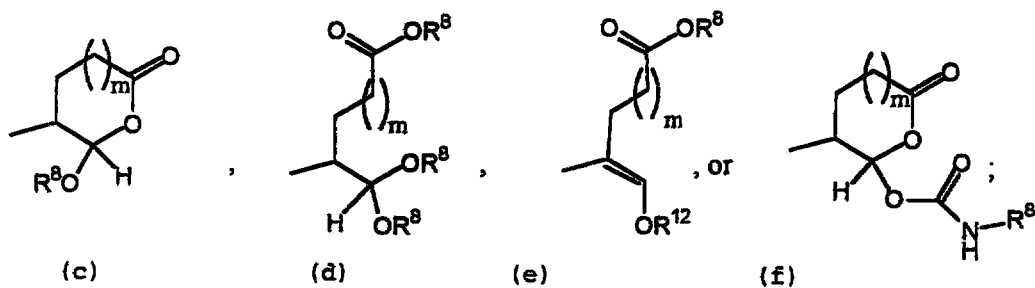
10 R⁴ and one R⁵ together with the atoms to which they are bound form a 3 to 7 membered cyclic or heterocyclic ring system, wherein any hydrogen atom bound to a carbon atom of the ring system is optionally replaced by R¹⁰ and any hydrogen atom bound to a nitrogen atom of the ring system is optionally replaced by R¹, selected from:

- 21 -



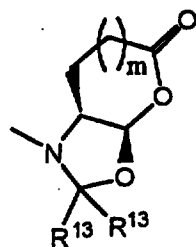
In an alternative most preferred embodiment,
 one R³ is -H and the other R³ is -CH₃, -C(H)(CH₃)₂ or
 5 -C(CH₃)₃ and R⁴ and R⁵ are as defined directly above.

According to another embodiment B, the
 present invention provides a compound of formula I,
 wherein Y is:



provided that when R⁶ is not hydrogen, R⁶ and Y,
 together with the nitrogen to which they are bound,
 form a ring (g):

- 22 -



(g) ;

R^{12} is -C(O)alkyl, -C(O)cycloalkyl, -C(O)alkenyl, -C(O)alkylaryl, -C(O)alkylheteroaryl, -C(O)heterocyclyl, or -C(O)alkylheterocyclyl;

5

R^{13} is -H, -alkyl, -aryl, -alkylaryl or -alkylheteroaryl; and

the other substituents are as described above.

10

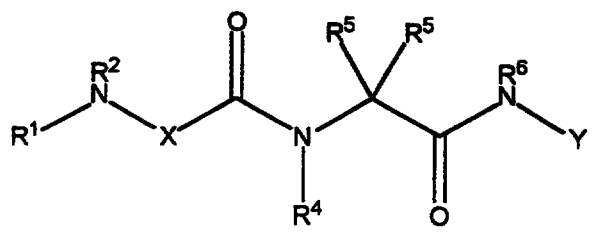
Preferably, in (c), (d), (e), or (f), R^8 is methyl, ethyl, n-propyl, isopropyl, cyclopentyl, phenethyl, or benzyl.

15

Preferred definitions for the other individual components of embodiment B are the same as those set forth above for embodiment A.

A preferred embodiment C of this invention provides compounds of formula I:

20

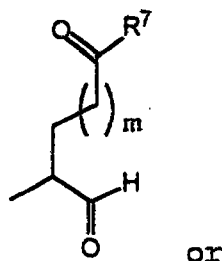


wherein:

Y is:

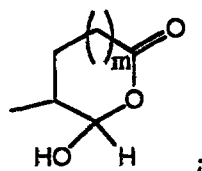
-23-

(a)



or

(b)



5

m is 0 or 1;

X is -C(R³)₂-

10

R¹ is H, -R⁸, -C(O)R⁸, -C(O)C(O)R⁸, -S(O)₂R⁸,
 -S(O)R⁸, -C(O)OR⁸, -C(O)N(H)R⁸, -S(O)₂N(H)-R⁸,
 -S(O)N(H)-R⁸, -C(O)C(O)N(H)R⁸, -C(O)CH=CHR⁸,
 -C(O)CH₂OR⁸, -C(O)CH₂N(H)R⁸, -C(O)N(R⁸)₂, -S(O)₂N(R⁸)₂,
 15 -S(O)N(R⁸)₂, -C(O)C(O)N(R⁸)₂, -C(O)CH₂N(R⁸)₂, -CH₂R⁸,
 -CH₂-alkenyl-R⁸, or -CH₂-alkynyl-R⁸;

R² is -H and each R³ is independently -H, an amino acid side chain, -R⁸, alkenyl-R⁹, or alkynyl-R⁹,
 20 or each R³ together with the atom to which they are bound, form a 3 to 7 membered cyclic or heterocyclic ring system, wherein a hydrogen atom bound to any -alkyl or -cycloalkyl carbon atom is optionally replaced by -R¹⁰, a hydrogen atom bound to any -aryl or
 25 -heteroaryl carbon atom is optionally replaced by -R¹¹, a hydrogen atom bound to any nitrogen atom of the ring system is optionally replaced by -R¹;

- 24 -

R⁴ is -H and each R⁵ is independently -H, an amino acid side chain, -R⁸, -alkenyl-R⁹, or -alkynyl-R⁹, or R⁴ and one R⁵ together with the atoms to which they are bound form a 3 to 7 membered cyclic or heterocyclic ring system, wherein a hydrogen atom bound to any -alkyl or -cycloalkyl carbon atom is optionally replaced by R¹⁰, a hydrogen atom bound to any -aryl or -heteroaryl carbon atom is optionally replaced by R¹¹, and a hydrogen atom bound to any nitrogen atom of the ring system is optionally replaced with R¹;

R⁶ is -H;

R⁷ is -OH, -OR⁸, -N(H)OH, or -N(H)S(O)₂R⁸;

each R⁸ is independently -alkyl, -cycloalkyl, -aryl, -heteroaryl, -heterocyclyl, -alkylcycloalkyl, -alkylaryl, -alkylheteroaryl, or -alkylheterocyclyl, wherein a hydrogen atom bound to any -alkyl or -cycloalkyl carbon atom is optionally replaced by R¹⁰, a hydrogen atom bound to any -aryl or -heteroaryl carbon atom is optionally replaced by R¹¹, and a hydrogen atom bound to any nitrogen atom is optionally replaced by R¹;

each R⁹ is independently -aryl, -heteroaryl, cycloalkyl, or -heterocyclyl, wherein a hydrogen atom bound to any -alkyl or -cycloalkyl carbon atom is optionally replaced by R¹⁰, a hydrogen atom bound to any -aryl or -heteroaryl carbon atom is optionally replaced by R¹¹, and a hydrogen atom bound to any nitrogen atom is optionally replaced by R¹;

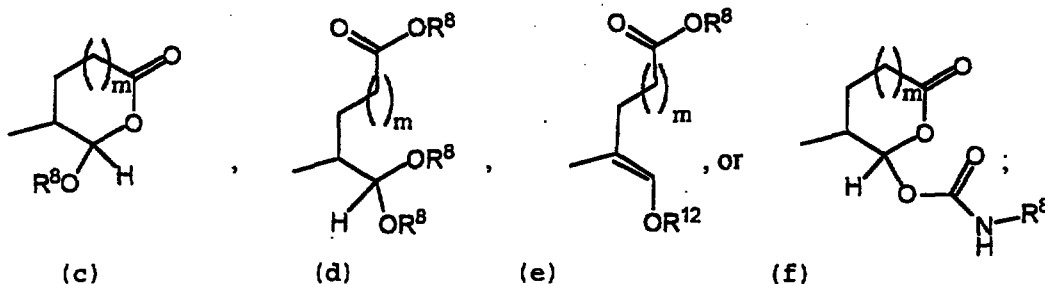
- 25 -

each R¹⁰ is independently -OH, -SH, -F, -Cl, -Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H, -N(H)C(O)NH₂, -perfluoroalkyl, -O-alkyl, -O-aryl, -O-alkylaryl, -N(H)alkyl, -N(H)aryl, -N(H)-alkylaryl, -N(alkyl)₂, -C(O)N(H)alkyl, -C(O)N(alkyl)₂, -N(H)C(O)alkyl, -N(H)C(O)Oalkyl, -N(H)C(O)Oaryl, -N(H)C(O)Oalkylaryl, -N(H)C(O)Oheteroaryl, -N(H)C(O)Oalkylheteroaryl, -N(H)C(O)Ocycloalkyl, -N(H)C(O)N(H)alkyl, -N(H)C(O)N(alkyl)₂, -N(H)C(O)N(H)aryl, -N(H)C(O)N(H)alkylaryl, -N(H)C(O)N(H)heteroaryl, -N(H)C(O)N(H)alkylheteroaryl, -N(H)C(O)N(H)cycloalkyl, -S-alkyl, -S-aryl, -S-alkylaryl, -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl, -CH₂NH₂, -CH₂N(H)alkyl, or -CH₂N(alkyl)₂, -alkyl, -cycloalkyl, -aryl, -heteroaryl, -heterocyclyl, -alkylcycloalkyl -alkylaryl, -alkylheteroaryl, or -alkylheterocyclyl, wherein a hydrogen atom bound to any -aryl or -heteroaryl carbon atom is optionally replaced by R¹¹ and a hydrogen atom bound to any nitrogen atom is optionally replaced by R¹; and

each R¹¹ is independently -OH, -SH, -F, -Cl, -Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H, -N(H)C(O)NH₂, -alkyl, -cycloalkyl, -perfluoroalkyl, -O-alkyl, -O-aryl, -O-alkylaryl, -N(H)alkyl, -N(H)aryl, -N(H)-alkylaryl, -N(alkyl)₂, -C(O)N(H)alkyl, -C(O)N(alkyl)₂, -N(H)C(O)alkyl, -N(H)C(O)N(H)alkyl, -N(H)C(O)N(alkyl)₂, -S-alkyl, -S-aryl, -S-alkylaryl, -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl, -CH₂NH₂, -CH₂N(H)alkyl, or -CH₂N(alkyl)₂;

provided that if one R³ is -H, then the other R³ is not -H.

Another preferred embodiment D of the present invention provides a compound of formula I, wherein Y is:



R^{12} is $-C(O)alkyl$, $-C(O)cycloalkyl$, $-C(O)alkenyl$, $-C(O)alkylaryl$, $-C(O)alkylheteroaryl$, $-C(O)heterocyclyl$, or $-C(O)alkylheterocyclyl$; and

10 the other substituents are as described above except that both of the R^3 groups may be $-H$.

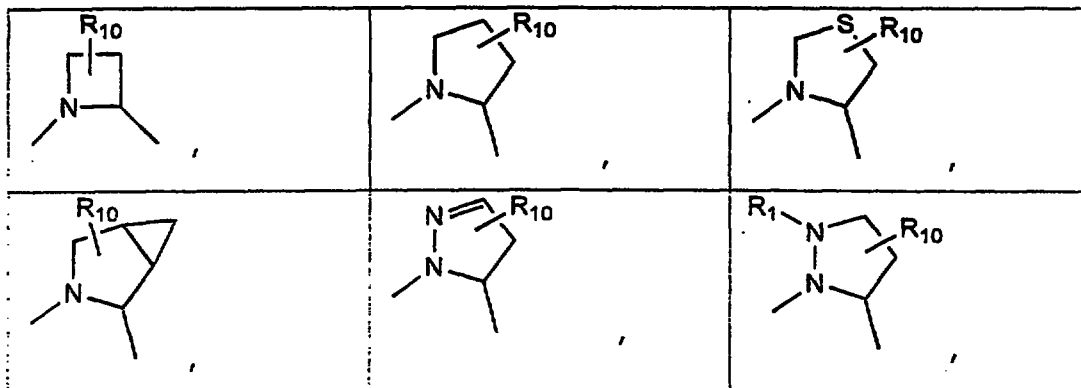
In any of embodiments A-D, preferred compounds are those wherein:

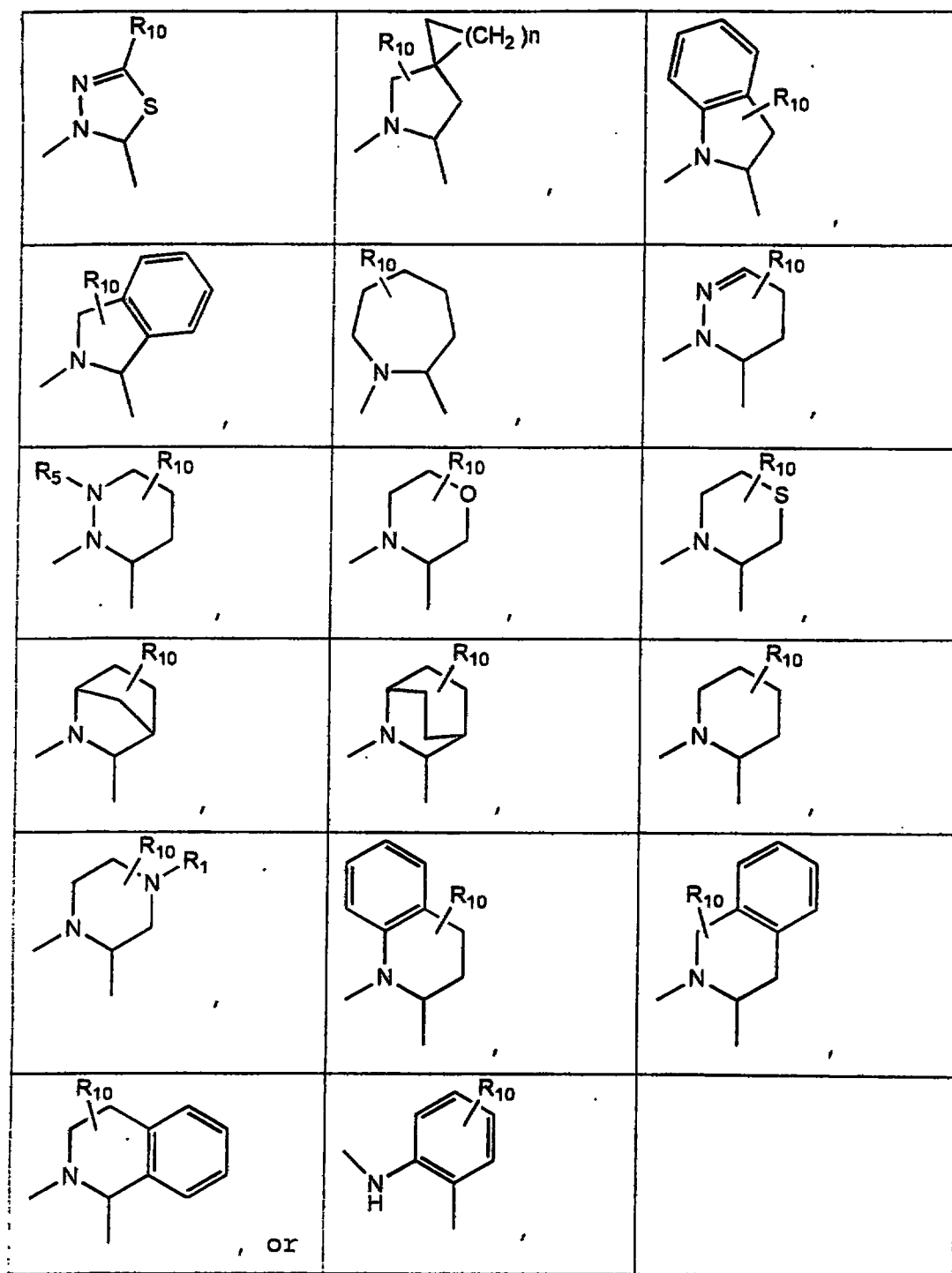
R^1 is $-C(O)R^8$ or $-C(O)(O)R^8$;

15 R^2 and one R^3 are both $-H$ and the other R^3 is an amino acid side chain, $-R^8$, alkenyl- R^9 , or alkynyl- R^9 ; or

R^4 and one R^5 together with the atoms to which they are bound form a ring system selected from:

20



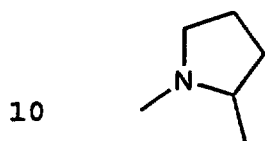


provided that each of the ring systems are optionally substituted with one or more R^{10} groups.

-28-

Alternatively, preferred compounds of
embodiments A-D are those wherein R^3 is -H and the
other R^3 is methyl, isopropyl, tert-butyl, $-\text{CH}_2\text{SR}^8$,
 $-\text{CH}_2\text{SO}_2\text{R}^8$, $-\text{CH}_2\text{CH}_2\text{SR}^8$, $-\text{CH}_2\text{CH}_2\text{SO}_2\text{R}^8$.

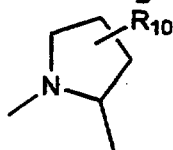
5 More preferred compounds of embodiments A-D
are those wherein R^4 and one R^5 together with the atoms
to which they are bound form the ring system:



and the other R^5 is H; or

one R^3 is -H and the other R^3 is methyl.

15 Alternatively, more preferred compounds of
embodiments A-D are those wherein R^4 and one R^5
together with the atoms to which they are bound form
the ring system:



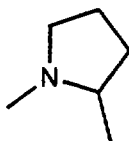
20

and the other R^5 is H.

In the above alternative embodiment, R^{10} is
preferably, 4-fluoro or 4,4-difluoro.

25 Most preferred compounds of this invention
are those wherein R^3 is methyl; and

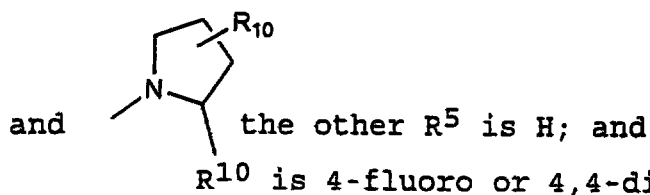
R^4 and one R^5 together with the atoms to
which they are bound form the ring system:



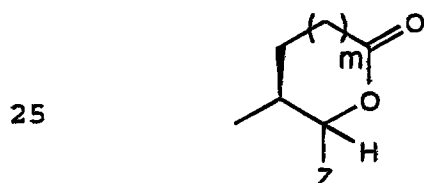
- 29 -

and the other R⁵ is H.

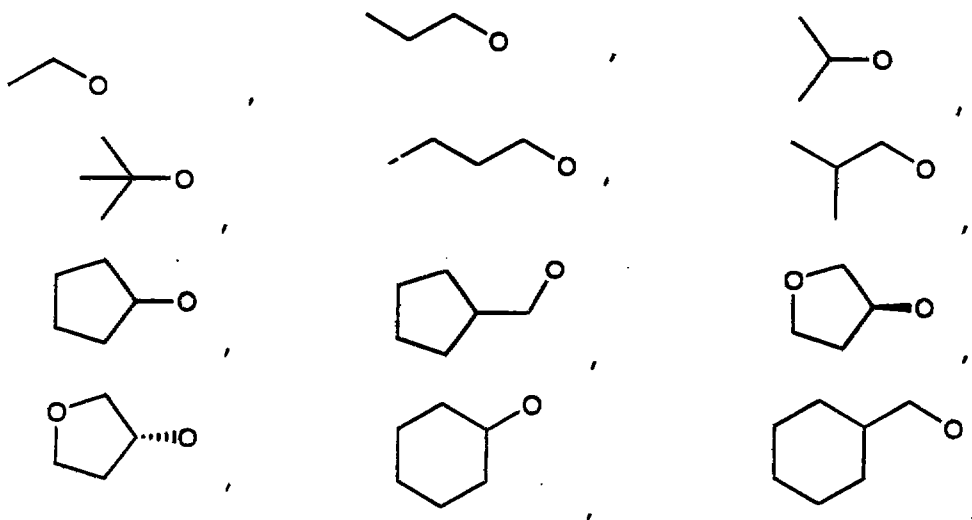
Alternatively, most preferred compounds of
embodiments A-D are those wherein R³ is methyl; and
5 R⁴ and one R⁵ together with the atoms to which they are
bound form the ring system:



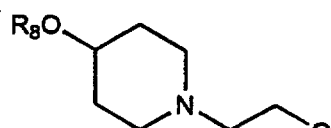
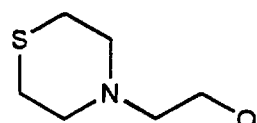
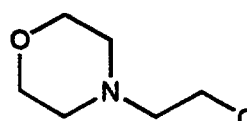
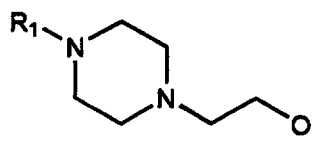
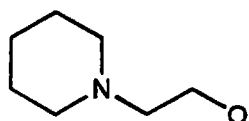
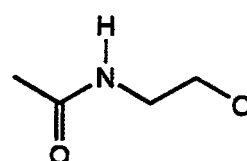
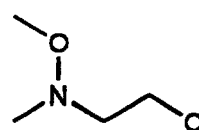
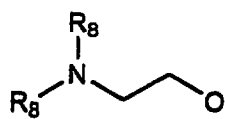
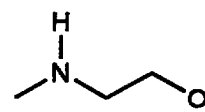
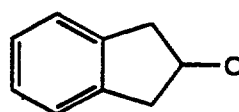
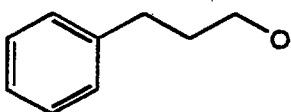
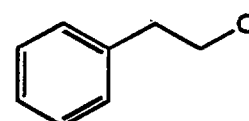
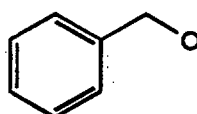
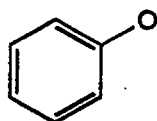
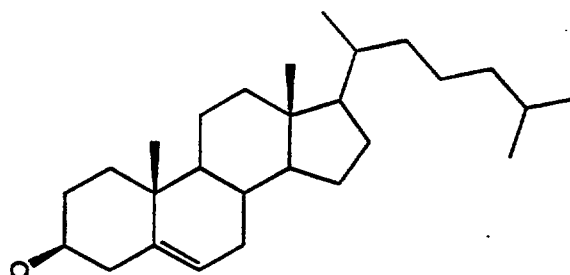
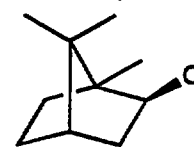
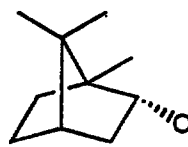
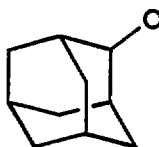
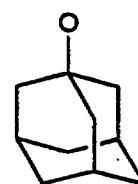
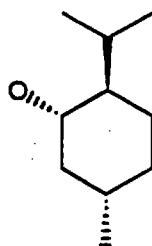
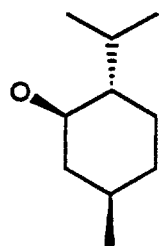
Preferred compounds of embodiments (B) or (D)
are those wherein Y is:



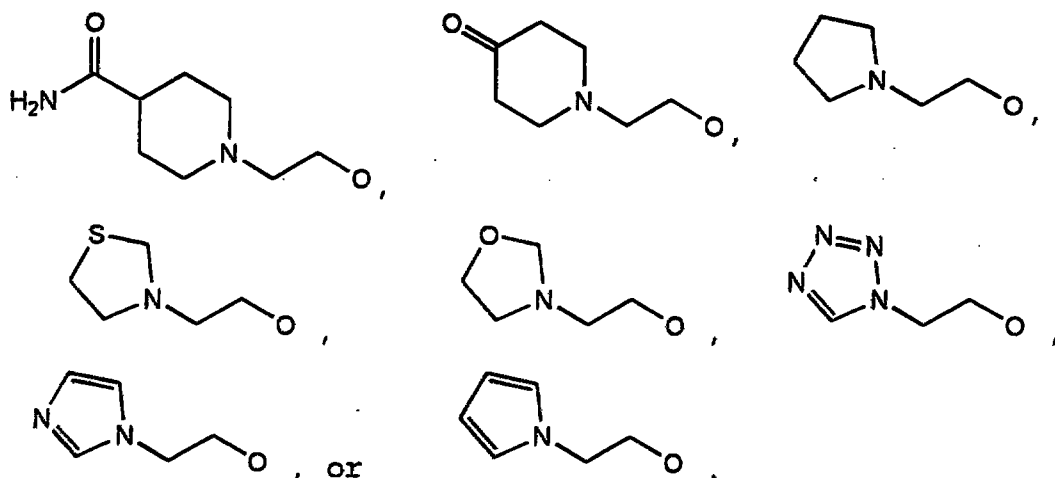
wherein Z represents -OR⁸ and Z is: CH₃O,



- 30 -



- 31 -



Specific compounds of this invention include, but are not limited to, Examples 5a-5bd, 7a-7at, 9a-9g, 15a-15f, 16a-16b, 17a-17e, 18a-18f, 20a-20t, 23a-23i, 24a-24e, 25a-25e, 26a-26h, 27a-27n, 28a-28c, 29a-29s, 32a-32e, 34, G1, G2, 41, 42, 45, 46, 51, 52, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76-93, 98a-z, aa-az, and ba-bb, 101, 102a, 102b, 108a-d, 110, 111, 116a-h, 120a and b, 121, 122 a-v, and 123 a-c.

The compounds of this invention may contain one or more "asymmetric" carbon atoms and thus may occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. Each stereogenic carbon may be of the R or S configuration. Although specific compounds and scaffolds exemplified in this application may be depicted in a particular stereochemical configuration, compounds and scaffolds having either the opposite stereochemistry at any given chiral center or mixtures thereof are also envisioned.

All such isomeric forms of these compounds are expressly included in the present invention, as well as pharmaceutically acceptable derivative thereof.

- 32 -

The term "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, ester, or salt of such ester, of a compound of this invention or any other compound which, upon
5 administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention or an active metabolite or residue thereof.

Pharmaceutically acceptable salts of the compounds of this invention include, for example, those
10 derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic,
15 tartaric, acetic, citric, methanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic and benzenesulfonic acids. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as
20 intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts. Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and N-(C₁₋₄ alkyl)₄⁺
25 salts.

This invention also envisions the "quaternization" of any basic nitrogen-containing groups of the compounds disclosed herein. The basic nitrogen can be quaternized with any agents known to
30 those of ordinary skill in the art including, for example, lower alkyl halides, such as methyl, ethyl, propyl and butyl chloride, bromides and iodides; dialkyl sulfates including dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl,
35 lauryl, myristyl and stearyl chlorides, bromides and

- 33 -

iodides; and aralkyl halides including benzyl and phenethyl bromides. Water or oil-soluble or dispersible products may be obtained by such quaternization.

5 When multiply substituted, each substituent may be picked independently of any other substituent as long as the combination of substituents results in the formation of a stable compound.

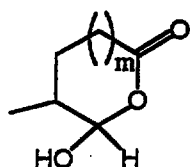
 Combinations of substituents and variables
10 envisioned by this invention are only those that result in the formation of stable compounds. The term "stable", as used herein, refers to compounds which possess stability sufficient to allow manufacture and administration to a mammal by methods known in the art.
15 Typically, such compounds are stable at a temperature of 40 °C or less, in the absence of moisture or other chemically reactive conditions, for at least a week.

 Preferred compounds of this invention may be readily absorbed by the bloodstream of patients upon
20 oral administration. This oral availability makes such compounds excellent agents for orally-administered treatment and prevention regimens against IL-1-, apoptosis-, IGIF-, or IFN- γ -mediated diseases.

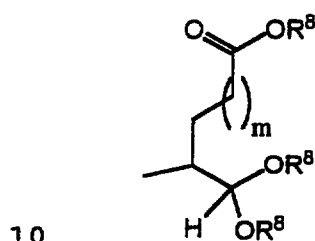
 It should be understood that the compounds of
25 this invention may exist in various equilibrium forms, depending on conditions including choice of solvent, pH, and others known to the practitioner skilled in the art. All such forms of these compounds are expressly included in the present invention. In particular, many
30 of the compounds of this invention, especially those which contain aldehyde or ketone groups and carboxylic acid groups in Y, may take hemi-acetal or hydrated forms. For example, compounds of embodiment A are in a hemiacetal form when Y is:

35

- 34 -

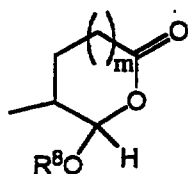


Depending on the choice of solvent and other conditions known to the practitioner skilled in the art, compounds of this invention may also take hydrated, acyloxy acetal, acetal, or enol forms. For example, compounds of this invention are in hydrated forms when Y is:

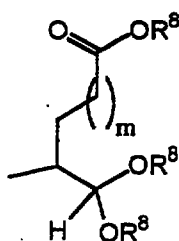


and R⁸ is H;

acyloxy acetal forms when Y is:

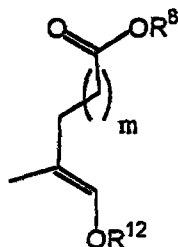


acetal forms when Y is and R⁸ is other than H:



and enol forms when Y is:

- 35 -



5 In addition, it should be understood that the equilibrium forms of the compounds of this invention may include tautomeric forms. All such forms of these compounds are expressly included in the present invention.

10 The compounds of formula I may be synthesized using conventional techniques. Advantageously, these compounds are conveniently synthesized from readily available starting materials.

15 Compounds of this invention may be prepared using the processes described herein. As can be appreciated by the skilled practitioner, these processes are not the only means by which the compounds described and claimed in this application may be synthesized. Further methods will be evident to those of ordinary skill in the art. Additionally, the
20 various synthetic steps described herein may be performed in an alternate sequence or order to give the desired compounds.

It should be understood that the compounds of this invention may be modified by appropriate
25 functionalities to enhance selective biological properties. Such modifications are known in the art and include those which increase biological penetration into a given biological system (e.g., blood, lymphatic system, central nervous system), increase oral
30 availability, increase solubility to allow

- 36 -

administration by injection, alter metabolism and alter rate of excretion. In addition, the compounds may be altered to pro-drug form such that the desired compound is created in the body of the patient as the result of the action of metabolic or other biochemical processes on the pro-drug. Such pro-drug forms typically demonstrate little or no activity in *in vitro* assays. Some examples of pro-drug forms include ketal, acetal, oxime, imine and hydrazone forms of compounds which contain ketone or aldehyde groups, especially where they occur in the Y group of the compounds of this invention. Other examples of pro-drug forms include the hemi-ketal, hemi-acetal, acyloxy ketal, acyloxy acetal, ketal, acetal and enol forms that are described herein.

Compositions and Methods

The compounds of this invention are caspase inhibitors, and in particular ICE inhibitors.

Accordingly, these compounds are capable of targeting
5 and inhibiting events in IL-1-, apoptosis-, IGIF-, and
IFN- γ -mediated diseases, and, thus, the ultimate
activity of that protein in inflammatory diseases,
autoimmune diseases, destructive bone, proliferative
10 disorders, infectious diseases, and degenerative
diseases. For example, the compounds of this invention
inhibit the conversion of precursor IL-1 β to mature IL-
1 β by inhibiting ICE. Because ICE is essential for the
production of mature IL-1, inhibition of that enzyme
effectively blocks initiation of IL-1-mediated
15 physiological effects and symptoms, such as
inflammation, by inhibiting the production of mature
IL-1. Thus, by inhibiting IL-1 β precursor activity,
the compounds of this invention effectively function as
IL-1 inhibitors.

20 Compounds of this invention also inhibit
conversion of pro-IGIF into active, mature IGIF by
inhibiting ICE. Because ICE is essential for the
production of mature IGIF, inhibition of ICE
effectively blocks initiation of IGIF-mediated
25 physiological effects and symptoms, by inhibiting
production of mature IGIF. IGIF is in turn essential
for the production of IFN- γ . ICE therefore effectively
blocks initiation of IFN- γ -mediated physiological
effects and symptoms, by inhibiting production of
30 mature IGIF and thus production of IFN- γ .

The compounds of this invention are
surprisingly bioavailable when compared with peptidyl
inhibitors, such as those described in, for example,
EP 618 223, EP 623 592, WO 93/09135, WO 93/16710, US
35 patent no. 5,434,248, WO 95/35308, or WO 96/33209.

- 38 -

Thus, the pharmaceutical compositions and methods of this invention will be useful for controlling caspase activity *in vivo*. The compositions and methods of this invention will therefore be useful for controlling IL-1, IGIF, or IFN- γ levels *in vivo* and for treating or
5 reducing the advancement, severity or effects of IL-1-, apoptosis-, IGIF-, or IFN- γ -mediated conditions, including diseases, disorders or effects.

Pharmaceutical compositions of this invention
10 comprise a compound of formula I or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier. Such compositions may optionally comprise an additional therapeutic agent. Such agents include, but are not limited to, an anti-inflammatory agent, a matrix metalloprotease inhibitor, a
15 lipoxxygenase inhibitor, a cytokine antagonist, an immunosuppressant, an anti-cancer agent, an anti-viral agent, a cytokine, a growth factor, an immunomodulator, a prostaglandin or an anti-vascular hyperproliferation compound.
20

The term "pharmaceutically acceptable carrier" refers to a non-toxic carrier that may be administered to a patient, together with a compound of this invention, and which does not destroy the
25 pharmacological activity thereof.

Pharmaceutically acceptable carriers that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum
30 proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium
35 hydrogen phosphate, potassium hydrogen phosphate,

- 39 -

sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes,
5 polyethylene-polyoxypropylene-block polymers, wool fat and self-emulsifying drug delivery systems (SEDDS) such as α -tocopherol, polyethyleneglycol 1000 succinate, or other similar polymeric delivery matrices.

In pharmaceutical composition comprising only
10 a compound of embodiments A-D as the active component, methods for administering these compositions may additionally comprise the step of administering to the subject an additional agent. Such agents include, but are not limited to, an anti-inflammatory agent, a
15 matrix metalloprotease inhibitor, a lipoxigenase inhibitor, a cytokine antagonist, an immunosuppressant, an anti-cancer agent, an anti-viral agent, a cytokine, a growth factor, an immunomodulator, a prostaglandin or an anti-vascular hyperproliferation compound.

20 The term "pharmaceutically effective amount" refers to an amount effective in treating or ameliorating an IL-1-, apoptosis-, IGIF-, or IFN- γ -mediated disease in a patient. The term "prophylactically effective amount" refers to an amount
25 effective in preventing or substantially lessening IL-1-, apoptosis-, IGIF-, or IFN- γ -mediated diseases in a patient.

The compounds of this invention may be employed in a conventional manner for controlling IGIF
30 and IFN- γ levels *in vivo* and for treating diseases or reducing the advancement or severity of effects which are mediated by IL-1, apoptosis, IGIF, or IFN- γ . Such methods of treatment, their dosage levels and requirements may be selected by those of ordinary skill
35 in the art from available methods and techniques.

For example, a compound of this invention may be combined with a pharmaceutically acceptable adjuvant for administration to a patient suffering from an IL-1-, apoptosis-, IGIF-, or IFN- γ -mediated disease in a pharmaceutically acceptable manner and in an amount effective to lessen the severity of that disease.

Alternatively, the compounds of this invention may be used in compositions and methods for treating or protecting individuals against IL-1, apoptosis-, IGIF, or IFN- γ mediated diseases over extended periods of time. The compounds may be employed in such compositions either alone or together with other compounds of this invention in a manner consistent with the conventional utilization of enzyme inhibitors in pharmaceutical compositions. For example, a compound of this invention may be combined with pharmaceutically acceptable adjuvants conventionally employed in vaccines and administered in prophylactically effective amounts to protect individuals over an extended period of time against IL-1-, apoptosis-, IGIF, or IFN- γ mediated diseases.

The compounds of formula I may also be co-administered with other caspase or ICE inhibitors to increase the effect of therapy or prophylaxis against various IL-1-, apoptosis-, IGIF-, or IFN- γ mediated diseases.

In addition, the compounds of this invention may be used in combination either conventional anti-inflammatory agents or with matrix metalloprotease inhibitors, lipoxygenase inhibitors and antagonists of cytokines other than IL-1 β .

The compounds of this invention can also be administered in combination with immunomodulators (e.g., bropirimine, anti-human alpha-interferon antibody, IL-2, GM-CSF, methionine enkephalin,

- 41 -

interferon-alpha, diethyldithiocarbamate, tumor
necrosis factor, naltrexone and EPO), with
prostaglandins, or with antiviral agents (e.g., 3TC,
polysulfated polysaccharides, ganciclovir, ribavirin,
5 acyclovir, alpha interferon, trimethotrexate and
fancyclovir) or prodrugs of these or related compounds
to prevent or combat IL-1-mediated disease symptoms
such as inflammation.

When the compounds of this invention are
10 administered in combination therapies with other
agents, they may be administered sequentially or
concurrently to the patient. Alternatively,
pharmaceutical or prophylactic compositions according
to this invention comprise a combination of a compound
15 of formula I and another therapeutic or prophylactic
agent.

The pharmaceutical compositions of this
invention may be administered orally, parenterally, by
inhalation spray, topically, rectally, nasally,
20 buccally, vaginally or via an implanted reservoir. We
prefer oral administration. The pharmaceutical
compositions of this invention may contain any
conventional non-toxic pharmaceutically-acceptable
carriers, adjuvants or vehicles. In some cases, the pH
25 of the formulation may be adjusted with
pharmaceutically acceptable acids, bases or buffers to
enhance the stability of the formulated compound or its
delivery form. The term parenteral as used herein
includes subcutaneous, intracutaneous, intravenous,
30 intramuscular, intra-articular, intrasynovial,
intrasternal, intrathecal, intralesional and
intracranial injection or infusion techniques.

The pharmaceutical compositions may be in the
form of a sterile injectable preparation, for example,
35 as a sterile injectable aqueous or oleaginous

- 42 -

suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as those described in Pharmacopeia Helvetica, or a similar alcohol.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, and aqueous suspensions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and solutions and propylene glycol are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired,

- 43 -

certain sweetening and/or flavoring and/or coloring agents may be added.

5 The pharmaceutical compositions of this invention may also be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound of this invention with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

10 Topical administration of the pharmaceutical compositions of this invention is especially useful when the desired treatment involves areas or organs readily accessible by topical application. For application topically to the skin, the pharmaceutical composition should be formulated with a suitable ointment containing the active components suspended or dissolved in a carrier. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical composition can be formulated with a suitable lotion or cream containing the active compound suspended or dissolved in a carrier. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. The pharmaceutical compositions of this invention may also be topically applied to the lower intestinal tract by rectal suppository formulation or in a suitable enema

15
20
25
30

- 44 -

formulation. Topically-administered transdermal patches are also included in this invention.

The pharmaceutical compositions of this invention may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

Dosage levels of between about 0.01 and about 100 mg/kg body weight per day, preferably between 0.5 and about 75 mg/kg body weight per day and most preferably between about 1 and 50 mg/kg body weight per day of the active ingredient compound are useful in a monotherapy for the prevention and treatment of IL-1-, apoptosis-, IGIF-, and IFN- γ mediated diseases, including inflammatory diseases, autoimmune diseases, destructive bone disorders, proliferative disorders, infectious diseases, degenerative diseases, necrotic diseases, inflammatory peritonitis, osteoarthritis, acute pancreatitis, chronic pancreatitis, asthma, adult respiratory distress syndrome, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Graves' disease, autoimmune gastritis, insulin-dependent diabetes mellitus (Type I), autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, myasthenia gravis, inflammatory bowel disease, Crohn's disease, psoriasis, atopic dermatitis, graft vs. host disease, osteoporosis, multiple myeloma-related bone disorder, leukemias and related disorders, myelodysplastic syndrome, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma,

- 45 -

Kaposi's sarcoma, multiple myeloma, sepsis, septic shock, Shigellosis, Alzheimer's disease, Parkinson's disease, cerebral ischemia, myocardial ischemia, spinal muscular atrophy, multiple sclerosis, AIDS-related encephalitis, HIV-related encephalitis, aging, alopecia, neurological damage due to stroke, ulcerative colitis, infectious hepatitis, juvenile diabetes, lichenplanus, acute dermatomyositis, eczema, primary cirrhosis, uveitis, Behcet's disease, atopic skin disease, pure red cell aplasia, aplastic anemia, amyotrophic lateral sclerosis, nephrotic syndrome and systemic diseases or diseases with effects localized in the liver or other organs having an inflammatory or apoptotic component caused by excess dietary alcohol intake or viruses, such as HBV, HCV, HGV, yellow fever virus, dengue fever virus, and Japanese encephalitis virus.

Typically, the pharmaceutical compositions of this invention will be administered from about 1 to 5 times per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (w/w). Preferably, such preparations contain from about 20% to about 80% active compound.

When the compositions of this invention comprise a combination of a compound of formula I and one or more additional therapeutic or prophylactic agents, both the compound and the additional agent should be present at dosage levels of between about 10%

- 46 -

to 80% of the dosage normally administered in a monotherapy regime.

Upon improvement of a patient's condition, a maintenance dose of a compound, composition or combination of this invention may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained when the symptoms have been alleviated to the desired level, treatment should cease. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence or disease symptoms.

As the skilled artisan will appreciate, lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the disease, and the patient's disposition to the disease and the judgment of the treating physician.

IL-1 or apoptosis mediated diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, inflammatory diseases, autoimmune diseases, proliferative disorders, infectious diseases, and degenerative diseases. The apoptosis-mediated diseases which may be treated or prevented by the compounds of this invention include degenerative diseases.

IL-1 or apoptosis mediated inflammatory diseases which may be treated or prevented include, but are not limited to osteoarthritis, acute pancreatitis, chronic pancreatitis, asthma, and adult respiratory

- 47 -

distress syndrome. Preferably the inflammatory disease is osteoarthritis or acute pancreatitis.

IL-1 or apoptosis mediated autoimmune diseases which may be treated or prevented include, but are not limited to, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Graves' disease, autoimmune gastritis, insulin-dependent diabetes mellitus (Type I), autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, myasthenia gravis, multiple sclerosis, inflammatory bowel disease, Crohn's disease, psoriasis, atopic dermatitis and graft vs. host disease. Preferably the autoimmune disease is rheumatoid arthritis, inflammatory bowel disease, Crohn's disease, psoriasis, or atopic dermatitis.

IL-1 or apoptosis mediated destructive bone disorders which may be treated or prevented include, but are not limited to, osteoporosis and multiple myeloma-related bone disorder.

IL-1 or apoptosis mediated proliferative diseases which may be treated or prevented include, but are not limited to, leukemias and related disorders, such as myelodysplastic syndrome, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, and multiple myeloma.

IL-1 or apoptosis mediated infectious diseases which may be treated or prevented include, but are not limited to, sepsis, septic shock, and Shigellosis.

IL-1 or apoptosis mediated degenerative or necrotic diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, Alzheimer's disease, Parkinson's disease, cerebral ischemia, and myocardial ischemia.

Preferably, the degenerative disease is Alzheimer's disease.

IL-1 or apoptosis-mediated degenerative diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, Alzheimer's disease, Parkinson's disease, cerebral ischemia, myocardial ischemia, spinal muscular atrophy, multiple sclerosis, AIDS-related encephalitis, HIV-related encephalitis, aging, alopecia, and neurological damage due to stroke.

Other diseases having an inflammatory or apoptotic component may be treated or prevented by the compounds of this invention. Such diseases may be systemic diseases or diseases with effects localized in the liver or other organs and may be caused by, for example, excess dietary alcohol intake or viruses, such as HBV, HCV, HGV, yellow fever virus, dengue fever virus, and Japanese encephalitis virus.

IGIF- or IFN- γ -mediated diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, inflammatory, infectious, autoimmune, proliferative, neurodegenerative and necrotic conditions.

IGIF- or IFN- γ -mediated inflammatory diseases which may be treated or prevented include, but are not limited to osteoarthritis, acute pancreatitis, chronic pancreatitis, asthma, rheumatoid arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, cerebral ischemia, myocardial ischemia and adult respiratory distress syndrome. Preferably, the inflammatory disease is rheumatoid arthritis, ulcerative colitis, Crohn's disease, hepatitis or adult respiratory distress syndrome.

IGIF- or IFN- γ -mediated infectious diseases which may be treated or prevented include, but are not

-49-

limited to infectious hepatitis, sepsis, septic shock and Shigellosis.

IGIF- or IFN- γ -mediated autoimmune diseases which may be treated or prevented include, but are not limited to glomerulonephritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Graves' disease, autoimmune gastritis, insulin-dependent diabetes mellitus (Type I), juvenile diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, myasthenia gravis, multiple sclerosis, psoriasis, lichenplanus, graft vs. host disease, acute dermatomyositis, eczema, primary cirrhosis, hepatitis, uveitis, Behcet's disease, atopic skin disease, pure red cell aplasia, aplastic anemia, amyotrophic lateral sclerosis and nephrotic syndrome. Preferably, the autoimmune disease is glomerulonephritis, insulin-dependent diabetes mellitus (Type I), juvenile diabetes, psoriasis, graft vs. host disease or hepatitis.

More preferred diseases which may be treated or prevented include rheumatoid arthritis, inflammatory bowel disease, including Crohn's disease and ulcerative colitis, inflammatory peritonitis, septic shock, pancreatitis, traumatic brain injury, organ transplant rejection, osteoarthritis, asthma, psoriasis, Alzheimer's disease, atopic dermatitis, or leukemias and related disorders, such as myelodysplastic syndrome or multiple myeloma.

Accordingly, one embodiment of this invention provides a method for treating or preventing an IL-1 or apoptosis mediated disease in a subject comprising the step of administering to the subject any compound, pharmaceutical composition, or combination described herein and a pharmaceutically acceptable carrier.

-50-

Another embodiment of this invention provides a method for decreasing IGIF production in a subject comprising the step of administering to the subject any compound, pharmaceutical composition, or combination described herein and a pharmaceutically acceptable carrier.

Yet another embodiment of this invention provides a method for decreasing IFN- γ production in a subject comprising the step of administering to the subject any compound, pharmaceutical composition, or combination described herein and a pharmaceutically acceptable carrier.

Although this invention focuses on the use of the compounds disclosed herein for preventing and treating IL-1, apoptosis-, IGIF, and IFN- γ -mediated diseases, the compounds of this invention can also be used as inhibitory agents for other cysteine proteases.

The compounds of this invention are also useful as commercial reagents which effectively bind to caspases or other cysteine proteases including, but not limited to ICE. As commercial reagents, the compounds of this invention, and their derivatives, may be used to block proteolysis of a target peptide in biochemical or cellular assays for ICE and ICE homologs or may be derivatized to bind to a stable resin as a tethered substrate for affinity chromatography applications. These and other uses which characterize commercial cysteine protease inhibitors will be evident to those of ordinary skill in the art.

In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

35

- 51 -

GENERAL METHODSAnalytical HPLC conditions:

Column: C-18, Particle size: 5 μ , Pore size: 100Å,
Column size: 4.6 x 150 mm

5 Solvent A: 0.1% TFA / 1% MeCN / 98.9% water

Solvent B: 0.1% TFA / 99.9% MeCN

Gradient: A to B over 20 min at a flow rate of 1
mL/min

10 Column: Cyano, Particle size: 5 μ , Pore size: 100Å,
Column size: 4.6 x 150 mm

Solvent A: 0.1% TFA / 1% MeCN / 98.9% water

Solvent B: 0.1% TFA / 99.9% MeCN

15 Gradient: A / B = 99% / 1% to 50% / 50% over 20 min at
a flow rate of 1 mL/min

HPLC Mass Spectral Analysis

20 Mass Spectral Analysis: All mass spectral
data were collected using a Micromass Quattro II triple
quadrupole mass spectrometer (Beverly, MA) equipped
with a cross-flow electrospray ionization source. The
mass spectrometer was coupled to a HPLC system
manufactured by Hewlett-Packard (HP1100). The
25 autosampler for the system was a Gilson 215 (Middleton,
WI) liquid handler. All of the equipment was
controlled by the MassLynx software package purchased
from Micromass.

30 Mass spectral analysis was performed by
liquid chromatography-MS to determine purity and
confirm molecular weight simultaneously. In instances

- 52 -

where the sample purity had been determined by other means, a flow injection analysis (FIA) was used instead of the full chromatography analysis. In all cases, both positive and negative ion spectra were collected.

5 Mass Spectrum Acquisition Conditions: For all experiments, the mass spectrometer was configured in electrospray mode with the cross-flow counter electrode. A flow splitter was used to reduce the flow from the HPLC to 40% of the original flow. The inlet
10 temperature was set to 140 °C and the drying gas flow was set to maximize signal. The resolution of the mass spectrometer was adjusted to 0.65 amu FWHM and data was collected in centroid mode. In positive ion mode, the
15 cone voltage was set to 25V, the capillary voltage was 3.8 kV. In negative ion mode, the cone voltage was set to 25 V and the capillary voltage was set to 3.5 kV. In both positive and negative ion mode, the time to acquire a full spectrum was 1s with a switching time of 0.25 seconds between scans. The mass range scanned for
20 molecules with an expected molecular weight of less than 350 amu was 70-500 m/z while for molecules with a expected mass of more than 350 amu the mass to charge ratio scanned was 200-1000 m/z.

Chromatography Conditions: Liquid
25 chromatography was performed using a YMC AQ C18 column (150 mm X 3mm with 5µm particle and a 120Å pore size). For all analysis, MeCN with 0.2 % formic acid was combined with water with 0.2% formic acid to form the elution gradient. The gradient profile consisted of
30 starting with 15 % MeCN: water and increasing the amount of MeCN linearly over ten minutes to 90%. That concentration was held constant for 2 minutes before returning to initial conditions. During the entire analysis the flow rate was 0.9mL/min.

- 53 -

Flow Injection Conditions: A 1:1 mixture of the water to MeCN (both with 0.2% formic acid added) was used to acquire the FIA data. The flow rate was set to 0.3 ml/min.

5

¹H NMR

All ¹H NMR spectra were acquired using a Bruker Instruments AMX-500 NMR spectrometer in the solvent given.

10

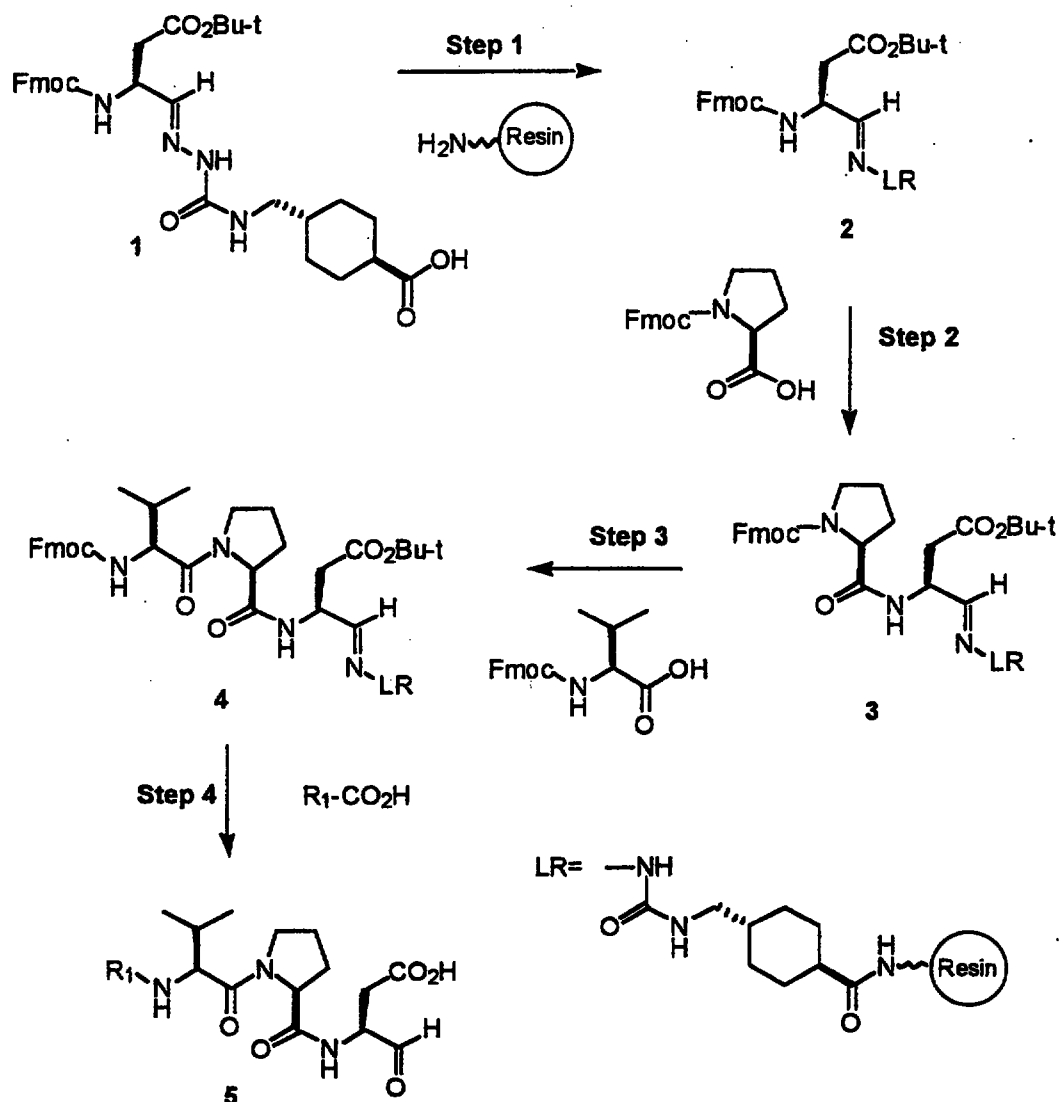
SYNTHETIC METHODS

General Procedure for the Preparation of Compounds of Formula I, Embodiment C (Schemes I-VI)

- 54 -

Procedure for the preparation of analogs 5a-5bd.

Scheme I



In Schemes I-VIII, the variable LR refers to the
 5 linker-resin and is defined as shown above in Scheme I.

Step 1: A 6.7 g portion (0.8 mmol/gram loading, 5.36
 mmol) of 4-methyl benzhydrylamine hydrochloride salt
 resin (Scheme I) was washed with DMF (3 x 50 mL), 10%
 10 DIEA/DMF (3 x 50 mL) and N-methylpyrrolidinone (NMP) (3

- 55 -

x 50 mL). To a suspension of the washed resin in 25 mL of NMP was added successively compound 1 (1.1 eq, 3.5 g, 5.90 mmol) DIEA (3.33 eq, 3.1 mL, 17.70 mmol), 1-hydroxybenzotriazole hydrate (HOBt) (1.1 eq, 797 mg, 5.90 mmol), and O-benzotriazole-N,N,N,N'-tetramethyluronium hexafluorophosphate (HBTU) (1.1 eq, 2.24 g, 5.90 mmol). Compound 1 was prepared according to the literature procedure of A. M. Murphy et al, J. Am. Chem. Soc., 114, pp. 3156-3157 (1992). The mixture was rotated at room temperature overnight using a wrist arm shaker.

The resulting mixture was filtered, and the resin was rinsed with DMF then treated with 12 mL of a 20% solution of acetic anhydride in DMF for 30 minutes at room temperature. The mixture was filtered, and the resin was washed successively with DMF (2 x 50mL), CH₃OH (50mL), 1:1 DMF/ CH₂Cl₂ (2 x 50mL), CH₃OH (50mL) and CH₂Cl₂ (3 x 50mL). After drying in vacuo, 9.0 grams of resin 2 were obtained (0.48 mmol/gram loading).

Step 2: To 4.5 g of resin 2 (0.48 mmol/gram, 2.16 mmol) was added 25 mL of a 20% solution of piperidine in DMF. The suspension was rotated at room temperature for 5 minutes and drained. The procedure was repeated over 20 minutes. The resin was then washed successively with DMF (2 x 40 mL), CH₃OH (40 mL), CH₂Cl₂ (2 x 40 mL), CH₃OH (40 mL) and NMP (40 mL). To a suspension of resin in 40 mL of NMP was added successively 2.92 g of N-Fmoc-proline (4 eq, 8.64 mmol), 3.0 mL of DIEA (8 eq, 17.28 mmol), 1.17 g of HOBt (4 eq, 8.64 mmol) and 3.27 g of HBTU (4 eq, 8.64 mmol). The mixture was rotated at room temperature overnight and drained. This

- 56 -

coupling procedure was repeated over 3 hours. The resin was then washed successively with DMF (2 x 40 mL), CH₃OH (40 mL), 1:1 DMF/ CH₂Cl₂ (2 x 40 mL), CH₃OH (40 mL) and CH₂Cl₂ (3 x 40 mL), and briefly dried in vacuo to afford resin 3.

Step 3: A suspension of resin 3 in 25 mL of a 20% solution of piperidine in DMF was rotated at room temperature for 5 minutes. The suspension was drained. The procedure was repeated over 20 minutes. The resin was washed successively with DMF (2 x 40 mL), CH₃OH (40 mL), CH₂Cl₂ (2 x 40 mL), CH₃OH (40 mL) and NMP (2 x 40 mL). To a suspension of resin in 40 mL of NMP was added successively 2.93 g of N-Fmoc-valine (4 eq, 8.64 mmol), 3.0 mL of DIEA (8 eq, 17.28 mmol), 1.17 g of HOBt (4 eq, 8.64 mmol) and 3.27 g of HBTU (4 eq, 8.64 mmol). The mixture was rotated at room temperature overnight and drained. This coupling procedure was repeated over 3 hours. The resin was then washed successively with DMF (2 x 40 mL), CH₃OH (40 mL), 1:1 DMF/ CH₂Cl₂ (2 x 40 mL), CH₃OH (40 mL) and CH₂Cl₂ (3 x 40 mL), and dried in vacuo to afford resin 4 (0.45 mmol/gram).

Step 4: To a 0.05 mmol portion of resin 4 was added 2 mL of a 20% solution of piperidine in DMF. The suspension was rotated at room temperature for 5 minutes, and drained. The procedure was repeated over 20 minutes. The resulting resin was washed successively with DMF (3 x 5 mL), CH₃OH (5 mL), and NMP (3 x 5 mL). The desired carboxylic acid was then added (4 eq, 0.2 mmol), followed by 0.8 mL of a 0.25M solution of HOBt in NMP, 0.14 mL of DIEA (8 eq, 0.4

- 57 -

mmol) and 0.8 mL of a 0.25M solution of HBTU in NMP. The mixture was rotated at room temperature overnight and drained. The resin was washed successively with DMF (2 x 5 mL), CH₃OH (5 mL), 1:1 DMF/ CH₂Cl₂ (2 x 5 mL), CH₃OH (5 mL) and CH₂Cl₂ (3 x 5 mL), and dried in vacuo. A 2 mL portion of a 95% solution of TFA in water was then added to the resin. The mixture was stirred at room temperature for one hour, and filtered. The filtrate was evaporated, and the residue was taken up in acetonitrile-water and purified by preparative HPLC to afford compounds 5a-5bd.

Product yield, analytical HPLC conditions, HPLC retention time, product purity, and mass spectral data obtained for examples 5a-5bd, 7a-7at, 9a-9g, 15a-15f, 16a-16b, 17a-17e, 18a-18f, 20a-20t, 23a-23i, 24a-24e, 25a-25e, 26a-26h, 27a-27n, 28a-28c, 29a-29s, 32a-32e are provided in Table 1 unless noted otherwise.

Table 1. Physical Data for Selected Examples

Ex.	Yield (mg)	HPLC Gradient /time (min)	RT (min)	Purity (%)	Mass Spec (M+H or M+Na)
5a	1.0	5-45%/10'	4.66	92	475.2
5b	1.0	5-45%/10'	6.86	85	457.2
5c	4.0	5-45%/10'	5.98	85	469.2
5d	1.5	5-45%/10'	6.82	95	467.5
5e	1.0	5-45%/10'	5.52	95	418.2
5f	0.6	5-45%/10'	4.28	93	434.2
5g	6.4	10-60%/10'	8.57	97	504.7 (+Na)
5h	15.6	10-60%/10'	4.51	99	459.2
5i	6.6	5%-90%/10'	9.34	90	506.1
5j	5.1	5%-90%/10'	10.04	95	506.1
5k	10.7	5%-90%/10'	8.64	85	520.1
5l	6.6	5%-90%/10'	8.72	85	540.1
5m	6.8	5%-90%/10'	7.89	85	502.0
5n	1.9	5%-0%/10'	6.46	85	494.1
5o	3.8	5%-90%/10'	7.04	85	506.1
5p	6.8	5%-90%/10'	11.62	95	576.0
5q	2.2	5%-90%/10'	9.72	90	508.1
5r	3.8	5%-90%/10'	7.27	85	462.1
5s	4.3	5%-90%/10'	6.46	90	470.1

Ex.	Yield (mg)	HPLC Gradient /time (min)	RT (min)	Purity (%)	Mass Spec (M+H or M+Na)
5t	5.9	5%-60%/9' 60%-90%/2'	10.27	90	486.2
5u	3.1	5%-60%/9' 60%-90%/2'	9.09	80	522.1
5v	1.0	5%-60%/9' 60%-90%/2'	11.63	85	502.2
5w	10.3	5%-60%/9' 60%-90%/2'	8.75	95	470.2
5x	8.8	5%-60%/9' 60%-90%/2'	8.88	95	565.1
5y	6.6	5%-60%/9' 60%-90%/2'	12.32	95	518.2
5z	10.2	5%-60%/9' 60%-90%/2'	12.63	95	502.2
5aa	2.5	5%-60%/9' 60%-90%/2'	9.57	95	554.1
5ab	7.8	5%-60%/9' 60%-90%/2'	10.54	85	538.2
5ac	1.4	5%-60%/9' 60%-90%/2'	9.28	95	476.2
5ad	5.3	5%-60%/9' 60%-90%/2'	6.51	85	469.2
5ae	4.3	5%-60%/9' 60%-90%/2'	9.81	95	551.1
5af	0.9	5%-60%/9' 60%-90%/2'	9.98	90	547.4
5ag	5.7	5%-60%/9' 60%-90%/2'	10.31	90	526.2
5ah	1.4	5%-60%/9' 60%-90%/2'	8.13	85	542.1
5ai	10.9	10%-90%/10'	5.88	85	584.2
5aj	4.2	10%-90%/10'	5.89	90	556.2
5ak	7.8	10%-90%/10'	5.54	85	568.3
5al	8.4	10%-90%/10'	6.25	95	516.2
5am	7.6	10%-90%/10'	6.49	95	474.3
5an	6.2	10%-90%/10'	6.00	95	500.2
5ao	9.4	10%-90%/10'	6.68	95	581.3
5ap	6.4	10%-90%/10'	4.30	90	500.3
5aq	5.2	10%-90%/10'	6.45	95	559.2
5ar	1.4	10%-90%/10'	5.38	90	561.3
5as	16.2	10%-90%/10'	4.25	95	475.2
5at	15.4	10%-90%/10'	6.68	95	560.3
5au	5.9	10%-90%/10'	6.70	90	498.3
5av	4.1	10%-90%/10'	5.13	85	531.2
5aw	5.5	10%-90%/10'	6.53	85	570.3
5ax	14.0	10%-90%/10'	6.26	90	557.3

- 59 -

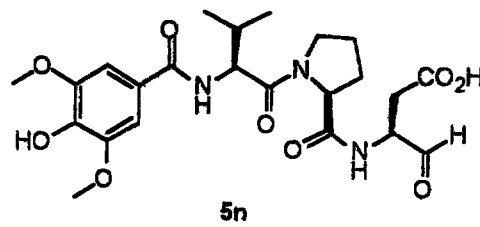
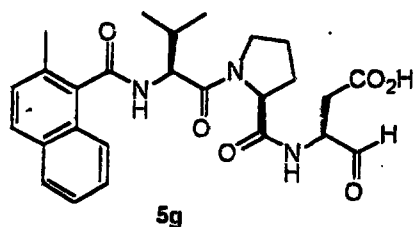
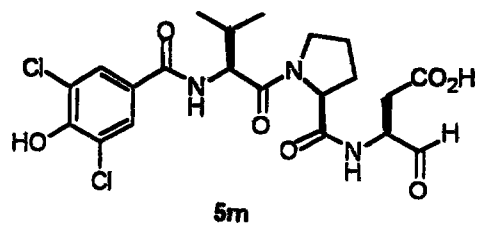
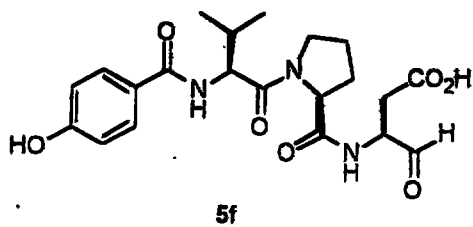
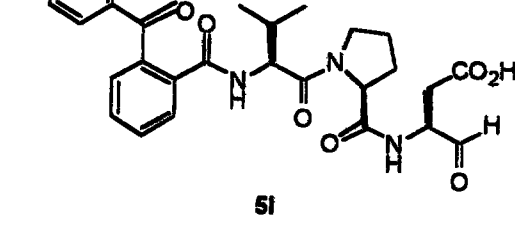
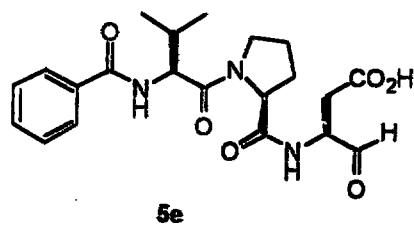
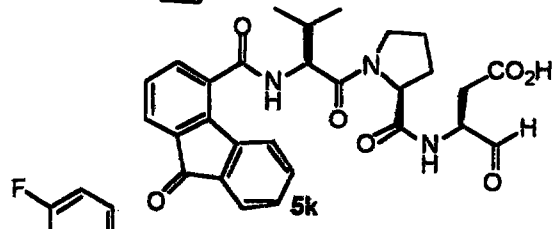
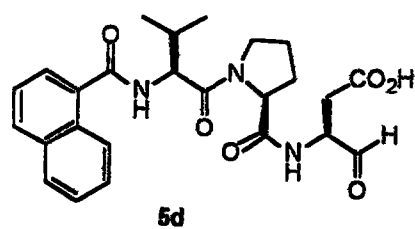
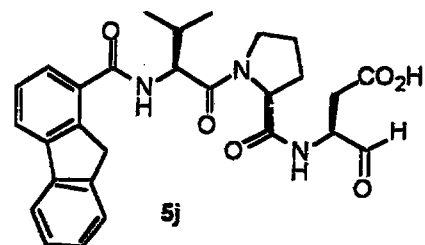
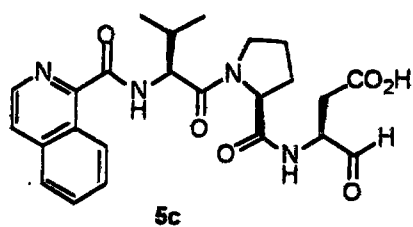
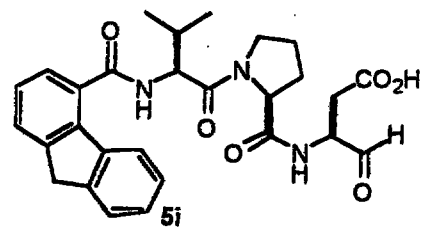
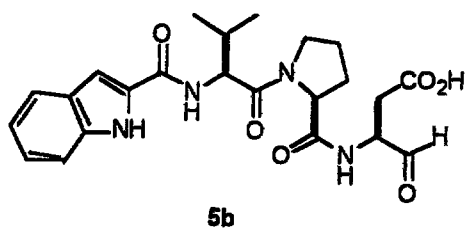
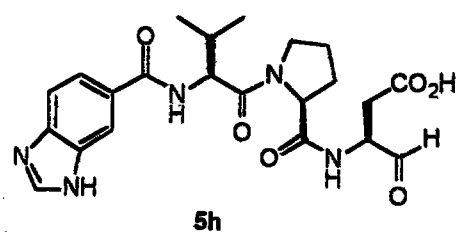
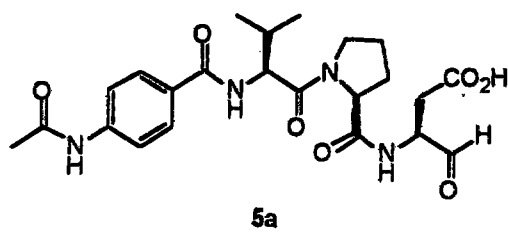
Ex.	Yield (mg)	HPLC Gradient /time (min)	RT (min)	Purity (%)	Mass Spec (M+H or M+Na)
5ay	10.4	10%-90%/10'	6.52	90	510.3
5az	9.2	10%-90%/10'	5.96	95	522.3
5ba	8.5	10%-90%/10'	6.69	95	562.3
5bb	4.6	10%-90%/10'	6.00	85	520.2
5bc	8.8	10%-90%/10'	4.96	90	546.2
5bd	8.2	10%-90%/10'	8.01	95	536.3
7a	2.1	5-45%/10'	5.28	86	440.2
7b	1.7	5-45%/10'	4.12	94	390.2
7c	0.5	5-45%/10'	4.04	94	434.2
7d	1.1	5-45%/10'	4.29	95	441.2
7e	1.1	5-45%/10'	3.28	98	447.2
7f	1.0	5-45%/10'	3.96	97	420.2
7g	11.0	10%-90%/10'	4.00	95	483.4
7h	6.0	10%-90%/10'	4.90	95	439.3
7i	12.0	10%-90%/10'	6.40	95	474.3
7j	6.6	10%-90%/10'	4.50	95	461.4
7k	4.0	10%-90%/10'	5.40	95	480.4
7l	8.6	10%-90%/10'	2.61	95	435.3
7m	6.0	10%-90%/10'	4.29	95	438.4
7n	15.4	10%-90%/10'	2.00	85	433.4
7o	8.4	10%-90%/10'	4.01	90	470.0
7p	3.0	10%-90%/10'	4.61	95	434.4
7q	5.7	10%-90%/10'	3.89	95	434.3
7r	8.8	10%-90%/10'	2.94	95	425.3
7s	10.5	10%-90%/10'	3.89	90	433.4
7t	6.9	10%-90%/10'	2.45	85	419.3
7u	11.6	10%-90%/10'	1.98	85	433.3
7v	4.6	10%-90%/10'	4.60	95	489.4
7w	13.8	10%-90%/10'	4.20	95	453.3
7x	6.0	10%-90%/10'	3.90	95	483.2
7y	12.0	10%-90%/10'	5.40	95	489.2
7z	10.0	10%-90%/10'	5.40	95	517.2
7aa	11.0	10%-90%/10'	5.30	95	453.8
7ab	10.0	10%-90%/10'	5.60	95	595.1
7ac	3.9	10-60%/10'	4.59	88	469.2
7ad	13.0	5-45%/10'	4.48	88	415.2
7ae	4.9	5-45%/10'	4.37	88	426.2
7af	20.6	5-45%/10'	4.68	86	405.3
7ag	14.9	5-45%/10'	4.78	82	406.3
7ah	9.5	5-45%/10'	7.40	91	447.3 (+Na)
7ai	10.8	5-45%/10'	9.21	95	480.8 (+Na)
7aj	8.3	5-45%/10'	9.14	92	481.1 (+Na)
7ak	13.6	5-45%/10'	8.05	96	464.8 (+Na)
7al	8.1	5-45%/10'	7.04	91	448.8 (+Na)
7am	7.8	5-45%/10'	8.54	90	480.4 (+Na)

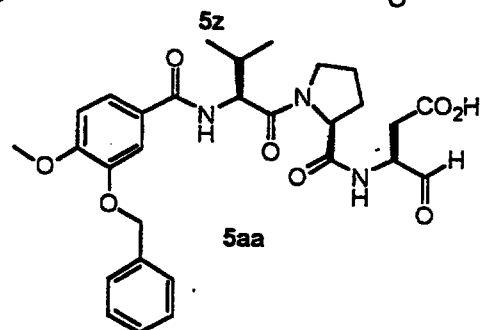
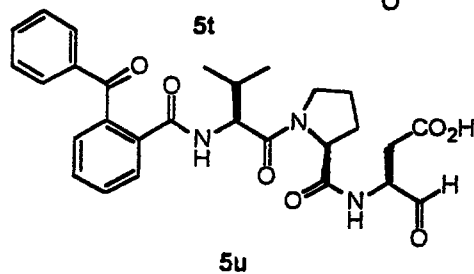
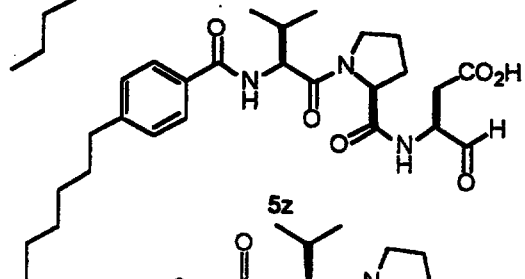
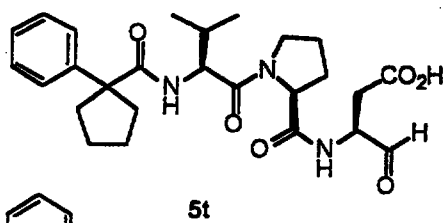
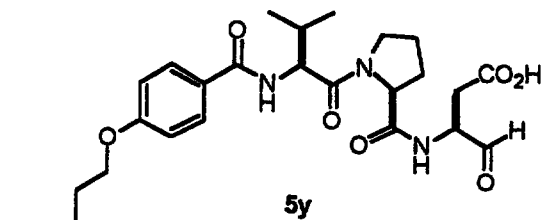
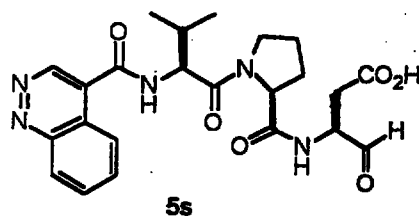
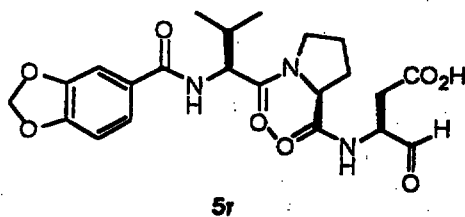
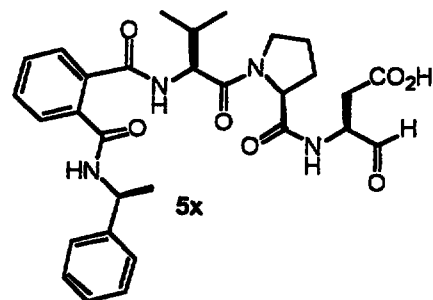
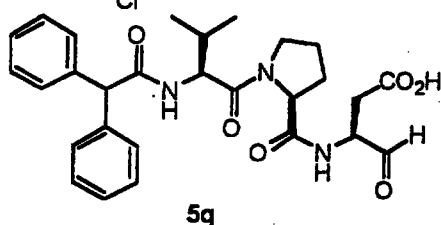
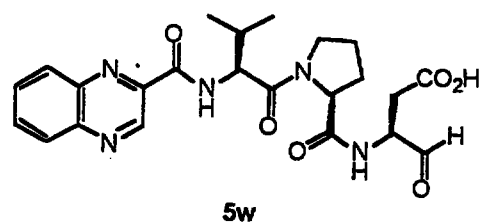
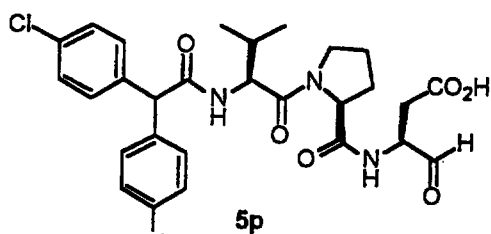
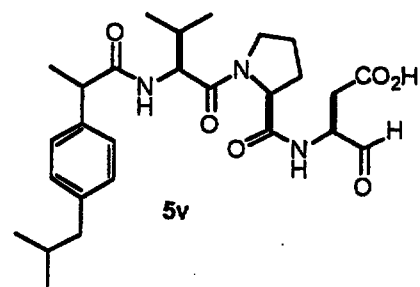
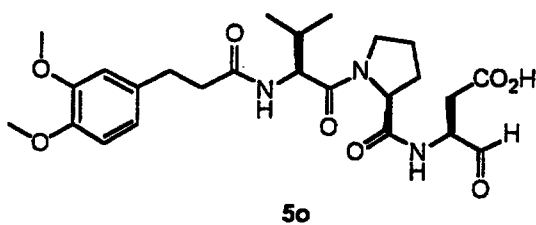
- 60 -

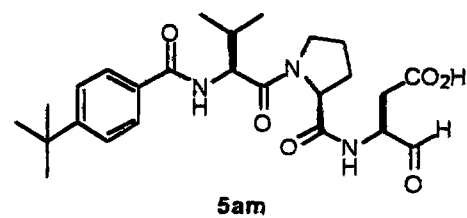
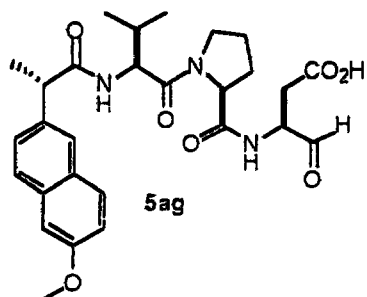
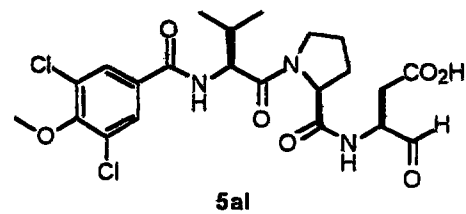
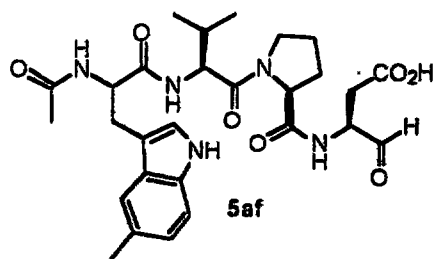
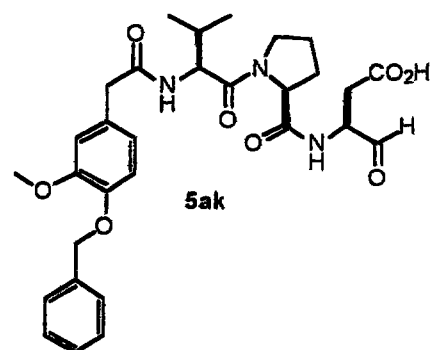
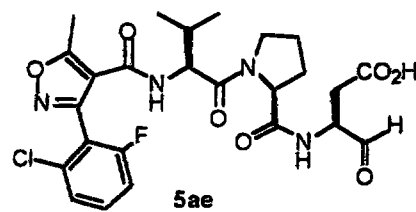
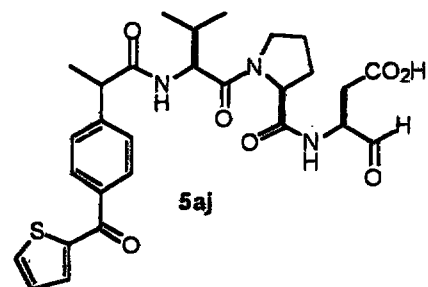
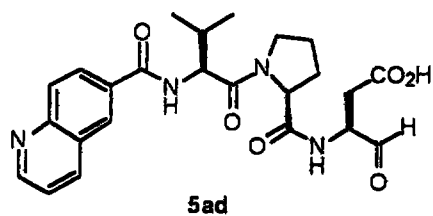
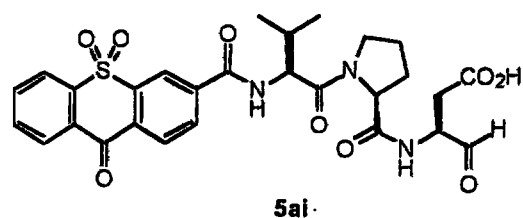
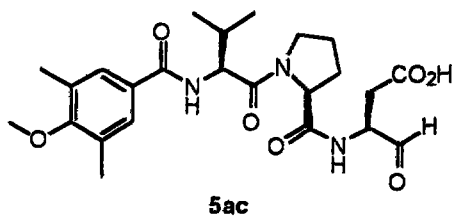
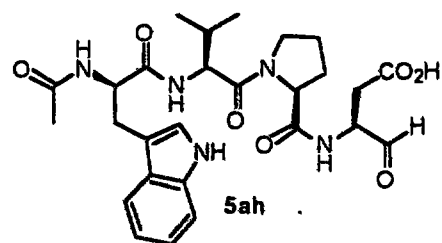
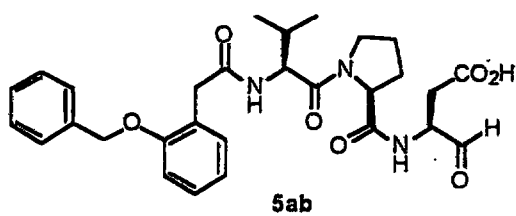
Ex.	Yield (mg)	HPLC Gradient /time (min)	RT (min)	Purity (%)	Mass Spec (M+H or M+Na)
7an	4.3	5-45%/10'	7.48	88	460.9 (+Na)
7ao	13.5	5-45%/10'	7.45	92	460.7 (+Na)
7ap	2.5	5-45%/10'	6.52	93	440.3 (+Na)
7aq	1.4	5-45%/10'	3.30	84	405.2
7ar	15.10	5-45%/10'	3.79	97	413.30
7as	15.70	5-45%/10'	5.50	88	441.30
7at	22.20	5-45%/10'	4.49	94	415.30
9a	0.5	10-60%/10'	7.08	98	527.4 (+Na)
9b	1.2	10-60%/10'	8.31	93	570.5 (+Na)
9c	2.5	10-60%/10'	8.46	97	569.6
9d	1.2	10-60%/10'	7.22	85	562.1
9e	0.4	10-60%/10'	7.86	95	543.2 (+Na)
9f	4.3	10-60%/10'	8.11	96	542.5
9g	2.1	10-60%/10'	7.18	93	526.3
15a	1.0	10%-60%/10'	5.76	95	477.6
15b	3.9	10%-60%/10'	6.32	91	483.8
15c	2.9	10%-90%/10'	3.30	85	463.3
15d	1.3	10%-90%/10'	4.26	95	531
15e	1.0	10%-90%/10'	3.10	85	482.3
15f	1.2	10%-90%/10'	3.60	95	484.6
16a	2.5	10%-90%/10'	2.80	95	456.3
16b	6.0	10%-90%/10'	1.90	95	454.2
17a	1.0	10%-90%/10'	3.30	85	496.8
17b	1.1	10%-90%/10'	3.62	85	498.3
17c	2.3	10%-90%/10'	5.80	95	573.2
17d	1.6	10%-90%/10'	1.60	95	525.1
17e	1.5	10%-90%/10'	2.62	95	526.3
18a	1.0	10%-90%/10'	3.90	95	528.3
18b	1.2	10%-90%/10'	5.27	95	562.3
18c	2.2	10%-90%/10'	4.09	95	499.2
18d	1.5	10%-90%/10'	3.78	95	498.3
18e	1.2	10%-90%/10'	4.78	95	541.3
18f	7.1	10%-90%/10'	3.84	98	526.1
20a	2.0	5-45%/10'	6.95	91	483.2
20b	1.3	5-45%/10'	7.58	99	482.2
20c	2.5	10%-90%/10'	5.89	85	483.3
20d	4.3	10%-90%/10'	4.09	90	471.3
20e	3.6	10%-90%/10'	4.65	95	455.3
20f	12.2	10%-90%/10'	3.25	95	518.3
20g	12.1	10%-90%/10'	5.01	90	487.2
20h	3.3	10%-90%/10'	4.30	90	533.2
20i	5.0	10%-90%/10'	4.16	90	485.2
20j	1.3	10%-90%/10'	3.45	85	531.2
20k	9.7	10%-90%/10'	5.41	90	516.2
20l	3.8	10%-90%/10'	3.73	85	504.2

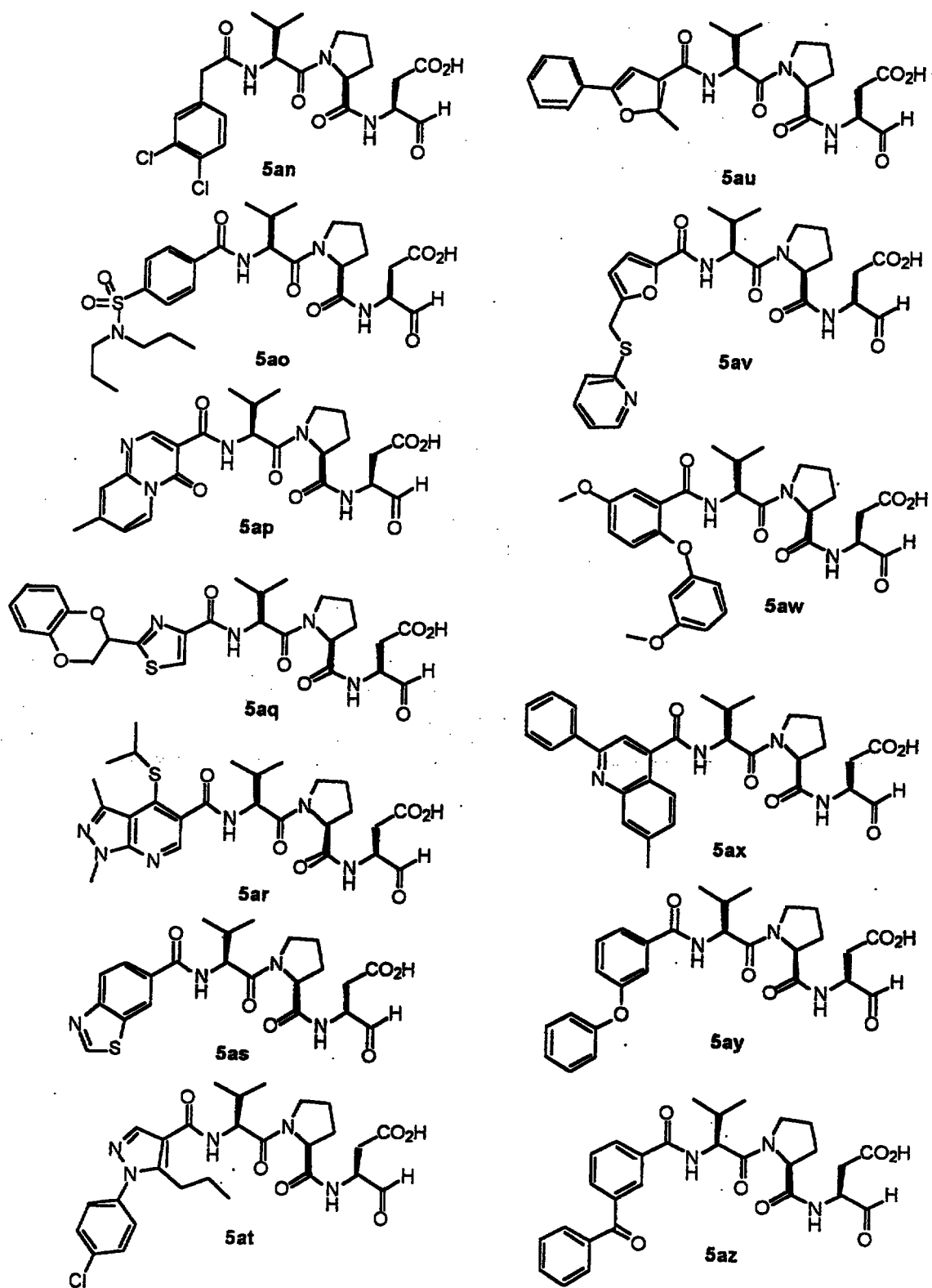
Ex.	Yield (mg)	HPLC Gradient /time (min)	RT (min)	Purity (%)	Mass Spec (M+H or M+Na)
20m	6.6	10%-90%/10'	4.52	90	488.2
20n	1.8	10%-90%/10'	2.85	90	551.2
20o	7.0	10%-90%/10'	4.70	95	520.2
20p	1.2	10%-90%/10'	4.00	95	566.2
20q	2.3	10%-90%/10'	4.93	95	481.3
20r	3.6	10%-90%/10'	4.45	95	519.3
20s	3.0	10%-90%/10'	4.18	95	552.2
20t	6.0	10%-90%/10'	3.80	90	517.4
23a	21.0	10-60%/10'	8.11	99	495.4
23b	25.0	10-60%/10'	8.94	99	539.8 (+Na)
23c	26.0	10-60%/10'	8.55	99	539.9 (+Na)
23d	12.6	10%-90%/10'	3.90	85	453.3
23e	8.3	10%-90%/10'	5.16	85	501.3
23f	12.5	10%-90%/10'	3.41	80	425.3
23g	1.5	10%-90%/10'	3.34	85	452.3
23h	8.4	10%-90%/10'	3.84	90	451.3
23i	9.0	10%-90%/10'	3.78	85	469.4
24a	1.1	10-60%/12'	8.76	90	480.5
24b	3.1	10-60%/10'	5.14	90	375.4
24c	7.2	10-60%/10'	10.33	96	531.0
24d	3.4	10-60%/10'	6.51	95	426.5 (+Na)
24e	6.9	10-60%/10'	7.22	99	455.5
25a	1.9	5-45%/10'	5.38	85	455.2
25b	1.5	5-45%/10'	6.90	97	483.2
25c	1.0	5-45%/10'	8.09	94	497.2
25d	12.8	5-45%/10'	5.75	88%	453.3
25e	9.5	5-45%/10'	7.76	90%	495.2
26a	10.2	5-45%/10'	7.36	95	455.1 (+Na)
26b	1.1	5-45%/10'	7.38	89	476.3
26c	13.8	5-45%/10'	8.13	98	483.2
26d	2.3	5-45%/10'	10.35	99	503.0
26e	12.8	5-45%/10'	11.11	99	523.2
26f	13.2	10-60%/10'	12.11	99	545.0
26g	0.7	10-60%/10'	10.89	87	523.2
26h	4.4	10-60%/10'	11.62	99	545.8
27a	5.0	10%-90%/10'	4.42	95	475.3
27b	16.4	10-60%/10'	5.20	92	505.1
27c	2.7	5-45%/10'	7.50	82	476.6 (+Na)
27d	1.6	5-45%/12'	8.70	90	503.2
27e	4.4	5-45%/12'	7.80	82	489.2
27f	1.2	5-45%/12'	6.95	85	476.3
27g	2.5	5-45%/12'	6.67	82	510.1
27h	1.1	5-45%/12'	8.49	95	524.1
27i	0.9	5-45%/12'	7.34	90	484.3
27j	4.3	5-45%/12'	5.77	82	470.3

Ex.	Yield (mg)	HPLC Gradient /time (min)	RT (min)	Purity (%)	Mass Spec (M+H or M+Na)
27k	1.3	5-45%/12'	5.33	95	551.1
27l	16.6	5-45%/10'	5.90	91	477.2
27m	7.0	5-45%/10'	7.70	93	494.2
27n	15.1	5-45%/10'	3.99	86	466.2
28a	1.2	5-45%/10'	5.91	86	487.1
28b	0.5	5-45%/10'	6.86	98	486.1
28c	1.5	5-45%/10'	7.47	93	515.1
29a	4.5	5-45%/12'	8.21	98	392
29b	28.0	5-45%/12'	11.80	96	443.3
29c	1.7	5-45%/10'	3.73	97	415.2
29d	1.7	5-45%/10'	4.62	89	414.2
29e	0.6	5-45%/10'	4.94	85	436.2
29f	1.1	5-45%/10'	6.23	97	442.2
29g	1.7	5-45%/10'	6.39	90	457.2
29h	0.7	5-45%/10'	3.56	92	408.2
29i	0.7	5-45%/10'	6.50	96	431.2
29j	0.4	5-45%/10'	7.24	89	445.2
29k	1.6	5-45%/10'	7.07	90	456.2
29l	0.9	5-45%/10'	3.08	99	408.2
29m	1.5	10-60%/10'	6.68	90	406.3
29n	6.4	10-60%/10'	4.27	87%	422.3
29o	8.4	10-60%/10'	4.42	86%	422.3
29p	13.2	10-60%/10'	5.62	83%	450.3
29q	15.1	5-45%/10'	3.79	97%	413.3
29r	15.7	5-45%/10'	5.50	88%	441.3
29s	19.9	5-45%/10'	4.30	95%	394.3
32a	7.7	5-60%/20'	14.2	90	469.3
32b	4.0	5-60%/20'	13.4	85	485.1 (+Na)
32c	2.5	5-60%/20'	11.1	90	459.3
32d	3.0	5-60%/20'	8.19	95	471.3
32e	4.9	5-60%/20'	14.2	90	486.2

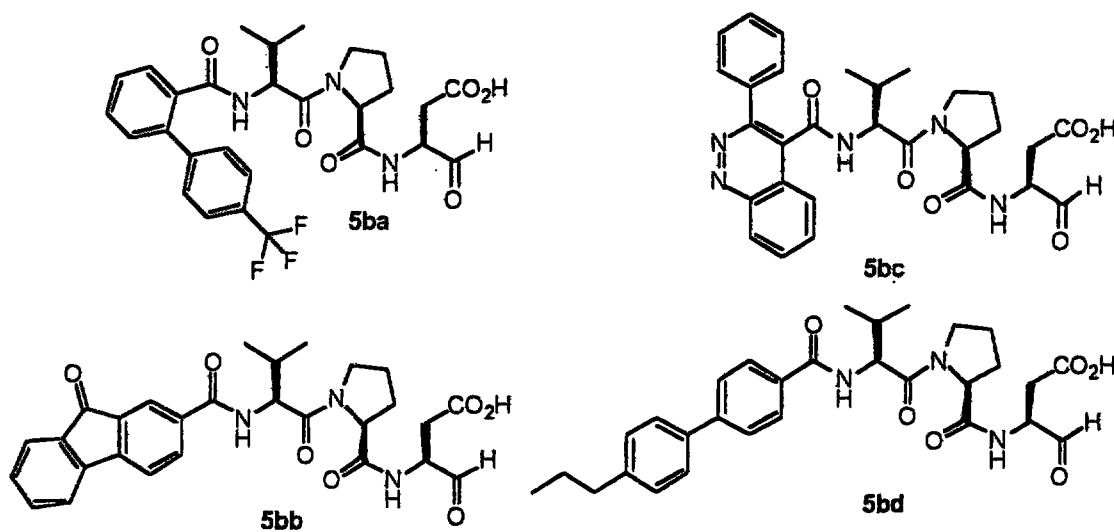








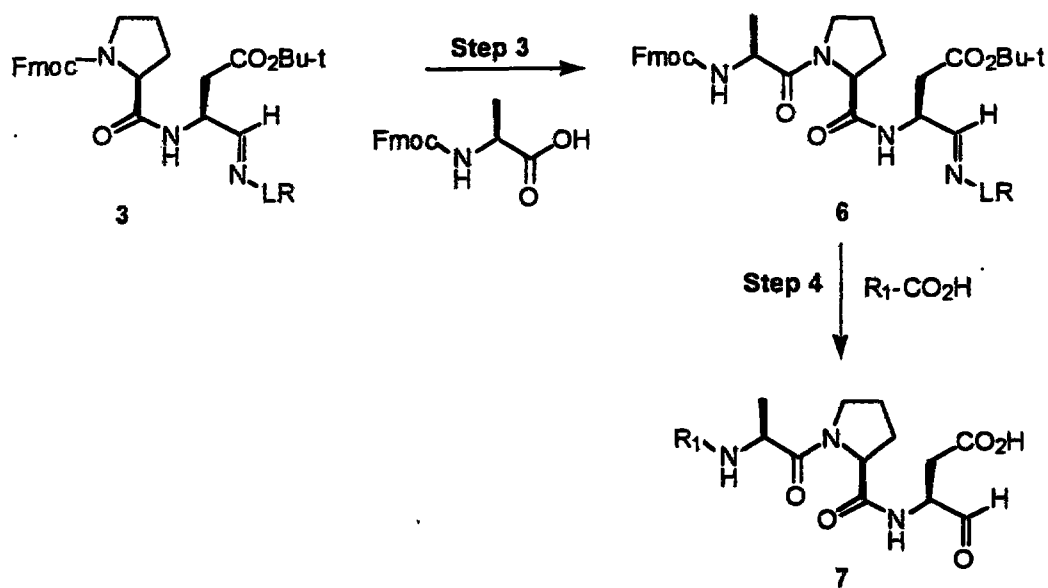
- 67 -



Procedure for the preparation of analogs 7a-7at.

5

Scheme II



Analogues of 7a-7at were prepared as described above in Scheme I only substituting Fmoc-alanine for Fmoc-valine in Step 3 (Scheme II).

10

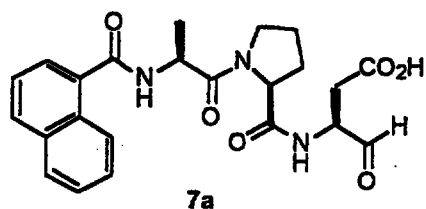
- 68 -

Step 3: A suspension of resin 3 (3.5 g, 1.75 mmol) in 20 mL of a 20% solution of piperidine in DMF was rotated at room temperature for 5 minutes. The suspension was drained. The procedure was repeated over 5 20 minutes. The resin was washed successively with DMF (2 x 30 mL), CH₃OH (30 mL), CH₂Cl₂ (2 x 30 mL), CH₃OH (30 mL) and NMP (2 x 30 mL). To a suspension of resin in 30 mL of NMP was added successively 1.44 g of N-Fmoc-alanine (4 eq, 7.0 mmol), 2.4 mL of DIEA (8 eq, 10 14.0 mmol), 0.95 g of HOBt (4 eq, 7.0 mmol) and 2.66 g of HBTU (4 eq, 7.0 mmol). The mixture was rotated at room temperature overnight and drained. This coupling procedure was repeated over 3 hours. The resin was then washed successively with DMF (2 x 30 mL), CH₃OH 15 (30 mL), 1:1 DMF/ CH₂Cl₂ (2 x 30 mL), CH₃OH (30 mL) and CH₂Cl₂ (3 x 30 mL), and dried *in vacuo* to afford resin 6 (0.50 mmol/gram).

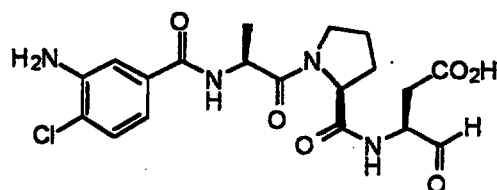
Step 4: To a 0.125 mmol portion of resin 6 was added 5 20 mL of a 20% solution of piperidine in DMF. The suspension was rotated at room temperature for 5 minutes, and drained. The procedure was repeated over 20 minutes. The resulting resin was washed successively with DMF (3 x 5 mL), CH₃OH (5 mL), and NMP 25 (3 x 5 mL). The desired carboxylic acid was then added (4 eq, 0.6 mmol), followed by 2.0 mL of a 0.25M solution of HOBt in NMP, 0.35 mL of DIEA (8 eq, 1.0 mmol) and 2.0 mL of a 0.25M solution of HBTU in NMP. The mixture was rotated at room temperature overnight 30 and drained. The resin was washed successively with DMF (3 x 5 mL), CH₃OH (5 mL), 1:1 DMF/ CH₂Cl₂ (2 x 5 mL), CH₃OH (5 mL) and CH₂Cl₂ (3 x 5 mL), and dried *in vacuo*. A 5 mL portion of a 95% solution of TFA in water was then added to the resin. The mixture was

- 69 -

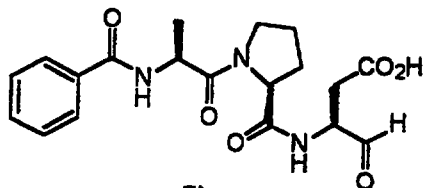
stirred at room temperature for one hour, and filtered. The filtrate was evaporated, and the residue was dissolved in acetonitrile-water and purified by preparative HPLC to afford compounds 7a-7at.



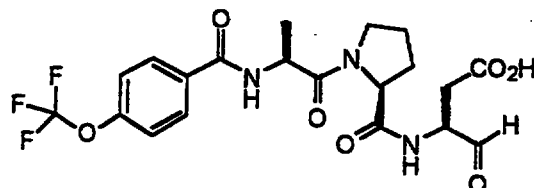
7a



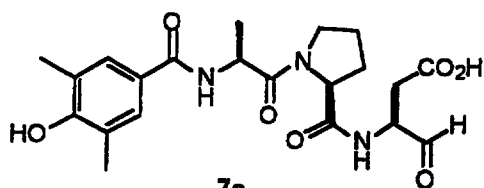
7h



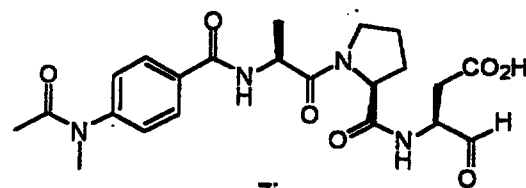
7b



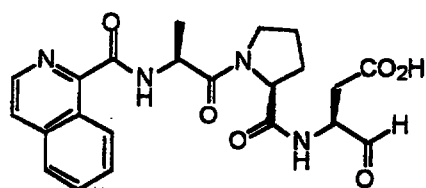
7i



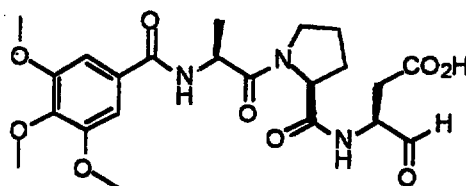
7c



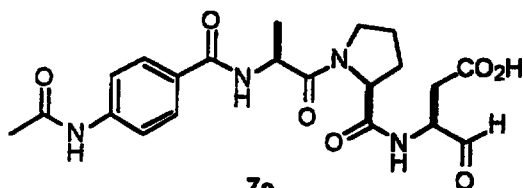
7j



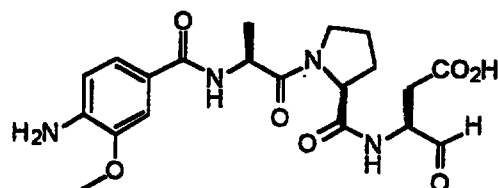
7d



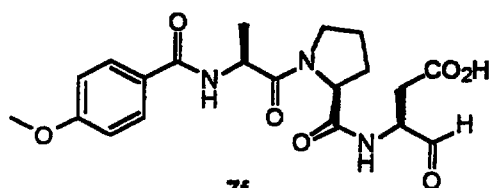
7k



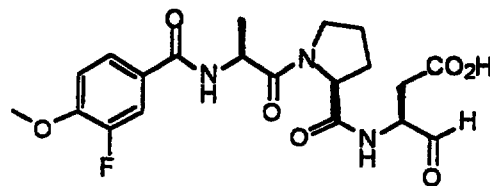
7e



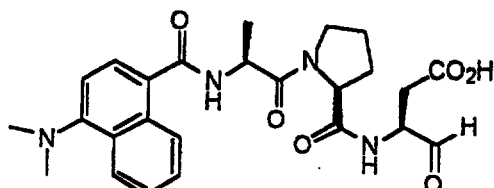
71



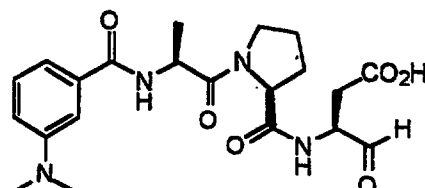
7f



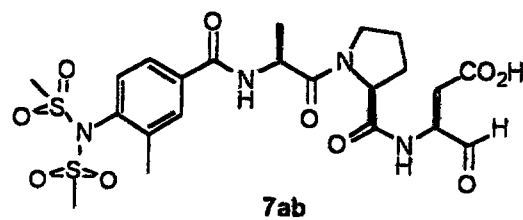
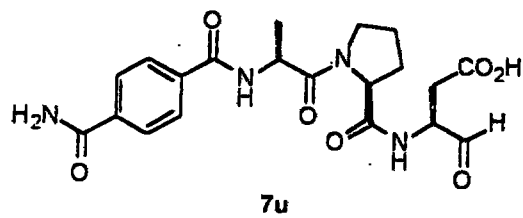
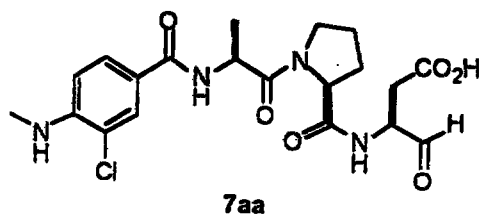
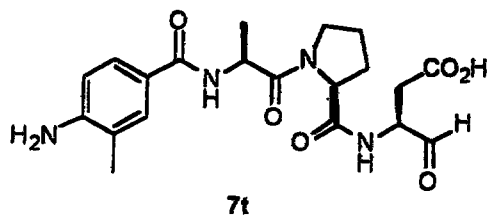
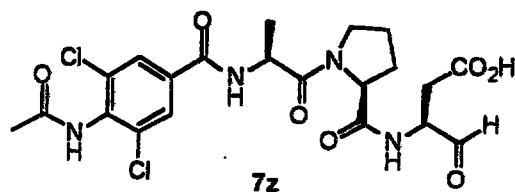
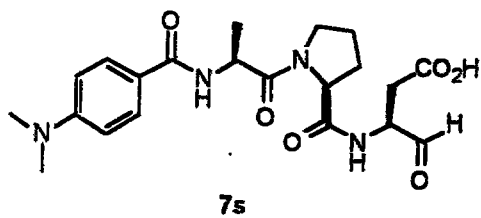
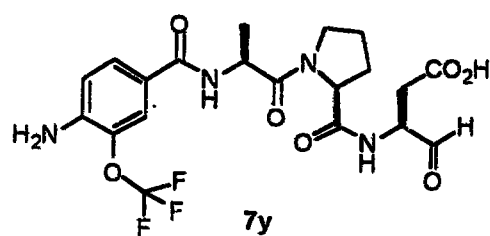
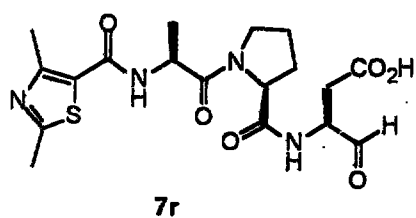
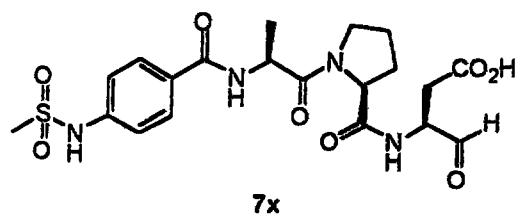
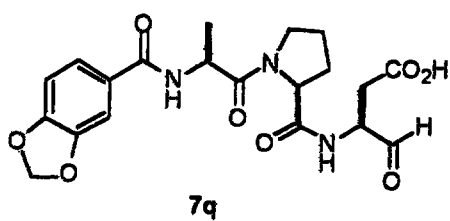
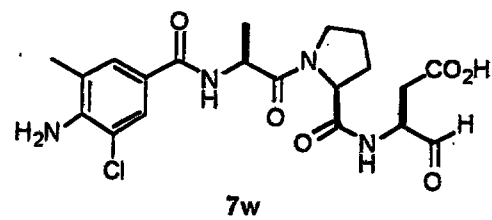
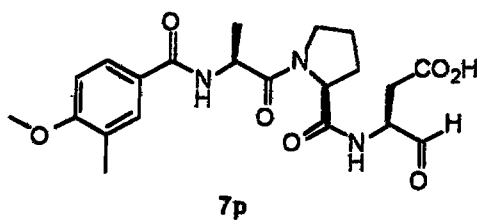
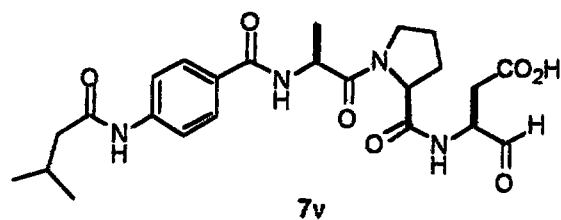
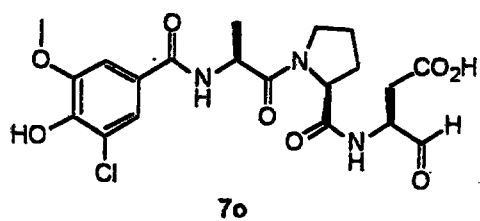
7m

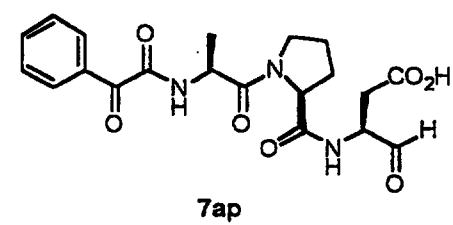
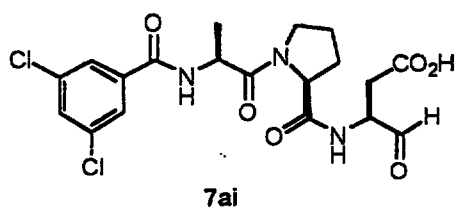
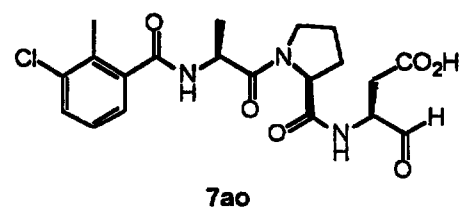
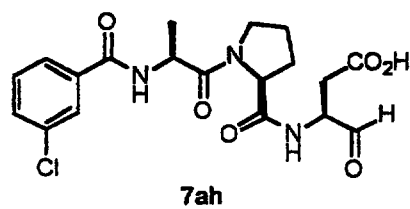
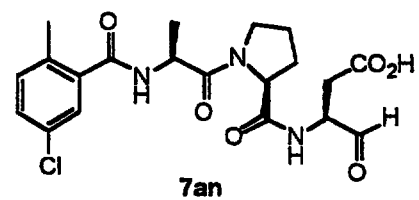
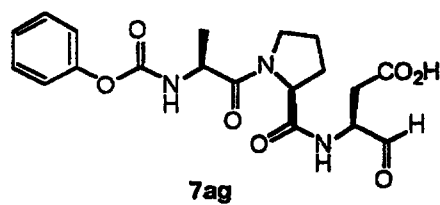
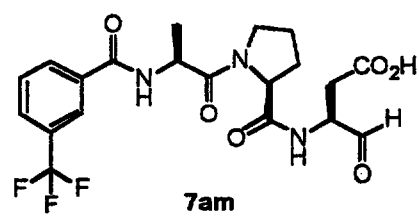
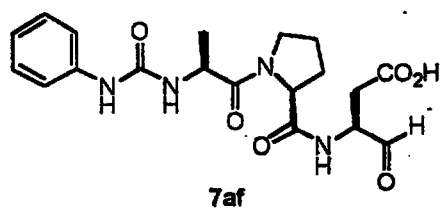
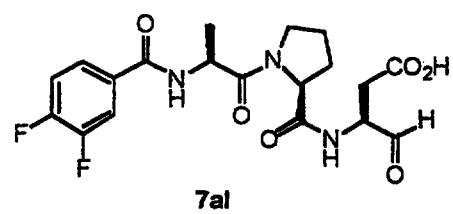
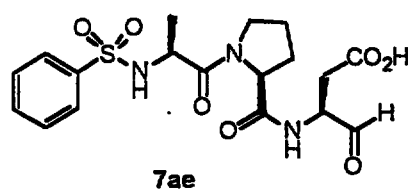
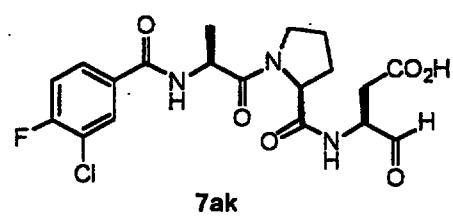
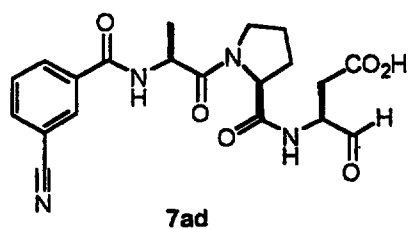
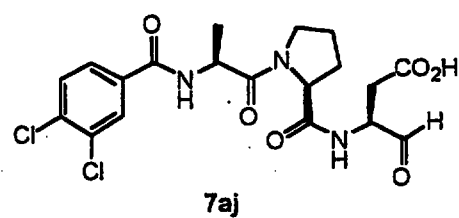
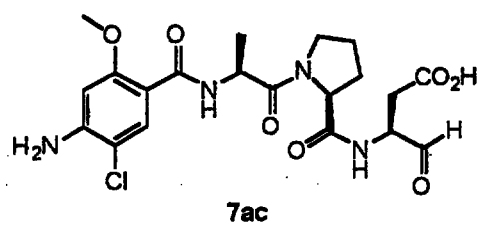


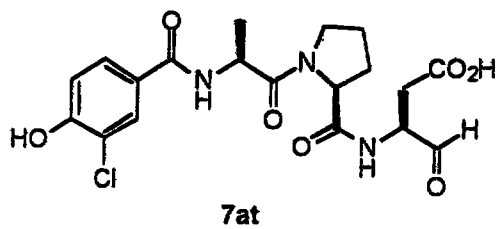
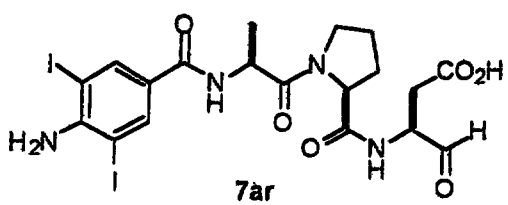
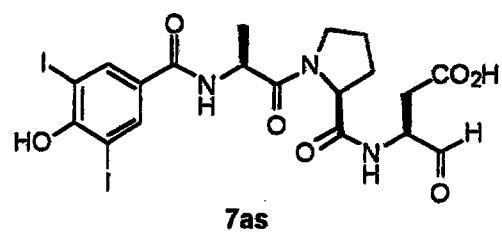
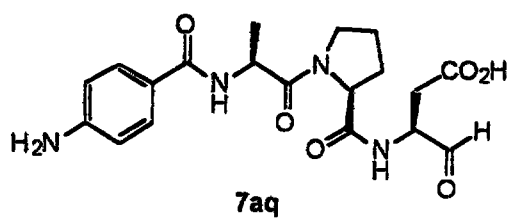
7g



7n

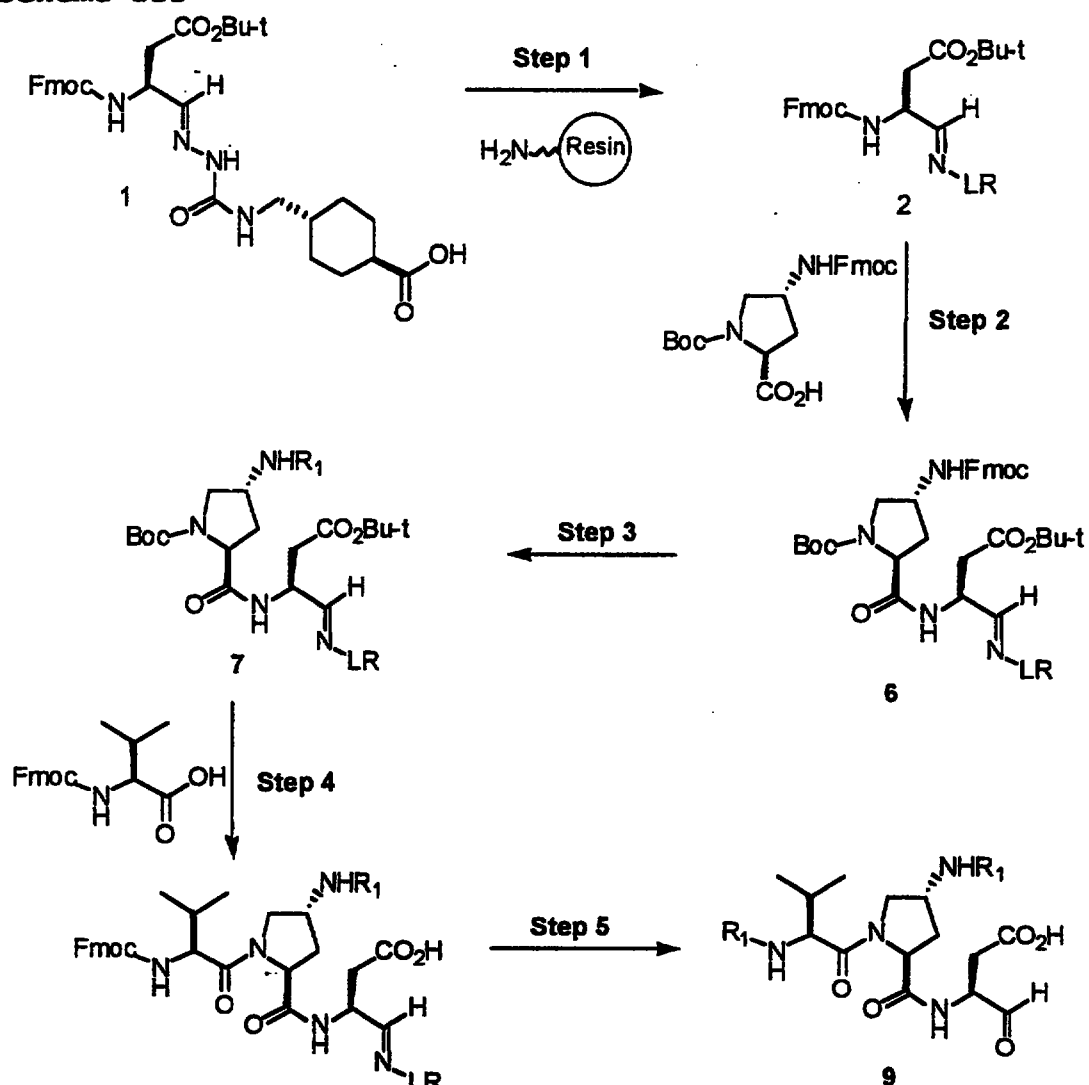






Procedure for the preparation of analogs 9a-9g.

Scheme III



5 **Step 1:** A 10.0 g portion (0.75 mmol/gram loading, 7.5 mmol) Agropore-aminomethyl resin (catalog number 800047) was washed with DMF (3 x 40 mL), 10% DIEA/DMF (3 x 40 mL), DMF and NMP (3 x 40 mL). To above resin was added successively compound 1 (0.87 eq, 3.88 g, 6.55 mmol), HBTU (1.14 eq, 3.13 g, 8.25 mmol), HOBT

- 75 -

(1.14 eq, 1.26 g, 8.25 mmol), and NMP (40 mL). The reagents were then mixed by bubbling nitrogen through the bottom of the flask for two minutes at room temperature. N,N-diisopropylethylamine (3.33 eq, 4.35 mL, 25 mmol) was added and the resulting suspension mixed at room temperature overnight, filtered, then washed successively with NMP (3x 40 mL) and DMF (3x 40 mL). The resin was then treated with 50 mL of a 20% solution of acetic anhydride in DMF for 38 minutes at room temperature. The mixture was filtered, and the resin was washed successively with NMP (3x 40 mL) CH₂Cl₂ (3x 40 mL), 1:1 CH₃OH / CH₂Cl₂ (3x 40 mL), and CH₃OH (3x 40 mL). After drying in vacuo, 13.76 grams of resin 2 were obtained (0.35 mmol/gram loading).

Step 2: Seven reaction vessels were each charged with 181 mg of resin 2 (0.48 mmol/gram, 0.063 mmol) then washed with CH₂Cl₂ (3 x 1 mL) and NMP (3 x 1 mL). Then each vessel was treated with 1 mL of a 25% solution of piperidine in DMF and mixed (vortex) at room temperature for 15 minutes. This procedure was repeated in triplicate. Each vessel was then washed three times with NMP (3 x 1 mL). The vessels were then treated with 500 μ l of a solution of 0.4 M (2S,4R)-Fmoc-4-amino-1-Boc-pyrrolidine-2-carboxylic acid /0.4 M HOBt/ NMP, 500 μ l of a solution of 0.4 M HBTU/ NMP, and 250 μ l of a solution of 1.6 M DIEA/ NMP and mixed for 3 hours at room temperature. After mixing, the vessels were drained and the procedure was repeated.

Step 3: The resulting resin was washed with NMP (3 x 1mL) and then treated with 1 mL of a 25% solution of piperidine in DMF and mixed (vortex) at room temperature for 15 minutes. The procedure was repeated

- 76 -

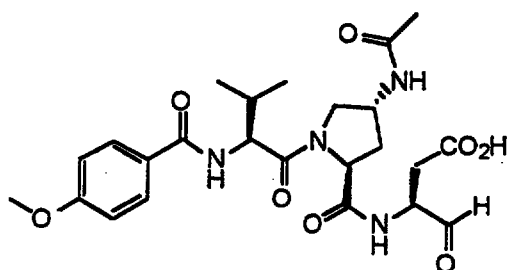
in triplicate. The resulting resin was washed with NMP (3 x 1mL) then treated with either acetic anhydride, or isopropyl isocyanate, or methane sulphonyl chloride, or methyl chloroformate. For acetic anhydride: add 300 μ l of a 1.6 M DIEA/ NMP solution and 1 mL of a solution of 0.5 M acetic anhydride/0.125 M DIEA/ 0.015 M HOBt in NMP. For isopropyl isocyanate: add 300 μ l of a 1.6 M DIEA/ NMP solution and 1 mL of a solution of 1 M isopropyl isocyanate in NMP. For methane sulphonyl chloride: add 600 μ l of a solution of 1 M pyridine in CH_2Cl_2 and 600 μ l of a solution of 1M methane sulphonyl chloride in CH_2Cl_2 . For methyl chloroformate: add 500 μ l of a 1.6 M DIEA/ NMP solution and 1 mL of a solution of 0.7 M methyl chloroformate in CH_2Cl_2 . The resulting suspensions were mixed for 6 hours at room temperature, the solvent drained and the coupling procedure repeated.

Step 4: The resulting resin was washed with NMP (3 x 1mL) then treated with a 1:1 mixture of TFA/ CH_2Cl_2 at room temperature for 30 minutes. The resulting resin was then washed with CH_2Cl_2 (3 x 1mL) and NMP (3 x 1mL). The resin was then treated with 500 μ l of a solution of 0.4 M Fmoc-valine-carboxylic acid/0.4 M HOBt/ NMP, 500 μ l of a solution of 0.4 M HBTU/ NMP, and 250 μ l of a solution of 1.6 M DIEA/ NMP and mixed for 3 hours at room temperature. After mixing, the vessels were drained and the coupling procedure was repeated.

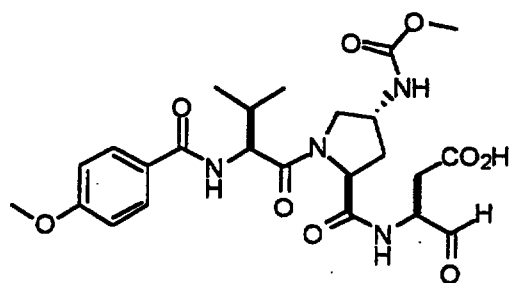
Step 5: The resulting resin was washed with NMP (3 x 1mL) then treated with 1 mL of a 25% solution of piperidine in DMF and mixed (vortex) at room temperature for 15 minutes. This procedure was repeated in triplicate. The resulting resin was washed with NMP

- 77 -

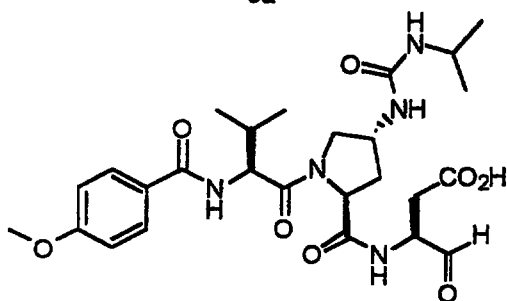
(3 x 1mL) then treated with either 500 μ l of a solution of 0.4 M 1-isoquinoline carboxylic acid/0.4 M HOBt/ NMP or 500 μ l of a solution of 0.4 M p-anisic acid/0.4 M HOBt/ NMP. The resulting mixtures were treated with
5 500 μ l of a solution of 0.4 M HBTU/ NMP and 250 μ l of a solution of 1.6 M DIEA/ NMP then mixed for 3 hours at room temperature, the solvent drained and the procedure repeated. The resulting resin was treated with 1.5 mL of a 95% solution of TFA in water and stirred at room
10 temperature for one hour then filtered. The filtrate was evaporated, and the residue was taken up in a 2:1:2 mixture of DMF /acetonitrile/water and purified by preparative HPLC to afford compounds 9a-9g.



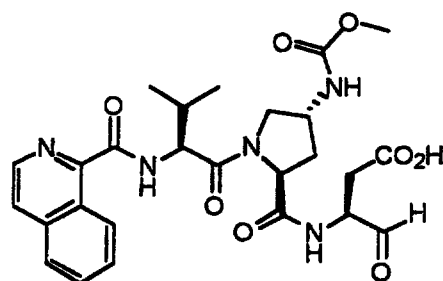
9a



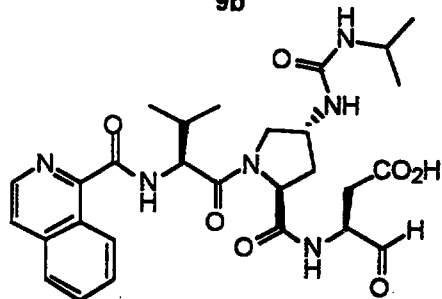
9e



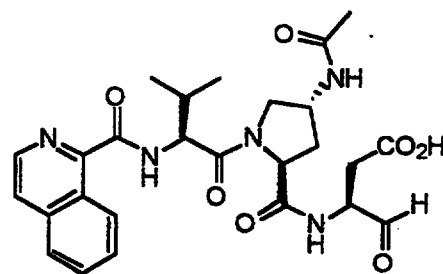
9b



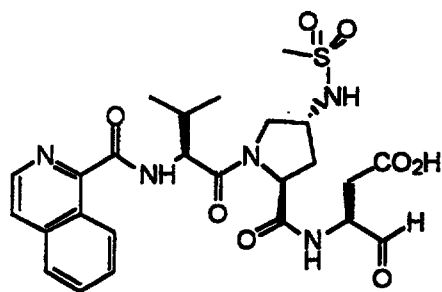
9f



9c



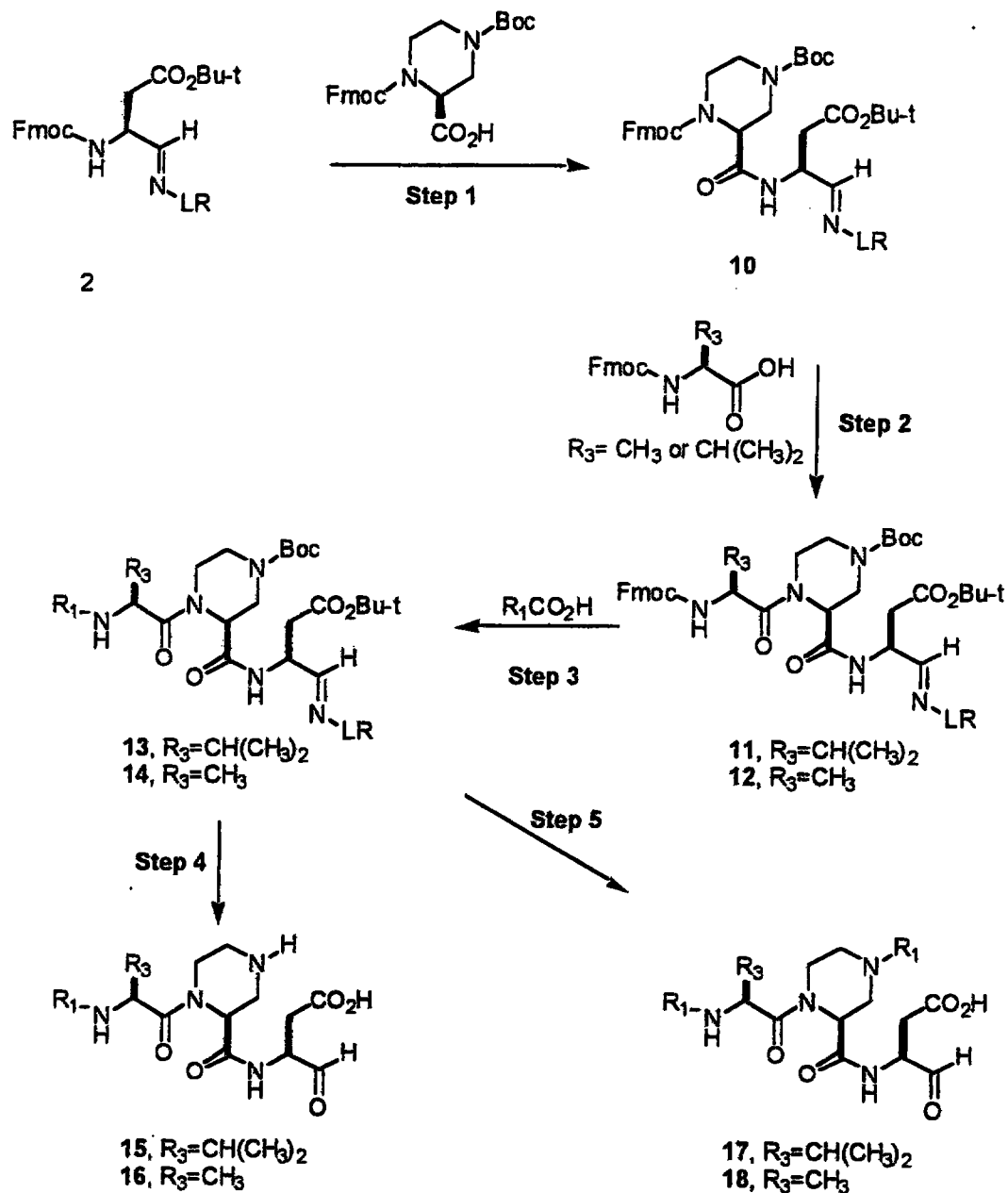
9g



9d

Procedure for the synthesis of analogs 15-18:

Scheme IV



Preparation of analogs 15 and 16 (Scheme IV):

- 5 Synthesis of 2-(S)-piperazine-1,2,4-tricarboxylic acid 4-tert-butyl ester 1-(9H-fluoren-9-ylmethyl) ester.

- 80 -

To a solution of 2-(S)-piperazine carboxylic acid (Lonza) (3g, 15 mmol) in 1:1 H₂O:dioxane (30 mL) was added a solution of (Boc)₂O in dioxane (3.3g, 15mmol, in 5mL dioxane) while maintaining the pH at 11 with 1N NaOH . The pH was maintained over 3 hours at room temperature. The solution was adjusted to pH9.5 with 1N HCl, cooled to 0°C and treated with Fmoc-Cl (3.87g, 15 mmol). The pH was maintained at 9.5 for 1 hour and the mixture stirred at room temperature overnight. The resulting suspension was filtered and the filtrate treated with 1N KHSO₄ to pH 2 then extracted with ethyl acetate (2 x 75 mL). The organic layer was dried with brine and MgSO₄ filtered, and concentrated to give colorless oil. The oil was dissolved in ethyl acetate and added to hexane to give 3.5g (51% yield) of white solid after isolation. ¹H NMR (500 MHz, DMSO-d₆) δ 1.55 (s, 9H) 2.80- 3.5 (m, 3H), 3.8-4.9 (m, 5H), 5.7 (bs, 1H), 7.3 (m, 2 H), 7.3- 7.9 ppm (m, 8H), LC/MS (ES⁻) m/e 451.3 (M-H)

20

Step 1: To 5 g of resin 2 (0.375 mmol/gram 1.82 mmol) was added 25 mL of a 20% solution of piperidine in DMF. The suspension was rotated at room temperature for 5 minutes and drained. The procedure was repeated over 20 minutes. The resin was then washed successively with DMF (2 x 50 mL), CH₃OH (50 mL), CH₂Cl₂ (2 x 50 mL), CH₃OH (50 mL) and NMP (50 mL). To a suspension of resin in 25 mL of NMP was added successively 3.5g of N-Fmoc-Boc piperazine carboxylic acid (4 eq, 7.48 mmol), 1.0 mL of DIEA (8 eq, 14.96 mmol), 1.01 g of HOBt (4 eq, 7.48 mmol) and 2.83g of HBTU (4 eq, 7.48 mmol). The mixture was rotated at room temperature overnight and drained. This coupling procedure was repeated over 3 hours. The resin was then washed successively with

30

- 81 -

DMF (2 x 50 mL), CH₃OH (50 mL), 1:1 DMF/ CH₂Cl₂ (2 x 50 mL), CH₃OH (1 x 50 mL) and CH₂Cl₂ (3 x 50 mL), and briefly dried in vacuo to afford resin 10.

5 Step 2: To 5 g (0.335 mmol/gram loading, 1.675 mmol) of
10 resin 10 was added 25 mL of a 20% solution of piperidine in
DMF. The suspension was rotated at room temperature
for 5 minutes and drained. The procedure was repeated
over 20 minutes. The resin was then washed
10 successively with DMF (2 x 50 mL), CH₃OH (50 mL), CH₂Cl₂
(2 x 50 mL), CH₃OH (50 mL) and NMP (2 x 50 mL). To a
suspension of resin in 25 mL of NMP was added
successively 2.08g of N-Fmoc-valine or N-Fmoc-alanine
(4 eq, 6.7 mmol), 1.17mL of DIEA (4 eq, 6.7 mmol),
15 0.905 g of HOBt (4 eq, 6.7mmol) and 1.38 g of HBTU (4
eq, 3.66 mmol). The mixture was rotated at room
temperature overnight and drained. This coupling
procedure was repeated over 3 hours. The resin was
then washed successively with DMF (2 x 50 mL), CH₃OH
20 (50 mL), 1:1 DMF/ CH₂Cl₂ (2 x 50 mL), CH₃OH (50 mL) and
CH₂Cl₂ (3 x 50 mL), and dried in vacuo to afford resin
11 or 12 respectively (0.35 mmol/gram, 5g).

25 Step 3: To a 1.5g (0.165 mmol) portion of resin 11 or
12 was added 2 mL of a 20% solution of piperidine in
DMF. The suspension was rotated at room temperature
for 5 minutes, and drained. The procedure was repeated
over 20 minutes. The resulting resin was washed
successively with DMF (3 x 15 mL), CH₃OH (15 mL), and
30 NMP (3 x 15 mL). The desired carboxylic acid was then
added (4 eq, 0.66 mmol), followed by 0.25g HOBt
(0.66mmol), 0.12 mL of DIEA (4 eq, 0.66 mmol) and 0.89g
(0.66mmol) HBTU in NMP. The mixture was rotated at
room temperature overnight and drained. The resin was

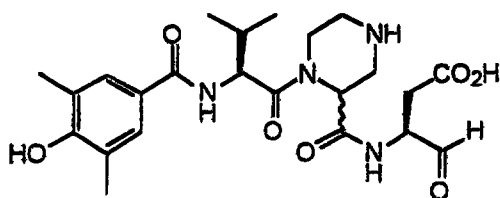
- 82 -

washed successively with DMF (2 x 15 mL), CH₃OH (15 mL), 1:1 DMF/ CH₂Cl₂ (2 x 15 mL), CH₃OH (15 mL) and CH₂Cl₂ (3 x 15 mL), and dried in vacuo to afford 13 or 14.

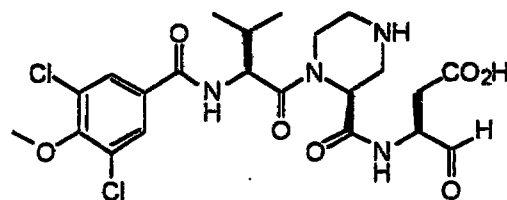
5

Step 4: A 2 mL portion of a 95% solution of TFA in water was then added to the resin. The mixture was stirred at room temperature for one hour, and filtered. The filtrate was evaporated, and the residue was taken up in acetonitrile-water and purified by preparative HPLC to afford compounds 15 and 16.

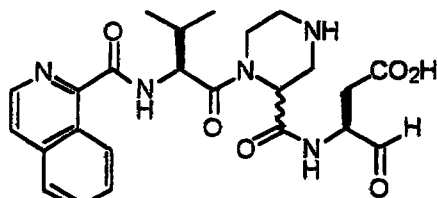
10



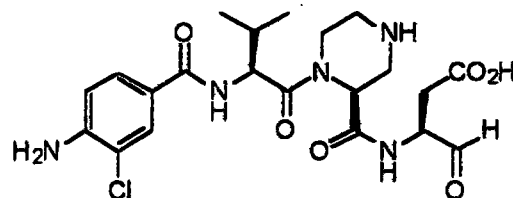
15a



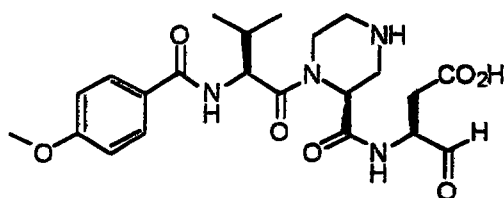
15d



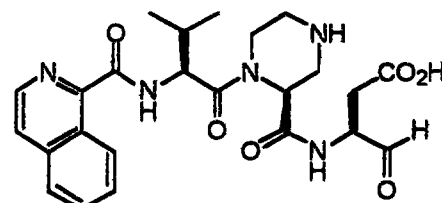
15b



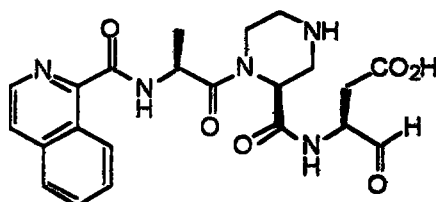
15e



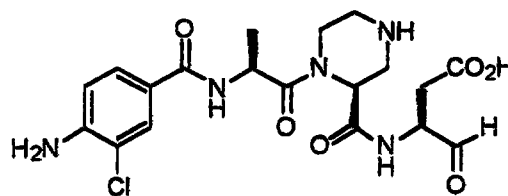
15c



15f



16a



16b

Procedure for the synthesis of analogs 17 and 18 (see Scheme IV):

5

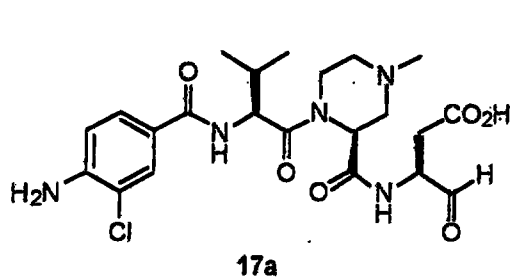
Step 5: Resin 13 or 14 was treated with 2 mL 25% TFA/CH₂Cl₂ for 30 min and washed with DMF (2 x 5 mL), 10% DIEA/CH₂Cl₂ (2 x 5 mL) DMF/CH₂Cl₂ (2 x 5 mL), CH₃OH (5 mL) and CH₂Cl₂ (3 x 5 mL) and dried for five minutes. The resulting resin was washed with NMP (3 x 1 mL) then treated with acetic anhydride, or methoxacetic

10

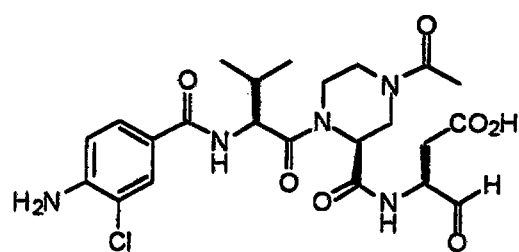
- 84 -

acid, or 2-propanesulfonyl chloride, or isopropyl isocyanate, or methane sulphonyl chloride, or methyl chloroformate according to the procedure used to prepare analogs 9 (Scheme III). Compounds 17 and 18
5 were obtained as described in Step 4 for compounds 15 and 16.

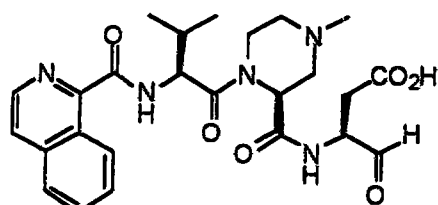
Compounds 17a and 17b were prepared by reductive amination using $\text{Na(OAc)}_3\text{BH}$ and HCHO (38% in H_2O , 0.2
10 mL) and CH_3COOH (0.02 mL) prior to Step 4 and compound 18c was prepared by treatment with phosgene followed by ammonia prior to Step 4.



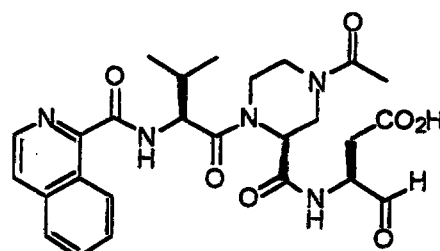
17a



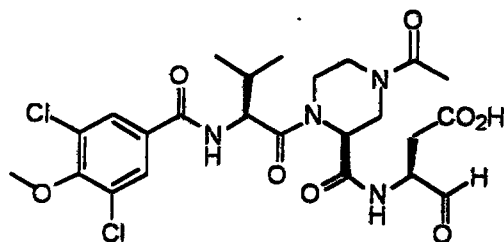
17d



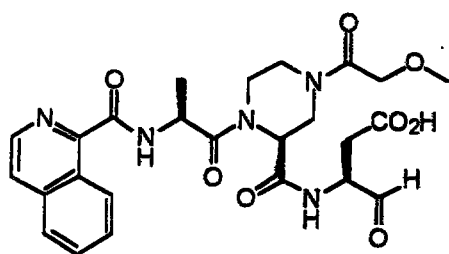
17b



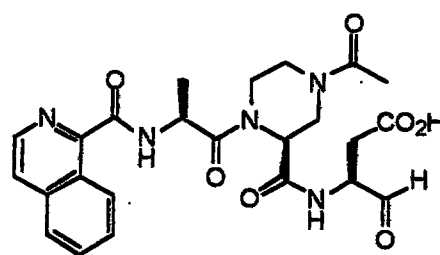
17e



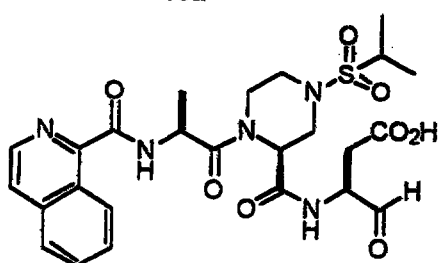
17c



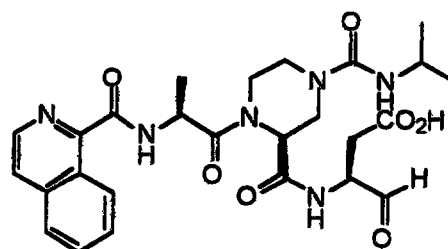
18a



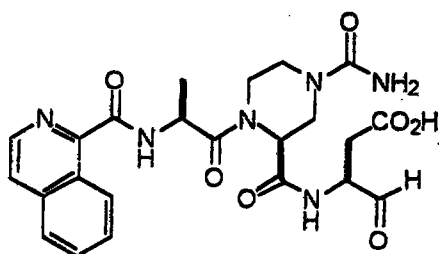
18d



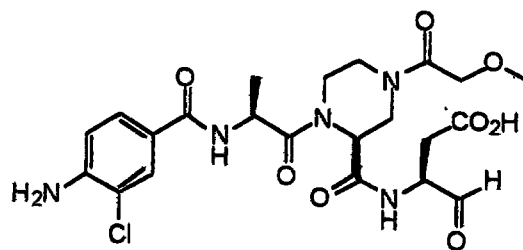
18b



18e



18c

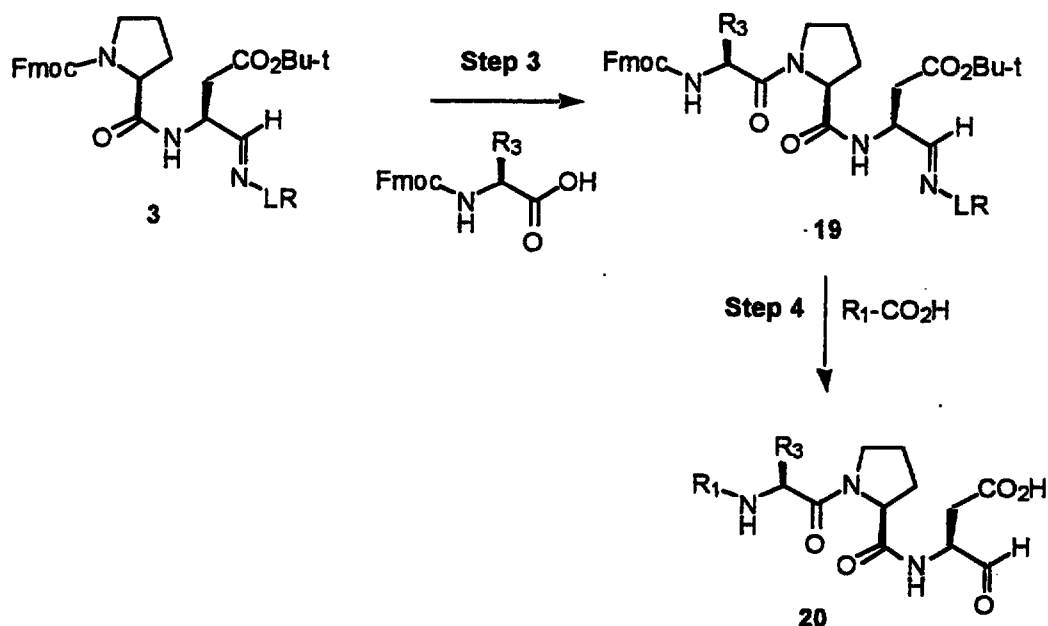


18f

- 86 -

Procedure for the preparation of analogs 20.

Compounds 20a-20t were prepared according to the procedure described for compounds 5 (Scheme I) only substituting the appropriate Fmoc-amino acid for Fmoc-Valine in Step 3 (Scheme V).

Scheme V

Preparation of 3-({1-[2-(4-amino-3-chloro-benzoylamino)-3-methylsulfonyl-propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (20i).

A suspension of 0.132 mmol of resin 3 in 4 mL of 20% piperidine in DMF was rotated at room temperature for 5 minutes, and the mixture was drained. The procedure was repeated over 20 minutes. The resin was washed successively with DMF (twice), CH₃OH (once), CH₂Cl₂ (twice), CH₃OH (once) and NMP (twice). To a suspension of the resin in 4 mL of NMP was added successively 189 mg of N-Fmoc-methyl cysteine (4 eq, 0.528 mmol), 0.185 mL of DIEA (8 eq, 1.056 mmol), 71 mg of HOBT (4 eq,

- 87 -

0.528 mmol) and 200 mg of HBTU (4 eq, 0.528 mmol). The mixture was rotated at room temperature overnight and drained. This coupling procedure was repeated over 3 hours. The resin was then washed successively with DMF (twice), CH₃OH (once), and 1:1 DMF/ CH₂Cl₂ (twice), CH₃OH (once) and CH₂Cl₂ (three times), and dried in vacuo.

A suspension of 100 mg of this resin in 2 mL of 20% piperidine in DMF was rotated at room temperature for 5 minutes, and drained. The procedure was repeated over 20 minutes. The resin was washed successively with DMF (twice), CH₃OH (once), CH₂Cl₂ (twice), CH₃OH (once) and NMP (twice). To a suspension of resin in 2 mL of NMP was added successively 38 mg of 4-amino-3-chlorobenzoic acid (4 eq, 0.2 mmol), 0.140 mL of DIEA (8 eq, 0.4 mmol), 27 mg of HOBt (4 eq, 0.2 mmol) and 76 mg of HBTU (4 eq, 0.4 mmol). The mixture was rotated at room temperature overnight and drained. The resin was then washed successively with DMF (twice), CH₃OH (once), and 1:1 DMF/ CH₂Cl₂ (twice), CH₃OH (once) and CH₂Cl₂ (three times), and dried in vacuo. The resin was then treated with 2 mL of 95% TFA in water for 1h. The suspension was filtered, the filtrate was concentrated in vacuo and purified by preparative HPLC to afford the title compound (20i).

Preparation of 3-({1-[2-(3,5-dichloro-4-hydroxy-benzoylamino)-4-methanesulfonyl-butyryl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (20p).

Compound 20p was prepared according to the procedure used for the preparation of 20i using N-Fmoc-methionine

- 88 -

as the first component coupled to resin 3, and 3,5-dichloro-4-hydroxybenzoic acid as the second component.

Preparation of 3-[(1-{2-[(isoquinoline-1-carbonyl)-amino]-3-methanesulfonyl-propionyl}-pyrrolidine-2-carbonyl)-amino]-4-oxo-butyric acid (20r).

N-Fmoc methyl cysteine was oxidized to the corresponding sulfone using the method of B. M. Trost and D. P. Curran, Tetrahedron Lett. 22, pp. 1287-190 (1981). To a solution of 0.714 g (2 mmol) of N-Fmoc methyl cysteine in 24 mL of a 1:1 solution of CH₃OH - water stirred at 0°C was added 3.68 g (3 eq, 6 mmol) of OxoneTM. The mixture was stirred at room temperature for 48 h, diluted with water, acidified to pH 2 using 6N HCl, and extracted with three 100 mL portions of ethyl acetate. The combined organic extracts were dried (MgSO₄) and concentrated in vacuo to afford 0.700 g (89% yield) of sulfone: ¹H NMR (DMSO-d₆, 500 MHz) δ 2.97 (s, 3H), 3.49-3.59 (m, 2H), 4.25 (m, 1H), 4.30-4.38 (m, 2H), 4.46 (m, 1H), 7.33 (t, 2H), 7.42 (t, 2H), 7.70-8.00 (m, 4H); exact mass calculated for C₁₉H₁₉NO₆S m/e 389.09, found m/e 390.2.

A suspension of 0.250 mmol of resin 3 in 10 mL of 20% piperidine in DMF was rotated at room temperature for 5 minutes, and the mixture was drained. The procedure was repeated over 20 minutes. The resin was washed successively with DMF (twice), CH₃OH (once), CH₂Cl₂ (twice), CH₃OH (once) and NMP (twice). To a suspension of the resin in 6 mL of NMP was added successively 200 mg of N-Fmoc-methyl cysteine sulfone (4 eq, 0.50 mmol), 0.175 mL of DIEA (8 eq, 1.00 mmol), 70 mg of HOBT (4 eq, 0.50 mmol) and 188 mg of HBTU (4 eq, 0.50 mmol). The mixture was rotated at room temperature overnight

- 89 -

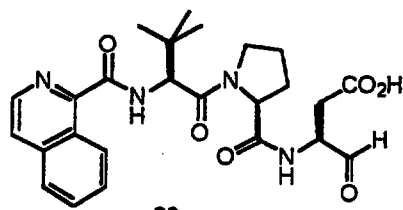
and drained. This coupling procedure was repeated over 3 hours. The resin was washed successively with DMF (twice), CH₃OH (once), 1:1 DMF/ CH₂Cl₂ (twice), CH₃OH (once) and CH₂Cl₂ (three times), and dried in vacuo .

5

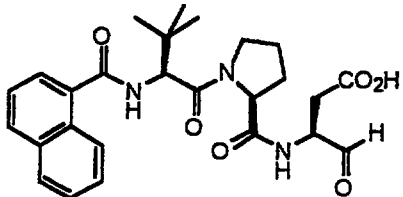
A suspension of 150 mg of this resin in 4 mL of 20% piperidine in DMF was rotated at room temperature for 5 minutes, and drained. The procedure was repeated over 20 minutes. The resin was washed successively with DMF (twice), CH₃OH (once), CH₂Cl₂ (twice), CH₃OH (once) and NMP (twice). To a suspension of resin in 3 mL of NMP was added successively 52 mg of 1-
10 isoquinolinecarboxylic acid (4 eq, 0.3 mmol), 0.104 mL of DIEA (8 eq, 0.6 mmol), 37 mg of HOBt (4 eq, 0.3
15 mmol) and 104 mg of HBTU (4 eq, 0.3 mmol). The mixture was rotated at room temperature overnight and drained. The resin was washed successively with DMF (twice), CH₃OH (once), and 1:1 DMF/ CH₂Cl₂ (twice), CH₃OH (once) and CH₂Cl₂ (three times), and dried in vacuo. The
20 resin was then treated with 2 mL of 95% TFA in water for 1 hour. The suspension was filtered, the filtrate was concentrated in vacuo and purified by preparative HPLC to afford the title compound (20r).

25 **Preparation of 3-({1-[2-(3,5-dichloro-4-hydroxy-benzoylamino)-3-methanesulfonyl-propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (20s).**

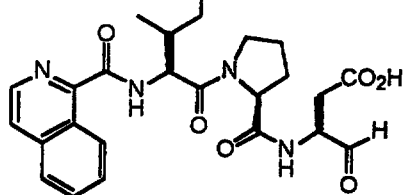
Compound 20s was prepared according to the procedure used for the preparation of 20i, using 3,5-dichloro-4-
30 hydroxybenzoic acid in place of 1-isoquinolinecarboxylic acid.



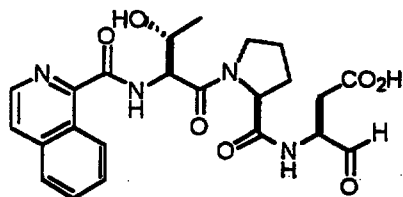
20a



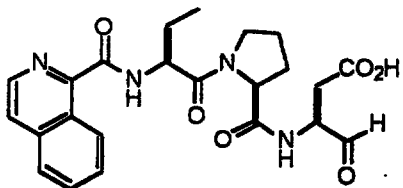
20b



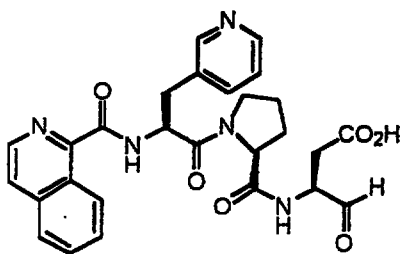
20c



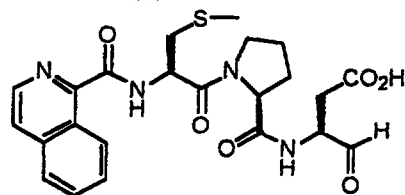
20d



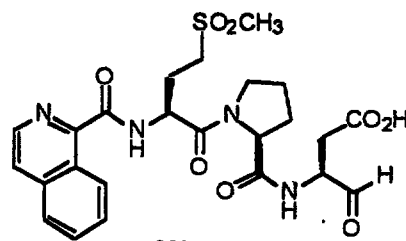
20e



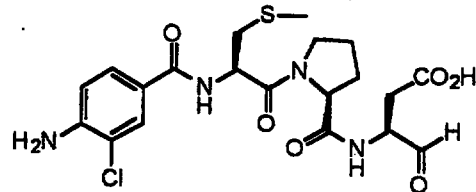
20f



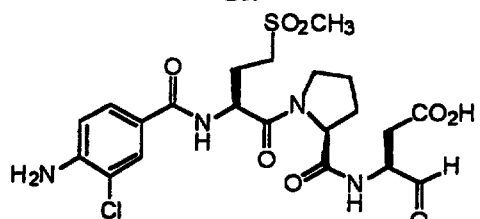
20g



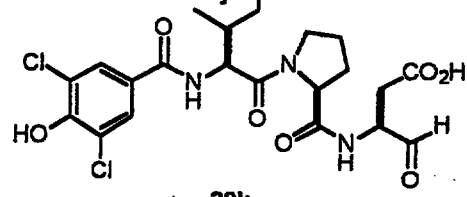
20h



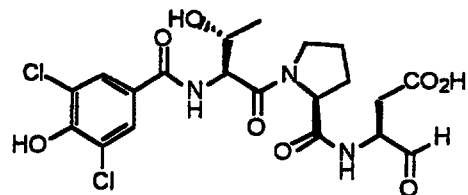
20i



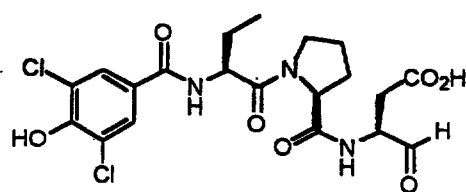
20j



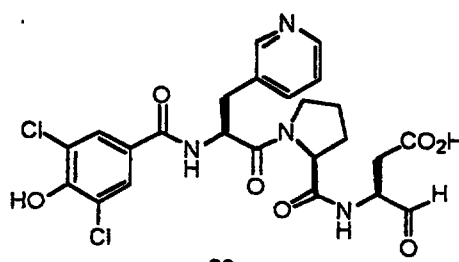
20k



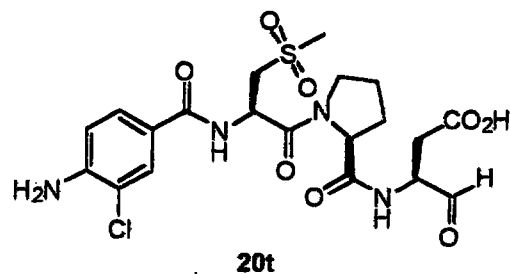
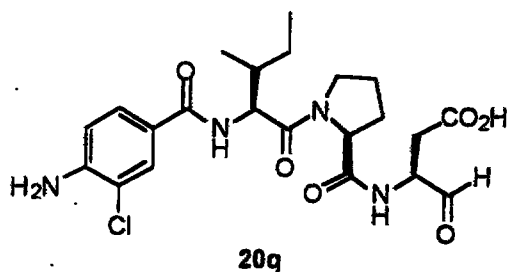
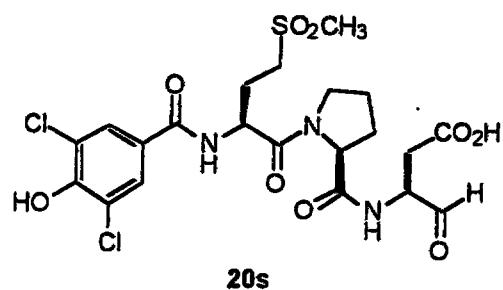
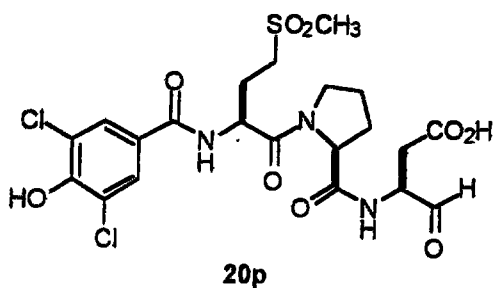
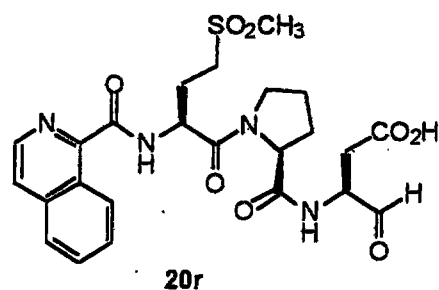
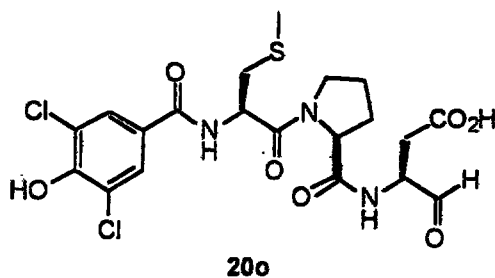
20l



20m



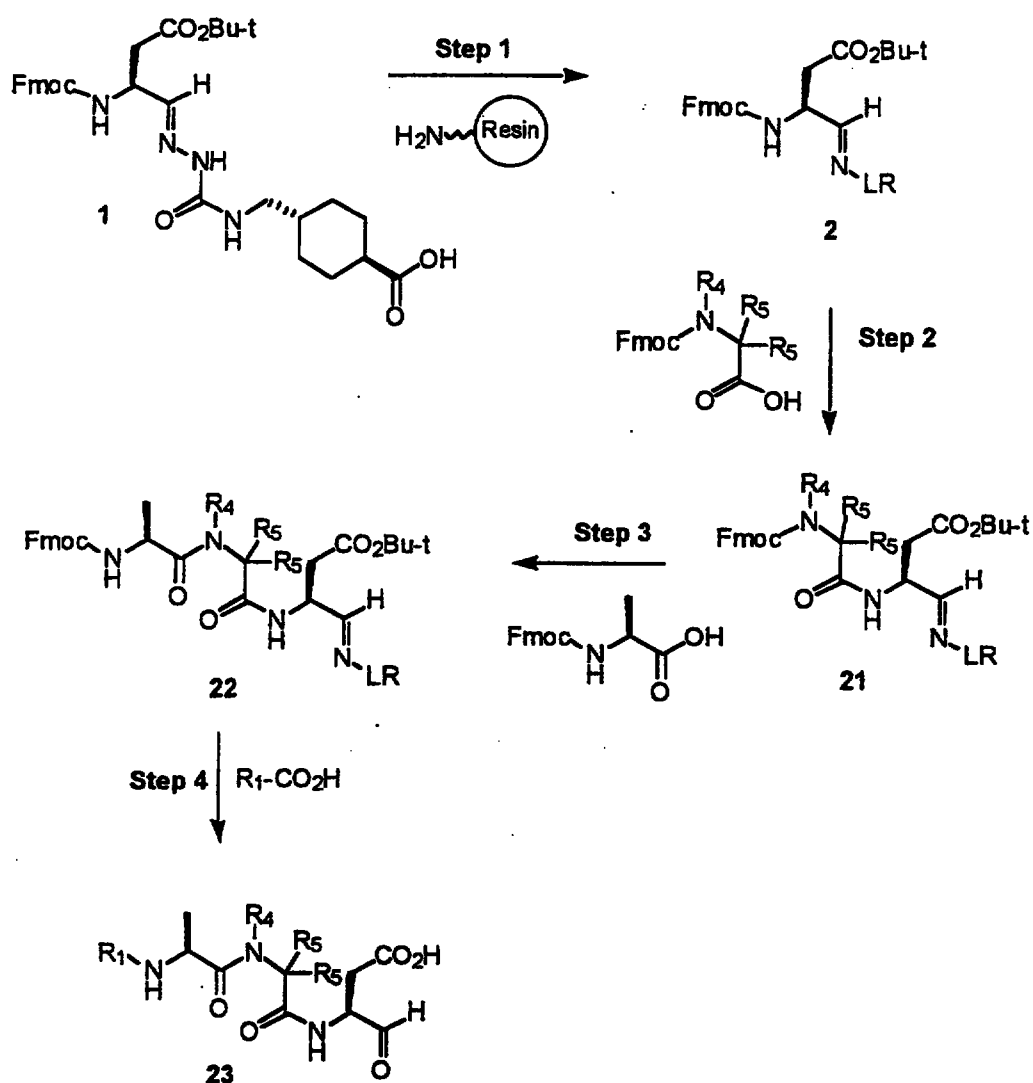
20n



Procedure for the preparation of analogs 23.

- 5 Compounds 23a-23i were prepared according to the procedure described for compounds 7 (Scheme II) only substituting the appropriate Fmoc-amino acid for Fmoc-proline in Step 2 (Scheme VI).

Scheme VI



Preparation of 3-({2-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-4-methyl-3,4-dihydro-2H-pyrazole-3-carbonyl}-amino)-4-oxo-butyrlic acid (23g).

Compound 23g was prepared according to the procedure described for compounds 7 only substituting 4-methyl-4,5-dihydro-pyrazole-1,5-dicarboxylic acid 1-(9H-

- 93 -

fluoren-9-ylmethyl) ester for Fmoc-proline (Scheme II) in Step 2.

5 Preparation of 4-methyl-4,5-dihydro-pyrazole-1,5-dicarboxylic acid 1-(9H-fluoren-9-ylmethyl) ester:

To a solution of 650 mg (2 mmol) of (10,10-dimethyl-3,3-dioxo- λ^6 -thia-4-aza-tricyclo[5.2.1.0^{0,0}]dec-4-yl)-(4-methyl-3,4-dihydro-2H-pyrazol-3-yl)-methanone (J. Am. Chem. Soc., 119, pp. 8379-8380 (1997)) in 6 mL of water and 14 mL of THF stirred at 0°C was added 420 mg (10 mmol, 5 eq) of lithium hydroxide. The mixture was stirred at 0°C for 2 hours and at room temperature for 30 minutes, diluted with 20 mL of water and washed with ether (20 mL). The pH of the solution was then adjusted to 9, and a solution of 519 mg (2 mmol, 1 eq) of Fmoc-Cl in 3 mL of dioxane was added. The mixture was stirred at room temperature overnight, washed with ether, acidified to pH 2-3 and extracted with 3 40-mL portions of ethyl acetate. The combined organic extracts were washed with brine, dried (MgSO₄) and concentrated in vacuo to afford 690 mg (98% yield) of a colorless foam which was identified as the title compound. ¹H NMR (DMSO-d₆, 500 MHz) δ 1.2 (d, 3H), 3.2 (m, 1H), 4.2-4.6 (m, 3H), 7.1 (s, 1H), 7.2-7.5 (m, 5H), 7.7-8.0 (m, 4H). Exact mass calculated for C₂₀H₁₈N₂O₄ m/e 350.13, found m/e 351.3

30 Preparation of 3-{1-[2-(4-amino-3-chloro-benzoylamino)-propionyl]-4-methoxy-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (23i).

- 94 -

Compound 23i was prepared according to the procedure described for compounds 7 only substituting N-Fmoc-4-methoxyproline for Fmoc-proline (Scheme II) in Step 2.

5 Preparation of N-Fmoc-4-methoxyproline:

To a solution of 735 mg (3 mmol) of N-Boc-4-hydroxyproline methyl ester in 20 mL of THF stirred at 0°C was added 79 mg (1.1 eq, 3.3 mmol) of 60% sodium hydride in mineral oil. The mixture was stirred at 0°C
10 for 1 hour, and methyl iodide (0.56 mL, 3 eq, 9 mmol) was added. The mixture was stirred at room temperature overnight, quenched by addition of saturated aqueous ammonium chloride, diluted with water, and extracted with three 80mL portions of ethyl acetate. The
15 combined organic extracts were washed with brine, dried (MgSO₄), and concentrated *in vacuo* to afford a pale yellow oil. The oil was taken up in 9 mL of CH₃OH and 3 mL of water, and 378 mg (3 eq, 9 mmol) of lithium hydroxide was added. The mixture was stirred at room
20 temperature overnight, diluted with water, acidified to pH 3 and extracted with three 80 mL portions of ethyl acetate. The combined organic extracts were washed with brine, dried (MgSO₄) and concentrated *in vacuo*. The residual oil was taken up in 10 mL of TFA and the
25 solution was stirred at room temperature for 2 hours, and concentrated *in vacuo*. The residual oil was diluted with 6 mL of 10% aqueous sodium carbonate and 3 mL of dioxane, and a solution of 9-fluorenylmethyl chloroformate (779 mg, 1eq, 3 mmol) in 5 mL of dioxane
30 was added. The mixture was stirred at room temperature overnight, diluted with water, acidified to pH 3 and extracted with three 80 mL portions of ethyl acetate. The combined organic extracts were washed with brine, dried (MgSO₄) and concentrated *in vacuo* to afford an

- 95 -

oil, which was purified by column chromatography over silica gel eluted with $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ 20:1, to afford 600 mg (55%) of N-Fmoc-4-methoxyproline: exact mass calculated for $\text{C}_{21}\text{H}_{21}\text{NO}_5$ m/e 367.14 found m/e 368.4.

5

To a 0.125 mmol portion of resin 2 was added 4 mL of 20% piperidine in DMF. The mixture was rotated at room temperature for 5 minutes and drained. The procedure was repeated over 20 minutes. The resin was washed successively with DMF (twice), CH_3OH (once), CH_2Cl_2 (twice), CH_3OH (once) and NMP (twice). To a suspension of the resin in 4 mL of NMP was added successively 184 mg of N-Fmoc-4-methoxyproline (4 eq, 0.50 mmol), 0.175 mL of DIEA (8 eq, 1.00 mmol), 70 mg of HOBt (4 eq, 0.50 mmol) and 188 mg of HBTU (4 eq, 0.50 mmol). The mixture was rotated at room temperature overnight and drained. This coupling procedure was repeated over 3 hours. The resin was washed successively with DMF (twice), CH_3OH (once), 1:1 DMF/ CH_2Cl_2 (twice), CH_3OH (once) and CH_2Cl_2 (three times), and dried in vacuo.

To the resin was added 4 mL of 20% piperidine in DMF. The mixture was rotated at room temperature for 5 minutes and drained. The procedure was repeated over 20 minutes. The resin was washed successively with DMF (twice), CH_3OH (once), CH_2Cl_2 (twice), CH_3OH (once) and NMP (twice). To a suspension of the resin in 4 mL of NMP was added successively 156 mg of N-Fmoc-alanine (4 eq, 0.50 mmol), 0.175 mL of DIEA (8 eq, 1.00 mmol), 70 mg of HOBt (4 eq, 0.50 mmol) and 188 mg of HBTU (4 eq, 0.50 mmol). The mixture was rotated at room temperature overnight and drained. This coupling procedure was repeated over 3 hours. The resin was

- 96 -

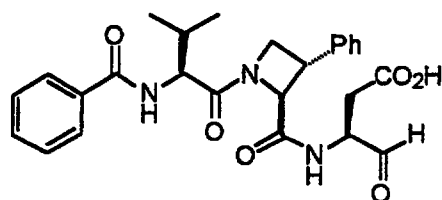
washed successively with DMF (twice), CH₃OH (once), 1:1 DMF/ CH₂Cl₂ (twice), CH₃OH (once) and CH₂Cl₂ (three times), and dried *in vacuo*.

- 5 To the resin was added 4 mL of 20% piperidine in DMF. The mixture was rotated at room temperature for 5 minutes and drained. The procedure was repeated over 20 minutes. The resin was washed successively with DMF (twice), CH₃OH (once), CH₂Cl₂ (twice), CH₃OH (once) and
- 10 NMP (twice). To a suspension of the resin in 4 mL of NMP was added successively 80 mg of 4-amino-3-chlorobenzoic acid (4 eq, 0.50 mmol), 0.175 mL of DIEA (8 eq, 1.00 mmol), 70 mg of HOBt (4 eq, 0.50 mmol) and 188 mg of HBTU (4 eq, 0.50 mmol). The mixture was
- 15 rotated at room temperature overnight and drained. The resin was washed successively with DMF (twice), CH₃OH (once), 1:1 DMF/ CH₂Cl₂ (twice), CH₃OH (once) and CH₂Cl₂ (three times), and dried *in vacuo*.
- 20 The resin was treated with 4 mL of 95% TFA in water for 1 hour. The mixture was filtered. The filtrate was concentrated *in vacuo* to afford an oil, which was purified by HPLC to afford the title compound (23i).

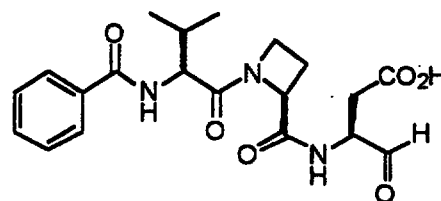
Compounds 24a-24e were prepared according to the procedure described for compounds 5 (Scheme I) only substituting either Fmoc-azetidine carboxylic acid or

- 98 -

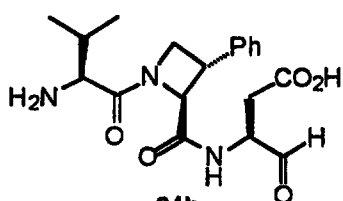
trans-2-phenyl- Fmoc-azetidine carboxylic acid for
Fmoc-proline in Step 2.



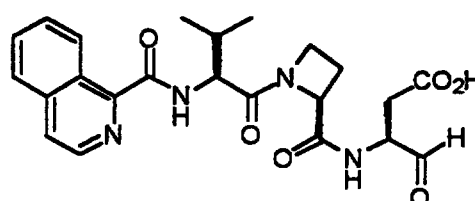
24a



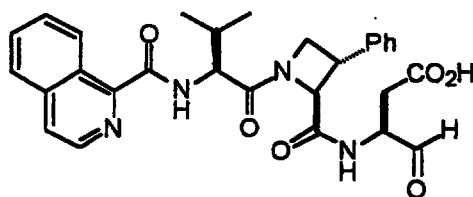
24d



24b



24e



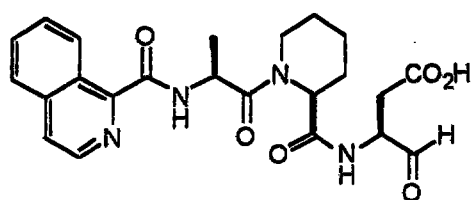
24c

5

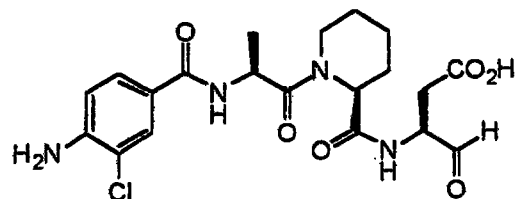
Procedure for the preparation of analogs 25.

Compounds 25a-25e were prepared according to the
procedures described for compounds 5 and 7 (Scheme I
and Scheme II) only substituting Fmoc-2(S)-pipecolic
acid for Fmoc-proline in Step 2 and coupling either
Fmoc-valine or Fmoc-alanine or Fmoc-tert-leucine in
Step 3.

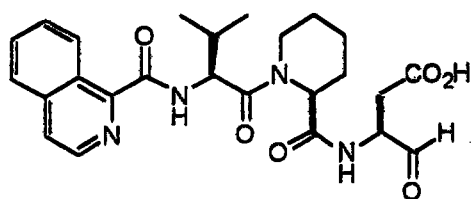
15



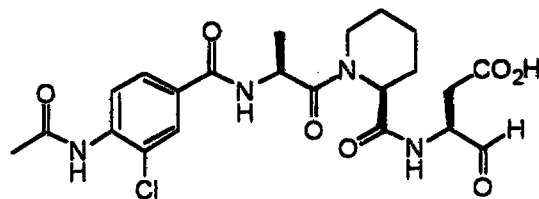
25a



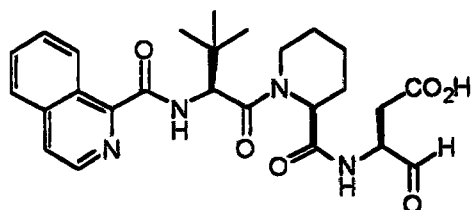
25d



25b



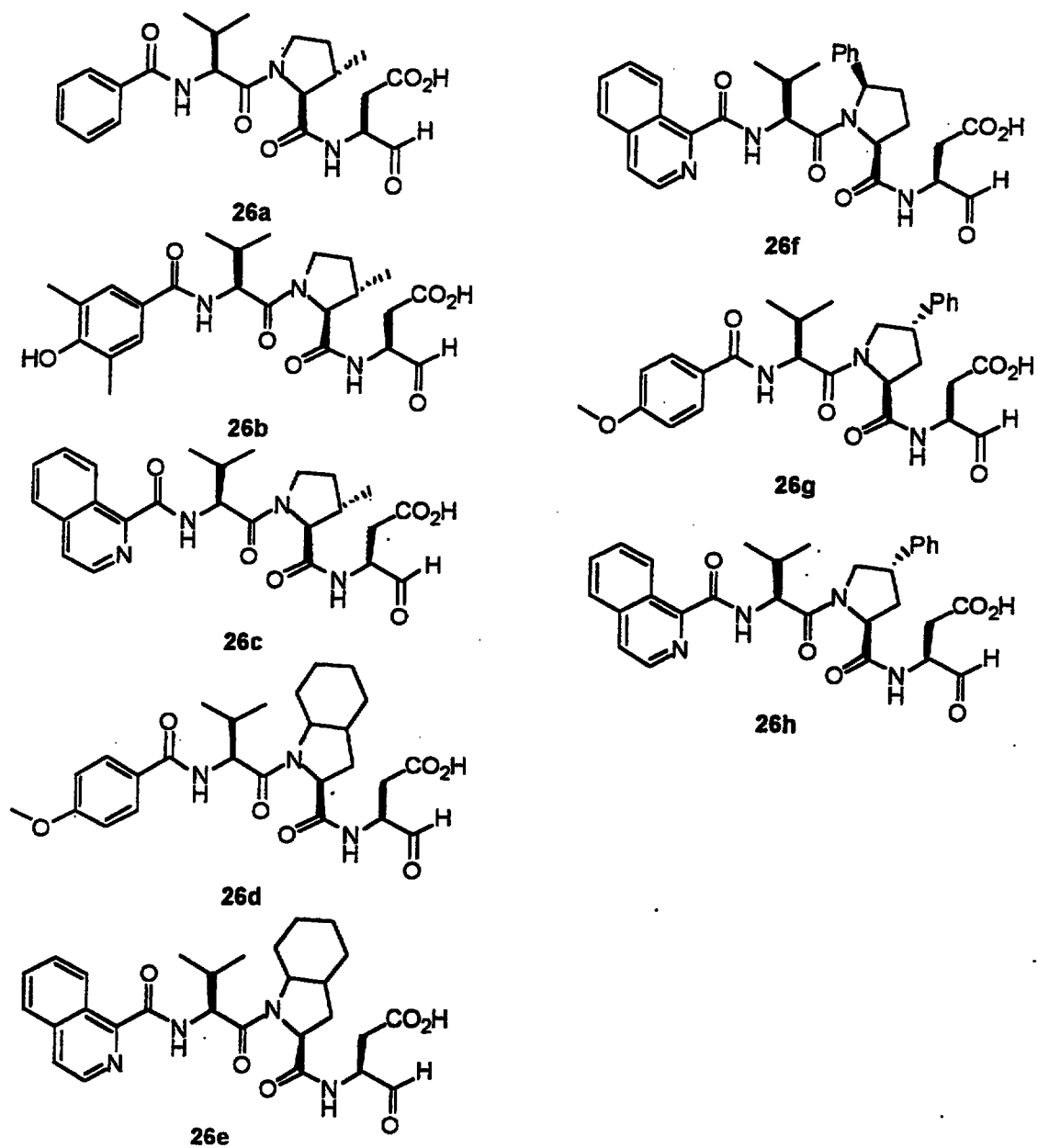
25e



25c

5 Procedure for the preparation of analogs 26a-h.

Compounds 26a- 26h were prepared according to the procedure described for compounds 23 (Scheme VI) only substituting Fmoc-valine for Fmoc-alanine in Step 3.



Procedure for the preparation of analogs 27.

- 101 -

Compounds 27a- 27n were prepared according to the procedure described for compounds 7 (Scheme II) only substituting Fmoc-4,4-difluoroproline for Fmoc-proline in Step 2.

5

Preparation of N-Boc 4,4-difluoroproline methyl ester:

To a solution of 9.63 mL (7.2 mmol) of oxalyl chloride in 10.6 mL of CH₂Cl₂ stirred at -78 °C was added a solution of 0.94 mL (13.2 mmol) of methyl sulfoxide in 10 15 mL of CH₂Cl₂. The solution was stirred at -78 °C for 30 min. A solution of 1.47 g (6 mmol) of N-Boc-4-hydroxyproline methyl ester in 19 mL of CH₂Cl₂ was then added dropwise. The mixture was stirred at -78°C for 1.5 h, and 3.34 mL (24 mmol) of triethylamine was 15 added. The solution was allowed to warm up to room temperature and stirred overnight. It was then diluted with 100 mL of CH₂Cl₂, washed successively with 100 mL of water, 100 mL of 1N HCl, and 100 mL of brine, dried (MgSO₄) and concentrated in vacuo. The residue was 20 purified by column chromatography over silica gel (eluted with ethyl acetate/hexanes, 1:3), to afford 1.294 g (89% yield) of N-Boc-4-oxo-proline methyl ester. ¹H NMR (500 MHz, CDCl₃) δ 1.45 (m, 9H), 2.60 (m, 1H), 2.95 (m, 1H), 3.75 (m, 3H), 3.90 (m, 2H), 4.80 (m, 25 1H).

To a solution of 808 mg (3.33 mmol) of N-Boc-4-oxo-proline methyl ester in 13 mL of CH₂Cl₂ stirred at 0°C was added 0.88 mL (7.19 mmol, 2.2 eq) of DAST. The 30 mixture was stirred at 0°C for 2 hours, at room temperature for 16 hours, and poured into ice water. The mixture was stirred at room temperature for 2 hours. The organic phase was separated, washed with water, dried (MgSO₄) and concentrated in vacuo. The

- 102 -

residue was purified by column chromatography over silica gel (eluted with ethyl acetate-hexanes, 1:8), to afford 754 mg (79% yield) of difluorinated derivative as a pale yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 1.50 (m, 9H), 2.45 (m, 1H), 2.70 (m, 1H), 3.75 (m, 3H), 3.80 (m, 2H), 4.50 (m, 1H).

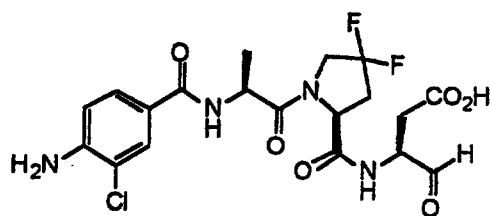
Preparation of N-Fmoc-4,4-difluoroproline:

To a solution of 754 mg (2.85 mmol) of N-Boc 4,4-difluoroproline methyl ester in 5 mL of THF stirred at 0°C was added a solution of 179 mg (4.27 mmol) of lithium hydroxide in 5 mL of water. The solution was stirred at 0°C for 3 h, at room temperature for 1 hour, diluted with water, extracted with ether, acidified to pH 2-3, and extracted with two 30 mL portions of ethyl acetate. The combined organic extracts were washed with brine, dried (MgSO₄) and concentrated in vacuo to afford 652 mg (91%) of acid as a pale yellow solid.

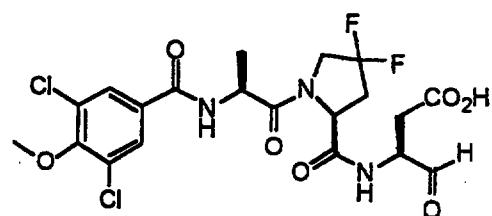
A solution of 652 mg (2 mmol) of N-Boc-4,4-difluoroproline in 10 mL of 1:1 TFA/ CH₂Cl₂ was stirred at 0°C for 45 min, and concentrated in vacuo. The residue was taken up in 3 mL of dioxane, and 5 mL of 10% aqueous sodium carbonate was added, followed by a solution of 675 mg (1 eq) of Fmoc-Cl in 5 mL of dioxane. The mixture was stirred at room temperature for 16 h, diluted with 20 mL of water, extracted with 2 20-mL portions of diethyl ether, acidified to pH 2, and extracted with three 30-mL portions of ethyl acetate. The combined organic extracts were washed with 50 mL of brine, dried (MgSO₄) and concentrated in vacuo. The residue was purified by column chromatography over silica gel (eluted with CH₂Cl₂/ CH₃OH 10:1), to afford 850 mg (88%) of N-Fmoc-4,4-difluoroproline as a

- 103 -

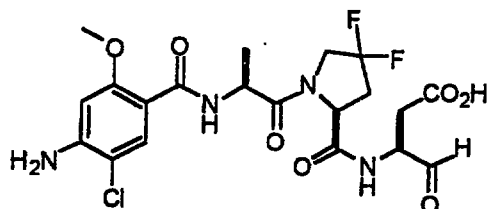
brownish solid. ^1H NMR (500 MHz, CDCl_3) δ 2.55 (m, 1H),
2.95 (m, 1H), 3.80 (m, 2H), 4.20 (m, 1H), 4.30 (m, 2H),
4.55 (m, 1H), 7.32 (m, 2H), 7.45 (m, 2H), 7.70 (m, 2H),
7.90 (m, 2H). Exact mass calculated for $\text{C}_{20}\text{H}_{17}\text{F}_2\text{NO}_4$ m/e
5 373.11, found m/e 374.4.



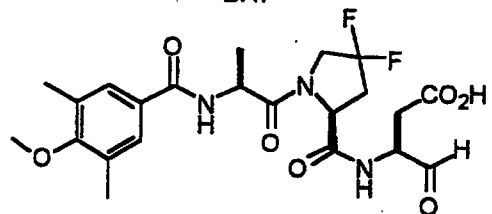
27a



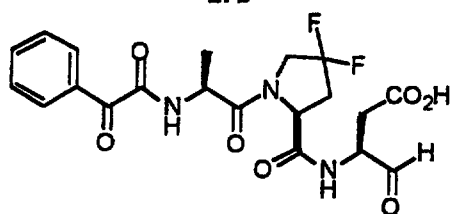
27h



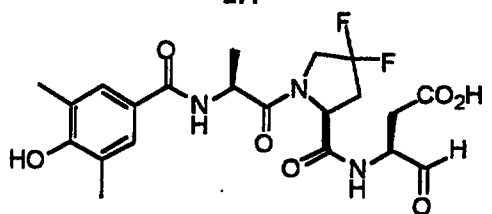
27b



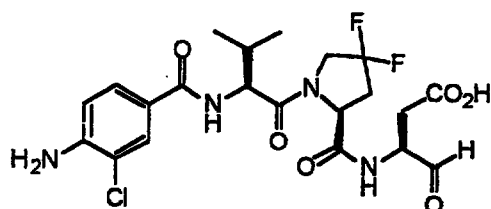
27i



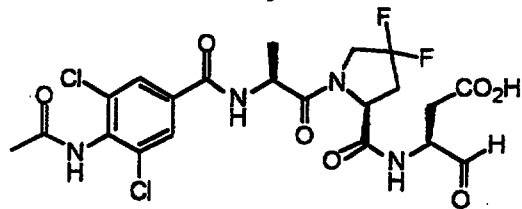
27c



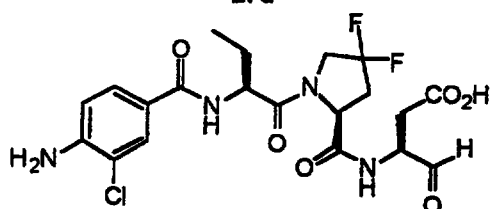
27j



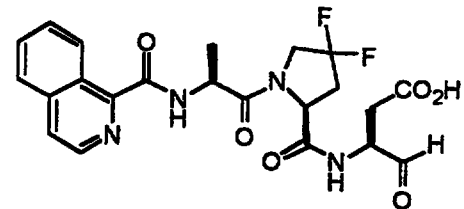
27d



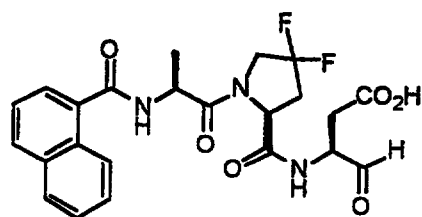
27k



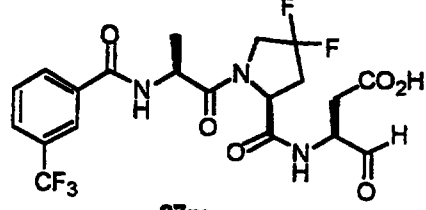
27e



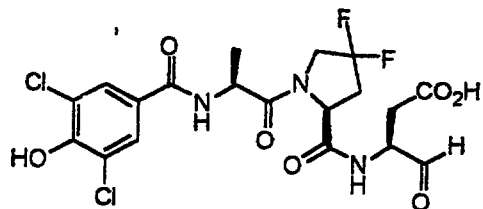
27l



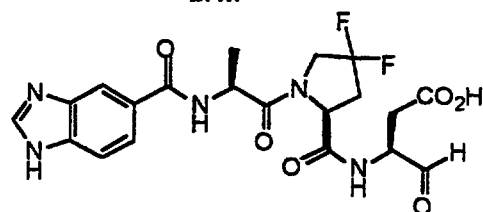
27f



27m



27g

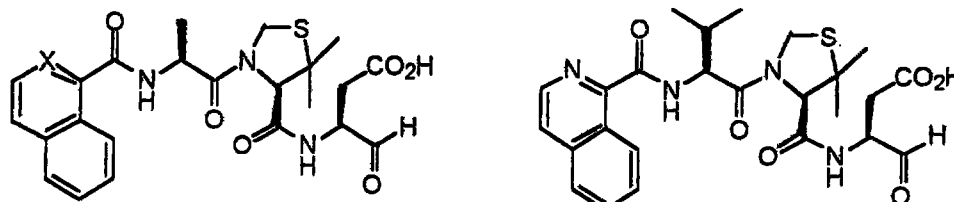


27n

- 105 -

Compounds 28a- 28c were prepared according to the procedure described for compounds 5 and 7 (Scheme I and Scheme II) only substituting Fmoc-dimethylthioproline for Fmoc-proline in Step 2.

5



28a, X=N
28b, X=C

28c

GENERAL PROCEDURES FOR THE PREPARATION OF COMPOUNDS OF
EMBODIMENT A FORMULA I (SCHEMES VII-VIII)

10

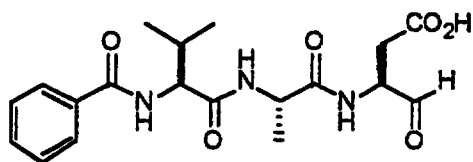
Compounds of Embodiment A Formula I where $R_4=H$ and one $R_5=H$:

15

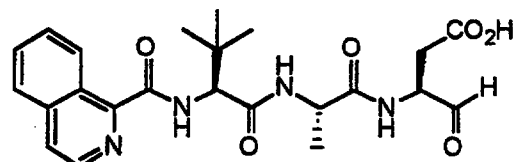
Procedure for the preparation of analogs 29.

Compounds 29a- 29s were prepared according to the procedure described for compounds 5 (Scheme I) only substituting Fmoc-alanine for Fmoc-proline in Step 2 and using either Fmoc-valine or Fmoc-alanine or Fmoc-tert-leucine in Step 3.

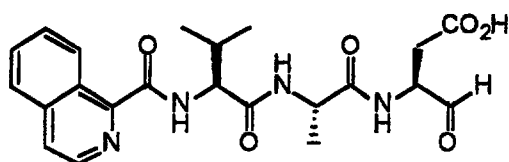
20



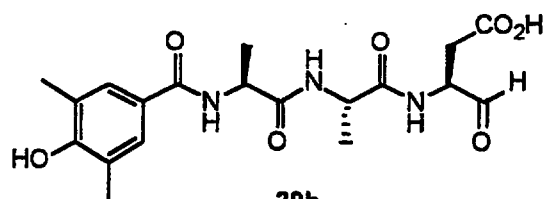
29a



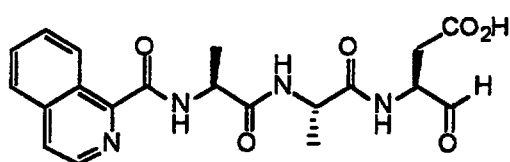
29g



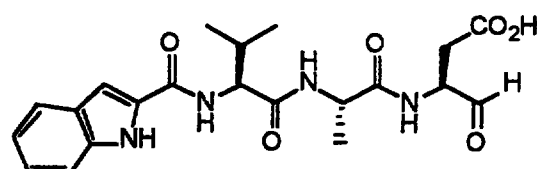
29b



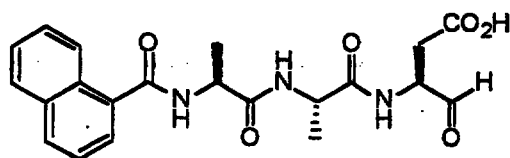
29h



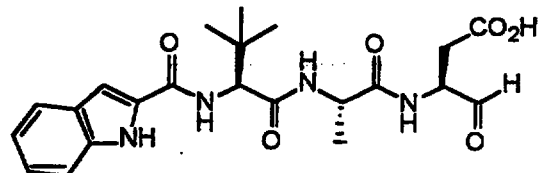
29c



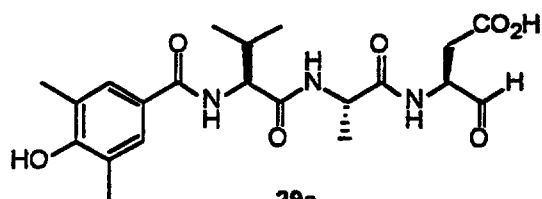
29i



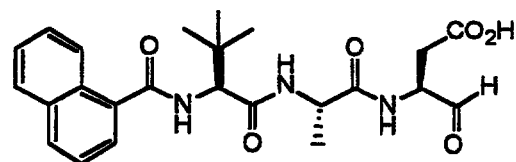
29d



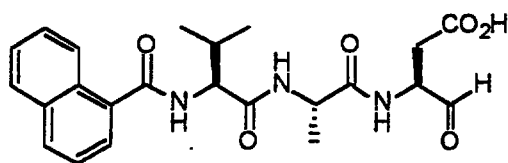
29j



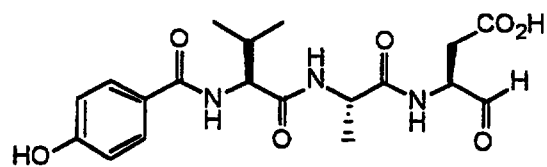
29e



29k

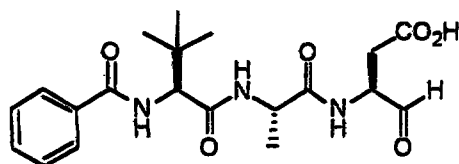


29f

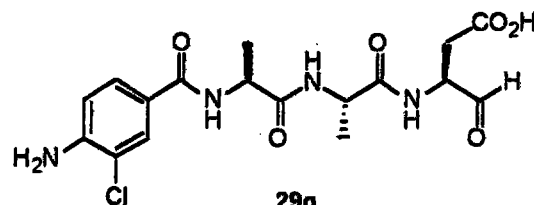


29l

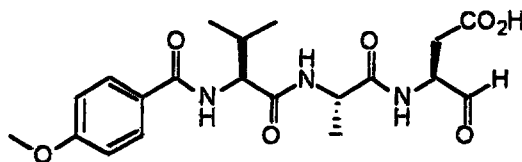
- 107 -



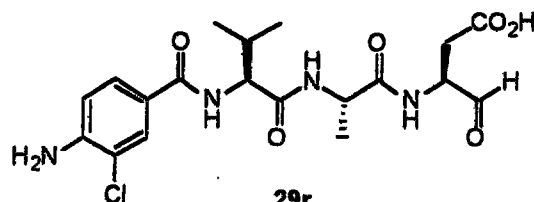
29m



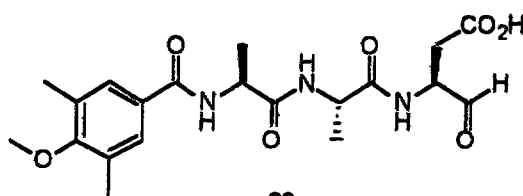
29q



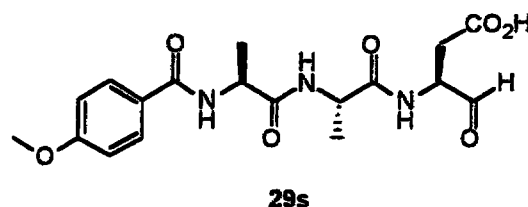
29n



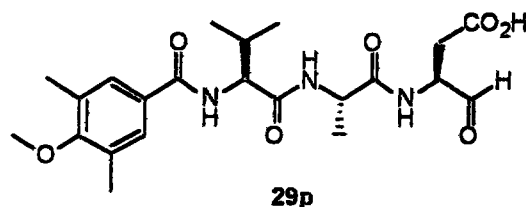
29r



29o



29s



29p

Procedure for the preparation of analogs 32.

Compounds 32a-32e were prepared according to the
 5 procedure described for compounds 5 (Scheme I) only
 substituting 2-(3-*tert*-butoxycarbonylamino-2-oxo-
 pyrrolidin-1-yl)-4-methyl-pentanoic acid (30)
 (Neosystem catalog number BB02101) for Fmoc-proline in
 Step 2 followed by Step 4 (Scheme VII).

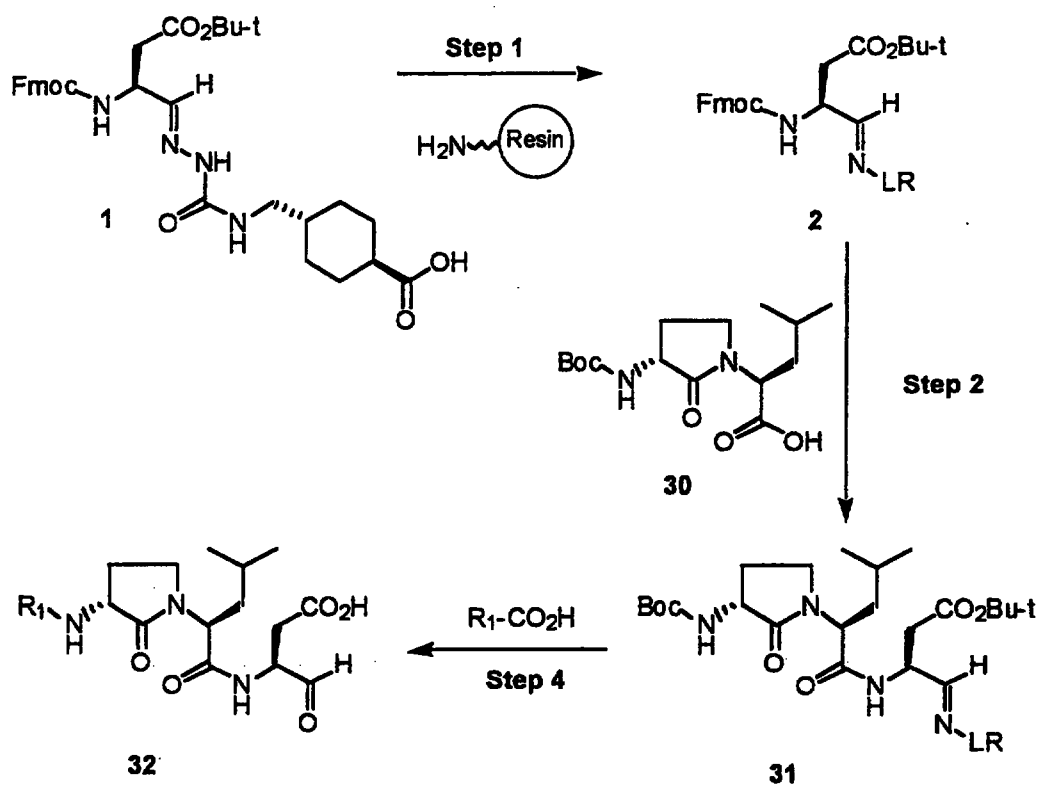
10

15

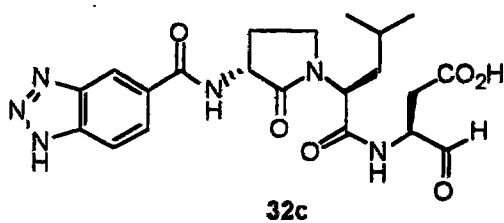
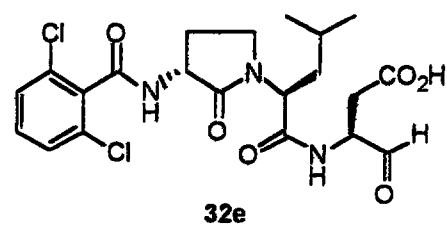
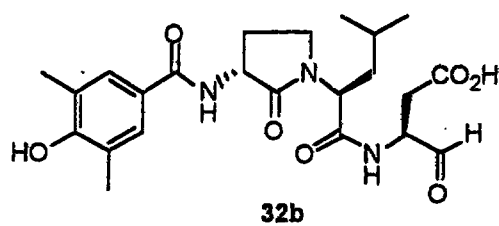
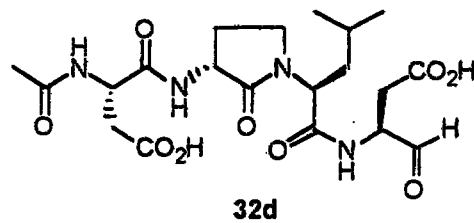
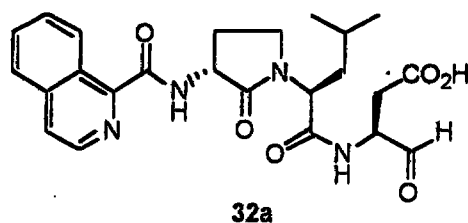
- 108 -

Compounds of Embodiment A Formula I wherein R_2 and R_3 together with the atoms to which they are bound form a 5 membered ring.

Scheme VII



- 109 -

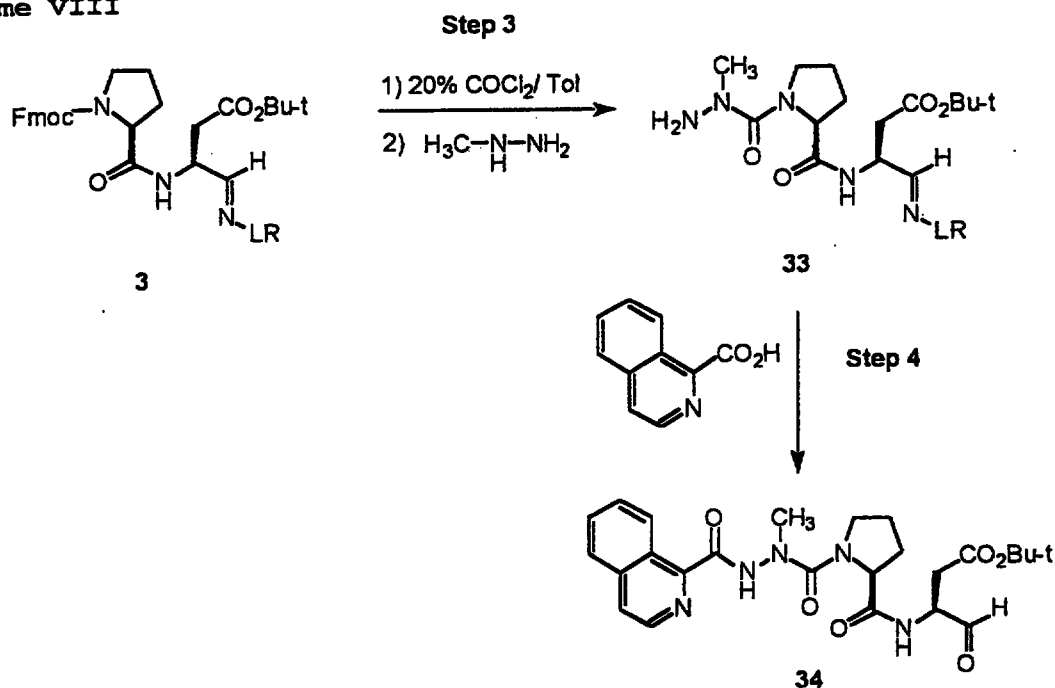


5

Compound of Embodiment A Formula I wherein X=N-CH₃

- 110 -

Scheme VIII



Preparation of 3-({1-[N-(isoquinoline-1-carbonyl)-N-methyl-hydrazinocarbonyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyrac acid (34).

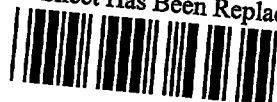
5 A suspension of 0.250 mmol of resin 3 (Scheme VIII) in 10 mL of 20% piperidine in DMF was rotated at room temperature for 5 minutes and drained. The procedure was repeated over 20 minutes. The resin was washed

10 successively with DMF (twice), CH₃OH (once), 1:1 DMF/CH₂Cl₂ (twice), CH₃OH (once) and CH₂Cl₂ (three times), and briefly dried. To the resin was added 5 mL of dry CH₂Cl₂, 0.128 mL of DIEA (3eq, 0.75 mmol) and 0.400 mL

15 of a 20% solution of phosgene in toluene (3 eq, 0.75 mmol). The suspension was rotated at room temperature for 1.5 hours. The mixture was drained, and the resin was washed with CH₂Cl₂ several times. To a suspension of resin in 5 mL of CH₂Cl₂ was added 0.133 mL of methyl hydrazine (10 eq, 2.5 mmol). The mixture was rotated

20 at room temperature overnight and drained. The resin

This Sheet Has Been Replaced



- 111 -

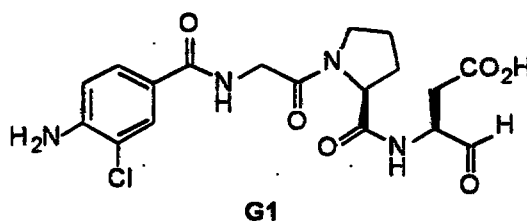
was washed successively with DMF (twice), CH₃OH (once), 1:1 DMF/ CH₂Cl₂ (twice), CH₃OH (once) and CH₂Cl₂ (three times), and dried in vacuo.

5 To a 0.075 mmol portion of the resin in 3 mL of NMP was added successively 52 mg of 1-isoquinolinecarboxylic acid (4 eq, 0.3 mmol), 0.19 mL of DIEA (8 eq, 0.6 mmol), 37mg of HOBt (4 eq, 0.3 mmol) and 104 mg of HBTU (4 eq, 0.3 mmol). The mixture was rotated at room
10 temperature overnight and drained. The resin was washed successively with DMF (twice), CH₃OH (once), 1:1 DMF/ CH₂Cl₂ (twice), CH₃OH (once) and CH₂Cl₂ (three times), and dried in vacuo.

15 The resin was treated with 4 mL of 95% TFA in water for 1 hour. The mixture was filtered. The filtrate was concentrated in vacuo to afford an oil, which was purified by HPLC to afford the title compound (34).

20

Compounds of Embodiment A Formula I wherein R₃ = R₃ = H:

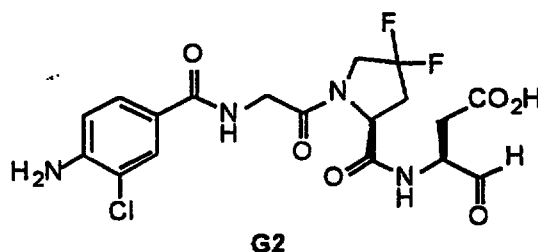


25 3-({1-[(4-Amino-3-chloro-benzoylamino)-acetyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (G1).

Prepared as described for compounds 7 only substituting Fmoc-glycine for Fmoc-alanine in Step 3 (Scheme II) to afford 4.3mg of the title compound. LC-MS (ES⁺)

30 m/e=425.2 (M+H)

- 112 -



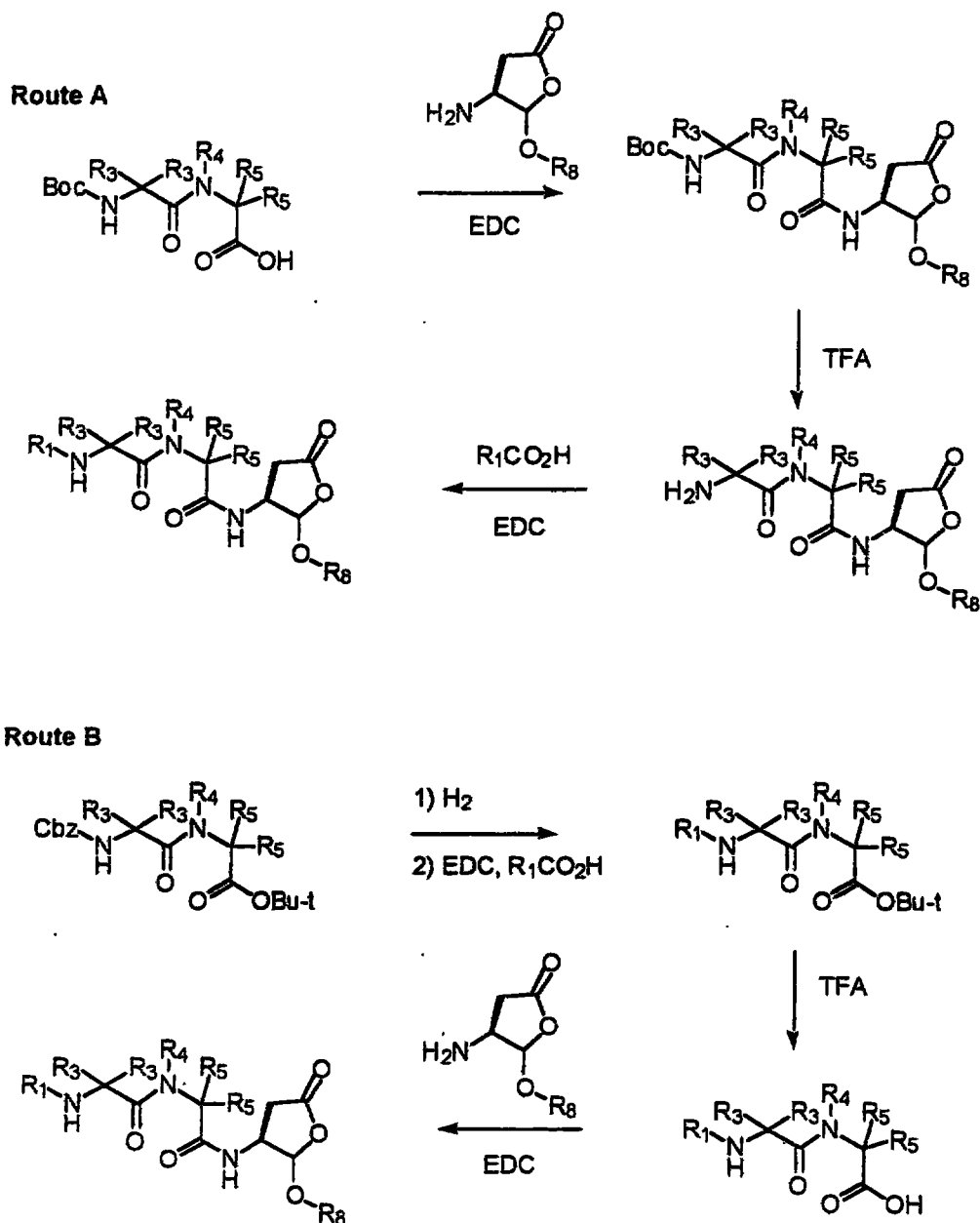
5 3-({1-[(4-Amino-3-chloro-benzoylamino)-acetyl]-4,4-difluoro-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (G2).

Prepared as described for compounds 7 and 27 only substituting Fmoc-glycine for Fmoc-alanine in Step 3 (Scheme II) to afford 10.0mg of the title compound. LC-
10 MS (ES⁺) m/e=461.2 (M+H)

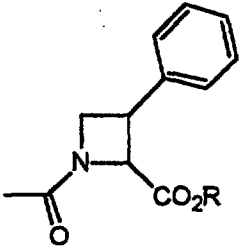
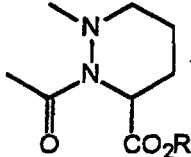
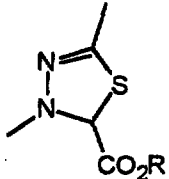
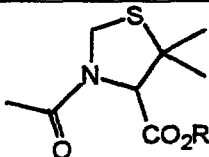
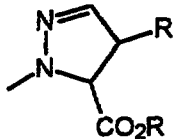
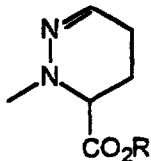
GENERAL PROCEDURES FOR THE PREPARATION OF COMPOUNDS OF
 EMBODIMENT C FORMULA I AND EMBODIMENT D FORMULA I
 WHEREIN Y= C (SCHEMES IX-XXII)

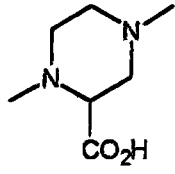
5

Scheme IX

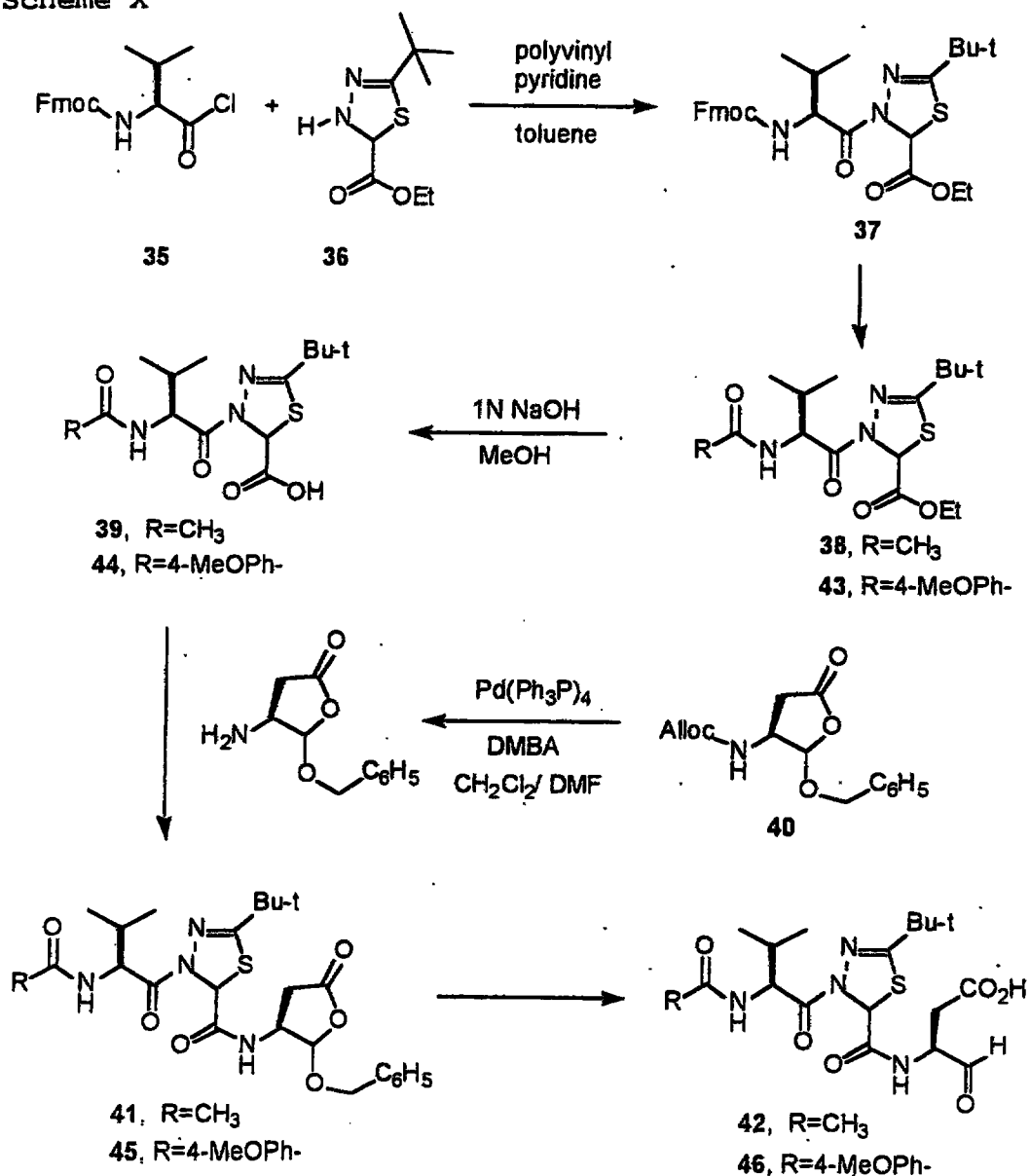


Sources for Selected Ring Systems

Structure	Reference
	Blythin, D.J., <u>J. Org. Chem.</u> , 59, pp. 6098-6100 (1994).
	Decicco, C.P. et al., <u>Syn. Lett.</u> , pp. 615-616, (1995).
	Bennion, C. et al., <u>J. Med. Chem.</u> , 34, pp. 439-447 (1991); Jensen, K.A. et al., <u>Acta Chemica Scand.</u> , 13, pp. 1097-1103 (1961).
	commercially available
	Mish, M. R., <u>J. Am. Chem. Soc.</u> , 119, pp. 8379-8380 (1997).
	Xi, N. et al., <u>J. Am. Chem. Soc.</u> , 120 pp. 80-86 (1998).

Structure	Reference
	Merour, J.Y. et al., <u>Tetrahedron Lett.</u> , 32, pp. 2469-2470 (1991); Rossen, K., <u>Tetrahedron Lett.</u> , 36, pp. 6419-6422 (1995).

Scheme X



- 116 -

5-tert-Butyl-3-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl butyryl]-2,3-dihydro-[1,3,4]thiadiazole-2-carboxylic acid ethyl ester (37).

5 A stirred suspension of polyvinylpyridine (2.63g, 25mmol) in a solution of 5-tert-butyl-2,3-dihydro-[1,3,4]thiadiazole-2-carboxylic acid ethyl ester (36), (J. Med. Chem., 34, p. 439 (1991)), (2.16g, 10mmol) in dry toluene was treated with the dropwise addition of
10 (1-chlorocarbonyl-2-methyl-propyl)-carbamic acid 9H-fluoren-9-ylmethyl ester (4.76g, 12.1mmol) in 20mL of anhydrous toluene. After stirring for 16 hours, the suspension was filtered and the filtrate was washed with saturated sodium bicarbonate solution. The
15 organic layer was separated, washed with water, dried over anhydrous sodium sulfate, and evaporated to give a yellow oil. Purification by flash chromatography eluting with 9/1 hexane/ ethyl acetate gave 2.66 g (49% yield) of the title compound (37) as a clear, viscous
20 oil. ¹H NMR (500MHz, CD₃OD) δ 0.89(d, 1.5H), 0.93 (d, 1.5H), 1.00 (d, 1.5H), 1.06 (d, 1.5H), 1.22 (t, 3H), 1.28 (s, 9H), 2.12-2.22 (m, 0.5H), 2.32-2.42 (m, 0.5H), 4.18-4.28 (m, 2H), 4.31-4.45 (m, 2H), 4.96-5.01 (m, 0.5H), 5.02-5.10 (m, 0.5H), 5.52 (d, 0.5H), 5.61 (d, 0.5H),
25 6.10 (s, 0.5H), 6.13 (s, 0.5H), 7.27-7.34 (m, 2H), 7.35-7.42 (m, 2H), 7.56-7.64 (m, 2H), 7.73-7.78 (m, 2H).

30 3-(2-Acetylamino-3-methylbutyryl)-5-tert-butyl-2,3-dihydro-[1,2,4] thiadiazole-2-carboxylic acid ethyl ester (38).

To a solution of (37) (Scheme IX) (0.508g, 0.94 mmol) in CH₃CN (10 mL) was added diethylamine (1 mL). The solution was stirred at room temperature for 2 hours,

- 117 -

the solvent removed in vacuo and the resultant oil azeotroped with CH_2Cl_2 (4x). The crude oil was dissolved in CH_2Cl_2 (5 mL) and triethylamine (0.26mL, 1.86mmol) and acetyl chloride (80 μ L, 1.1mmol) were added. The solution was stirred at room temperature under an N_2 atmosphere for 2 hours. The solvent was evaporated, and the crude material dissolved in EtOAc and washed with 0.5N NaHSO_4 (2x), saturated NaHCO_3 (2x) and brine and was dried over anhydrous Na_2SO_4 , filtered and evaporated to give a yellow oil. Purification by flash column chromatography on silica gel using hexanes/EtOAc (95/5 to 90/10%) yielded the product as a yellow oil (0.301g, 89% yield). $^1\text{H-NMR}$ (500MHz, CDCl_3) δ 0.88 (dd, 3H), 0.99 (dd, 3H), 1.16-1.45 (m, 12H), 2.02 (s, 3H), 2.09-2.19 (m, 0.5H), 2.30-2.40 (m, 0.5H), 4.12-4.29 (m, 2H), 5.20-5.27 (m, 0.5H), 5.30-5.36 (m, 0.5H), 6.60 (s, 0.5H), 6.90 (s, 0.5H), 6.20-6.31 (m, 1H). Analytical HPLC (C18 column), (mixture of diastereomers) 7.77, 7.98min. LC-MS (ES^+) $m/e=358.3$ (M+H).

3-(2-Acetylamino-3-methylbutyryl)-5-tert-butyl-2,3-dihydro-[1,2,4]thiadiazole-2-carboxylic acid (39). To a solution of 38 (0.301g, 0.84mmol) in MeOH (10mL) was added 1N NaOH solution (1.7mL, 1.7mmol). The reaction was stirred at room temperature for 2 hours and solvent was evaporated. The residue was dissolved in EtOAc and washed with 0.5N NaHSO_4 (2x) and brine and was dried over anhydrous Na_2SO_4 , filtered and evaporated to give the title compound as a yellow solid (0.277g, quantitative).

Preparation of 2-(Benzyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester (40).

- 118 -

Compound 40 was prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester by a modification of the procedure described in Bioorg. Med. Chem. Lett. Vol. 2, No. 6, pp. 613-618, (1992).

5

To a solution of DMSO (27.52 g, 352 mmol) in CH₂Cl₂ (240 mL) at -78°C was added oxalyl chloride (24.4 g, 192 mmol). After 15 min, a solution of 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester (41.44 g, 160 mmol) in CH₂Cl₂ (100 mL) was slowly added and the mixture was stirred at -78°C for an additional 1.5 hours. DIEA (62.0 g, 480 mmol) was added and the mixture allowed to warm to room temperature for 15 min. The resulting solution was diluted with CH₂Cl₂ (300 mL), washed with 0.5 N NaHSO₄ (500 mL x 2), water (300 mL x 2), and brine (400 mL x 2). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to 200mL volume. To this solution was added, benzyl alcohol (48 g, 444 mmol), followed by 3Å molecular sieves (30 g) and p-toluenesulfonic acid (0.8 g). The reaction mixture was allowed to stir for 4 days and TFA (96 mL) was added. The resulting suspension was stirred for one hour then evaporated *in vacuo*. Ethyl acetate (500 mL) was added and the mixture was filtered through Celite. The filtrate was washed with saturated NaHCO₃ (500 mL x 2), water (400 mL x 2), and brine (300 mL x 2). The organic solution was dried over anhydrous Na₂SO₄, filtered and evaporated *in vacuo* to give 90 g of pale yellow oil, which was stirred with hexane (400 mL x 2) to give 31 g of crude product from the lower layer residue. Chromatography using ethyl acetate/hexane (4/96 to 22/78) afforded 6.97 g of *anti*-2-(benzyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester

- 119 -

(higher Rf), 4.53 g of *syn* diastereomer and 12.97 g of the mixture of the diastereomers (overall yield 53%).
¹H-NMR (500 MHz, CDCl₃) for *anti* diastereomer: δ 2.41-2.45 (m, H), 3.02-3.07 (m, H), 4.28 (br, H), 4.50-4.80 (m, 3H), 4.80-5.15 (m, 2H), 5.24-5.32 (m, 2H), 5.48 (s, H), 5.88-6.00 (m, H), 7.31-7.56 (m, 5H); for *syn* diastereomer: δ 2.49-2.53 (m, H), 2.83-2.89 (m, H), 4.57-4.65 (m, 4H), 4.87-4.90 (m, H), 5.12-5.30 (m, 3H), 5.52-5.53 (d, H), 5.88-6.00 (m, H), 7.31-7.39 (m, 5H); retention time on analytical HPLC: 10.49 min for *anti* diastereomer and 10.37 min for *syn* diastereomer; LC-MS: m/z = 292 (M+H⁺).

3-(2-Acetylamino-3-methylbutyryl)-5-*tert*-butyl-2,3-dihydro-[1,2,4] thiadiazole-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydrofuran-3-yl)-amide (41).
To a solution of (2-benzyloxy-5-oxo-tetrahydrofuran-3-yl)-carbamic acid allyl ester (40) (0.385g, 1.32mmol) in DMF (2ml) and CH₂Cl₂ (2ml) was added DMBA (0.456g, 2.92mmol) and Pd(PPh₃)₄ (0.136g, 0.12mmol) and the solution was stirred at room temperature for 15min. A solution of (39) in CH₂Cl₂ (4.5ml) and DMF (0.5ml) was added, followed by HOBT (0.168g, 1.24mmol) and EDC (0.256g, 1.33mmol). The reaction was stirred at room temperature for 18hours under N₂. The solvent was evaporated. The crude material was dissolved in EtOAc and washed with 0.5N NaHSO₄ (2x), saturated NaHCO₃ (2x) and brine and was dried over anhydrous Na₂SO₄, filtered and evaporated to give a yellow solid. Purification by flash column chromatography gave the title compound (41) as a mixture of diastereomers (374mg, 88% yield).
¹H-NMR (500MHz, CDCl₃) δ 0.75-1.05 (m, 6H), 1.19-1.34 (m, 9H), 1.93-2.08 (m, 3H), 2.19-2.50 (m, 2H), 2.80-

- 120 -

3.03 (m, 1H), 4.56-4.93 (m, 3H), 5.02-5.20 (m, 1H),
5.46-5.56 (m, 1H), 5.95-6.16 (m, 2H), 6.86-6.95 (m,
1H), 7.20-7.43 (m, 5H). Analytical HPLC (C18
column), (mixture of diastereomers) 8.58min. LC-MS
5 (ES⁺) m/e=519.2 (M+H).

**Preparation of 3-{[3-(2-acetylamino-3-methyl-butyryl)-
5-tert-butyl-2,3-dihydro-[1,3,4]thiadiazole-2-
carbonyl]-amino}-4-oxo-butyric acid (42).**

10 A 45 mg (0.087mmol) sample of 41 was hydrolyzed
according to method A (see Scheme XXIII) to give 17 mg
(45% yield) of the title compound. Analytical HPLC
(C18 column): 5.15min. LC-MS (ES⁺) m/e=429.3 (M+H).

15 **5-tert-Butyl-3-[2-(4-methoxy-benzoylamino)-3-methyl-
butyryl]-2,3-dihydro-[1,3,4]thiadiazole-2-carboxylic
acid ethyl ester (43)..**

Was prepared by the method reported above for compound
38 using anisoyl chloride to give 216mg (50%) of the
20 title compound as an amorphous solid. ¹H NMR (500 MHz,
CDCl₃) δ 0.92 (d, 1.5H), 0.98 (d, 1.5H), 1.03 (d, 1.5H),
1.07 (d, 1.5H), 1.21 (t, 3H), 1.28 (s, (H), 2.21-2.28
(m, 0.5H), 2.41-2.48 (m, 0.5H), 3.83 (s, 3H), 4.15-4.28
(m, 2H), 5.41-5.46 (m, 0.5H), 5.48-5.53 (m, 0.5H), 6.08
25 (s, 0.5H), 6.13 (s, 0.5H), 6.75 (d, 0.5H), 6.85 (d,
0.5H), 6.91 (d, 2H), 7.59 (d, 2H).

**5-tert-Butyl-3-[2-(4-methoxy-benzoylamino)-3-methyl-
butyryl]-2,3-dihydro-[1,3,4]thiadiazole-2-carboxylic
30 acid (44).**

Prepared by the procedure described for 39 to give
180mg (quantitative) of the title compound as a white
solid. ¹H NMR (500 MHz, CDCl₃) δ 0.92 (d, 1.5H), 0.96
(d, 1.5H), 1.03 (d, 1.5H), 1.07 (d, 1.5H), 2.22-2.30

- 121 -

(m, 0.5H), 2.37-2.45 (m, 0.5H), 3.83 (s, 1.5H) 3.84 (s, 1.5H), 5.41-5.48 (m, 1H), 6.14 (s, 0.5H), 6.15 (s, 0.5H), 6.87-6.95 (m, 2H), 7.75-7.83 (m, 3H).

5

5-tert-Butyl-3-[2-(4-methoxy-benzoylamino)-3-methyl-buteryl]-2,3-dihydro-[1,3,4]thiadiazole-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (45a and 45b).

10 Was prepared by the procedure reported for compound 41 to give the crude title compound as 4 diastereomers. The crude material was purified by flash chromatography, eluting with a gradient of CH₂Cl₂ to CH₂Cl₂/ethyl acetate (6/4) to give 31mg of the higher R_f component as a single diastereomer (45a). Analytical HPLC (Microsorb C18 column) 19.87 min. ¹H NMR (500 MHz, CDCl₃) (single diastereomer) δ 1.04 (d, 3H), 1.14 (d, 3H), 1.28 (s, 9H), 2.77 (d, 0.5H), 2.81 (d, 0.5H), 2.90 (d, 0.5H), 2.95 (d, 0.5H), 3.84 (s, 3H), 4.44-4.49 (m, 1H), 4.53 (d, 1H), 4.85 (d, 1H), 5.02-5.08 (m, 1H), 6.37 (s, 1H), 6.41 (d, 1H), 6.93 (d, 2H), 7.26-7.40 (m, 5H), 7.75 (d, 2H), 7.92-7.96 (m, 1H).

25 The lower R_f fraction contained 185mg of a solid as a 3:1:2 mixture of diastereomers (45b). Analytical HPLC: Microsorb C18 column. 19.00, 19.26, 20.02 mins, ¹H NMR (500 MHz, CDCl₃) (3:1:2 mixture of 3 diastereomers) δ 0.89 (d, 2.25 H), 0.98 (d, 0.75H), 1.02 (d, 0.5H), 1.03 (d, 1.5H), 1.08 (d, 0.25H), 1.10 (d, 0.75H), 1.16 (s, 0.75H), 1.17 (s, 2.25H), 1.23 (s, 0.375H), 1.24 (s, 1.125H), 1.28 (s, 1.125 H), 1.29 (s, 3.375H), 2.12-2.18 (m, 0.33H), 2.32-2.42 (m, 0.67H), 2.43-2.51 (m, 0.5H), 2.61-2.67 (m, 0.5H), 2.84-2.92 (m, 0.5H), 2.96-3.07 (m, 0.5H), 3.85 (s, 3H), 4.58-4.71 (m, 2H), 4.81 (d,

30

- 122 -

0.16H), 4.86 (d, 0.32H), 4.91 (d, 0.52H), 5.09-5.13 (m, 0.33H), 5.14-5.18 (m, 0.67H), 5.35 (dd, 1H), 5.46 (s, 0.16H), 5.53 (d, 0.32H), 5.58-5.62 (d, 0.52H), 6.17 (s, 0.52H), 6.20 (s, 0.16H), 6.34 (s, 0.32H), 6.50 (d, 0.32H), 6.62 (d, 0.16H), 6.67 (d, 0.52H), 6.86 (d, 0.33H), 6.91 (d, 0.67H), 6.94 (d, 1.0H), 7.24-7.43 (m, 5H), 7.61 (d, 1H), 7.70 (d, 0.33H), 7.71 (d, 0.67H), 7.76 (d, 1H).

10 **Preparation of 3-({5-tert-butyl-3-[2-(4-methoxy-benzoylamino)-3-methyl-butyryl]-2,3-dihydro-[1,3,4]thiadiazole-2-carbonyl}-amino)-4-oxo-butyric acid (46a).**

A 30mg sample of 45a was hydrolyzed according to method
15 B (see Scheme XXIII) to give 8mg (30% yield) of the desired product. Analytical HPLC (Microsorb C-18 column, acetonitrile/ water, with TFA buffer) 12.85min, ¹H NMR (500 MHz, CD₃OD) δ 0.98-1.1 (m, 6H), 1.28 (s, 9H), 2.20-2.31 (m, 1H), 2.40-2.48 (m, 1H), 2.6-2.72 (m, 1H), 3.84 (s, 3H), 4.18-4.26 (m, 1H), 4.56-4.62 (m, 1H), 5.25-5.32 (m, 1H), 6.24-6.28 (m, 1H), 6.98 (d, 2H), 7.85 (d, 2H).

25 **Preparation of 3-({5-tert-butyl-3-[2-(4-methoxy-benzoylamino)-3-methyl-butyryl]-2,3-dihydro-[1,3,4]thiadiazole-2-carbonyl}-amino)-4-oxo-butyric acid (46b).**

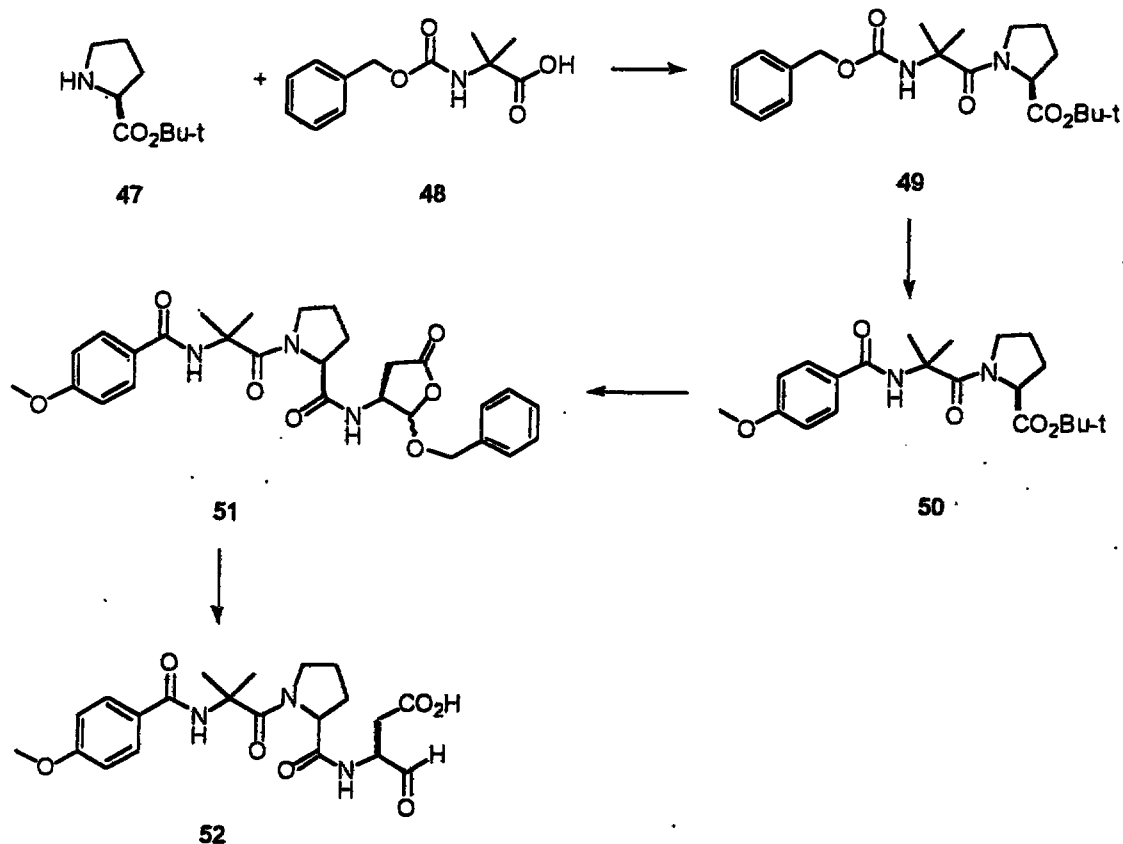
A 30mg sample of 45b (mixture of 3 diastereomers) was hydrolyzed according to method B (see Scheme XXIII) to
30 give 22mg (84% yield) of the desired product as a 3:2 mixture of diastereomers. Analytical HPLC (Microsorb cyano column) 7.08, 7.78min. ¹H NMR (500MHz, CD₃OD) δ 0.98-1.08 (m, 4H), 1.09-1.12 (m, 2H), 1.29 & 1.31 (2 singlets, 9H), 2.23-2.30 (m, 0.5H), 2.36-2.55 (m,

- 123 -

1.5H), 2.62-2.72 (m, 1H), 3.85 (s, 3H), 4.18-4.27 (m, 1H), 4.58-4.65 (m, 1H), 5.27-5.33 (m, 1H), 6.23-6.27 (m, 1H), 7.00 (d, 2H), 7.70-7.88 (m, 2H).

5

Scheme XI



1-(2-Benzyloxycarbonylamino-2-methyl-propionyl)-pyrrolidine-2-carboxylic acid tert-butyl ester (49).

To a solution of proline-tert-butyl ester (47) (2.00g, 12mmol, in CH_2Cl_2 (15ml) was added N-carbobenzyloxy-2-methylalanine (3.05g, 13mmol), HOBT (2.36g, 17mmol) and EDC (3.43g, 18mmol) and the solution was stirred at room temperature under N_2 for 48hours. The solvent was evaporated, the crude material dissolved in EtOAc and

- 124 -

washed with 0.5N NaHSO₄ (2x), saturated NaHCO₃ (2x) and brine and was dried over anhydrous Na₂SO₄, filtered and evaporated to give a white solid (4.68g, 100%). ¹H-NMR (500MHz, CDCl₃) δ 1.20-2.15 (m, 4H), 1.43 (s, 9H), 1.59 (d, 6H), 3.21-3.79 (m, 2H), 4.35 (br s, 1H), 4.82-5.19 (m, 3H), 5.74 (br s, 1H), 7.17-7.49 (m, 5H). Analytical HPLC (C18 column) 10.66min. LC-MS (ES⁺) m/e= 391.3 (M+H).

10 1- [2- (4-Methoxy-benzoylamino)-2-methyl-propionyl]-pyrrolidine-2-carboxylic acid tert-butyl ester (50).
To solution of 49 (1.00g, 2.56mmol) in MeOH (20ml) was added 10% Pd/C (200mg) and the mixture was stirred under H₂ for 2 hours. The mixture was filtered through
15 a 0.45µm PTFE filter and the solvent removed in vacuo to yield a colorless oil. This oil was dissolved in CH₂Cl₂ (25mL) and DIEA (660µl, 3.79mmol) and p-anisoyl chloride (480mg, 2.8mmol) were added. The solution was stirred at room temperature under N₂ for 18hours. The
20 solvent was removed in vacuo and the oil dissolved in EtOAc. The organic phase was washed with 0.5N NaHSO₄ (2x), water, saturated NaHCO₃ (2x) and brine. The organic phase was dried over Na₂SO₄, filtered and evaporated to give a white solid which was purified by
25 flash column chromatography, eluting with CH₂Cl₂/MeOH (99/1 to 98/2%) to give the title compound as a white solid (655mg, 65% yield). ¹H-NMR (500MHz, CDCl₃) δ 1.47 (s, 9H), 1.68-2.24 (m, 5H), 1.80 (d, 6H), 3.55-3.68 (m, 1H), 3.72-3.93 (m, 1H), 3.84 (s, 3H), 4.43-4.55 (m, 1H), 6.90 (d, 2H), 7.60 (br s, 1H), 7.77 (d, 2H).
30 Analytical HPLC (C18 column) 8.98min.

- 125 -

1-[2-(4-Methoxy-benzoylamino)-2-methyl-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (51).

To a solution of 50 (325mg, 0.83mmol) in dioxane (5mL) was added triethylamine (463μl, 3.32mmol) and TMS-triflate (642μl, 3.32mmol) and the solution was stirred at 100°C for 5 hours, then at room temperature for 18 hours. The reaction was diluted with water, adjusted to pH 8 with saturated NaHCO₃ and extracted with Et₂O, dried over Na₂SO₄, filtered and evaporated to give a white solid (230mg, 83% yield) which was used directly in the next step.

To a solution of (2-benzyloxy-5-oxo-tetrahydrofuran-3-yl)-carbamic acid allyl ester (40) (1.027g, 3.5mmol) in CH₂Cl₂ (20ml) was added DMBA (543mg, 3.48mmol) and Pd(PPh₃)₄ (280mg, 0.24mmol) and the solution was stirred at room temperature under N₂ for 20 minutes. A solution of 1-[2-(4-methoxy-benzoylamino)-2-methyl-propionyl]-pyrrolidine-2-carboxylic acid (818mg, 2.45mmol) in CH₂Cl₂ (5ml) was added, followed by HOBT (0.534g, 3.95mmol) and EDC (738mg, 3.84mmol). The reaction was stirred at room temperature for 18 hours under N₂. The solvent was evaporated, the crude material dissolved in EtOAc and washed with 0.5N NaHSO₄ (2x), saturated NaHCO₃ (2x) and brine and was dried over anhydrous Na₂SO₄, filtered and evaporated to give a yellow solid. Purification by flash column chromatography, eluting with ethyl acetate/hexanes (20/80 to 50/50%), gave the product as pale yellow solid (760mg, 61% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.53 (d, 6H), 1.65-1.93 (m, 3H), 1.96-2.14 (m, 1H), 2.60 (dd, 0.1H), 2.77 (dd, 0.85H), 2.94 (dd, 0.85H), 3.04-3.11 (m, 0.2H), 3.42-3.52 (m, 1H), 3.57-3.67 (m, 1H), 3.84 (s, 3H), 4.38-4.76 (m,

- 126 -

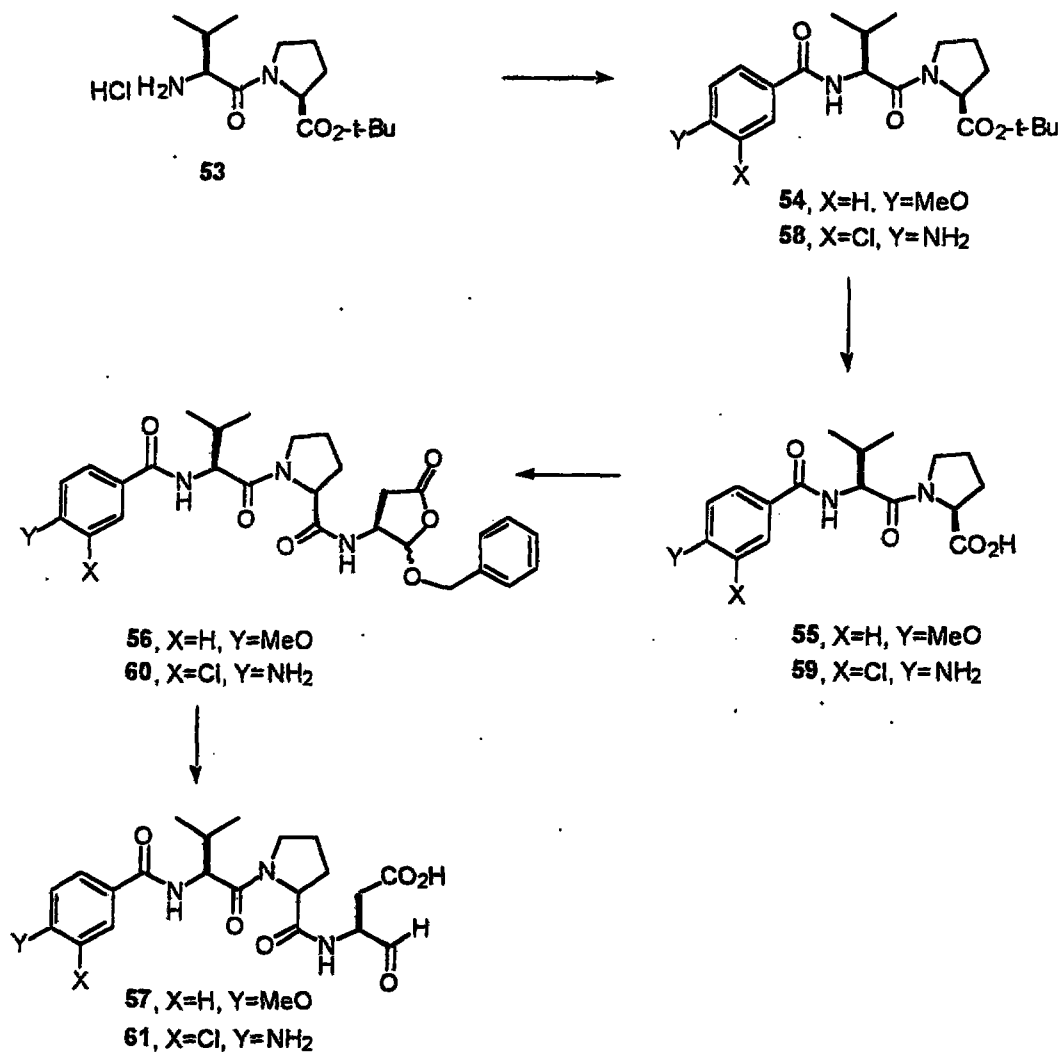
3H), 4.84 (d, 1H), 5.64-5.70 (m, 1H), 6.96-7.03 (m, 2H), 7.23-7.43 (m, 5H), 7.78-7.97 (m, 2H). Analytical HPLC (C18 column) 13.32, 14.37min. LC-MS (ES⁺) m/e=524.3 (M+H).

5

Preparation of 3-({1-[2-(4-methoxy-benzoylamino)-2-methyl-propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (52).

10 A 61mg (0.14mmole) sample of 51 was hydrolyzed according to method C (see Scheme XXIII) to afford 30mg (60% yield) of the title compound: Analytical HPLC (C18 column) 6.79min. LC-MS (ES⁺) m/e=434.3 (M+H).

Scheme XII



1- [2- (4-Methoxy-benzoylamino)-3-methylbutyryl]-
pyrrolidine-2-carboxylic acid tert-butyl ester (54).

- 5 To a suspension of H-val-pro-OtBu.HCl (53) (2.011g,
7.44mmol) in CH₂Cl₂ (20ml) was added DIEA (3.2ml,
18.4mmol) followed by a solution of 4-methoxy-benzoyl
chloride (1.26g, 7.4mmol) in CH₂Cl₂ (5ml). The solution
was stirred at room temperature under nitrogen for
10 1hour then concentrated. The resulting oil was
dissolved in EtOAc and washed with 0.5N KHSO₄ (2x),

- 128 -

saturated NaHCO_3 (2x) and brine, then concentrated in vacuo to give the title compound as a white solid (2.814g, 94% yield). $^1\text{H-NMR}$ (500MHz, CDCl_3) δ 1.05 (dd, 6H), 1.46 (s, 9H), 1.88-2.29 (m, 5H), 3.65-3.74 (m, 1H), 3.81-3.92 (m, 1H), 3.85 (s, 3H), 4.32-4.42 (m, 1H), 4.81-4.91 (m, 1H), 6.79-6.86 (m, 1H), 6.91 (d, 2H), 7.78 (d, 2H). Analytical HPLC (cyano column) 10.18min.

10 1-[2-(4-Methoxy-benzoylamino)-3-methylbutyryl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydrofuran-3-yl) amide (56).

15 A 1.079g (2.67mmol) sample of 54 was dissolved in 15% TFA in CH_2Cl_2 (40mL) and stirred at room temperature for 4hours. The solvent was concentrated in vacuo to give 55 as a white solid (0.93g, 100%) which was used in the next step.

20 To a solution of 40 (1.796g, 6.17mmol) in CH_2Cl_2 (20ml) was added DMBA (1.119g, 7.17mmol) and $\text{Pd}(\text{PPh}_3)_4$ (0.683g, 0.59mmol) and the solution stirred at room temperature for 20 minutes. A solution of 55 (0.928g, 2.67mmol) in CH_2Cl_2 (17ml) and DMF (2ml) was added, followed by HOBT (0.811g, 6.01mmol) and EDC (1.16g, 6.04mmol). The
25 reaction was stirred at room temperature for 18hours under N_2 . The solvent was evaporated, the crude material dissolved in EtOAc and washed with 0.5N NaHSO_4 (2x), saturated NaHCO_3 (2x) and brine and was dried over anhydrous Na_2SO_4 , filtered and evaporated to give
30 a yellow solid. Purification by flash chromatography eluting with ethyl acetate/ CH_2Cl_2 (10/90 to 40/60%) gave the title compound as pale yellow solid (910mg, 63% yield). $^1\text{H-NMR}$ (500MHz, CDCl_3) δ 0.96 (dd, 6H), 1.84-2.19 (m, 4H), 2.25-2.38 (m, 1H), 2.45 (dd, 1H),

- 129 -

2.80-2.98 (m, 1H), 3.60-3.72 (m, 1H), 3.82-3.95 (m, 1H), 3.86 (s, 3H), 4.26-4.95 (m, 6H), 5.41 (s, 0.2H), 5.53 (d, 0.8H), 6.67-6.77 (m, 1H), 6.88-6.99 (d, 2H), 7.22-7.57 (m, 5H), 7.71-7.82 (d, 2H).

- 5 Analytical HPLC (cyano column) (mixture of 2 diastereomers) 9.21min. LC-MS (ES⁺) m/e= 538.3 (M+H).

3-({1-[2-(4-Methoxy-benzoylamino)-3-methyl-butyryl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (57).

- 10 A 125mg (0.23mmol) sample of 56 was hydrolyzed according to method A (see Scheme XXIII) to afford 60mg (58% yield) of the title compound: Analytical HPLC 5.71min. LC-MS (ES⁺) m/e=448.2 (M+H).

15 **Preparation of 4-Amino-3-chloro-benzoic acid:**

- A suspension of 4-amino-3-chlorobenzonitrile (4.82g, 31.58mmol) was heated to reflux in 6N HCl (140ml). The precipitate dissolved upon heating to give a colorless solution. Upon further heating the solution became
20 cloudy. After 9 hours the reaction was cooled to room temperature. The resulting precipitate was filtered, then dissolved in THF and the solvent evaporated. The residue was repeatedly concentrated from toluene to give a white solid (3.18g, 59% yield). ¹H-NMR (500MHz, CD₃OD:CDCl₃ 1:4) δ 6.80 (d, 1H), 7.75 (dd, 1H), 7.94 (d, 1H). Analytical HPLC (cyano column) 8.73min.
25

1-[2-(4-Amino-3-chloro-benzoylamino)-3-methylbutyryl]-pyrrolidine-2-carboxylic acid tert-butyl ester (58).

- 30 To a suspension of 53 (1.707g, 6.31mmol) in CH₂Cl₂ (25ml) at 0°C was added DIEA (3.2ml, 18.4mmol) followed by a solution of 4-amino-3-chlorobenzoic acid (1.298g, 7.56mmol), HOBT (1.005g, 7.44mmol) and EDC (1.456g, 7.58mmol). The resulting mixture was stirred at 0°C

- 130 -

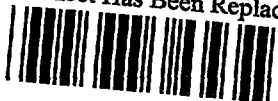
for 15minutes then allowed to warm to room temperature and stir for 18hours. The solvent was evaporated and the resulting oil dissolved in EtOAc, washed with 0.5N NaHSO₄ (2x), saturated NaHCO₃ (2x) and brine to give a white solid (2.68g). Flash chromatography using MeOH/CH₂Cl₂ (1/99 to 2/98%) gave 2.04g (76% yield) of 58 as a white solid. ¹H-NMR (500MHz, CDCl₃) δ 1.05 (dd, 6H), 1.47 (s, 9H), 1.86-2.29 (m, 5H), 3.62-3.78 (m, 1H), 3.78-3.94 (m, 1H), 4.39 (dd, 1H), 4.79-4.89 (dd, 1H), 6.73 (d, 1H), 6.78 (d, 1H), 7.52 (dd, 1H), 7.75 (d, 1H). Analytical HPLC (cyano column) 16.18min. LC-MS (ES⁺) m/e=424.3 (M+H).

1- [2- (4-Amino-3-chloro-benzoylamino)-3-methylbutyryl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (60).

A 0.632g (1.49mmol) sample of 59 was dissolved in 50% TFA in CH₂Cl₂ (20mL) and the solution stirred at room temperature for 2hours. Residual TFA was removed by repeated concentration from CH₂Cl₂ (3x) to give the product as a white solid.

A 385mg (1.04mmol) sample was allowed to react with 40 by the method used for compound 56. The title compound (60) was isolated as a yellow solid (265mg, 45% yield). ¹H-NMR (500MHz, CD₃OD) δ 0.89-1.12 (m, 6H), 1.72-2.26 (m, 5H), 2.49 (dd, 0.25H), 2.60 (dd, 0.7H), 2.80 (dd, 0.75H), 2.96-3.09 (m, 0.3H), 3.64-3.77 (m, 1H), 3.94-4.10 (m, 1H), 4.20-4.74 (m, 4H), 4.76-4.95 (m, 1H), 5.51 (s, 0.5H), 5.61-5.70 (m, 1.5H), 6.79 (dd, 1H), 7.23-7.43 (m, 5H), 7.48-7.61 (m, 1.4H), 7.68-7.81 (m, 1H), 7.99-8.12 (m, 0.6H).

This Sheet Has Been Replaced



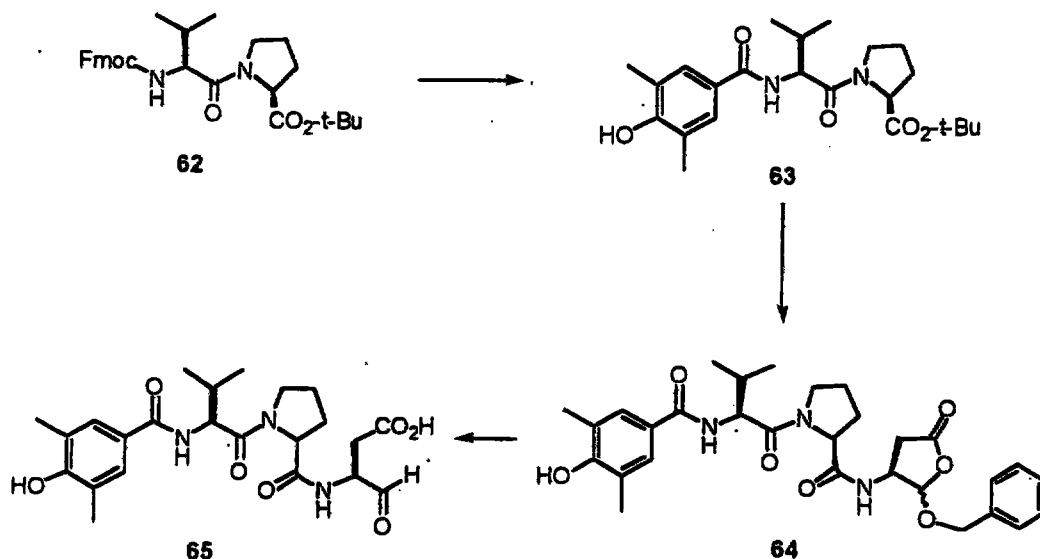
- 131 -

Analytical HPLC (cyano column) (mixture of 2 diastereomers) 14.90, 15.20min. LC-MS (ES⁺) m/e=557.2 (M+H).

5 3-({1-[2-(4-Amino-3-chloro-benzoylamino)-3-methyl-buteryl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyrlic acid (61).

A 45mg (0.08mmol) sample of 60 was hydrolyzed according to method A (see Scheme XXIII) to afford 30mg (80% yield) of the title compound: ¹H NMR (500MHz, CD₃OD) δ 1.06 (dd, 6H), 1.78-2.38 (m, 5H), 2.38-2.86 (m, 2H), 3.62-3.83 (m, 1H), 4.12-4.76 (m, 4H), 7.04-7.21 (m, 1H), 7.58-8.01 (m, 2H); Analytical HPLC 8.16min. LC-MS (ES⁺) m/e=467.3 (M+H).

Scheme XIII



20 1-[2-(4-Hydroxy-3,5-dimethyl-benzoylamino)-3-methyl-buteryl]-pyrrolidine-2-carboxylic acid tert-butyl ester (63).

To a solution of 62 (prepared from 53 and Fmoc-Cl) (600mg, 1.22mmol) in anhydrous DMF (10ml) was added

- 132 -

diethylamine (3ml). The solution was stirred at room temperature under N₂ for 3hours and the solvent was evaporated. The resulting oil was dissolved in CH₂Cl₂ (8ml) and 3,5-dimethyl-4-hydroxybenzoic acid (0.302g, 1.82mmol), HOBT (338mg, 2.5mmol) and EDC (0.456g, 2.43mmol) were added and the solution stirred at room temperature under N₂ for 18hours. The solvent was concentrated in vacuo and the resulting oil dissolved in EtOAc, washed with 0.5N NaHSO₄ (2x), saturated NaHCO₃ (2x) and brine to give the crude product as a white solid (0.80g). Flash chromatography eluting with MeOH/CH₂Cl₂ (1/99 to 2/98%) gave 380mg (75% yield) of a white solid. ¹H-NMR (500MHz, CDCl₃) δ 1.06 (dd, 6H), 1.47 (s, 9H), 1.90-2.32 (m, 5H), 2.24 (s, 6H), 3.65-3.75 (m, 1H), 3.84-3.92 (m, 1H), 4.36-4.42 (m, 1H), 4.82-4.88 (m, 1H), 5.53-5.61 (m, 1H), 6.77-6.85 (m, 1H), 7.42 (s, 2H). Analytical HPLC (cyano column) 17.53min. LC-MS (ES⁺) m/e=419.3 (M+H).

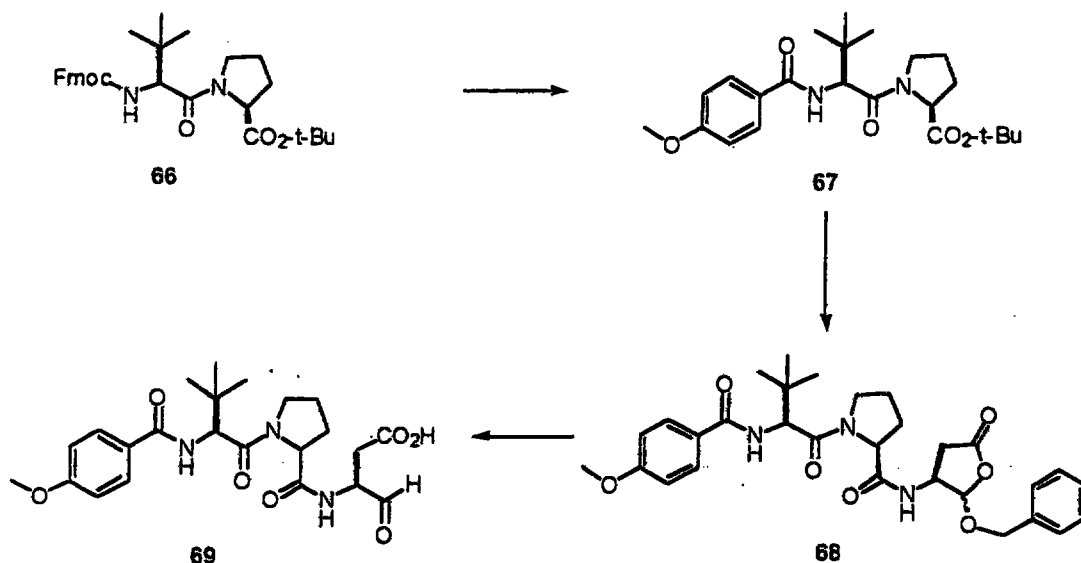
1- [2- (4-Hydroxy-3,5-dimethyl-benzoylamino)-3-methyl-butyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (64). Prepared from 63 and 40 by the method used to prepare 56 to give title compound (64) as a pale yellow solid (352mg, 72% yield). ¹H-NMR (500MHz, CD₃OD) δ 0.83-1.28 (m, 6H), 1.66-2.37 (m, 3H), 2.23 (s, 6H), 2.48-2.54 (m, 0.2H), 2.61 (ddd, 0.8H), 2.72 (ddd, 0.9H), 3.01-3.09 (m, 1H), 3.66-3.76 (m, 1H), 3.95-4.07 (m, 1H), 4.48-4.73 (m, 3H), 4.75-4.92 (m, 1H), 5.45-5.48 (m, 0.1H), 5.61-5.64 (m, 0.1H), 5.64-5.70 (m, 0.8H), 7.21-7.62 (m, 6H), 7.88-8.04 (m, 1H). Analytical HPLC (cyano column) (mixture of 2 diastereomers) 17.73min. LC-MS (ES⁺) m/e= 552.3 (M+H).

- 133 -

3-({1-[2-(4-Hydroxy-3,5-dimethyl-benzoylamino)-3-methyl-butyryl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (65).

A 160mg (0.29mmol) sample of 64 was hydrolyzed according to method A (see Scheme XXIII) to afford 13.1mg (10% yield) of the title compound: Analytical HPLC (cyano column) 10.28min. LC-MS (ES⁺) m/e=462.2 (M+H).

Scheme XIV



10

1-[2-(2-9H-Fluoren-9-yl-acetylamino)-3,3-dimethyl-butyryl]-pyrrolidine-2-carboxylic acid tert-butyl ester (66).

To a solution of H-pro-OtBu (53) (1.033g, 6.0mmol, II, Scheme 5) in CH₂Cl₂ (20ml) and DMF (5ml) was added Fmoc-tLeu-OH (2.337g, 6.60mmol, I, Scheme 5), HOBT (1.63g, 12.1mmol) and EDC (2.30g, 12.0mmol) and the solution stirred at room temperature under N₂ for 18hours. The solvent was removed in vacuo, and the residue dissolved in EtOAc then washed with 0.5N NaHSO₄ (2x), saturated NaHCO₃ (2x) and brine. The organic

20

- 134 -

layer was dried over anhydrous Na_2SO_4 and evaporated to give a pale yellow solid (3.65g). Flash chromatography using EtOAc/hexanes (10/90 to 20/80%) give the title compound (66) (2.25g, 74% yield). ^1H -NMR (500MHz, CDCl_3) δ 1.09 (s, 9H), 1.47 (s, 9H), 1.79-2.28 (m, 3H), 3.62-3.72 (m, 1H), 3.76-3.83 (m, 1H), 4.18-4.43 (m, 4H), 5.48-5.67 (m, 1H), 7.28-7.44 (m, 4H), 7.55-7.64 (m, 2H), 7.72-7.82 (m, 2H). Analytical HPLC (cyano column) 11.95min. LC-MS (ES^+) $m/e=507.3$ (M+H).

1- [2- (4-Methoxy-benzoylamino) -3,3-dimethyl-butyryl] -pyrrolidine-2-carboxylic acid tert-butyl ester (67).

To a solution of 66 (0.503g, 0.99mmol) in DMF (8ml) was added diethylamine (2.5ml) and the solution stirred at room temperature for 1hour and the solvent evaporated. The resulting residue was repeatedly concentrated from CH_2Cl_2 (3x). The resulting oil was dissolved in CH_2Cl_2 (9ml) and DIEA (260 μl , 1.49mmol) and 4-methoxy-benzoyl chloride (190mg, 1.05mmol) was added. The solution was stirred under N_2 for 18hours and the solvent concentrated in vacuo. The residue was dissolved in EtOAc and washed with 0.5N NaHSO_4 (2x), saturated NaHCO_3 (2x) and brine then dried over anhydrous Na_2SO_4 and evaporated to give a white solid (0.529g). Flash chromatography on silica gel using MeOH/ CH_2Cl_2 (1/99 to 2/98%) gave the title compound (2.25g, 74% yield). ^1H -NMR (500MHz, CD_3OD) δ 1.01 (s, 1.4H), 1.11 (s, 7.6H), 1.73-2.25 (m, 4H), 2.47-2.77 (m, 1H), 2.81 (dd, 0.7H), 2.91-3.11 (m, 0.3H), 3.61-4.03 (m, 3H), 3.84 (s, 3H), 4.29-4.49 (m, 1H), 4.49-5.00 (m, 5H), 5.46 (s, 0.15H), 5.58-5.73 (m, 0.85H), 6.94-7.04 (m, 2H), 7.27-7.41 (m, 4H), 7.61-7.73 (m, 1H), 7.74-7.84 (m, 2H). Analytical HPLC (cyano column) 13.10min.

- 135 -

1- [2- (4-Methoxy-benzoylamino)-3,3-dimethyl-butyryl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (68).

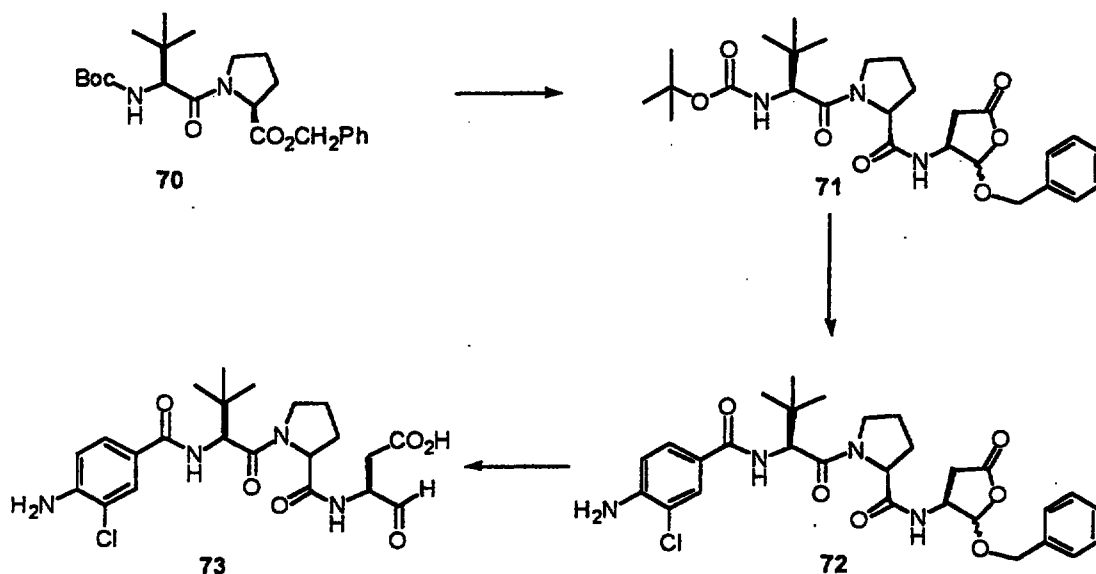
To a solution of 67 (0.90g, 1.74mmol) in CH₂Cl₂ (25ml) was added 2,6-lutidine (2.1ml, 18.0mmol) and TMS-triflate (2.3ml, 11.9mmol) and the reaction stirred at room temperature under N₂ for 1.5hours. The resulting mixture was diluted with CH₂Cl₂, washed with 10% NaHCO₃ (2x) and brine then dried over Na₂SO₄, filtered and evaporated. The residue was dissolved in CH₂Cl₂ then treated with DIEA (0.6ml, 3.5mmol) and 4-methoxy-benzoyl chloride (0.355g, 2.09mmol) and allowed to stir under N₂ at room temperature for 18hours. The crude product was purified by flash chromatography, eluting with CH₂Cl₂/MeOH (99/1) to yield the title compound (274mg, 28% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.01 (s, 1.4H), 1.11 (s, 7.6H), 1.73-2.25 (m, 4H), 2.47-2.77 (m, 1H), 2.81 (dd, 0.7H), 2.91-3.11 (m, 0.3H), 3.61-4.03 (m, 3H), 3.84 (s, 3H), 4.29-4.49 (m, 1H), 4.49-5.00 (m, 5H), 5.46 (s, 0.15H), 5.58-5.73 (m, 0.85H), 6.94-7.04 (m, 2H), 7.27-7.41 (m, 4H), 7.61-7.73 (m, 1H), 7.74-7.84 (m, 2H). Analytical HPLC (cyano column) (mixture of 2 diastereomers) 17.03, 17.39min. LC-MS (ES⁺) m/e=552.3 (M+H).

3- ({1- [2- (4-Methoxy-benzoylamino)-3,3-dimethyl-butyryl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (69).

A 117mg (0.21mmol) sample of 68 was hydrolyzed according to method C (see Scheme XXIII) to afford 40mg (41% yield) of the title compound: Analytical HPLC 7.16min. LC-MS (ES⁺) m/e=462.3 (M+H).

- 136 -

Scheme XV



1- (2-tert-Butoxycarbonylamino-3,3-dimethyl-butyryl)-pyrrolidine-2-carboxylic acid benzyl ester (70).

5 To a suspension of H-pro-OBzl.HCl (2.00g, 8.66mmol) in CH₂Cl₂ (20ml) was added DIEA (2.25ml, 12.92mmol) to give a colorless solution. Boc-tLeu-OH (1.95g, 9.52mmol), HOBT (1.76g, 13.03mmol) and EDC (2.49g, 12.95mmol) were added and the solution stirred under N₂ at room temperature for 18hours. Removed solvent in vacuo, dissolved in EtOAc and washed with H₂O, 0.5N NaHSO₄ (2x), saturated NaHCO₃ (2x) and brine. Dried over anhydrous Na₂SO₄ and evaporated to give the title compound. (3.57g, 99% yield). ¹H-NMR (500MHz, CDCl₃) δ

10 0.99 (s, 9H), 1.40 (s, 9H), 1.88-2.33 (m, 4H), 3.58-3.90 (m, 2H), 4.21-4.35 (d, 1H), 4.53-4.66 (m, 1H), 5.04-5.38 (m, 3H), 7.14-7.42 (m, 5H). LC-MS (ES⁺) m/e=419.4 (M+H).

15

20 {1-[2-(2-Benzylloxy-5-oxo-tetrahydro-furan-3-ylcarbonyl)-pyrrolidine-1-carbonyl]-2,2-dimethyl-propyl}-carbamic acid tert-butyl ester (71).

- 137 -

An 871mg (2.08mmol) sample of 70 was dissolved in MeOH (15mL) and 10% Pd/C (200mg) added. The suspension was stirred under H₂ for 1hour then filtered through Celite and the solvent evaporated. This resulting residue was
5 reacted with 40 according to the procedure used to prepare 56 to give 889mg (71% yield) of the title compound (71). ¹H-NMR (500MHz, CDCl₃) δ 0.93 (s, 9H), 1.44 (s, 9H), 1.78-2.18 (m, 4H), 2.29-2.49 (m, 2H), 2.76-3.04 (m, 1H), 3.50-3.70 (m, 1H), 3.70-3.85 (m,
10 1H), 4.20-4.37 (m, 1H), 4.49-4.78 (m, 3H), 4.78-4.98 (m, 1H), 5.12-5.26 (m, 1H), 5.40-5.59 (m, 1H), 7.10-7.78 (m, 5H). Analytical HPLC (cyano column) 11.17min. LC-MS (ES⁺) m/e=518.3 (M+H).

15 1-[2-(4-Amino-3-chloro-benzoylamino)-3,3-dimethyl-butryrl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (72).
A solution of 456mg (0.088mmol) of 71 in CH₂Cl₂ (20ml) was treated with anhydrous TFA (5mL) then stirred at
20 room temperature under N₂ for 1 hour and evaporated to dryness. The residue was repeatedly concentrated from CH₂Cl₂ (3x) then dried under vacuum. The resulting residue was dissolved in CH₂Cl₂ (20ml), cooled to 0°C, then treated with DIEA (1.3ml, 8eq, 2.46mmol) followed
25 by 4-amino-3-chloro-benzoic acid (202mg, 1.17mmol), HOBT (183mg, 1.35mmol), and EDC (279mg, 1.45mmol). The resulting mixture was allowed to warm to room temperature and stir for 18hours. The solvent was removed in vacuo and the residue dissolved in EtOAc
30 then washed with distilled water (3x), 0.5N NaHSO₄ (2x), saturated NaHCO₃ (2x) and brine. The organic layer was dried over Na₂SO₄, filtered and evaporated to give a residue that was purified by flash chromatography, eluting with CH₂Cl₂/MeOH (99/1 to

- 138 -

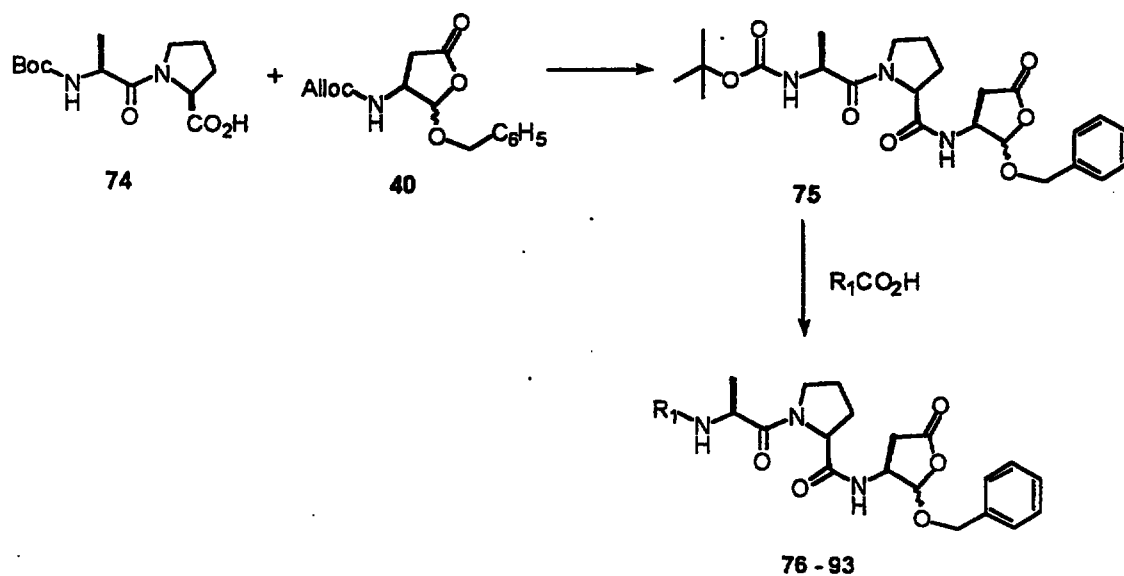
97/3%), affording 285mg (57% yield) of the title compound (72) as a yellow solid.

¹H-NMR (500MHz, CD₃OD) δ 0.91-1.24 (m, 9H), 1.70-2.27 (m, 4H), 2.47-2.85 (m, 1.5H), 2.99-3.13 (m, 0.5H), 3.39-3.53 (m, 0.5H), 3.60-3.78 (m, 1.5H), 3.85-4.04 (m, 1H), 4.24-4.47 (m, 2H), 4.53-4.97 (m, 4H), 5.46 (s, 0.3H), 3.88-4.02 (m, 0.1H), 5.60-5.69 (m, 0.6H), 6.80 (d, 1H), 7.22-7.77 (m, 7H). Analytical HPLC (cyano column) (mixture of 2 diastereomers) 15.90, 16.23min. LC-MS (ES⁺) m/e=571.2 (M+H).

3-({1-[2-(4-Amino-3-chloro-benzoylamino)-3,3-dimethylbutyryl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (73).

A 40mg (0.07mmol) sample of 72 was hydrolyzed according to method A (see Scheme XXIII) to afford 25mg (74% yield) of the title compound: Analytical HPLC (cyano column) 10.66min. LC-MS (ES⁺) m/e=481.3 (M+H).

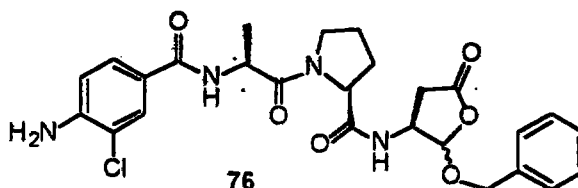
Scheme XVI



- 139 -

{2-[2-(2-Benzoyloxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl}-carbamic acid tert-butyl ester (75).

To a solution of 40 (6.69g, 23.0mmol) in anhydrous
5 CH₂Cl₂ was added 1,3-dimethylbarbituric acid (DMBA) (3.97g, 25.4mmol) and Pd(PPh₃)₄ (1.12g, 0.97mmol). The solution was stirred under N₂ at room temperature for 15min, cooled to 0°C, followed by the addition of Boc-ala-pro-OH (BaChem) (5.087g, 17.8mmol), HOBT (3.60g,
10 26.7mmol) and EDC (5.12g, 26.7mmol). The resulting solution was allowed to warm to room temperature and stir under N₂ for 18hours. The solvent was concentrated in vacuo and the residue dissolved in EtOAc, then washed with 0.5N NaHSO₄ (2x), saturated
15 NaHCO₃ (2x) and brine. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to give an orange oil (12.23g). Flash column chromatography on silica gel using CH₂Cl₂/EtOAc (80/20 to 60/40) gave the title compound 75 as a yellow solid (7.28g, 86% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.19-1.31 (m, 3H), 1.42 (s, 9H), 1.69-2.29 (m, 4H), 2.45-2.67 (m, 0.9H), 2.71-2.86 (m, 0.5H), 2.99-3.10 (m, 0.6H), 3.49-3.84 (m, 2H), 4.24-4.45 (m, 2.5H), 4.57-4.73 (m, 1.5H), 4.76-4.92 (m, 1H), 5.45 (s, 0.45H), 5.63-5.68 (m, 0.55H), 7.25-7.40 (m,
20 5H). Analytical HPLC (cyano column) (mixture of 2 diastereomers) 15.99, 16.33min. LC-MS (ES⁺) m/e=476.3 (M+H).



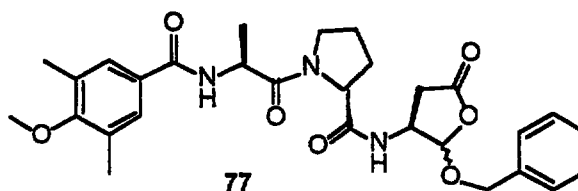
30

- 140 -

[1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide] (76).

A 1.899g (3.99mmol) sample of 75 in CH₂Cl₂ (20ml) was
5 treated with anhydrous TFA (5ml) then stirred at room
temperature under N₂ for 1 hour and evaporated to
dryness. The residue was repeatedly concentrated from
CH₂Cl₂ (3x) then dried under vacuum. The resulting
residue was dissolved in CH₂Cl₂ (20ml), cooled to 0°C,
10 then treated with DIEA (5.6ml, 8eq, 32.1mmol), 4-amino-
3-chloro-benzoic acid (0.910g, 5.3mmol), HOBT (0.824g,
6.1mmol), and EDC (1.197g, 6.23mmol). The resulting
mixture was warmed to room temperature and stirred for
18hours. The solvent was removed in vacuo and the
15 residue dissolved in EtOAc then washed with distilled
water (3x), 0.5N NaHSO₄ (2x), saturated NaHCO₃ (2x) and
brine. The organic layer was dried over Na₂SO₄,
filtered and evaporated to give a residue that was
purified by flash chromatography using CH₂Cl₂/MeOH
20 (99/1 to 97/3%). The title compound was obtained as a
white solid (1.221g, 58% yield). ¹H-NMR (500MHz, CD₃OD)
δ 1.15 (d, 0.25H), 1.29-1.60 (m, 2.75H), 2.41-2.54 (m,
0.5H), 2.55-2.70 (m, 0.5H), 2.77 (dd, 0.5H), 3.03 (ddd,
0.5H), 3.59-3.75 (m, 1H), 3.75-3.98 (m, 1H), 4.26-5.01
25 (m, 5H), 5.41-5.57 (m, 1H). 5.60-5.76 (m, 0.5H), 6.70-
6.92 (m, 0.5H), 7.15-7.48 (m, 5H), 7.48-7.68 (m, 1H),
7.68-7.88 (m, 1H), 8.15-8.34 (m, 1H). Analytical HPLC
(cyano column) (mixture of 2 diastereomers) 14.44,
14.89min. LC-MS (ES⁺) m/e=529.3 (M+H).

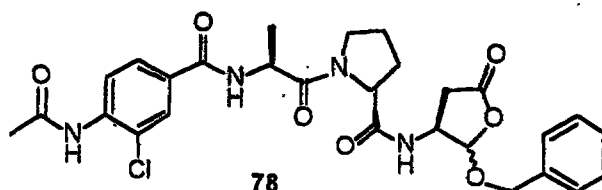
30



- 141 -

[1-[2-(4-Methoxy-3,5-dimethyl-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide] (77) was
5 synthesized from 75 and 3,5-dimethyl-4-methoxy benzoic acid according to the procedure used to prepare 76 to afford the title compound (1.18g, 44% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.40 (m, 3H), 1.67-2.41 (m, 4H), 2.28 (s, 6H), 2.48 (ddd, 0.5H), 2.62 (dd, 0.5H), 2.78 (ddd, 0.5H), 3.04 (ddd, 0.5H), 3.62-3.94 (m, 3H), 3.71 (s, 3H), 4.21-4.51 (m, 2H), 4.59-4.85 (m, 4H), 5.46 (s, 0.25H), 5.52 (s, 0.25H), 5.63 (d, 0.4H), 5.67 (d, 0.1H), 7.17-7.45 (m, 5H), 7.45-7.65 (m, 2H).
10 Analytical HPLC (cyano column) (mixture of 2 diastereomers) 15.06, 15.39min. LC-MS (ES⁺) m/e=538 (M+H)

Preparation of 4-Acetylamino-3-chlorobenzoic acid
To a solution of 4-amino-3-chloro-benzoic acid (10.0g, 58.3mmol) in anhydrous THF (100mL) was added acetyl
20 chloride (20.7ml, 291.1mmol) and the solution stirred at room temperature for 48hours. The solvent was evaporated and the product precipitated from hexanes then filtered and dried to give a white solid (11.73g, 94% yield). ¹H-NMR (500MHz, CD₃OD) δ 2.28 (s, 3H), 7.92 (dd, 1H), 7.99-8.16 (m, 2H). Analytical HPLC (cyano
25 column) 7.84min.

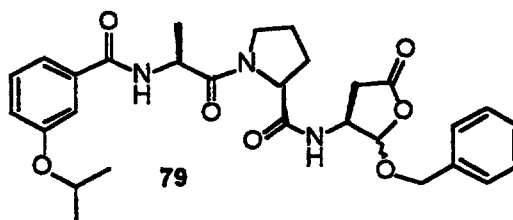


30

- 142 -

1- [2- (4-Acetylamino-3-chloro-benzoylamino)-propionyl]-
pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-
tetrahydro-furan-3-yl)-amide (78).

Prepared from 75 and 4-acetylamino-3-chloro-benzoic
acid according to the procedure used to prepare 76 to
afford the title compound (146mg, 19% yield). ¹H-NMR
(500MHz, CD₃OD) δ 1.28-1.52 (m, 3H), 1.68-2.38 (m, 4H),
2.20 (s, 3H), 2.41-2.88 (m, 1.5H), 2.96-3.10 (m, 0.5H),
2.96-3.10 (m, 0.5H), 3.43-3.75 (m, 1H), 3.80-3.96 (m,
1H), 4.25-5.00 (m, 2H), 5.42-5.54 (m, 0.5H), 5.63-5.78
(m, 0.5H), 7.13-7.48 (m, 0.5H), 7.79-8.14 (m, 2.5H),
8.56-8.70 (m, 0.5H). Analytical HPLC (cyano column)
(mixture of 2 diastereomers) 8.64min. LC-MS (ES⁺)
m/e=571.2 (M+H).

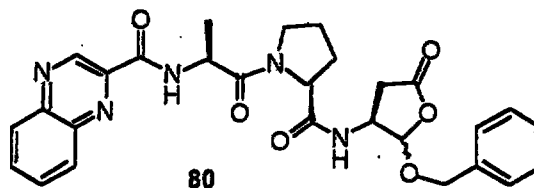


1- [2- (3-Isopropoxy-benzoylamino)-propionyl]-
pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-
tetrahydro-furan-3-yl)-amide (79).

Prepared from 75 and 3-isopropoxybenzoic acid according
to the procedure used to prepare 76 to afford the title
compound (120mg, 58% yield). ¹H-NMR (500MHz, CD₃OD) δ
1.27 (d, 6H), 1.33-1.52 (m, 3H), 1.69-2.31 (m, 4H),
2.49 (dd, 0.3H), 2.63 (dd, 0.7H), 2.78 (dd, 0.7H), 3.03
(dd, 0.3H), 3.43-3.73 (m, 1H), 3.78-3.94 (m, 1H), 4.27-
4.47 (m, 2H), 4.47-4.87 (m, 4H), 5.47 (s, 0.7H), 5.53
(d, 0.3H), 5.64 (d, 0.8H), 5.72 (d, 0.2H), 6.98-7.12
(m, 1H), 7.19-7.47 (m, 9H). Analytical HPLC (cyano

- 143 -

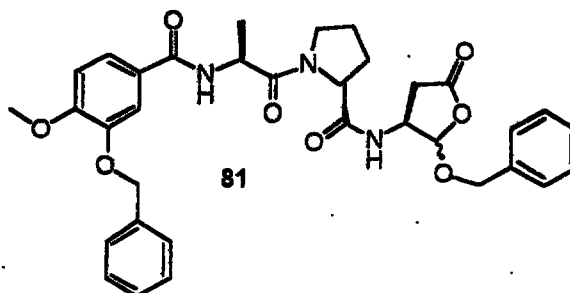
column) (mixture of 2 diastereomers) 14.54, 14.85min.
LC-MS (ES⁺) m/e=538 (M+H).



5

Quinoxaline-2-carboxylic acid {2-[2-(2-benzyloxy-5-oxo-tetrahydrofuran-3-ylcarbamoyl)-pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl}-amide (80).

- 10 Prepared from 75 and 2-quinoxaline carboxylic acid according to the procedure used to prepare 76 to afford the title compound (122mg, 60% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.12-1.67 (m, 3H), 1.68-2.34 (m, 4H), 2.35-2.70 (m, 0.85H), 2.70-2.95 (m, 0.75H), 3.06 (dd, 0.4H),
- 15 3.41-3.49 (m, 2H), 4.18-5.03 (m, 6H), 5.47 (d, 0.5H), 5.55, (d, 2H), 5.67 (dd, 1H), 5.71 (dd, 0.3H), 7.03-7.53 (m, 5H), 7.80-8.06 (m, 2H), 8.06-8.34 (m, 2H), 9.43-9.48 (m, 1H). Analytical HPLC (cyano column) (mixture of 2 diastereomers) 9.06min. LC-MS (ES⁺)
- 20 m/e=532.3 (M+H).



- 144 -

1- [2- (3-Benzyloxy-4-methoxy-benzoylamino) -propionyl] -
pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-
tetrahydro-furan-3-yl) -amide (81) .

Prepared from 75 and 3-benzyloxy-4-methoxy benzoic acid
5 according to the procedure used to prepare 76 to afford
the title compound (142mg, 58% yield). ¹H-NMR (500MHz,
CD₃OD) δ 1.14 (d, 0.3H), 1.27-1.52 (m, 2.7H), 1.66-2.30
(m, 4H), 2.47 (dd, 0.4H), 2.59 (dd, 0.6H), 2.77 (dd,
0.6H), 3.02 (dd, 0.4H), 3.41-3.72 (m, 1H), 3.72-3.99
10 (m, 2H), 3.86 (s, 3H), 4.19-4.86 (m, 5H), 4.99-5.15 (m,
2H), 5.45 (m, 0.8H), 5.65 (m, 1.2H), 6.98 (dd, 1H),
7.11-7.63 (m, 12H). Analytical HPLC (cyano
column) (mixture of 2 diastereomers) 12.28, 12.44min.
LC-MS (ES⁺) m/e=616.3 (M+H).

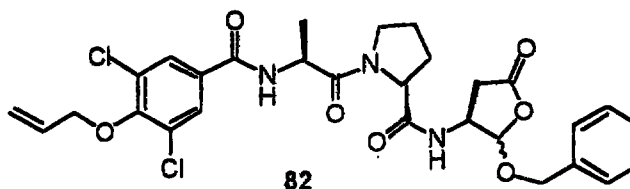
15

4-Allyloxy-3,5-dimethyl-benzoic acid.

A mixture of 4-hydroxy-3,5-dimethyl-benzoic acid (3.32
20 g, 20 mmol), allyl bromide (7.26 g, 60 mmol),
benzyltriethylammonium chloride (455 mg, 2 mmol) and
K₂CO₃ (6.9 g, 50 mmol) in DMF (50 mL) was stirred at
room temperature for 16 hours. The mixture was diluted
with ethyl acetate (200 mL), washed with water, brine.
25 The organic layer was dried over Na₂SO₄, filtered and
evaporated in vacuo to give 5.3 g of the ester as an
oil. The ester was refluxed with NaOH (5 g, 125 mmol)
in water/methanol (50 mL/50 mL) for 6 hours. The
mixture was evaporated in vacuo to remove methanol and
30 the resulted solution was diluted with water (200 mL),
washed with ethyl acetate/hexane (30 mL/70 mL). The
aqueous layer was acidified at 0°C with concentrated
HCl solution to pH 2. The resulted precipitate was
collected by filtration and washed with water, dried

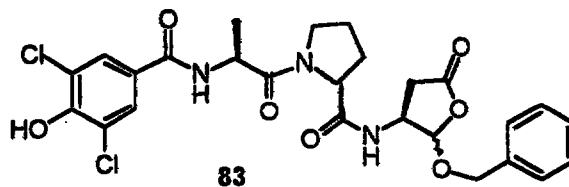
- 145 -

over high vacuum to afford 3.86 g (yield 94%) of the title compound. $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 2.33 (s, 6H), 4.35-4.37 (m, 2H), 5.28-5.30 (m, H), 5.42-5.46 (m, H), 6.07-6.15 (m, H), 7.79 (s, 2H); retention time on analytical HPLC: 11.28 min; LC-MS: $m/z = 205$ (M-H^+).



1- [2- (4-Allyloxy-3,5-dichloro-benzoylamino) -propionyl] -pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (82).

Prepared from 75 and 4-allyloxy-3,5-dichloro-benzoic acid according to the procedure used to prepare 76 to afford the title compound (208mg. 47% yield). $^1\text{H-NMR}$ (500MHz, CDCl_3) δ 1.05-1.58 (m, 3H), 1.68-3.21 (m, 7H), 3.39-3.90 (m, 3H), 4.05-5.01 (m, 6H), 5.22-5.62 (m, 3H), 6.04-6.25 (m, 1H), 6.94-7.63 (m, 8H). Analytical HPLC (cyano column) (mixture of 2 diastereomers) 9.69, 9.89min. LC-MS (ES^+) $m/e=604.2$ ($\text{M}+\text{H}$).

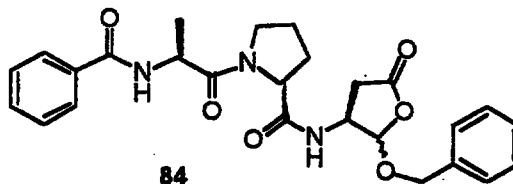


1- [2- (3,5-Dichloro-4-hydroxy-benzoylamino) -propionyl] -pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (83).

A 140 mg sample of 82 (0.23mmol) was dissolved in CH_2Cl_2 (4mL) and treated with DMBA (35.4mg, 0.26mmol) and $\text{Pd}(\text{PPh}_3)_4$ (32mg, 0.028mmol). The solution was

- 146 -

stirred at 0°C for 15mins, warmed to room temperature for 2hours, then diluted with CH₂Cl₂ and washed with water (2x) and brine. The solvent was concentrated in vacuo and the residue purified by flash chromatography on silica gel using MeOH/CH₂Cl₂ (1/99 to 3/97) to give the title compound (93.2mg, 71% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.16 (d, 0.25H), 1.28-1.49 (m, 2.75H), 1.63-2.33 (m, 4H), 2.48 (dd, 0.4H), 3.39-3.59 (m, 0.2H), 3.60-3.73 (m, 0.8H), 3.73-3.96 (m, 1H), 4.24-4.48 (m, 2H), 4.57-4.92 (m, 7H), 5.44 (s, 0.4H), 5.50 (d, 0.4H), 5.64 (d, 0.8H), 5.75 (d, 0.5H), 7.16-7.43 (m, 5H), 7.78-7.89 (m, 1.6H), 8.40-8.63 (m, 0.4H). Analytical HPLC (cyano column) (mixture of 2 diastereomers) 11.57, 11.82min. LC-MS (ES⁺) m/e=564.1 (M+H)

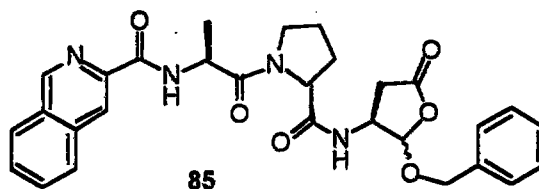


1-(2-Benzoylamino-propionyl)-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (84).

Prepared from 75 and benzoyl chloride according to the procedure used to prepare 76 to afford the title compound as a colorless oil (8mg, 38% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.35-1.54 (m, 3H), 1.72-2.30 (m, 4H), 2.42-2.70 (m, 1.3H), 2.74-2.84 (m, 0.5H), 3.03 (dd, 0.2H), 3.41-3.75 (m, 2H), 3.81-3.96 (m, 1H), 4.22-4.86 (m, 4H), 5.46 (s, 0.3H), 5.51-5.54 (m, 0.1H), 5.66 (d, 0.5H), 5.72 (d, 0.1H), 7.20-7.57 (m, 7H), 7.77-7.89 (m, 2H), 8.42-8.67 (m, 1H). Analytical HPLC (cyano

- 147 -

column) (mixture of 2 diastereomers) 15.23, 15.67min.
LC-MS (ES⁺) m/e=481.2 (M+H)

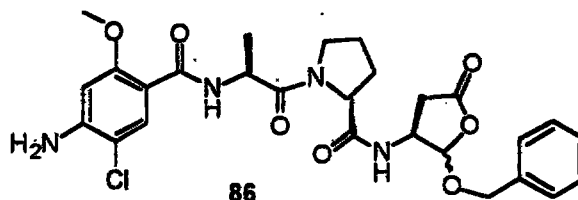


5

Isoquinoline-1-carboxylic acid {2-[(2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)carbamoyl]-pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl}-amide (85).

Prepared from 75 and 1-isoquinolinecarboxylic acid according to the procedure used to prepare 76 to afford the title compound (732mg, 53% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.22-1.56 (m, 3H), 1.70-2.34 (m, 4H), 2.43-2.71 (m, 0.9H), 2.73-2.89 (m, 0.5H), 3.06 (ddd, 0.6H), 3.42-3.81 (m, 2H), 3.84-4.01 (m, 1H), 4.29-5.00 (m, 5H), 5.47 (d, 0.65H), 5.55 (s, 0.3H), 5.67 (d, 0.8H), 5.72 (d, 0.25H), 7.21-7.43 (m, 5H), 7.49-7.83 (m, 2.8H), 7.88-8.04 (m, 1.8H), 8.45-8.54 (m, 0.8H), 8.97-9.06 (m, 0.6H). Analytical HPLC (mixture of 2 diastereomers) 15.71, 16.04min. LC-MS (ES⁺) m/e=531.2 (M+H).

20



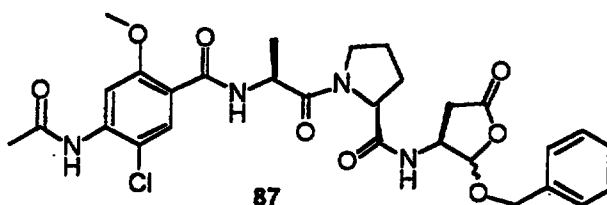
1-[2-(4-Amino-5-chloro-2-methoxy-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (86).

25

Prepared from 75 and 4-amino-5-chloro-2-methoxy benzoic acid according to the procedure used for 76 to afford

- 148 -

the title compound (330mg, 61% yield). $^1\text{H-NMR}$ (500MHz, CD_3OD) δ 1.22 (d, 0.25H), 1.29-1.50 (m, 0.75H), 1.68-2.36 (m, 4H), 2.38-2.89 (m, 1.5H), 2.94-3.14 (m, 0.5H), 3.37-3.98 (m, 6H), 4.27-4.98 (m, 6H), 5.44-5.50 (m, 0.4H), 5.53-5.56 (s, 0.1H), 5.60-5.75 (m, 0.5H), 6.50 (s, 1H), 7.17-7.45 (m, 4H), 7.73-7.90 (m, 1H), 8.49-8.70 (m, 1H). Analytical HPLC (cyano column) (mixture of 2 diastereomers) 16.39, 16.82min. LC-MS (ES^+) m/e = 559.2 ($\text{M}+\text{H}$).

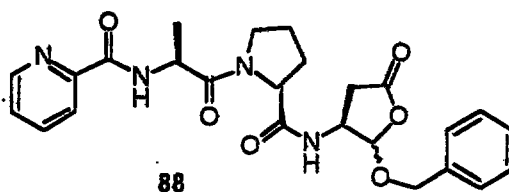


1-[2-(4-Acetylamino-5-chloro-2-methoxy-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (87).

Prepared from 75 and 4-acetylamino-5-chloro-2-methoxy benzoic acid according to the procedure used for 76 to afford the title compound (364mg, 64% yield). $^1\text{H-NMR}$ (500MHz, CD_3OD) δ 1.20-1.27 (m, 0.25), 1.35-1.49 (m, 0.75H), 1.72-2.30 (m, 4H), 2.23 (s, 3H), 2.42-2.58 (m, 0.6H), 2.59-2.68 (m, 0.5H), 2.73-2.86 (m, 0.7H), 2.99-3.11 (m, 0.7H), 3.41-4.07 (m, 5H), 4.29-4.97 (m, 5H), 4.79-5.56 (m, 0.5H), 5.65-5.73 (m, 0.5H), 7.18-7.44 (m, 4.3H), 7.90-8.09 (m, 2H), 8.71-8.85 (m, 0.7H).

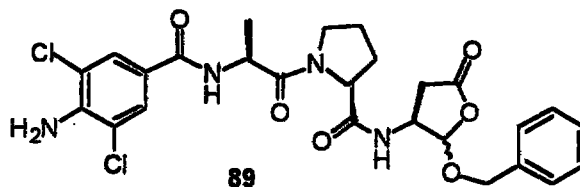
Analytical HPLC (cyano column) (mixture of 2 diastereomers) 15.61, 16.01min. LC-MS (ES^+) m/e = 601.1 ($\text{M}+\text{H}$).

- 149 -



5 **Pyridine-2-carboxylic acid{2-[2-(2-benzyloxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl}-amide (88).**

Prepared from 75 and pyridine-2-carboxylic acid according to the procedure used for 76 to afford the title compound (233mg, 42% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.30-1.59 (m, 3H), 1.68-2.36 (m, 4H), 2.39-2.57 (m, 0.6H), 2.57-2.69 (m, 0.35H), 2.71-2.87 (m, 0.4H), 10 3.05 (dd, 0.65H), 3.39-3.93 (m, 3H), 4.24-4.99 (m, 5H), 5.49-5.55 (m, 0.8H), 5.63-5.77 (m, 1.2H), 7.17-7.46 (m, 5H), 7.49-7.60 (m, 1H), 7.89-7.99 (m, 1H), 8.03-8.12 (m, 1H), 8.58-8.67 (m, 1H). Analytical HPLC (cyano 15 column) (mixture of 2 diastereomers) 8.63min. LC-MS (ES⁺) m/e=481.3 (M+H).



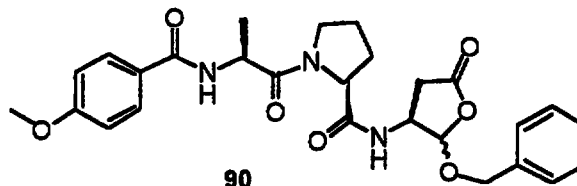
20 **[1-[2-(4-Amino-3,5-dichloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide] (89).**

Prepared from 75 and 3,5-dichloro-4-aminobenzoic acid according to the procedure used for 76 to afford the 25 title compound (162mg, 70% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.21-1.58 (m, 3H), 1.58-2.37 (m, 4H), 2.37-3.13 (m, 2H), 3.43-3.74 (m, 1.5H), 3.77-3.94 (m, 1H), 4.28-

- 150 -

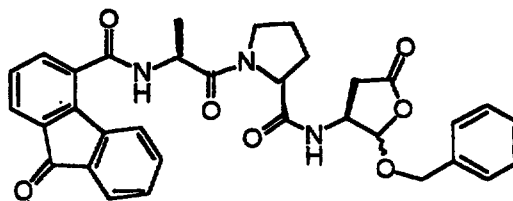
4.51 (m, 1.5H), 4.50-5.01 (m, 3H), 5.41-5.77 (m, 1H),
7.15-7.49 (m, 5H), 7.66-7.88 (m, 2H). Analytical HPLC
(cyano column) (mixture of 2 diastereomers) 8.36min. LC-
MS (ES⁺) m/e=563.2 (M+H).

5



10 1-[2-(4-Methoxy-benzoylamino)-propionyl]-pyrrolidine-2-
carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-
yl)-amide (90).

Prepared from 75 and 4-methoxy-benzoylchloride
according to the procedure used for 76 to afford the
title compound (404mg, 50%). ¹H-NMR (500MHz, CD₃OD) δ
15 1.19 (d, 0.3H), 1.29-1.58 (m, 2.7H), 1.58-2.38 (m, 4H),
2.43-2.69 (m, 1H), 2.74-2.86 (m, 0.6H), 2.99-3.11 (m,
0.4H), 3.39-3.75 (m, 1.5H), 3.77-3.94 (m, 1H), 3.84 (s,
3H), 4.29-4.94 (m, 4.5H), 5.45-5.55 (m, 4.5H), 5.63-
5.71 (m, 0.5H), 5.73 (d, 0.1H), 6.85-7.09 (m, 2H),
20 7.19-7.44 (m, 4H), 7.73-7.92 (m, 2H), 8.26-8.44 (m,
1H). Analytical HPLC (cyano column) (mixture of 2
diastereomers) 15.18, 15.65min. LC-MS (ES⁺) m/e=510.2
(M+H).

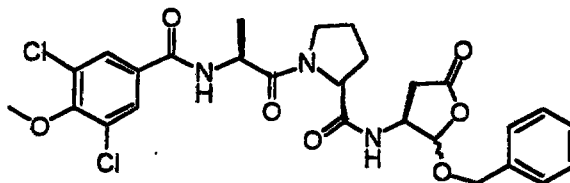


25

- 151 -

1-{2-[(9-Oxo-9H-fluorene-4-carbonyl)-amino]-propionyl}-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (91).

Prepared from 75 and 9-oxo-9H-fluorene-carboxylic acid according to the procedure used for 76 to afford the title compound (403mg, 44% yield). ¹H-NMR (500MHz, CDCl₃) δ 1.38-1.59 (m, 3H), 1.75-2.37 (m, 4H), 2.43-2.59 (m, 0.65H), 2.59-2.72 (m, 0.35H), 2.79-2.89 (m, 0.35H), 3.01-3.11 (m, 0.65H), 3.68-3.86 (m, 1H), 3.92-4.09 (m, 1H), 4.35-5.03 (m, 7H), 5.42-5.90 (m, 1H), 7.06-8.00 (m, 12H). Analytical HPLC (cyano column) (mixture of 2 diastereomers) 12.30min. LC-MS (ES⁺) m/e=582.1 (M+H).



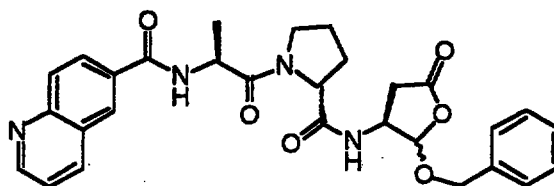
92

15

1-[2-(3,5-Dichloro-4-methoxy-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (92).

Prepared from 75 and 3,5-dichloro-4-methoxy-benzoic acid according to the procedure used for 76 to afford the title compound (364mg, 46% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.17 (d, 0.25H), 1.28-1.53 (m, 2.75H), 1.64-2.33 (m, 4H), 2.39-2.94 (m, 1.5H), 2.94-3.12 (m, 0.5H), 3.41-3.74 (m, 2H), 3.74-4.00 (m, 1H), 3.91 (s, 3H), 4.26-5.02 (m, 5H), 5.42-5.81 (m, 1H), 7.08 (d, 0.4H), 7.21-7.43 (m, 4.6H), 7.53-7.69 (m, 0.8H), 7.85-7.97 (m, 1.2H). Analytical HPLC (cyano column) (mixture of 2 diastereomers) 10.79min. LC-MS (ES⁺) m/e=578.2 (M+H).

- 152 -



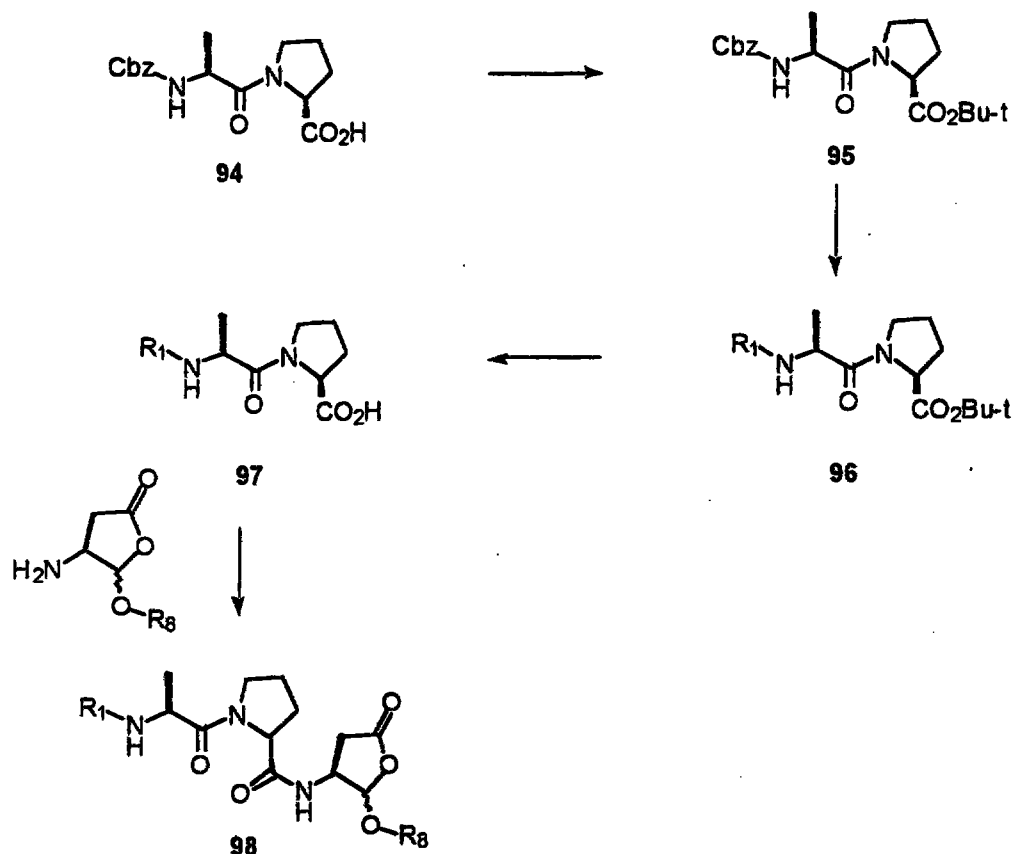
93

Quinoline-6-carboxylic acid {2-(2-benzyloxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-pyrrolidin-1-yl}-1-methyl-2-oxo-ethyl}-amide (93).

Prepared from 75 and 6-quinolinecarboxylic acid according to the procedure used for 76 to afford the title compound (344mg, 71% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.11-1.58 (m, 3H), 1.69-2.40 (m, 4H), 2.42-3.15 (m, 2H), 3.80-4.01 (m, 1H), 4.29-4.99 (m, 5H), 5.44-5.54 (m, 0.5H), 5.63-5.73 (d, 0.4H), 5.73-5.79 (d, 0.1H), 7.18-7.43 (m, 5H), 7.56-7.67 (m, 1H), 8.08 (d, 1H), 8.13-8.25 (m, 1H), 8.40-8.56 (m, 2H), 8.88-8.99 (m, 1H). Analytical HPLC (cyano column) (mixture of 2 diastereomers) 10.27, 10.50min. LC-MS (ES⁺) m/e=531.2 (M+H).

- 153 -

Scheme XVII

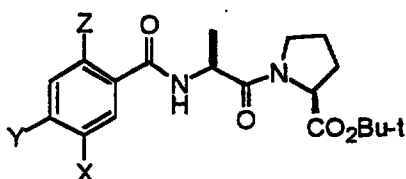


1- (2-Benzyloxycarbonylamino-propionyl)-pyrrolidine-2-carboxylic acid tert-butyl ester (95).

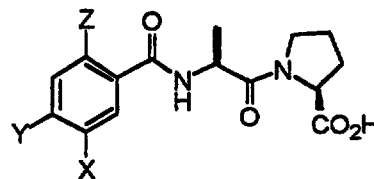
- 5 Prepared according to the method described in Pierre Chevallet, Patrick Garrouste, Barbara Malawaska & Jean Martinez in Tetrahedron Letters, Vol. 34, pp. 7409-7412, (1993). A mixture of Cbz-ala-pro-OH (10.0 g, 31.2 mmol), tert-butyl bromide (180 g, 1.31 mol),
- 10 benzyltriethylammonium chloride (7.11 g, 31.2 mmol) and K_2CO_3 (180 g, 1.30 mol) in N,N-dimethylacetamide (DMA) (225 mL) was stirred at 55°C for 24 hours. The reaction mixture was cooled to room temperature and diluted with one liter of ice-water, extracted with
- 15 ethyl acetate (200 mL x 3). The organic layer was dried over anhydrous Na_2SO_4 , filtered and evaporated in

- 154 -

vacuo to give 14 g of oil, which was purified by flash chromatography using hexane/ethyl acetate (95/5 to 50/50) to afford 11.73 g (yield 99.7%) of the title compound as a clear oil. $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 1.25-1.50 (m, 12 H), 1.85-2.25 (m, 4H), 3.42-3.70 (m, 2H), 4.25-4.57 (m, 2H), 5.07-5.11 (m, 2H), 5.69 (d, H), 7.28-7.38 (m, 5H); retention time on analytical HPLC: 11.07 min; LC-MS: m/z = 377 ($M+H^+$).

96a, X=Cl, Y=NH₂, Z=H

96b, X=Cl, Y=AcNH, Z=H

96c, X=Cl, Y=AcNH, Z=CH₃O97a, X=Cl, Y=NH₂, Z=H

97b, X=Cl, Y=AcNH, Z=H

97c, X=Cl, Y=AcNH, Z=CH₃O

1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid tert-butyl ester (96a).

To a solution of 95 (10.50g, 27.9mmol) in MeOH (100ml) was added a suspension of 10% Pd/C (5.00g) in EtOAc (50ml). The mixture was stirred under H₂ for 48hours, filtered through celite and the solvent evaporated to yield a waxy solid. This was dissolved in CH₂Cl₂ (100ml) and DMF (50ml) and the solution cooled to 0°C. 4-Amino-3-chlorobenzoic acid (5.82g, 27.2mmol), DIEA (14.58ml, 83.7mmol), HOBT (3.77g, 27.9mmol) and EDC (6.68g, 34.8mmol) were added and the solution stirred at 0°C for 15mins then at room temperature for 24hours. The reaction mixture was diluted with EtOAc, washed with NaHSO₄ (2x), 10% NaHCO₃ (2x) and brine then dried over MgSO₄, filtered and evaporated. The crude product was purified by flash column chromatography, using CH₂Cl₂/MeOH (99/1 to 97/3%) to yield the title compound

- 155 -

as a white solid (7.75g, 70% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.27-1.67 (m, 12H), 1.82-2.14 (m, 4H), 3.48-3.85 (m, 2H), 4.26-4.53 (m, 3H), 4.81-4.98 (m, 1H), 6.71 (d, 1H), 7.15 (m, 1H), 7.50 (dd, 1H), 7.75 (d, 1H). Analytical HPLC 10.83min. LC-MS (ES⁺) m/e=396.3 (MH⁺).

1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (97a).

Prepared from 96a by treatment with TFA/ CH₂Cl₂. After complete reaction, the solvent is removed in vacuo and the residue repeatedly concentrated from toluene. The resulting residue was dried under vacuum to a constant weight.

15

1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid tert-butyl ester (96b). Prepared from 95 and 4-acetylamino-3-chlorobenzoic acid according to the method used for 96a to afford the title compound as a white solid (9.18g, 77% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.30-1.62 (m, 12H), 1.85-2.16 (m, 3H), 2.16-2.44 (m, 1H), 2.27 (s, 3H), 3.47-3.83 (m, 2H), 4.34-4.54 (m, 1H), 4.89 (m, 1H), 7.27-7.39 (m, 1H), 7.59-7.71 (m, 2H), 7.83-7.97 (m, 1H), 8.47 (d, 1H). Analytical HPLC 9.43min.

25

1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (97b).

Prepared from 96b by treatment with TFA/ CH₂Cl₂. After complete reaction, the solvent is removed in vacuo and the residue repeatedly concentrated from toluene. The resulting residue was dried under vacuum to a constant weight.

30

- 156 -

4-Acetylamino-5-chloro-2-methoxy-benzoic acid.

4-Acetylamino-5-chloro-2-methoxy-benzoic acid methyl ester (2.09g, 8.11mmol) was dissolved in MeOH (110ml) and LiOH solution (25.48mmol in 30ml, 1:1 MeOH:H₂O) added and the solution stirred at room temperature for 6 hours. The solvent was concentrated in vacuo, EtOAc added and the organic phase was washed with 0.5N HCl then extracted with saturated NaHCO₃ (2x). The aqueous phase was acidified with 12N HCl to pH 1 and the resulting precipitate extracted into CH₂Cl₂. The combined extracts were dried over anhydrous Na₂SO₄, filtered and evaporated to give the title compound as a white solid (0.933g, 50% yield). ¹H-NMR (500MHz, CDCl₃) δ 2.31 (s, 3H), 4.10 (s, 3H), 7.78-7.92 (br s, 1H), 8.17 (s, 1H), 8.45 (s, 1H). Analytical HPLC 5.62min.

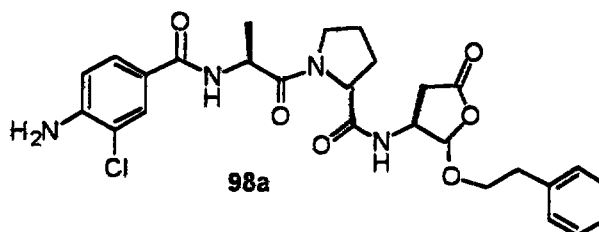
1-[2-(4-Acetylamino-5-chloro-2-methoxy-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid tert-butyl ester (96c).

To a solution of 95 (1.534g, 4.07mmol) in MeOH (40ml) was added 10%Pd/C (650mg) and the mixture stirred under H₂ for 2 hours. The suspension was filtered through celite and evaporated to give a yellow oil. This was allowed to react with 4-acetyl-5-chloro-2-methoxy benzoic acid following the procedure used for the preparation of 96a to give the title compound (497mg, 52% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.46 (d, 3H), 1.49 (s, 9H), 1.80-2.01 (m, 3H), 2.19-2.40 (m, 1H), 2.22 (s, 3H), 3.58-3.72 (m, 1H), 3.78-3.89 (m, 1H), 3.98-4.09 (s, 3H), 4.31-4.45 (s, 1H), 4.78-4.95 (m, 1H), 7.89-8.10 (m, 2H). Analytical HPLC 11.31min.

- 157 -

1- [2- (4-Acetylamino-5-chloro-2-methoxy-benzoylamino) -
propionyl]-pyrrolidine-2-carboxylic acid (97c).

Prepared from 96c by treatment with TFA/ CH₂Cl₂. After
complete reaction, the solvent is removed in vacuo and
the residue repeatedly concentrated from toluene. The
resulting residue was dried under vacuum to a constant
weight



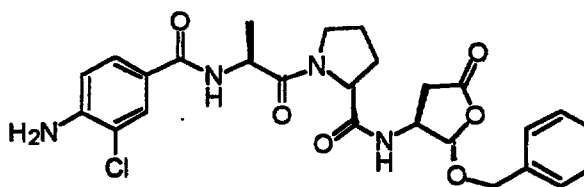
10

1- [2- (4-Amino-3-chloro-benzoylamino) -propionyl]-
pyrrolidine-2-carboxylic acid (5-oxo-2-phenethyloxy-
tetrahydro-furan-3-yl)-amide (98a).

To a solution of (5-oxo-2-phenethyloxy-tetrahydro-
furan-3-yl)-carbamic acid allyl ester (194mg, 0.54mmol)
(prepared as described for (40) using phenethyl
alcohol) in anhydrous CH₂Cl₂ (5mL) at 0°C was added DMBA
(196mg, 1.26mmol) and Pd(PPh₃)₄ (32mg, 0.03mmol). The
solution was stirred for 15min and a solution of 97a
(prepared from 96a by treatment with TFA in
CH₂Cl₂) (166mg, 0.49mmol) and DIEA (680μl, 3.90mmol) in
CH₂Cl₂ (2mL) was added followed by HOBT (98mg,
0.73mmol) and EDC (122mg, 0.63mmol). The solution was
stirred at 0°C for 15min then at room temperature for
18hours. The solvent was removed in vacuo and the
residue dissolved in EtOAc then washed with 0.5N NaHSO₄
(2x), saturated NaHCO₃ (2x) and brine. Dried over
anhydrous Na₂SO₄ and evaporated to give an orange solid
which was purified by flash column chromatography,

- 158 -

using $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (99/1 to 97/3%) to yield the title compound as a white solid (190mg, 73% yield). $^1\text{H-NMR}$ (500MHz, CD_3OD) δ 1.29 (d, 0.6H), 1.41 (d, 2.4H), 1.78 (m, 1H), 2.08 (m, 3H), 2.56 (m, 1H), 2.77 (dd, 1H), 2.94 (t, 2H), 3.53 (m, 0.3H), 3.67 (m, 0.8H), 3.85 (m, 2H), 3.96-4.08 (m, 1H), 4.40 (m, 2H), 4.62 (m, 1H), 4.67-4.79 (m, 1H), 5.57 (d, 0.7H), 5.60 (d, 0.3H), 6.78 (dd, 1H), 7.21 (m, 5H), 7.58 (m, 1H), 7.79 (m, 1H), 8.26 (d, 1H). Analytical HPLC 14.52min. LC-MS (ES^+) $m/e=543.2$ (MH^+).

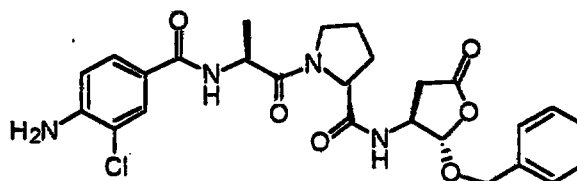


98b

1- [2- (4-Amino-3-chloro-benzoylamino) -propionyl] -pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl) -amide (98b).

Was prepared from the syn diastereomer of (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl) -carbamic acid allyl ester (40) and 97a following the method used for 98a. The title compound was isolated as a pale yellow solid (720mg, 51% yield). $^1\text{H-NMR}$ (500MHz, CD_3OD) δ 1.16 (d, 0.5H), 1.40 (d, 2.5H), 1.64-2.25 (m, 4H), 2.61 (dd, 1H), 2.79 (dd, 1H), 3.37-3.59 (m, 1H), 3.59-3.74 (m, 1H), 3.77-3.92 (m, 1H), 4.29-4.47 (m, 1H), 4.47-5.02 (m, 4H), 5.48 (s, 0.5H), 5.66 (d, 1H), 5.68 (d, 0.5H), 6.79 (d, 1H), 7.17-7.52 (m, 5H), 7.48-7.62 (m, 1H), 7.68-7.83 (m, 1H). Analytical HPLC 15.98min. LC-MS (ES^+) $m/e=529.2$ (MH^+).

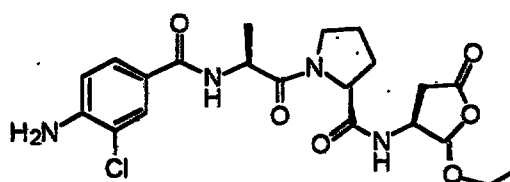
- 159 -



98c

1- [2- (4-Amino-3-chloro-benzoylamino)-propionyl]-
 5 pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-
 tetrahydro-furan-3-yl)-amide (98c).

Prepared from the anti-(2-benzyloxy-5-oxo-tetrahydro-
 furan-3-yl)-carbamic acid allyl ester (40) and 97a
 following the method used for 98a. The title compound
 10 was isolated as a white solid (186.6mg, 46% yield).
¹H-NMR (500MHz, CD₃OD) δ 1.30-1.52 (m, 3H), 1.76-2.33
 (m, 4H), 2.41-2.59 (m, 1H), 2.90 (dd, 0.15H), 3.04 (dd,
 0.85H), 3.44-3.75 (m, 1.5H), 3.82-3.95 (m, 1H), 4.27-
 4.42 (m, 2H), 4.42-4.56 (m, 0.5H), 4.56-4.86 (m, 4H),
 15 5.42-5.55 (m, 1H), 6.79 (d, 1H), 7.21-7.42 (m, 4.6H),
 7.54-7.63 (m, 1.4H), 7.76-7.83 (m, 0.65H), 8.60-8.68
 (m, 0.35H). Analytical HPLC 15.19min. LC-MS (ES⁺)
 m/e=529.3 (MH⁺).



98d

20

2- (Ethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid
 allyl ester.

Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric
 25 acid tert-butyl ester as described for (40) using

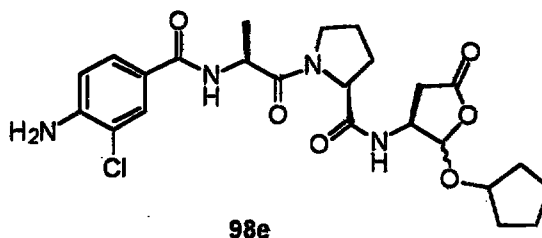
- 160 -

ethanol. Chromatography using hexane/ethyl acetate (95/5 to 80/20) gave 0.94 g of *anti*-2-(ethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester (higher Rf), 1.96 g of *syn* diastereomer (lower Rf) and 8.08 g of the mixture of the diastereomers (total overall yield 60%). ¹H-NMR (500 MHz, CDCl₃) for the *anti* diastereomer: δ 1.13-1.31 (m, 3H), 2.31-2.45 (m, 1H), 2.92-3.08 (m, 1H), 3.52-3.72 (m, 1H), 3.78-3.92 (m, 1H), 4.10-4.25 (m, 1H), 4.45-4.70 (m, 2H), 5.00 (bs, 1H), 5.12-5.45 (m, 3H), 5.80-5.95 (m, 1H); for *syn* diastereomer 1.13-1.35 (m, 3H), 2.38-2.50 (m, 1H), 2.75-2.92 (m, 1H), 3.60-3.73 (m, 1H), 3.82-3.95 (m, 1H), 4.40-4.70 (m, 3H), 5.10-5.52 (m, 4H), 5.80-5.94 (m, 1H); LC-MS: m/z = 230 (M+H⁺) for both diastereomers.

1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98d).

Prepared from (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester and 97a following the method used for 98a. The title compound was isolated as a white solid (175mg, 77% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.13 (t, 0.5H), 1.23 (t, 2.5H), 1.36 (d, 0.5H), 1.44 (d, 2.5H), 1.75-2.38 (m, 4H), 2.56 (dd, 1H), 2.76 (dd, 1H), 3.45-3.97 (m, 5H), 4.47 (dd, 1H), 4.59-4.67 (m, 1H), 4.74 (q, 1H), 5.55 (d, 0.2H), 5.56 (d, 0.8H), 6.75-6.82 (m, 1H), 7.56 (dd, 1H), 7.77 (d, 1H), 8.39 (d, 1H). Analytical HPLC 8.17min. LC-MS (ES⁺) m/e=467.4 (MH⁺).

- 161 -



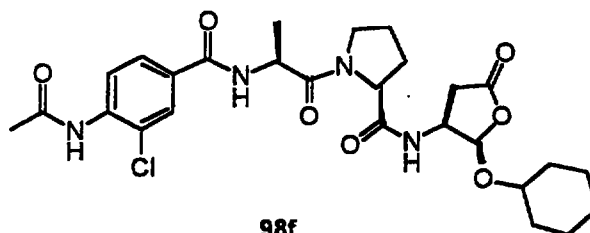
(2-Cyclopentyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester.

- 5 Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester as described for 40 using cyclopentanol to afford the title compound as a mixture of diastereomers. Flash column chromatography using hexanes/EtOAc (90/10 to 80/20) afforded the *syn*
- 10 diastereomer of the title compound: *syn* diastereomer ^1H NMR (500MHz, CDCl_3) δ 1.5-2.0 (m, 8H), 2.45 (dd, 1H), 2.81 (dd, 0.9H), 3.0 (dd, 0.1H), 4.31 (m, 1H), 4.59 (m, 4H), 5.23 (m, 1H), 5.32 (m, 1H), 5.45 (s, 0.1H), 5.51 (s, 0.9H), 5.92, (m, 1H) ppm; *anti* diastereomer ^1H -NMR
- 15 (500 MHz, CDCl_3) δ 1.50 (m, 2H), 1.67 (m, 6H), 2.36 (d, 1H), 2.8 (dd, 0.08H), 2.96 (dd, 0.92H), 4.13 (m, 1H), 4.25 (m, 1H), 4.55 (br, 2H), 5.20 (d, 1H), 5.30 (m, 2H), 5.43 (s, 0.92H), 5.5 (d, 0.08H), 5.89 (s, 1H) ppm.
- 20 1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-cyclopentyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98e).

- Prepared from (2-cyclopentyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester and 97a following the
- 25 method used for 98a to give the title compound (280mg, 51% yield). ^1H -NMR (500MHz, CD_3OD) δ 1.38 (d, 0.5H), 1.44 (d, 2.5H), 1.49-2.35 (m, 12H), 2.47 (dd, 0.7H), 2.56 (dd, 0.3H), 2.75 (dd, 0.3H), 2.81-2.88 (m, 0.1H), 2.97 (dd, 0.6H), 3.47-3.76 (m, 0.2H), 3.82-3.96 (m,

- 162 -

1H), 4.10-4.40 (m, 2H), 4.40-4.46 (m, 1H), 5.44 (d, 0.5H), 5.50 (d, 0.2H), 5.65 (d, 0.3H), 6.79 (d, 1H), 7.54-7.64 (m, 1H), 7.78 (d, 1H), 8.21-8.31 (m, 1H). Analytical HPLC 15.02, 15.34min. LC-MS (ES⁺) m/e=507.3 (MH⁺)



10 (2-Cyclohexyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester.

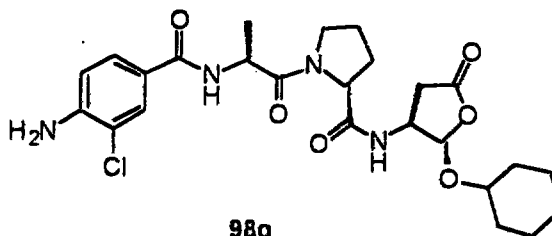
Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester as described for 40 using cyclohexanol to afford the title compound as a mixture of diastereomers (pale yellow oil) (4.62g, 85% yield). Flash column chromatography using hexanes/EtOAc (90/10 to 80/20) gave 394 mg (7% yield) of the syn diastereomer of the title compound. ¹H NMR (500MHz, CDCl₃) δ. 1.11-2.09 (m, 10H), 2.35-2.61 (dd, 1H), 2.72-2.98 (dd, 1H), 3.60-3.83 (m, 1H), 4.32-4.72 (m, 3H), 5.06-5.43 (m, 2H), 5.60 (d, 1H), 5.82-6.03 (m, 1H).

1- [2- (4-Acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-cyclohexyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98f).

25 Prepared from syn-(2-cyclohexyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester and 97b following the method used for 98a to give the title compound (121mg, 33% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.06-1.61 (m, 9H), 1.61-2.37 (m, 7H), 2.22 (s, 3H), 2.52-2.81 (m, 2H), 3.49-3.78 (m, 2H), 3.84-3.97 (m, 1H), 4.42-4.57

- 163 -

(m, 1H), 4.57-4.69 (m, 1H), 5.67-5.81 (m, 1H), 7.72-7.89 (m, 1H), 7.89-8.12 (m, 2H). Analytical HPLC 9.84min. LC-MS (ES⁺) m/e=563.3 (MH⁺).



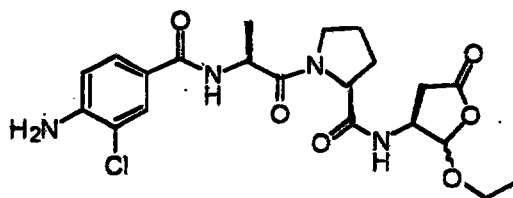
98g

5

1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-cyclohexyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98g).

10 Prepared from syn-(2-cyclohexyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester and 97a following the method used for 98a to give the title compound (153mg, 47% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.06-2.38 (m, 14H), 1.42 (d, 3H), 2.50-2.66 (m, 1H), 2.69-2.82 (dd, 1H), 3.06-3.75 (m, 2H), 3.80-3.94 (m, 1H), 4.40-4.52 (m, 1H), 4.57-4.65 (m, 1H), 4.70-4.80 (m, 1H), 5.72 (d, 1H), 6.71 (m, 1H), 7.50-7.63 (m, 1H), 7.78 (d, 0.6H), 8.42 (d, 0.4H). Analytical HPLC 10.30min. LC-MS (ES⁺) m/e=521.2 (MH⁺).

20



98h

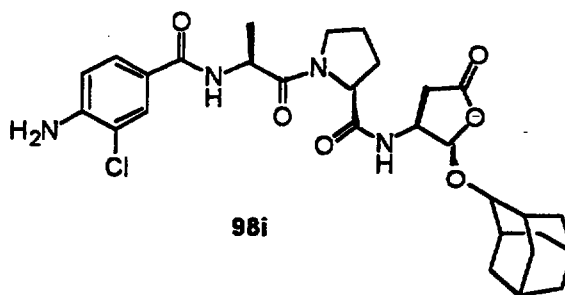
25 1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98h).

- 164 -

Prepared from (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester and 97a following the method used for 98a. The title compound was isolated as a white solid (195mg, 82% yield). ¹H-NMR (500MHz, CD₃OD)

5 δ 1.32-1.55 (m, 3H), 1.58-1.77 (m, 3H), 1.98-2.54 (m, 4H), 2.68-2.76 (d, 0.3H), 2.79-2.89 (m, 0.7H), 2.96-3.10 (m, 0.7H), 3.18-3.27 (dd, 0.3H), 3.72-4.18 (m, 4H), 4.46-5.12 (m, 3H), 5.60 (s, 0.4H), 5.74-5.84 (m, 0.6H), 7.03 (d, 0.8H), 7.75-7.86 (m, 1H), 8.01 (d, 0.7H), 8.35 (d, 0.3H), 8.74 (d, 0.2H). Analytical HPLC

10 8.31min. LC-MS (ES⁺) m/e=467.3 (MH⁺).



15 [5-Oxo-2-(tricyclo[3.3.1.1.0]dec-2-yloxy)-tetrahydro-furan-3-yl]-carbamic acid allyl ester.

Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester as described for 40 using 2-adamantanol (6.21g, 5 equivalents) to afford the title

20 compound as a pale yellow oil (1.52g, 61% yield). ¹H NMR (500MHz, CDCl₃) δ 1.38-2.22 (m, 14H), 2.40 (d, 0.2H), 2.53 (dd, 0.7H), 2.87 (dd, 0.7H), 2.87 (dd, 0.8H), 3.00-3.12 (m, 0.3H), 3.84-3.97 (m, 1H), 4.40-4.71 (m, 3H), 5.18-5.44 (m, 2H), 5.53-5.69 (m, 1H),

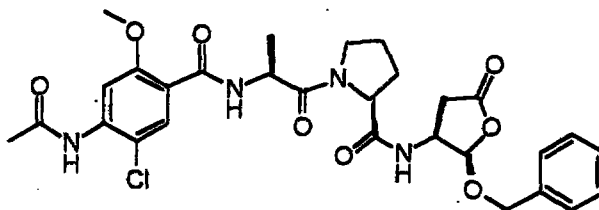
25 5.82-6.02 (m, 1H).

1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid [5-oxo-2-

- 165 -

(tricyclo[3.3.1.1^{0,0}]dec-2-yloxy)-tetrahydro-furan-3-yl] amide (98i).

Prepared from [5-oxo-2-(tricyclo[3.3.1.1^{0,0}]dec-2-yloxy)-tetrahydro-furan-3-yl]-carbamic acid allyl ester and 97a following the method used for 98a. The title compound was isolated as a white solid (76mg, 13% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.38-2.22 (m, 14H), 2.40 (d, 0.2H), 2.53 (dd, 0.7H), 2.87 (dd, 0.8H), 3.00-3.12 (m, 0.3H), 3.84-3.97 (m, 1H), 4.40-4.71 (m, 3H), 5.18-5.44 (m, 2H), 5.53-5.69 (m, 1H), 5.82-6.02 (m, 1H). Analytical HPLC. 11.89min. LC-MS (ES⁺) m/e = 573.2 (MH⁺).



98j

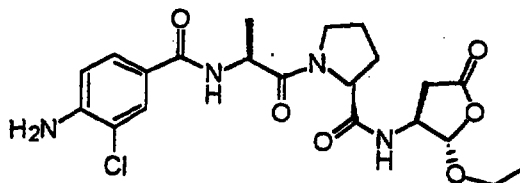
15

1-[2-(4-Acetylamino-5-chloro-2-methoxy-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98j).

Prepared from syn-{2-[2-(2-benzyloxy-5-oxo-tetrahydro-furan-3-ylcarbonyl)-pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl}-carbamic acid tert-butyl ester and 97c following the procedure used for 98a to afford the title compound (222mg, 82% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.23 (d, 0.6H), 1.42 (d, 2.4H), 1.72-2.27 (m, 4H), 2.23 (s, 3H), 2.63 (dd, 1H), 2.77-2.88 (m, 1H), 3.43-3.52 (m, 0.5H), 3.56-3.71 (m, 1.5H), 3.74-3.85 (m, 1H), 3.98 (s, 3H), 4.38-4.50 (m, 1.5H), 4.51-4.92 (m, 4.5H), 5.63-5.76 (m, 1H), 7.23-7.40 (m, 5H), 7.97 (s, 1H), 8.45 (d, 1H),

- 166 -

8.69-8.80 (m, 1H) Analytical HPLC 11.63min LC-MS (ES⁺)
m/e = 601.2 (MH⁺).



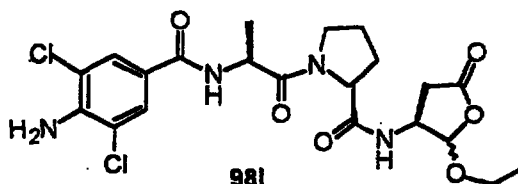
98k

5

Synthesis of 1-[2-(4-amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98k).

Prepared from anti-(2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester and 97a following the method used for 98a to afford 175 mg of title compound (59%). ¹H-NMR (500 MHz, 1:1 CDCl₃:CD₃OD) δ 1.10-1.28 (m, 3H), 1.42 (d, 0.6H), 1.46 (d, 2.4H), 1.75-2.45 (m, 4H), 2.45-2.70 (m, 1H), 2.80-3.05 (m, 1H), 3.50-3.95 (m, 4H), 4.20-4.75 (m, 3H), 4.75-4.90 (m, 1H), 5.32 (s, 0.8H), 5.38 (s, 0.2H), 6.80 (d, 1H), 7.55-7.84 (m, 2H). Analytical HPLC: 10.47 min. LC-MS (ES⁺): m/e = 467.3 (M+H⁺).

20



98l

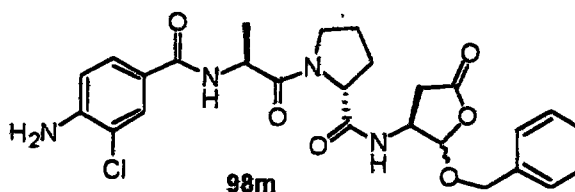
25

Synthesis of 1-[2-(4-amino-3,5-dichloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98l).

Prepared from (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester and 1-[2-(4-amino-3,5-dichloro-benzoylamino)-propionyl]-pyrrolidine-2-

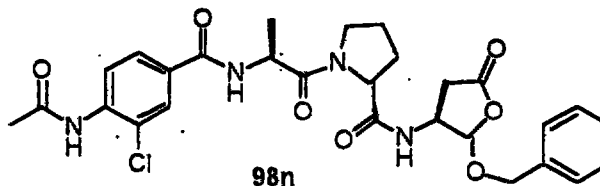
- 167 -

carboxylic acid tert-butyl ester according to the method used for 98a to afford 158 mg of title compound (54% yield). $^1\text{H-NMR}$ (500 MHz, 1:1 $\text{CDCl}_3:\text{CD}_3\text{OD}$) δ 1.08-1.30 (m, 3H), 1.32-1.52 (m, 3H), 1.72-2.44 (m, 4H), 2.40-3.05 (m, 2H), 3.50-3.97 (m, 4H), 4.25-4.70 (m, 3H), 4.70-4.86 (m, 1H), 5.33 (s, 0.4H), 5.47 (s, 0.1H), 5.56 (d, 0.4H), 5.62 (d, 0.1H), 7.50 (s, 1H), 7.80 (s, 1H). Analytical HPLC: 10.84 min. LC-MS (ES^+): m/e = 501.2 ($\text{M}+\text{H}^+$).



1-[2-(4-amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98m).

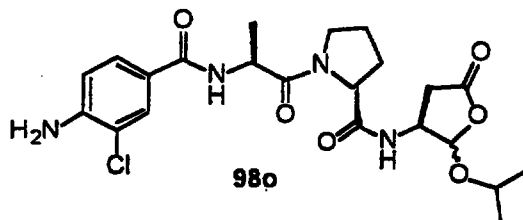
Prepared according to the procedure used to prepare 98a using Cbz-Ala-D-pro-OH to afford 230 mg of title compound (69% yield). $^1\text{H-NMR}$ (500 MHz, 1:1 $\text{CDCl}_3:\text{CD}_3\text{OD}$) δ 1.30 (d, 1.2H), 1.45 (d, 1.8H), 1.62-2.40 (m, 4H), 2.40-3.10 (m, 2H), 3.30-3.97 (m, 2H), 4.33-4.95 (m, 5H), 5.30 (s, 0.5H), 5.68 (d, 0.5H), 6.80 (d, 1H), 7.25-7.95 (m, 7H). Analytical HPLC: 11.56, 11.91 min. LC-MS (ES^+): m/e = 529.2 ($\text{M}+\text{H}^+$).



- 168 -

1-[2-(4-acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98n).

Prepared from 97b and syn-(2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl according to the procedure used to prepare 98a to afford 210 mg of title compound (64% yield). ¹H-NMR (500 MHz, 1:1 CDCl₃:CD₃OD) δ 1.33 (d, 0.6H), 1.44 (d, 2.4H), 1.68-2.40 (m, 4H), 2.26 (s, 3H), 2.55-3.05 (m, 2H), 3.40-3.90 (m, 2H), 4.20-4.95 (m, 5H), 5.68 (d, 0.8H), 5.84 (d, 0.2H), 7.15-8.30 (m, 8H). Analytical HPLC: 15.67 min. LC-MS (ES⁺): m/e = 571.1 (M+H⁺).



15

(2-Isopropoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester

Prepared as described for compound 40 using isopropanol to afford 3.80 grams (81% yield) of the title compound as a colorless oil. ¹H-NMR (500 MHz, CDCl₃) δ 1.10-1.35 (m, 6H), 2.32-2.60 (m, 1H), 2.82 (dd, 0.5H), 3.02 (dd, 0.5H), 3.82-4.11 (m, 1H), 4.48-4.66 (m, 3H), 5.20-5.36 (m, 2H), 5.54 (dd, 1H), 5.82-6.05 (m, 1H). LC-MS (ES⁺): m/e = 244.2 (M+H⁺).

25

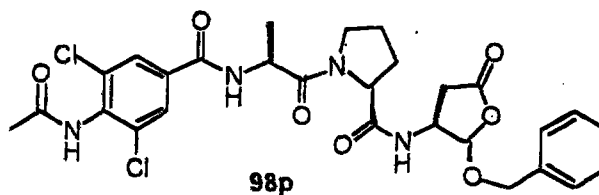
1-[2-(4-amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-isopropoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98o).

Prepared from 97a and (2-isopropoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the

30

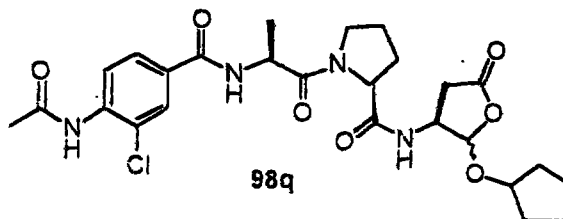
- 169 -

procedure used to prepare 98a to afford 200 mg of title compound (66% yield). $^1\text{H-NMR}$ (500 MHz, 1:1 $\text{CDCl}_3:\text{CD}_3\text{OD}$) δ 1.05-1.35 (m, 6H), 1.35-1.50 (m, 3H), 1.70-2.45 (m, 4H), 2.45-3.05 (m, 2H), 3.55-4.10 (m, 3H), 4.15-4.88 (m, 4H), 5.48 (s, 0.4H), 5.58 (s, 0.1H), 5.64 (d, 0.4H), 5.70 (d, 0.1H), 6.78 (d, 1H), 7.58 (d, 1H), 7.80 (s, 1H). Analytical HPLC: 12.19, 12.40 min. LC-MS (ES^+): $m/e = 581.2$ ($\text{M}+\text{H}^+$).



1-[2-(4-acetylamino-3,5-dichloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98p).

Prepared from 1-[2-(4-acetylamino-3,5-dichloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid tert-butyl ester and syn-(2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 230 mg of title compound (72% yield). $^1\text{H-NMR}$ (500 MHz, 1:1 $\text{CDCl}_3:\text{CD}_3\text{OD}$) δ 1.36 (d, 0.6H), 1.47 (d, 2.4H), 1.68-2.47 (m, 4H), 2.23 (s, 3H), 2.60-3.15 (m, 2H), 3.40-3.90 (m, 2H), 4.15-4.95 (m, 5H), 5.68 (d, 0.8H), 5.84 (d, 0.2H), 7.20-7.98 (m, 7H). Analytical HPLC: 13.07 min. LC-MS (ES^+): $m/e = 605.1$ ($\text{M}+\text{H}^+$).

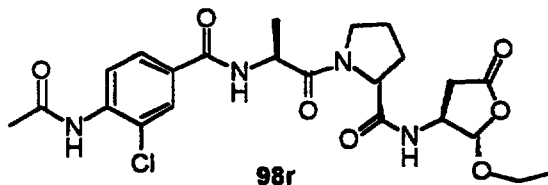


- 170 -

1-[2-(4-acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-cyclopentyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98q).

5 Prepared from 97b and (2-cyclopentyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 215 mg of title compound (69% yield). ¹H-NMR (500 MHz, 1:1 CDCl₃:CD₃OD) δ 1.35-1.90 (m, 11H), 1.90-2.35 (m, 4H), 2.24 (s, 3H), 2.40-3.10 (m, 2H), 3.50-3.95 (m, 3H), 4.15-4.90 (m, 3H), 5.44 (s, 0.55H), 5.56 (s, 0.15H), 5.64 (d, 0.22H), 5.71 (d, 0.08H), 7.70-8.25 (m, 3H). Analytical HPLC: 12.13 min. LC-MS (ES⁺): m/e = 549.2 (M+H⁺).

15

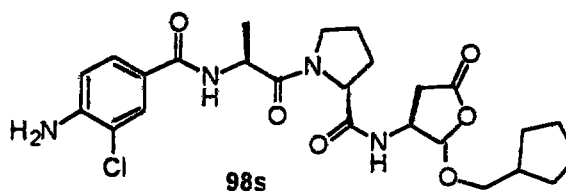


Synthesis of 1-[2-(4-acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98r).

20 Prepared from 97b and syn-(2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 68 mg of title compound (24%). ¹H-NMR (500 MHz, 1:1 CDCl₃:CD₃OD) δ 1.13 (t, 0.6H), 1.28 (t, 2.4H), 1.38 (d, 0.6H), 1.48 (d, 2.4H), 1.75-2.40 (m, 4H), 2.22 (s, 3H), 2.55-2.88 (m, 2H), 3.50-3.92 (m, 4H), 4.40-4.90 (m, 3H), 5.57 (d, 0.8H), 5.61 (d, 0.2H), 7.60-8.20 (m, 3H). Analytical HPLC: 8.64 min. LC-MS (ES⁺): m/e = 509.2 (M+H⁺).

30

- 171 -



Preparation of of (2-cyclopentylmethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester.

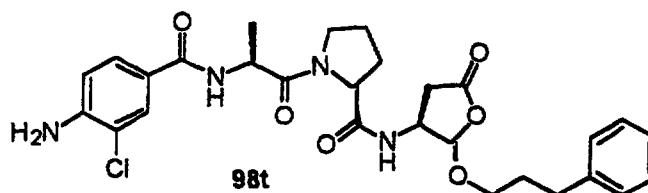
5 Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester as described for compound 40 using cyclopentylmethanol (6.5 mL, 60 mmol) to afford 2.98 grams (52% total yield) of the title compound as a mixture of epimers. Purification provided 0.97 grams (17% yield) of the 4(S), 5(R) as a colorless oil. ¹H NMR (500 MHz, CDCl₃) δ 1.19 (m, 2H), 1.54 (m, 4H), 1.71 (m, 2H), 2.16 (m, 1H), 2.44 (dd, J=17.2, 10.4Hz, 1H), 2.82 (dd, J=17.2, 8.4Hz, 1H), 3.44 (dd, J=9.3, 7.2Hz, 1H), 3.71 (dd, J=9.3, 7.2Hz, 1H), 4.57 (m, 3H), 5.32 (m, 3H), 5.41 (d, J=5.2Hz, 1H), 5.91 (ddt, J=17.1, 10.4, 5Hz, 1H) ppm. LC-MS. (ES⁺): m/e=284.

Also isolated was epimer mixture (0.66 gram, 11% yield) and the 4(S), 5(S) epimer (1.35 gram, 24% yield) as a waxy solid. ¹H-NMR (500MHz, CDCl₃) δ 1.20 (m, 2H), 1.54 (m, 4H), 1.69 (m, 2H), 2.10 (m, 1H), 2.37 (d, J=8.1Hz, 1H), 2.97 (dd, J=18.0, 7.6Hz, 1H), 3.42 (dd, J=7.3, 1.7Hz, 1H), 3.49 (m, 2H), 3.64 (dd, J=9.0, 7.3Hz, 1H), 4.19 (br, 1H), 4.55 (m, 2H), 5.25 (m, 2H), 5.36 (s, 1H), 5.87 (m, 1H) ppm. LC-MS (ES⁺): m/e=284 (M+H).

1-[2-(4-amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-cyclopentylmethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98s).

- 172 -

Prepared from 97a and syn-(2-cyclopentylmethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 195 mg of title compound (51% yield). ¹H-NMR (500 MHz, 1:1 CDCl₃:CD₃OD) δ 1.15-1.90 (m, 11H), 1.90-2.40 (m, 5H), 2.55-2.78 (m, 2H), 3.50-3.90 (m, 4H), 4.38-4.92 (m, 3H), 5.53 (d, 0.8H), 5.57 (d, 0.2H), 6.78 (d, 1H), 7.50-8.15 (m, 2H). Analytical HPLC: 10.48 min. LC-MS (ES⁺): m/e = 521.2 (M+H⁺).



(5-oxo-2-(3-phenyl-propoxy)-tetrahydro-furan-3-yl)-carbamic acid allyl ester.

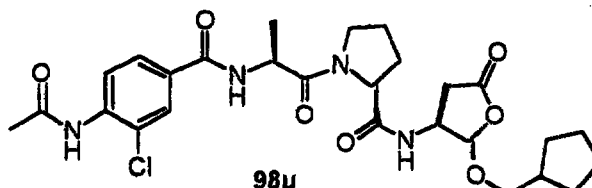
Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester as described for compound 40 using 3-phenylpropanol to afford 1.15 grams (32% yield) of the title compound as a colorless oil. ¹H-NMR (500 MHz, CDCl₃) δ 1.82-2.05 (m, 2H), 2.38 (dd, 1H), 2.68 (m, 2H), 2.82 (dd, 1H), 3.55-3.65 (m, 1H), 3.82-3.92 (m, 1H), 4.48-4.72 (m, 3H), 5.12-5.59 (m, 3H), 5.82-6.03 (m, 1H), 7.11-7.45 (m, 5H). Analytical HPLC: 9.08 min. LC-MS (ES⁺): m/e = 320.2 (M+H⁺).

1-[2-(4-amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (5-oxo-2-(3-phenyl-propoxyl)-tetrahydro-furan-3-yl)-amide (98t).

Prepared from 97b and syn-(5-oxo-2-(3-phenyl-propoxyl)-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 200 mg of title compound (57% yield). ¹H-NMR

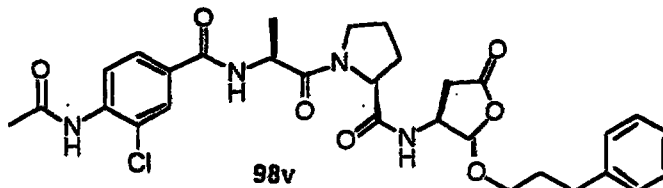
- 173 -

(500 MHz, 1:1 CDCl₃:CD₃OD) δ 1.34 (d, 0.6H), 1.44 (d, 2.4H), 1.75-2.40 (m, 6H), 2.50-2.95 (m, 4H), 3.47-3.95 (m, 4H), 4.38-4.82 (m, 3H), 5.52 (d, 0.8H), 5.56 (d, 0.2H), 6.75-8.25 (m, 8H). Analytical HPLC: 10.79 min.
 5 LC-MS (ES⁺): m/e = 557.2 (M+H⁺).



Synthesis of 1-[2-(4-acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid
 10 (2-cyclopentylmethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98u).

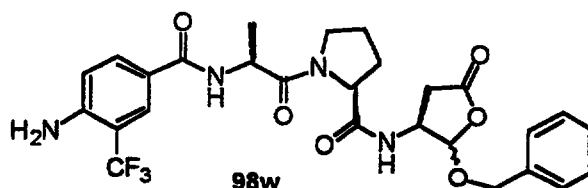
Prepared from 97b and *syn*-(2-cyclopentylmethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to
 15 afford 215 mg of title compound (67% yield). ¹H-NMR (500 MHz, 1:1 CDCl₃:CD₃OD) δ 1.38 (d, 0.6H), 1.47 (d, 2.4H), 1.11-1.88 (m, 8H), 1.92-2.40 (m, 5H), 2.24 (s, 3H), 2.53-2.86 (m, 2H), 3.30-3.90 (m, 4H), 4.38-4.89 (m, 3H), 5.53 (d, 0.8H), 5.60 (d, 0.2H), 7.68-8.22 (m, 3H).
 20 Analytical HPLC: 9.90 min. LC-MS (ES⁺): m/e = 563.3 (M+H⁺).



25 1-[2-(4-acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (5-oxo-2-(3-phenyl-propoxyl)-tetrahydro-furan-3-yl)-amide (98v).

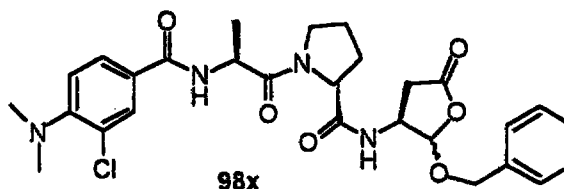
- 174 -

Prepared from 1-[2-(4-acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid tert-butyl ester and *syn*-(5-oxo-2-(3-phenyl-propoxyl)-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 238 mg of title compound (75% yield). ¹H-NMR (500 MHz, 1:1 CDCl₃:CD₃OD) δ 1.33 (d, 0.6H), 1.56 (d, 2.4H), 1.78-2.45 (m, 6H), 2.27 (s, 3H), 2.53-2.97 (m, 4H), 3.53-3.94 (m, 4H), 4.47-4.86 (m, 3H), 5.53 (d, 0.8H), 5.62 (d, 0.2H), 7.11-8.26 (m, 8H). Analytical HPLC: 10.27 min. LC-MS (ES⁺): m/e = 599.2 (M+H⁺).



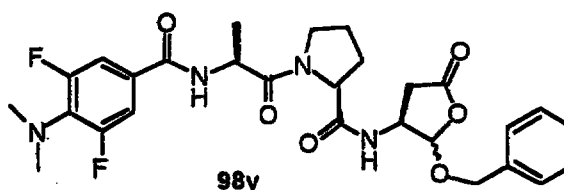
1-[2-(4-amino-3-trifluoromethyl-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98w). Prepared from {2-[2-(2-benzyloxy-5-oxo-tetrahydro-furan-3-ylcarbonyl)-pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl}-carbamic acid tert-butyl ester and 4-amino-3-trifluoromethyl-benzoic acid according to the procedure used to prepare 98a to afford 56 mg of title compound (48% yield). ¹H-NMR (500 MHz, 1:1 CDCl₃:CD₃OD) δ 1.20-1.55 (m, 3H), 1.75-2.50 (m, 4H), 2.50-3.10 (m, 2H), 3.50-4.00 (m, 2H), 4.30-5.00 (m, 5H), 5.42 (s, 0.4H), 5.51 (s, 0.2H), 5.62 (d, 0.3H), 5.78 (d, 0.1H), 6.84 (d, 1H), 7.20-8.15 (m, 7H). Analytical HPLC: 14.90, 15.20 min. LC-MS (ES⁺): m/e = 563.2 (M+H⁺).

- 175 -



1-[2-(3-chloro-4-dimethylamino-benzoylamino)-
propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-
5-oxo-tetrahydro-furan-3-yl)-amide (98x).

Prepared from {2-[2-(2-benzyloxy-5-oxo-tetrahydro-
furan-3-ylcarbmoyl)-pyrrolidin-1-yl]-1-methyl-2-oxo-
ethyl}-carbamic acid tert-butyl ester and 3-chloro-4-
dimethylamino-benzoic acid according to the procedure
used to prepare 98a to afford 82 mg of title compound
(44% yield). $^1\text{H-NMR}$ (500 MHz, 1:1 $\text{CDCl}_3:\text{CD}_3\text{OD}$) δ 1.18-
1.53 (m, 3H), 1.70-2.40 (m, 4H), 2.55-3.10 (m, 2H),
2.84 (s, 6H), 3.45-3.94 (m, 2H), 4.25-4.95 (m, 5H),
5.46 (s, 0.3H), 5.51 (s, 0.2H), 5.63 (d, 0.4H), 5.73
(d, 0.1H), 7.05 (d, 1H), 7.15-7.95 (m, 7H). Analytical
HPLC: 11.85, 12.19 min. LC-MS (ES^+): $m/e = 557.3$
($\text{M}+\text{H}^+$).

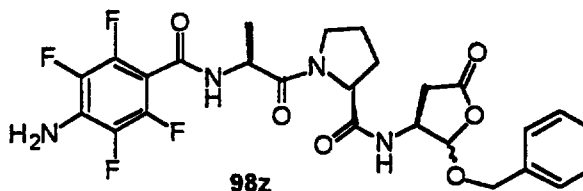


1-[2-(4-dimethylamino-3,5-difluoro-benzoylamino)-
propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-
5-oxo-tetrahydro-furan-3-yl)-amide (98y).

Prepared from {2-[2-(2-benzyloxy-5-oxo-tetrahydro-
furan-3-ylcarbmoyl)-pyrrolidin-1-yl]-1-methyl-2-oxo-
ethyl}-carbamic acid tert-butyl ester and 4-
dimethylamino-3,5-dichloro-benzoic acid according to

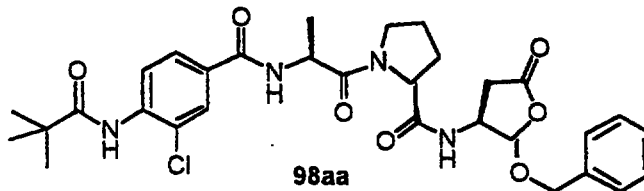
- 176 -

the procedure used to prepare 98a to afford 106 mg of title compound (65% yield). $^1\text{H-NMR}$ (500 MHz, 1:1 $\text{CDCl}_3:\text{CD}_3\text{OD}$) δ 1.10-1.55 (m, 3H), 1.75-2.30 (m, 4H), 2.45-3.15 (m, 2H), 2.84 (s, 6H), 3.40-3.95 (m, 2H), 4.15-4.95 (m, 5H), 5.47 (s, 0.35H), 5.54 (s, 0.15H), 5.67 (d, 0.4H), 5.77 (d, 0.1H), 7.20-7.70 (m, 7H). Analytical HPLC: 12.21, 12.51 min. LC-MS (ES^+): m/e = 559.2 ($\text{M}+\text{H}^+$).



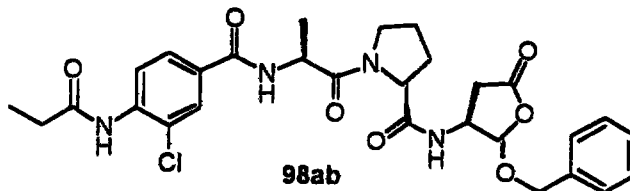
1- [2- (4-amino-2,3,5,6-tetrafluoro-benzoylamino) - propionyl] -pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl) -amide (98z).

Prepared from {2-[2-(2-benzyloxy-5-oxo-tetrahydro-furan-3-ylcarbmoyl)-pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl}-carbamic acid tert-butyl ester and 4-amino-2,3,5,6-tetrafluoro-benzoic acid according to the procedure used to prepare 98a to afford 58 mg of title compound (73% yield). $^1\text{H-NMR}$ (500 MHz, 1:1 $\text{CDCl}_3:\text{CD}_3\text{OD}$) δ 1.30-1.50 (m, 3H), 1.62-2.35 (m, 4H), 2.45-3.12 (m, 2H), 3.50-3.90 (m, 2H), 4.20-4.95 (m, 5H), 5.42 (s, 0.4H), 5.52 (s, 0.1H), 5.64 (d, 0.4H), 5.82 (d, 0.1H), 7.25-7.65 (m, 5H). Analytical HPLC: 16.56, 16.90 min. LC-MS (ES^+): m/e = 567.2 ($\text{M}+\text{H}^+$).



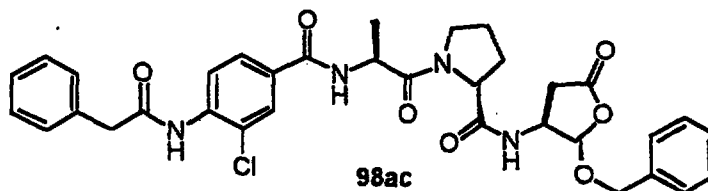
- 177 -

1-{2-[3-chloro-4-(2,2-dimethylpropionylamino)-benzoylamino]-propionyl}-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98aa). To a suspension of 98b (100 mg, 0.19 mmol) and poly(4-vinylpyridine) (200 mg) was added pivaloyl chloride (70 μ L, 0.57 mmol). The resulting suspension was stirred overnight at room temperature the filtered and diluted with EtOAc (25 mL). The organic layer was washed with 10% NaHCO₃ (2 x 25 mL), saturated NaCl (1 x 25 mL), dried (MgSO₄), and evaporated to dryness to afford 98 mg of title compound (85% yield) after chromatography. ¹H-NMR (500 MHz, 1:1 CDCl₃:CD₃OD) δ 1.10-1.55 (m, 3H), 1.38 (s, 9H), 1.65-2.40 (m, 4H), 2.60-3.10 (m, 2H), 3.46-3.88 (m, 2H), 4.20-4.95 (m, 5H), 5.62 (d, 0.8H), 5.78 (d, 0.2H), 7.15-8.30 (m, 8H). Analytical HPLC: 11.82 min. LC-MS (ES⁺): m/e = 613.2 (M+H⁺).



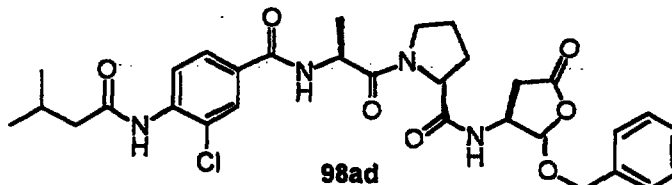
1-[2-(3-chloro-4-propionylamino)-benzoylamino]-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98ab). Prepared from 98b and propionyl chloride according to the procedure used to prepare 98aa to afford 104 mg of title compound (95% yield). ¹H-NMR (500 MHz, 1:1 CDCl₃: CD₃OD) δ 1.16 (t, 0.6H), 1.18 (d, 0.6H), 1.27 (t, 2.4H), 1.38 (d, 2.4H), 1.72-2.35 (m, 4H), 2.45-2.58 (m, 2H), 2.58-3.05 (m, 2H), 3.45-3.85 (m, 2H), 4.20-4.88 (m, 5H), 5.64 (d, 0.8H), 5.76 (d, 0.2H), 7.20-8.35 (m, 8H). Analytical HPLC: 9.89 min. LC-MS (ES⁺): m/e = 585.2 (M+H⁺).

- 178 -



1- [2- (3-chloro-4-phenylacetylamino) -benzoylamino) -
 5 propionyl] -pyrrolidine-2-carboxylic acid (2-benzyloxy-
 5-oxo-tetrahydro-furan-3-yl) -amide (98ac).

Prepared from 98b and phenylacetyl chloride according
 to the procedure used to prepare 98aa to afford 85 mg
 of title compound (77% yield). ¹H-NMR (500 MHz, 1:1
 CDCl₃: CD₃OD) δ 1.18 (d, 0.6H), 1.40 (d, 2.4H), 1.72-
 2.38 (m, 4H), 2.58-3.05 (m, 2H), 3.46-3.78 (m, 2H),
 3.85 (s, 2H), 4.18-4.92 (m, 5H), 5.63 (d, 0.8H), 5.75
 (d, 0.2H), 7.15-8.34 (m, 13H). Analytical HPLC: 11.63
 min. LC-MS (ES⁺): m/e = 647.2 (M+H⁺).

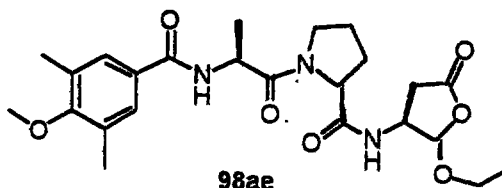


1- [2- (3-chloro-4-methyl-butrylamino) -benzoylamino) -
 5 propionyl] -pyrrolidine-2-carboxylic acid (2-benzyloxy-
 5-oxo-tetrahydro-furan-3-yl) -amide (98ad).

Prepared from 98b and isovaleryl chloride according to
 the procedure used to prepare 98aa to afford 60 mg of
 title compound (58% yield). ¹H-NMR (500 MHz, 1:1
 CDCl₃:CD₃OD) δ 1.07 (d, 5H), 1.15 (d, 0.8H), 1.27 (d,
 1H), 1.45 (d, 2.2H), 1.67-2.30 (m, 5H), 2.34 (d, 2H),
 2.58-3.05 (m, 2H), 3.48-3.88 (m, 2H), 4.10-4.98 (m,
 5H), 5.68 (d, 0.7H), 5.78 (m, 0.3H), 7.18-8.33 (m, 8H).

- 179 -

Analytical HPLC: 10.74 min. LC-MS (ES⁺): m/e = 613.2 (M+H⁺).



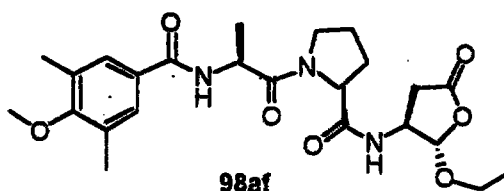
5

1-[2-(4-Methoxy-3,5-dimethyl-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98ae).

Prepared from 1-[2-(4-methoxy-3,5-dimethyl-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid tert-butyl ester and syn-(2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 174 mg (81% yield) of the title compound. ¹H-NMR (500 MHz, CDCl₃):

δ 1.04 (t, 0.45H), 1.27 (t, 2.55H), 1.34-1.45 (m, 3H), 1.95-2.45 (m, 10H), 2.78-2.84 (m, H), 3.60-3.90 (m, 8H), 4.50-4.70 (m, 2H), 4.90-4.94 (m, H), 5.45 (d, 0.85H), 5.61 (d, 0.15H), 6.99 (d, H), 7.15 (d, H), 7.45 (s, 2H); retention time on analytical HPLC: 10.09 min; LC-MS: m/z = 476 (M+H⁺).

20



1-[2-(4-Methoxy-3,5-dimethyl-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98af).

25

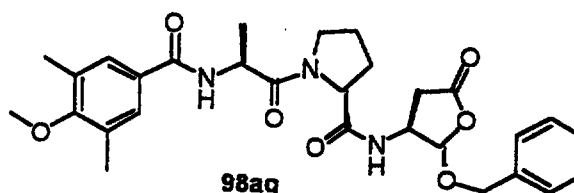
Prepared from 1-[2-(4-methoxy-3,5-dimethyl-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid

- 180 -

tert-butyl ester and anti-(2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 168 mg (77% yield) of the title compound. $^1\text{H-NMR}$ (500 MHz, CDCl_3):

5 δ 1.10-1.35 (m, 3H), 1.35-1.60 (m, 3H), 1.90-2.45 (m, 10H), 2.60-3.00 (m, H), 3.55-3.95 (m, 8H), 4.15-4.60 (m, 2H), 4.83-5.00 (m, H), 5.29 (s, H), 6.95-7.06 (m, H), 7.50 (s, 2H), 7.92 (d, H); retention time on analytical HPLC: 10.14 min; LC-MS: $m/z = 476$ ($\text{M}+\text{H}^+$).

10



1- [2- (4-Methoxy-3,5-dimethyl-benzoylamino) -propionyl] -pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl) -amide (98ag).

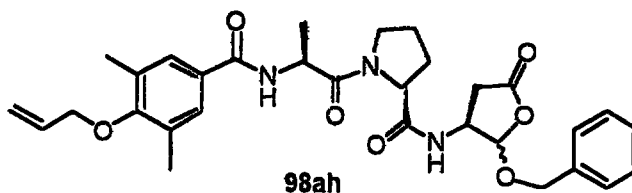
15

Prepared from 1- [2- (4-methoxy-3,5-dimethyl-benzoylamino) -propionyl] -pyrrolidine-2-carboxylic acid tert-butyl ester and syn-(2-benzyloxy-5-oxo-tetrahydro-furan-3-yl) -carbamic acid allyl ester (40) according to the procedure used to prepare 98a to afford 406 mg (yield 71%) of the title compound. $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 1.09 (d, 0.6H), 1.35 (m, 2.4H), 1.90-2.20 (m, 3H), 2.22-2.50 (m, 10H), 2.84-2.90 (m, H), 3.52-3.62 (m, 1.6H), 3.65-3.80 (m, 3.4H), 4.10-4.40 (m, H), 4.50-4.75 (m, 3H), 4.82-4.95 (m, 2H), 5.54 (d, 0.8H), 5.80 (d, 0.2H), 6.87 (d, H), 7.10-7.40 (m, 6H), 7.45 (s, 2H); retention time on analytical HPLC: 16.71 min¹; LC-MS: $m/z = 538$ ($\text{M}+\text{H}^+$).

20

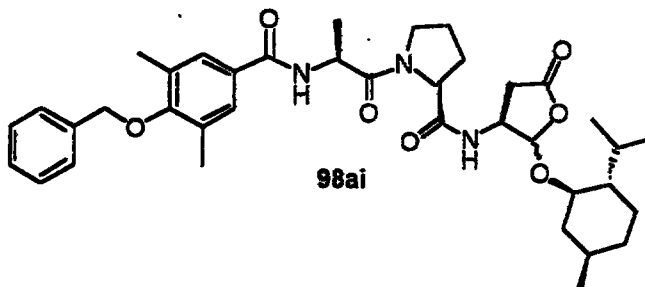
25

- 181 -



1- [2- (4-Allyloxy-3,5-dimethyl-benzoylamino) -propionyl] -
 pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-
 5 tetrahydro-furan-3-yl) -amide (98ah).

Prepared from 1- [2- (4-allyloxy-3,5-dimethyl-
 benzoylamino) -propionyl] -pyrrolidine-2-carboxylic acid
 tert-butyl ester and 40 according to the procedure used
 to prepare 98a to afford 264 mg (yield 46%) of the
 10 title compound. ¹H-NMR (500 MHz, CDCl₃): δ 1.09-1.43
 (m, 3H), 1.90-2.20 (m, 3H), 2.20-2.38 (m, 7H), 2.38-
 2.52 (m, H), 2.80-2.95 (m, H), 3.52-3.67 (m, H), 3.70-
 3.80 (m, H), 4.10-4.40 (m, 2H), 4.40-4.95 (m, 5H),
 5.26-5.55 (m, 3H), 6.00-6.14 (m, H), 6.87 (d, H), 7.10-
 15 7.70 (m, 8H); retention time on analytical HPLC: 18.56
 and 18.92 min¹; LC-MS: m/z = 564 (M+H⁺)



20 {2- [1R- (2S-Isopropyl-5R-methyl-cyclohexyloxy)] -5-oxo-
 tetrahydro-furan-3-yl} -carbamic acid allyl ester.

Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyrlic
 acid tert-butyl ester as described for 40 using (1R,
 2S, 5R) - (-) -menthol to afford 0.32 g of syn
 25 diastereomer (lower R_f) of the title compound and 4.25

- 182 -

g of the mixture of anti/syn diastereomers (overall yield 67%). ¹H-NMR (500 MHz, CDCl₃) mixture: δ 0.70-1.05 (m, 13H), 1.20-1.47 (m, 2H), 1.60-1.80 (m, 2H), 1.94-2.20 (m, 2H), 2.35-2.50 (m, H), 2.82-3.04 (m, H), 3.40-3.61 (m, H), 4.43-4.70 (m, 3H), 5.15-5.35 (m, 2H), 5.48-5.61 (m, H), 5.90-5.94 (m, H); for syn diastereomer 0.70-1.05 (m, 13H), 1.20-1.47 (m, 2H), 1.60-1.80 (m, 2H), 1.94-2.18 (m, 2H), 2.40-2.50 (m, H), 2.82-2.92 (m, H), 3.54-3.61 (m, H), 4.45-4.70 (m, 3H), 5.18-5.35 (m, 2H), 5.58-5.61 (m, H), 5.90-5.93 (m, H); LC-MS: m/z = 340 (M+H⁺) for the mixture of anti/syn diastereomers.

4-Benzyloxy-3,5-dimethyl-benzoic acid.

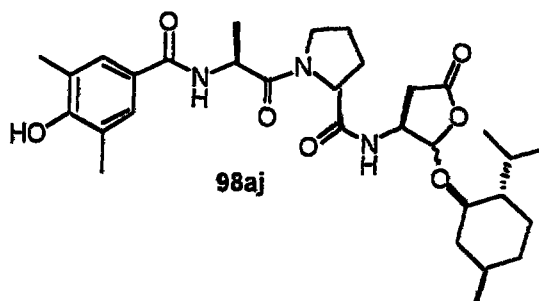
Prepared by the method used to synthesize 4-allyloxy-3,5-dimethyl-benzoic acid to afford 2.43 g (yield 56%) of the title compound. ¹H-NMR (500 MHz, CDCl₃): δ 4.87 (s, 2H), 7.36-7.48 (m, 5H), 7.92 (s, 2H); LC-MS: m/z = 255 (M-H⁺).

1-[2-(4-Benzyloxy-3,5-dimethyl-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid {2-[1R-(2S-isopropyl-5R-methyl-cyclohexyloxy)]-5-oxo-tetrahydro-furan-3-yl}-amide (98ai).

Prepared from 1-[2-(4-benzyloxy-3,5-dimethyl-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid tert-butyl ester {2-[1R-(2S-Isopropyl-5R-methyl-cyclohexyloxy)]-5-oxo-tetrahydro-furan-3-yl}-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 130 mg (yield 39%) of the title compound. ¹H-NMR (500 MHz, CDCl₃): δ 0.45-1.10 (m, 12H), 1.15-1.90 (m, 8H), 1.90-2.45 (m, 12H), 2.80-2.84 (m, H), 3.50-3.85 (m, 3H), 4.45-4.70 (m, 2H), 4.80-4.95 (m, 3H), 5.62 (d, H), 7.05 (d, H), 7.17 (d, H), 7.30-

- 183 -

7.60 (m, 7H), 7.62-7.75 (m, H); retention time on analytical HPLC: 15.90 and 16.08 min; LC-MS: m/z = 662 (M+H⁺).



5

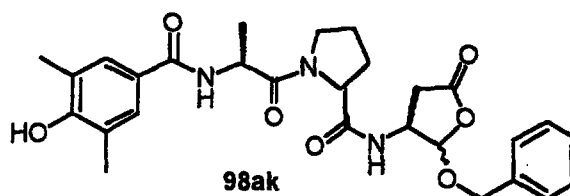
1- [2- (4-Hydroxy-3,5-dimethyl-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid {2-[1R-(2S-isopropyl-5R-methyl-cyclohexyloxy)]-5-oxo-tetrahydro-furan-3-yl}-amide (98aj).

10

A solution of 1- [2- (4-benzyloxy-3,5-dimethyl-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid {2-[1R-(2S-isopropyl-5R-methyl-cyclohexyloxy)]-5-oxo-tetrahydro-furan-3-yl}-amide (110 mg, 0.17 mmol) in ethyl acetate (2 mL) was stirred with 10% palladium on carbon (20 mg) under hydrogen atmosphere for 24 hours then filtered through Celite and evaporated in vacuo. The resulting residue was purified by chromatography using CH₂Cl₂/methanol (99/1 to 96/4) to afford 58 mg of the title compound. ¹H-NMR (500 MHz, CDCl₃): δ 0.70-1.00 (m, 10H), 1.20-1.80 (m, 10H), 1.90-2.40 (m, 11H), 2.82-2.86 (m, H), 3.57-3.78 (m, 3H), 4.55-4.67 (m, 2H), 4.90-4.94 (m, H), 5.29 (s, H), 5.62 (d, H), 6.90 (d, H), 7.14 (d, H), 7.42 (s, 2H); retention time on analytical HPLC: 12.84 and 13.05 min; LC-MS: m/z = 572 (M+H⁺).

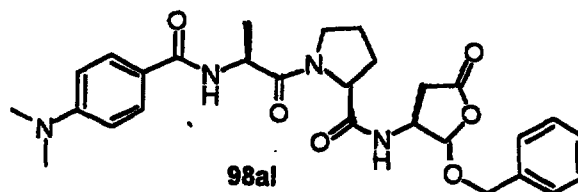
25

- 184 -



1- [2- (4-Hydroxy-3,5-dimethyl-benzoylamino) -propionyl] -
pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-
5 tetrahydro-furan-3-yl) -amide (98ak).

A solution of 98ah (230 mg, 0.41 mmol) in CH_2Cl_2 (10 mL) was treated with DMBA (65 mg, 0.42 mmol) and $\text{Pd}(\text{PPh}_3)_4$ (50 mg) at room temperature for 20 hours. The mixture was concentrated to dryness in vacuo and purified by flash chromatography using CH_2Cl_2 /methanol (99.5/0.5 to 97/3) to afford 181 mg of the title compound. $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 1.08 (d, 0.75H), 1.20-1.35 (m, 2.25H), 1.70-2.50 (m, 12H), 2.80-2.90 (m, H), 3.50-3.65 (m, H), 3.70-3.80 (m, H), 4.10-4.25 (m, H), 4.35-4.98 (m, 3H), 5.53 (d, 0.75H), 5.85 (d, 0.25H), 6.81 (d, H), 7.13-7.60 (m, 8H); retention time on analytical HPLC: 10.38 and 10.56 min; LC-MS: m/z = 524 ($\text{M}+\text{H}^+$).



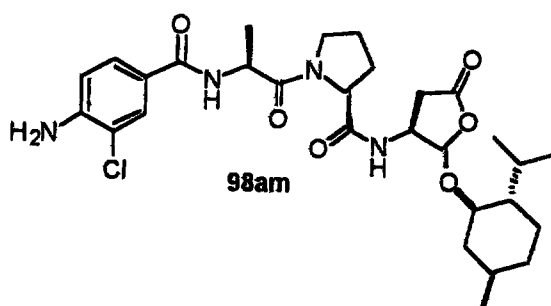
20

1- [2- (4-Dimethylamino-benzoylamino) -propionyl] -
pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-
25 tetrahydro-furan-3-yl) -amide (98al).

Prepared from 1- [2- (4-dimethylamino-benzoylamino) -
propionyl] -pyrrolidine-2-carboxylic acid tert-butyl

- 185 -

ester and *syn*-(2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 60 mg (45% yield). ¹H-NMR (500 MHz, CDCl₃): δ 1.04 (d, 0.75H), 1.35 (d, 2.25H), 1.80-2.50 (m, 5H), 2.75-3.20 (m, 8H), 3.45-3.75 (m, 2H), 4.05-4.20 (m, 0.5H), 4.30-4.80 (m, 3.5H), 4.80-4.95 (m, 1.5H), 5.52 (d, H), 5.75-6.00 (m, 0.5H), 6.60-6.90 (m, 3H), 7.10-7.50 (m, 4H), 7.50-7.80 (m, 2H); retention time on analytical HPLC: 10.46 min; LC-MS: m/z = 523 (M+H⁺).

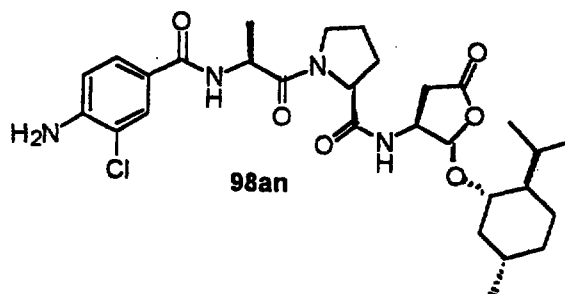


1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid {2R-[1R-(2S-isopropyl-5R-methyl-cyclohexyloxy)]-5-oxo-tetrahydro-furan-3-yl}-amide (98am).

Prepared from 1-[2-(4-amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid tert-butyl ester (97a) and *syn*-{2-[1R-(2S-isopropyl-5R-methyl-cyclohexyloxy)]-5-oxo-tetrahydro-furan-3-yl}-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 103 mg (yield 67%) of the title compound. ¹H-NMR (500 MHz, CDCl₃): δ 0.70-1.10 (m, 12H), 1.20-1.50 (m, 5H), 1.50-1.85 (m, 2H), 1.90-2.30 (m, 5H), 2.75-2.85 (m, H), 3.50-3.70 (m, 2H), 3.70-3.82 (m, H), 4.20-4.65 (m, 4H), 4.80-4.95 (m, H), 5.61 (d, H), 6.70-6.73 (m, H), 6.95 (d, H), 7.15 (d, H), 7.49-

- 186 -

7.51 (m, H), 7.73 (s, H); retention time on analytical HPLC: 12.88 min; LC-MS: $m/z = 577$ ($M+H^+$).



5

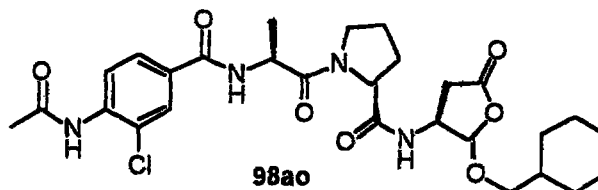
{2-[1S-(2R-Isopropyl-5S-methyl-cyclohexyloxy)]-5-oxo-tetrahydro-furan-3-yl}-carbamic acid allyl ester.

Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester as described for 40 using (1S, 2R, 5S)-(+)-menthol to afford 855 mg of anti diastereomer (higher Rf) of the title compound, 503 mg of syn diastereomer (lower Rf) and 459 mg of the mixture of anti/syn diastereomers (overall yield 66%).
¹H-NMR (500 MHz, CDCl₃) anti diastereomer: δ 0.74-1.00 (m, 12H), 1.20-1.45 (m, 2H), 1.58-1.72 (m, 2H), 1.98-2.12 (m, 2H), 2.18-2.40 (m, H), 2.98-3.03 (m, H), 3.49-3.54 (m, H), 4.17 (br, H), 4.59 (br, 2H), 4.97 (br, H), 5.22-5.33 (m, 2H), 5.58 (s, H), 5.87-5.93 (m, H); for syn diastereomer 0.75-1.02 (m, 12H), 1.25-1.45 (m, 2H), 1.57-1.70 (m, 2H), 2.00-2.16 (m, 2H), 2.40-2.52 (m, H), 2.78-2.90 (m, H), 3.40-3.50 (m, H), 4.58 (br, 2H), 5.24-5.35 (m, 2H), 5.51-5.52 (d, H), 5.85-5.98 (m, H); LC-MS: $m/z = 340$ ($M+H^+$) for both of diastereomers.

1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid {2R-[1S-(2R-isopropyl-5S-methyl-cyclohexyloxy)]-5-oxo-tetrahydro-furan-3-yl}-amide (98an).

- 187 -

Prepared from 97a and syn-{2-[1S-(2R-isopropyl-5S-methyl-cyclohexyloxy)]-5-oxo-tetrahydro-furan-3-yl}-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 88 mg (50% yield) of the title compound. ¹H-NMR (500 MHz, CDCl₃): δ 0.70-1.10 (m, 12H), 1.20-1.50 (m, 14H), 1.50-1.70 (br, 2H), 1.90-2.25 (m, 4H), 2.27-2.37 (m, H), 2.40-2.50 (m, H), 2.75-2.79 (m, H), 3.35-3.80 (m, 3H), 4.20-4.57 (m, 3H), 4.60-4.70 (m, H), 4.88-4.92 (m, H), 5.53 (d, H), 6.71-6.75 (m, H), 6.90 (d, H), 7.20 (d, H), 7.50-7.53 (m, H), 7.75 (d, H); retention time on analytical HPLC: 13.20 min; LC-MS: m/z = 577 (M+H⁺).



(2-Cyclohexylmethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester.

Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester as described for 40 using

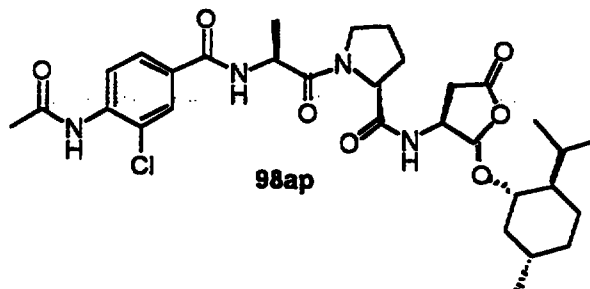
cyclohexylmethanol to afford 1.04 g (higher Rf) (35% yield) of anti diastereomer of the title compound and 1.295 g (lower Rf) (44% yield) of syn diastereomer. ¹H-NMR (500 MHz, CDCl₃) for anti diastereomer: δ 0.90-0.96 (m, 2H), 1.10-1.30 (m, 3H), 1.55-1.85 (m, 6H), 2.37-2.41 (d, H), 2.97-3.03 (m, H), 3.34-3.38 (m, H), 3.58-3.62 (m, H), 4.55-4.70 (m, 2H), 4.70-4.73 (m, H), 5.03 (bs, H), 5.22-5.37 (m, 3H), 5.87-5.93 (m, H); for syn diastereomer 0.91-0.97 (m, 2H), 1.10-1.31 (m, 3H), 1.56-1.90 (m, 7H), 2.44-2.48 (m, H), 2.81-2.87 (m, H), 3.35-3.39 (m, H), 3.63-3.67 (m, H), 4.53-4.70 (m, 3H),

- 188 -

5.20-5.50 (m, 3H), 5.89-5.95 (m, H); LC-MS: m/z = 298 ($M+H^+$) for both of diastereomers.

5 1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-cyclohexylmethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98ao).

Prepared from 97b and syn-(2-cyclohexylmethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 212 mg (64% yield) of the title compound. ^1H -NMR (500 MHz, CDCl_3): δ 0.70-1.30 (m, 5H), 1.30-1.85 (m, 9H), 1.85-2.60 (m, 8H), 2.75-3.00 (m, H), 3.10-3.80 (m, 4H), 4.30-4.95 (m, 3H), 5.42 (d, 0.85H), 5.62 (d, 0.15H), 6.87 (d, 0.15H), 7.08 (d, 0.85H), 7.25 (d, H), 7.60-7.90 (m, 3H), 8.08 (d, 0.15H), 8.50 (d, 0.85H); retention time on analytical HPLC: 11.81 min; LC-MS: m/z = 577 ($M+H^+$).



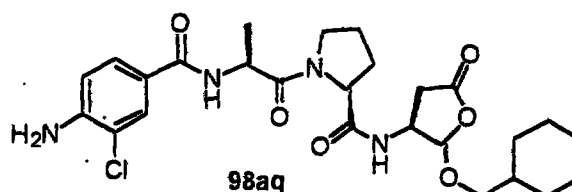
20

1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid {2R-[1S-(2R-isopropyl-5S-methyl-cyclohexyloxy)]-5-oxo-tetrahydro-furan-3-yl}-amide (98ap).

25 Prepared from 97b and syn-{2-[1S-(2R-isopropyl-5S-methyl-cyclohexyloxy)]-5-oxo-tetrahydro-furan-3-yl}-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 223 mg (63% yield) of the title compound. ^1H -NMR (500 MHz, CDCl_3): δ 0.70-1.15

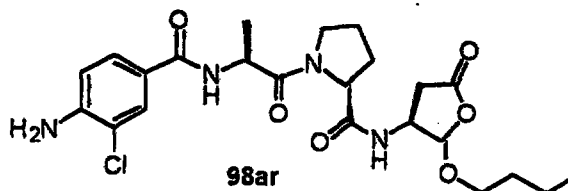
- 189 -

(m, 12H), 1.20-1.85 (m, 8H), 1.85-2.60 (m, 9H), 2.74-2.88 (m, H), 3.35-3.85 (m, 3H), 4.40-4.55 (m, H), 4.65-4.78 (m, H), 4.88-4.91 (m, H), 5.53 (d, H), 7.00-7.25 (m, 2H), 7.60-7.90 (m, 3H), 8.50 (d, H); retention time
 5 on analytical HPLC: 13.31 min; LC-MS: $m/z = 619$ ($M+H^+$).



1- [2- (4-Amino-3-chloro-benzoylamino)-propionyl]-
 10 pyrrolidine-2-carboxylic acid (2-cyclohexylmethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98aq).

Prepared from 97a and syn-(2-cyclohexylmethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to
 15 afford 113 mg (56% yield) of the title compound. 1H -NMR (500 MHz, $CDCl_3$): δ 0.70-1.35 (m, 5H), 1.35-1.90 (m, 8H), 1.90-2.20 (m, 3H), 2.30-2.60 (m, H), 2.80-3.00 (m, H), 3.15-3.80 (m, 4H), 4.28-4.75 (m, 4H), 4.89-4.93 (m, H), 5.42 (d, H), 6.74 (d, H), 6.87 (d, H), 7.30 (d, H),
 20 7.51-7.53 (m, H), 7.74 (d, H); retention time on analytical HPLC: 12.02 min; LC-MS: $m/z = 535$ ($M+H^+$).



25 (2-Butoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester.

- 190 -

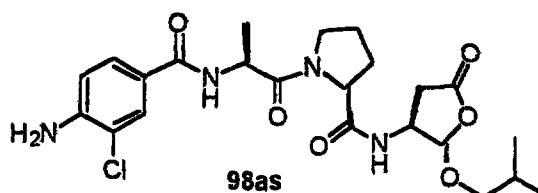
Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester as described for 40 using n-butanol to afford 878 mg (29% yield) of the title compound (313 mg of anti diastereomer, 260 mg of syn diastereomer and 305 mg of the mixture). ¹H-NMR (500 MHz, CDCl₃) for anti diastereomer: δ (higher Rf) 0.89-0.96 (t, 3H), 1.32-1.40 (m, 2H), 1.54-1.63 (m, 2H), 2.37-2.41 (d, H), 2.98-3.04 (q, H), 3.55-3.60 (m, H), 3.77-3.82 (m, H), 4.19-4.22 (m, H), 4.58 (br, 2H), 5.03 (br, H), 5.23-5.40 (m, 3H), 5.87-5.93 (M, H), for syn diastereomer (lower Rf) 0.91-0.95 (t, 3H), 1.34-1.39 (m, 2H), 1.56-1.63 (m, 2H), 2.42-2.50 (m, H), 2.83-2.87 (m, H), 4.07-4.11 (t, H), 4.45-4.50 (m, 0.5H), 4.51-4.70 (m, 2.5H), 5.23-5.35 (m, 2H), 5.42-5.43 (d, H), 5.80-5.95 (m, H); LC-MS: m/z = 258 (M+H⁺) for both of diastereomers.

1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-butoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98ar).

Prepared from 97a and syn-(2-butoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 118 mg (48% yield) of the title compound as a syn diastereomer.

¹H-NMR (500 MHz, CDCl₃): δ 0.80-1.02 (m, 2H), 1.35-1.51 (m, 5H), 1.51-1.70 (m, 2H), 1.90-2.27 (m, 3H), 2.30-2.46 (m, H), 2.80-2.90 (m, H), 3.55-3.94 (m, 4H), 4.30-4.75 (m, 4H), 4.90-5.00 (m, H), 5.44-5.46 (m, H), 6.73-6.80 (m, H), 6.80-6.93 (m, H), 7.16-7.25 (m, H), 7.49-7.60 (m, H), 7.70-7.84 (m, H); retention time on analytical HPLC: 9.71 min; LC-MS: m/z = 495 (M+H⁺).

- 191 -



(2-Isobutoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester.

5 Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester as described for 40 using isobutanol to afford 190 mg (yield 7.3%) of the title compound as an anti diastereomer and 290 mg (yield 11%) of the *syn* diastereomer. ¹H-NMR (500 MHz, CDCl₃) for
 10 anti diastereomer: δ (higher R_f) 0.85-1.05 (m, 6H), 1.82-1.98 (m, H), 2.37-2.42 (d, H), 2.98-3.04 (m, H), 3.31-3.35 (m, H), 3.55-3.58 (m, H), 4.20-4.30 (t, H), 4.58 (br, 2H), 5.07 (br, H), 5.22-5.43 (m, 3H), 5.84-5.96 (m, H), for *syn* diastereomer (lower R_f) 0.85-1.05
 15 (m, 6H), 1.88-1.95 (m, H), 2.40-2.51 (m, H), 2.83-2.90 (m, H), 3.33-3.36 (m, H), 3.61-3.65 (m, H), 3.87-3.88 (d, H), 4.40-4.68 (m, 3H), 5.20-5.40 (m, 2H), 5.42-5.43 (d, H), 5.80-5.97 (m, H); LC-MS: m/z = 258 (M+H⁺) for both of diastereomers.

20

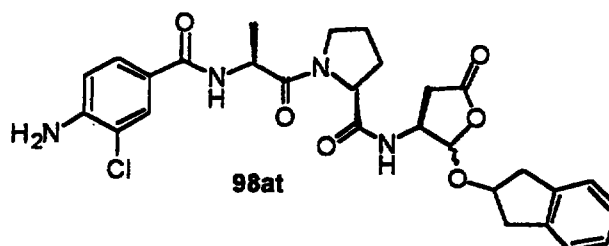
1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-isobutoxy-5-oxo-tetrahydro-furan-3-yl)-amide (98as).

Prepared from 97a and *syn*-(2-isobutoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester
 25 according to the procedure used to prepare 98a to afford 93 mg (38% yield) of the title compound. ¹H-NMR (500 MHz, CDCl₃): δ 0.74-0.76 (t, 0.6H), 0.80-1.00 (m, 5.4H), 1.40-1.50 (m, 3H), 1.90-2.22 (m, 3H), 2.33-2.45 (m, H), 2.80-2.90 (m, H), 3.32-3.38 (m, H), 3.55-3.80
 30

- 192 -

(m, 3H), 4.38 (br, H), 4.50-4.60 (m, H), 4.70-4.80 (m, H), 4.90-5.00 (m, H), 5.42-5.45 (m, H), 6.74-6.76 (d, H), 6.86-6.88 (d, H), 7.31-7.33 (d, H), 7.51-7.53 (m, H), 7.74-7.75 (d, H); retention time on analytical

5 HPLC: 9.63 & 9.80 min; LC-MS: m/z = 495 (M+H⁺).



[2-(indan-2-yl)oxy-5-oxo-tetrahydro-furan-3-yl]-
10 carbamic acid allyl ester.

Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester (5.2 gram, 20 mmol) as described for 40 using 2-indanol (8.05 gram, 60 mmol) to afford 4.10 grams (65% yield) of the title compound as a
15 mixture of epimers. Purification provided 1.76 gram (28% yield) of the 4(S), 5(R) epimer as a yellow oil.
¹H NMR (500 MHz, CDCl₃) δ 2.42 (dd, J=17.2, 10.5Hz, 1H), 2.79 (dd, J=17.2, 8.4Hz, 1H), 2.99 (dd, J=16.7, 4.1Hz, 1H), 3.04 (dd, J=16.7, 4.1Hz, 1H), 3.22 (dd, J=17.2, 6.6Hz, 1H), 3.26 (dd, J=17.2, 6.6Hz, 1H), 4.53 (m, 3H),
20 4.70 (m, 1H), 5.20 (m, 2H), 5.60 (d, J=5.3Hz, 1H), 5.87 (m, 1H), 7.17 (m, 4H) ppm. LC-MS (ES⁺): m/e=318 (M+H).
Analytical HPLC (C18 column): 17.094 min.

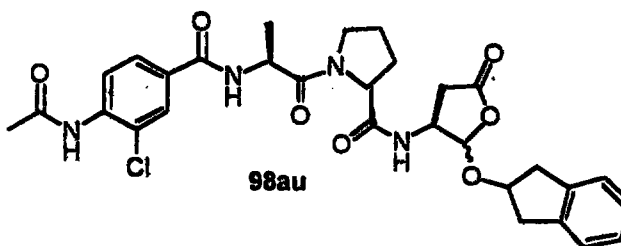
25 Also isolated was epimer mixture (0.75 gram, 12% yield), and the 4(S), 5(S) epimer (1.59 gram, 25%) as a white solid. ¹H-NMR (500MHz, CDCl₃) δ 2.38 (d, J=17.9Hz, 1H), 3.0 (m, 3H), 3.22 (m, 2H), 4.13 (m, 1H), 4.58 (m, 2H), 4.68 (m, 2H), 4.98 (br s, 1H), 5.26 (m, 1H), 5.57
30 (s, 1H), 5.88 (ddt, J=18.0, 11.1, 5.4Hz, 1H), 7.20 (m,

- 193 -

4H) ppm. LC-MS (ES⁺): m/e=318 (M+H). Analytical HPLC (C18 column): 17.025 (5.5%), 17.325 (94.5%) min.

5 1-[2-(4-amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid [2-(indan-2-yloxy)-5-oxo-tetrahydro-furan-3-yl]-amide (98at).

Prepared from 97a and [2-(indanol-2-yl)oxy-5-oxo-tetrahydro-furan-3-yl]-carbamic acid allyl ester according to the procedure used to prepare 98a to afford the title compound as a 71:29 mixture of epimers as an off-white solid (0.20g, 58% yield). ¹H-NMR (500 MHz, CDCl₃) δ 1.0-1.5 (m, 3H), 1.6-2.3 (m, 4H), 2.42 (m, 1H), 2.6-3.4 (m, 6H), 3.5-4.1 (m, 3H), 4.2-4.9 (m, 4H), 5.65 (d, J=5.0Hz, 0.80H), 5.8 (m, 0.07H), 5.85 (d, J=5.0Hz, 0.13H), 6.8-7.3 (m, 6H), 7.4-7.9 (m, 3H) ppm. Analytical HPLC (C18 column) 16.035 (71.4%), 16.476 (28.6%) min. LC-MS (ES⁺): m/e=555 (M+H).



20

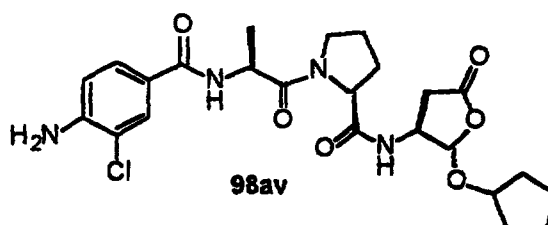
1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid [2-(indan-2-yloxy)-5-oxo-tetrahydro-furan-3-yl]-amide (98au).

Prepared from 97b and [2-(indanol-2-yl)oxy-5-oxo-tetrahydro-furan-3-yl]-carbamic acid allyl ester according to the procedure used to prepare 98a to afford the title compound as a 76:24 mixture of epimers as an off-white solid (0.22g, 57% yield). ¹H-NMR (500 MHz, CDCl₃) δ 1.08 (d, J=6.9Hz, 0.4H), 1.26 (d, J=6.9Hz,

25

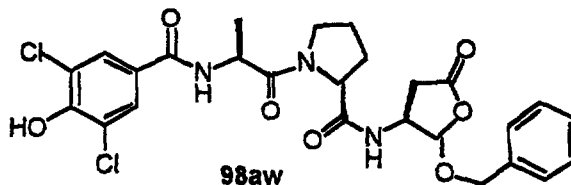
- 194 -

0.6H), 1.35 (d, J=6.9Hz, 2H), 1.8-2.3 (m, 3H), 2.28 (s, 2H), 2.29 (s, 1H), 2.4 (m, 1H), 2.8 (m, 1H), 3.10 (m, 2H), 3.27 (m, 2H), 3.58 (m, 2H), 3.69 (m, 1H), 4.5-4.9 (m, 4H), 5.65 (d, J=5.3Hz, 0.68H), 5.84 (d, J=5.3Hz, 0.18H), 6.38 (br, 0.14H), 6.9-7.7 (m, 6H), 7.6-7.9 (m, 3H), 8.33 (br d, J=6.8Hz, 0.18H), 8.51 (br d, J=8.0Hz, 0.82H) ppm. Analytical HPLC (C18 column) 15.596 (76.2%), 15.932 (23.8%) min. LC-MS (ES⁺): m/e=597 (M+H).



1- [2- (4-amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-cyclopentyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98av).

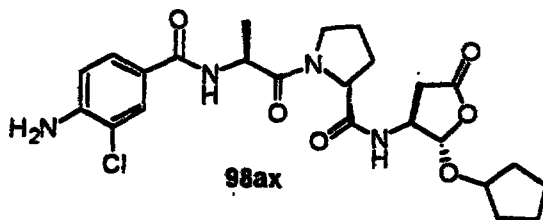
Prepared from 97a and syn-(2-cyclopentyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester according to the procedure used to prepare 98a to afford the title compound as an off-white solid (0.19g, 59% yield). ¹H-NMR (500 MHz, CDCl₃) δ 1.2-2.4 (m, 15H), 2.4-3.1 (m, 2H), 3.6-3.9 (m, 2H), 4.2-4.4 (m, 2H), 4.5-5.0 (m, 4H), 5.40 (d, J=5.0Hz, 0.35H), 5.55 (d, J=5.0Hz, 0.65H), 6.8-8.2 (m, 5H) ppm. Analytical HPLC (C18 column) 14.065 min. LC-MS (ES⁺): m/e=507 (M+H).



- 195 -

1-[2-(3,5-Dichloro-4-hydroxy-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98aw).

Prepared from 1-[2-(4-allyloxy-3,5-dimethyl-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid tert-butyl ester and syn-40 according to the procedure used to prepare 98a to afford the title compound as a pale yellow solid (1.087g, 64% yield). ¹H-NMR (500MHz, CDCl₃) δ 1.09 (d, J=6.9Hz, 0.6H), 1.33 (d, J=6.9Hz, 2.4H), 1.96 (m, 1H), 2.03 (m, 1H), 2.10 (m, 1H), 2.28 (m, 0.8H), 2.40 (dd, J=17.3, 10.2Hz, 0.8H), 2.56 (m, 0.2H), 2.85 (dd, J=17.3, 8.5Hz, 0.8H), 3.09 (dd, J=17.7, 10.2Hz, 0.2H), 3.57 (m, 1H), 3.73 (dt, J=9.2, 7.9Hz, 0.8H), 4.09 (m, 0.2H), 4.21 (d, J=7.9Hz, 0.2H), 4.44 (d, J=9.8Hz, 0.2H), 4.55 (dd, J=8.0, 3.0Hz, 0.8H), 4.62 (d, J=11.6Hz, 1H), 4.70 (m, 1H), 4.80 (m, 1H), 4.89 (d, J=11.6Hz, 0.8H), 5.52 (d, J=5.2Hz, 0.8H), 5.82 (d, J=5.2Hz, 0.2H), 6.51 (br, 0.2H), 6.62 (br, 0.8H), 7.0-7.4 (m, 7H), 7.43 (s, 0.4H), 7.66 (d, J=1.0Hz, 1.6H) ppm. Analytical HPLC (C18 column) 10.135 min. LC-MS (ES⁺): m/e= 564, 566 (6:4) (M+H).

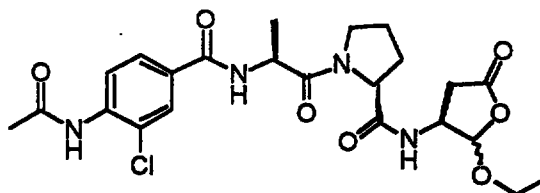


1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-cyclopentyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (98ax).

Prepared according to the procedure used to prepare (98av) using anti-(2-cyclopentyloxy-5-oxo-tetrahydro-furan-3-yl)-amide to afford the title compound as an

- 196 -

off-white solid (0.24 g, 74% yield). $^1\text{H-NMR}$ (500MHz, CDCl_3) δ 1.41 (d, $J=6.5\text{Hz}$, 3H), 1.7 (m, 7H), 1.98 (br, 2H), 2.13 (br, 2H), 2.27 (m, 1H), 2.69 (m, 1H), 2.86 (dd, $J=18.0$, 6.8Hz, 0.7H), 2.98 (dd, $J=18.3$, 8.2Hz, 0.3H), 3.60 (br, 1.4H), 3.77 (br, 0.6H), 4.1-4.6 (m, 5H), 4.82 (m, 1H), 5.27 (m, 0.65H), 5.51 (d, $J=5.3\text{Hz}$, 0.05H), 5.59 (br s, 0.3H), 6.76 (br, 1H), 7.00 (br, 1H), 7.49 (br, 1H), 7.74 (br, 1H), 7.89 (br, 1H) ppm. Analytical HPLC (C18 column) 9.756 min. LC-MS (ES $^+$): $m/e=507$ (M+H).

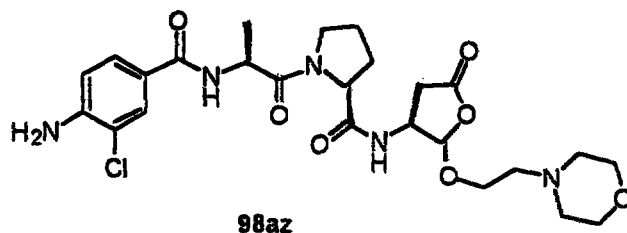


98ay

1- [2- (4-Acetylamino-3-chloro-benzoylamino) -propionyl] -pyrrolidine-2-carboxylic acid (2-ethoxy-5-oxo-tetrahydro-furan-3-yl) -amide (98ay).

Prepared from (2-ethoxy-5-oxo-tetrahydro-furan-3-yl) -carbamic acid allyl ester and 97b following the method used for 98a. The title compound was isolated as a white solid (51 mg, 18% yield). $^1\text{H-NMR}$ (500MHz, 1:1 $\text{CDCl}_3:\text{CD}_3\text{OD}$) δ 1.08-1.35 (m, 3H), 1.35-1.55 (m, 3H), 1.75-2.44 (m, 4H), 2.26 (s, 3H), 2.44-3.07 (m, 2H), 3.48-3.97 (m, 2H), 4.18-4.92 (m, 5H), 5.32 (d, 0.4H), 5.47 (d, 0.1H), 5.58 (d, 0.4H), 5.64 (d, 0.1H), 7.70-8.35 (m, 3H). Analytical HPLC 10.37, 10.54 min. LC-MS (ES $^+$) $m/e= 509.2$ (M+H $^+$).

- 197 -



[2-(2-Chloro-ethoxy)-5-oxo-tetrahydro-furan-3-yl]-carbamic acid allyl ester.

- 5 Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester (5.2g, 20 mmol) as described for 40 using chloroethanol (4.05mL, 60 mmol) to afford 1.84g (35% yield) of the title compound as a mixture of epimers. For the anti-diastereomer: ¹H-NMR (500 MHz, CDCl₃) δ 2.42 (dd, J=18.1Hz, 1H), 3.00 (dd, J=18.1, 7.8Hz, 1H), 3.63 (m, 2H), 3.85 (m, 1H), 4.02 (m, 1H), 4.23 (m, 1H), 4.57 (br s, 2H), 5.17 (br s, 1H), 5.22 (d, H=11.5Hz, 1H), 5.29 (d, J=16.8Hz, 1H), 5.44 (s, 1H), 5.89 (m, 1H) ppm; LC-MS (ES+) m/e= 264 (M+H). For the
- 10 15 syn-diastereomer: ¹H-NMR (500 MHz, CDCl₃) δ 2.47 (dd, J=17.3, 10.7Hz, 1H), 2.83 (dd, J=17.3, 8.4Hz, 1H), 3.65 (m, 2H), 3.83 (m, 1H), 4.11 (m, 1H), 4.57 (m, 3H), 5.22 (d, H=10.4Hz, 1H), 5.30 (d, J=17.2Hz, 1H), 5.33, (m, 1H), 5.47 (d, J=5.2Hz, 1H), 5.89 (ddt, J=17.1, 11.0, 5.4Hz, 1H) ppm; LC-MS (ES+) m/e= 264 (M+H).
- 20

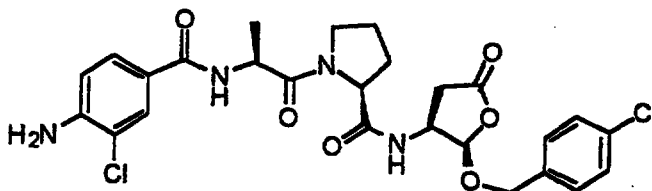
[2-(2-Morpholin-4-yl-ethoxy)-5-oxo-tetrahydro-furan-3-yl]-carbamic acid allyl ester.

- Is prepared from [2-(2-chloro-ethoxy)-5-oxo-tetrahydro-furan-3-yl]-carbamic acid allyl ester by reaction with
- 25 morpholine (2 eq) and KI (1 eq) in DMF.

- 198 -

1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid [2-(2-morpholin-4-ylethoxy)-5-oxo-tetrahydro-furan-3-yl]-amide (98az).

Is prepared from 97a and syn-[2-(2-morpholin-4-ylethoxy)-5-oxo-tetrahydro-furan-3-yl]-carbamic acid allyl ester following the method used for 98a.



98ba

[2-(4-Chloro-benzyloxy)-5-oxo-tetrahydro-furan-3-yl]-carbamic acid allyl ester

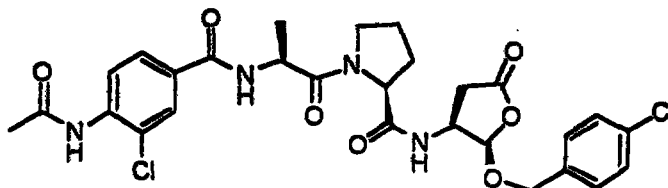
Prepared from 3-allyloxycarbonylamino-4-hydroxy-butyric acid tert-butyl ester as described for 40 using 4-chlorobenzylalcohol to afford the title compound as a white solid. Anti-diastereomer: HPLC (C18 column) 10.924 min; $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ 2.41 (d, $J=8.0\text{Hz}$, 1H), 3.02 (dd, $J=18.1$, 7.8Hz , 1H), 4.25 (br, 1H), 4.56 (m, 2H), 4.58 (d, $J=11.7\text{Hz}$, 1H), 4.79 (d, $J=11.7\text{Hz}$, 1H), 4.99 (br, 1H), 5.22 (dd, $J=10.4$, 1.1Hz , 1H), 5.28 (dd, $J=17.2$, 1.3Hz , 1H), 5.44 (s, 1H), 5.86 (m, 1H), 7.25 (d, $J=8.4\text{Hz}$, 2H), 7.32 (d, $J=8.4\text{Hz}$, 2H) ppm; LC-MS (ES+) $m/e=326$ (M+H); Syn-diastereomer: HPLC (C18 column) 10.780 min; $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ 2.47 (dd, $J=17.3$, 10.5Hz , 1H), 2.85 (dd, $J=17.3$, 8.4Hz , 1H), 4.55 (m, 3H), 4.58 (d, $J=11.7\text{Hz}$, 1H), 4.84 (d, $J=11.7\text{Hz}$, 1H), 5.23 (dd, $J=10.4$, 1.1Hz , 1H), 5.30 (d, $J=16.6\text{Hz}$, 1H), 5.49 (d, $J=5.0\text{Hz}$, 1H), 5.89 (ddt, $J=17.1$, 11.0 , 5.4Hz , 1H), 7.23 (d, $J=8.3\text{Hz}$, 2H), 7.31 (d, $J=8.3\text{Hz}$, 2H) ppm; LC-MS (ES+) $m/e=326$ (M+H).

30

- 199 -

1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid [2-(4-chloro-benzyloxy)-5-oxo-tetrahydro-furan-3-yl]-amide (98ba).

Prepared from 97a and syn-[2-(4-chloro-benzyloxy)-5-oxo-tetrahydro-furan-3-yl]-carbamic acid allyl ester following the method used for 98a to afford 154 mg (65% yield) of the title compound as a pale pink solid.
HPLC (C18 column) 10.597 min; $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ 1.14 (d, $J=6.8\text{Hz}$, 0.75H), 1.34 (d, $J=6.8\text{Hz}$, 2.25H), 1.6 (br, 0.25H), 1.91 (m, 1H), 2.03 (m, 1H), 2.10 (m, 1H), 2.29 (m, 0.75H), 2.40 (dd, $J=17.3$, 10.3Hz, 0.75H), 2.51 (m, 0.25H), 2.82 (dd, $J=17.3$, 8.5Hz, 0.75H), 3.08 (dd, $J=17.9$, 10.9Hz, 0.25H), 3.58 (m, 1H), 3.72 (dd, $J=16.5$, 8.7Hz, 0.75H), 4.10 (m, 0.25H), 4.22 (d, $J=8.0\text{Hz}$, 0.25H), 4.39 (d, $J=10.8\text{Hz}$, 0.25H), 4.54 (dd, $J=9.1$, 2.9Hz, 0.75H), 4.60 (d, $J=11.9\text{Hz}$, 0.75H), 4.68 (m, 1H), 4.85 (d, $J=11.7\text{Hz}$, 0.75H), 4.86 (m, 1H), 5.49 (d, $J=5.2\text{Hz}$, 0.75H), 5.81 (d, $J=5.2\text{Hz}$, 0.25H), 6.2 (br, 0.25H), 6.74 (m, 2H), 7.05 (d, $J=8.5\text{Hz}$, 0.5H), 7.17 (d, $J=8.4\text{Hz}$, 0.5H), 7.30 (m, 3.25H), 7.48 (dd, $J=8.4$, 2.0Hz, 0.75H), 7.56 (d, $J=1.9\text{Hz}$, 0.25H), 7.73 (d, $J=1.9\text{Hz}$, 0.75H), 8.42 (d, $J=5.7\text{Hz}$, 0.25H) ppm; LC-MS (ES+) $m/e=563$, 565 (M+H).



98bb

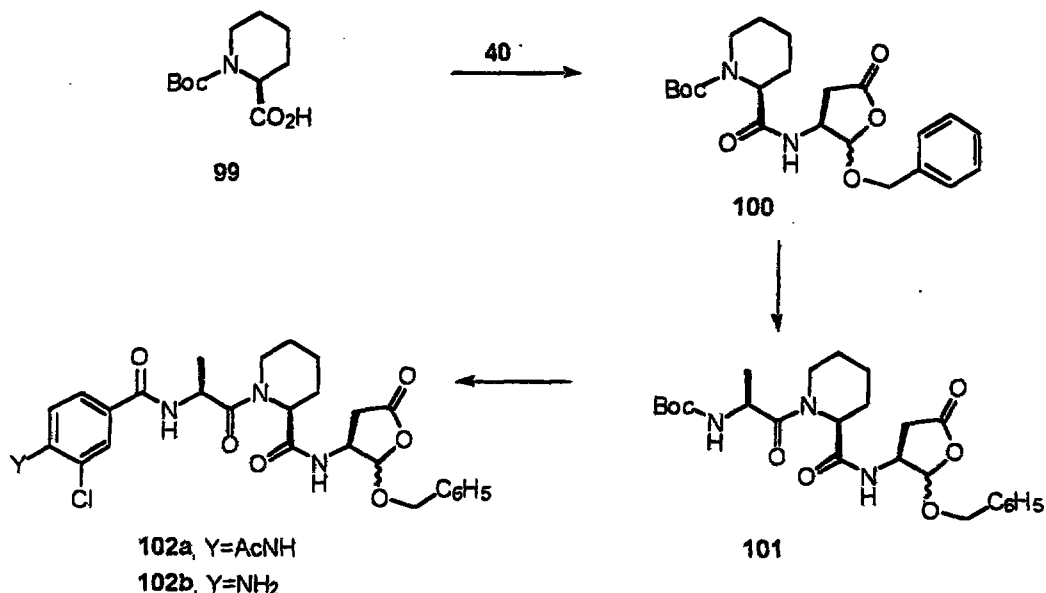
- 200 -

1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid [2-(4-chloro-benzyloxy)-5-oxo-tetrahydro-furan-3-yl]-amide (98bb).

Prepared from 97b and syn-[2-(4-chloro-benzyloxy)-5-oxo-tetrahydro-furan-3-yl]-carbamic acid allyl ester according to the procedure used to prepare 98a to afford 165mg (64% yield) of the title compound as pale yellow solid. HPLC (C18 column) 10.491 min; ¹H-NMR (500 MHz, CDCl₃) δ 1.16 (d, J=6.8Hz, 0.6H), 1.35, (d, J=6.8Hz, 2.4H), 1.94 (m, 1H), 2.04 (m, 1H), 2.10 (m, 1H), 2.25 (s, 3H), 2.28 (m, 1H), 2.40 (dd, J=17.3, 10.4Hz, 0.8H), 2.53 (m, 0.2H), 2.84 (dd, J=17.3, 8.5Hz, 0.8H), 3.02 (dd, J=17.5, 10.5Hz, 0.2H), 3.58 (m, 1H), 3.72 (ddd, J=17.2, 8.3, 8.3Hz, 0.8H), 4.13 (m, 0.2H), 4.22 (d, J=8.2Hz, 0.2H), 4.40 (d, J=10.9Hz, 0.2H), 4.54 (dd, J=8.1, 3.0Hz, 0.8H), 4.60 (d, J=11.8Hz, 0.8H), 4.69 (m, 1H), 4.85 (d, J=11.8Hz, 0.8H), 4.87 (m, 1H), 5.49 (d, J=5.2Hz, 0.8H), 5.80 (d, J=5.2Hz, 0.2H), 6.47 (br, 0.2H), 6.95 (d, J=8.3Hz, 0.8H), 7.05 (d, J=8.3Hz, 0.4H), 7.18 (d, J=8.3Hz, 0.4H), 7.29 (m, 3.2H), 7.49 (dd, J=8.6, 1.9Hz, 0.2H), 7.63 (dd, J=8.6, 1.9Hz, 0.8H), 7.74 (d, J=1.9Hz, 1H), 7.85 (d, J=1.9Hz, 0.8H), 8.25 (d, J=6.4Hz, 0.2H), 8.51 (m, 0.8H) ppm; LC-MS (ES+) m/e=605, 607 (M+H).

- 201 -

Scheme XVIII



2-(2-Benzyloxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-
piperidine-1-carboxylic acid-tert butyl ester (100).

5 Prepared from piperidine-1,2-dicarboxylic acid 1-tert-butyl ester 99 and 40 following the method used in the preparation of 75 to give the title compound as a yellow solid (2.63g, 57% yield). ¹H-NMR (500MHz, CDCl₃) δ 1.15-1.79 (m, 15H), 2.12-2.50 (m, 2H), 2.56-2.83 (m, 1H), 2.89 (dd, 0.5H), 3.05 (dd, 0.5H), 3.81-4.15 (br s, 1H), 4.36-4.97 (m, 3H), 5.37-5.61 (m, 1H), 6.42-6.89 (br s, 1H), 7.17-7.51 (m, 5H). LC-MS (ES⁺) m/e=419.4 (MH⁺).

15 {2-[2-(2-Benzyloxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-piperidin-1-yl]-1-methyl-2-oxo-ethyl}-carbamic acid tert-butyl ester (101).

2-(2-Benzyloxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-piperidine-1-carboxylic acid tert-butyl ester (100) was
20 dissolved in 20% TFA in CH₂Cl₂ (25mL) and stirred at

- 202 -

room temperature for 50min. The solvent was evaporated and the residual acid azeotroped with CH_2Cl_2 (4x). The resulting oil was dissolved in CH_2Cl_2 (20mL) and DMF (5mL), cooled to 0°C and treated with DIEA (4.7mL, 5 27.0mmol), Boc-alanine (970mg, 5.1mmol), HOBT (924mg, 6.8mmol) and EDC (1.31g, 6.8mmol) and the solution stirred under N_2 for 18hours. The solvent was concentrated in vacuo then dissolved in EtOAc and washed with 0.5N NaHSO_4 (2x), saturated NaHCO_3 (2x) and 10 brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to give an orange solid that was dissolved in CH_2Cl_2 and added dropwise to diethyl ether to afford a white precipitate. The title compound as a white solid (1.21g, 73% yield). $^1\text{H-NMR}$ (500MHz, CDCl_3) 15 δ 1.10-1.79 (m, 18H), 1.98-2.19 (m, 0.5H), 2.28-2.88 (m, 3H), 2.89-3.13 (m, 0.5H), 3.78-3.95 (m, 0.5H), 4.21-5.16 (m, 5.5H), 5.38-5.59 (m, 0.3H), 5.66 (d, 0.4H), 5.80 (d, 0.3H), 7.24-7.40 (m, 5H). LC-MS (ES^+) $m/e=490.3$ (MH^+).

20

1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-piperidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)amide (102a).

Prepared from {2-[2-(2-benzyloxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-piperidin-1-yl]-1-methyl-2-oxo-ethyl}-carbamic acid tert butyl ester and 4-acetylamino-3-chlorobenzoic acid by the procedure used in the preparation of 98a to give the title compound (71mg, 25 47% yield). $^1\text{H-NMR}$ (500MHz, CDCl_3) δ 1.10-1.97 (m, 10H), 2.10-2.68 (m, 5H), 2.73-3.24 (m, 2H), 3.62-3.92 (m, 1H), 4.24-5.27 (m, 5H), 5.48-5.59 (m, 0.5H), 5.75-5.85 (m, 0.5H), 6.51-6.61 (d, 1H), 7.05-7.45 (m, 4H), 30

- 203 -

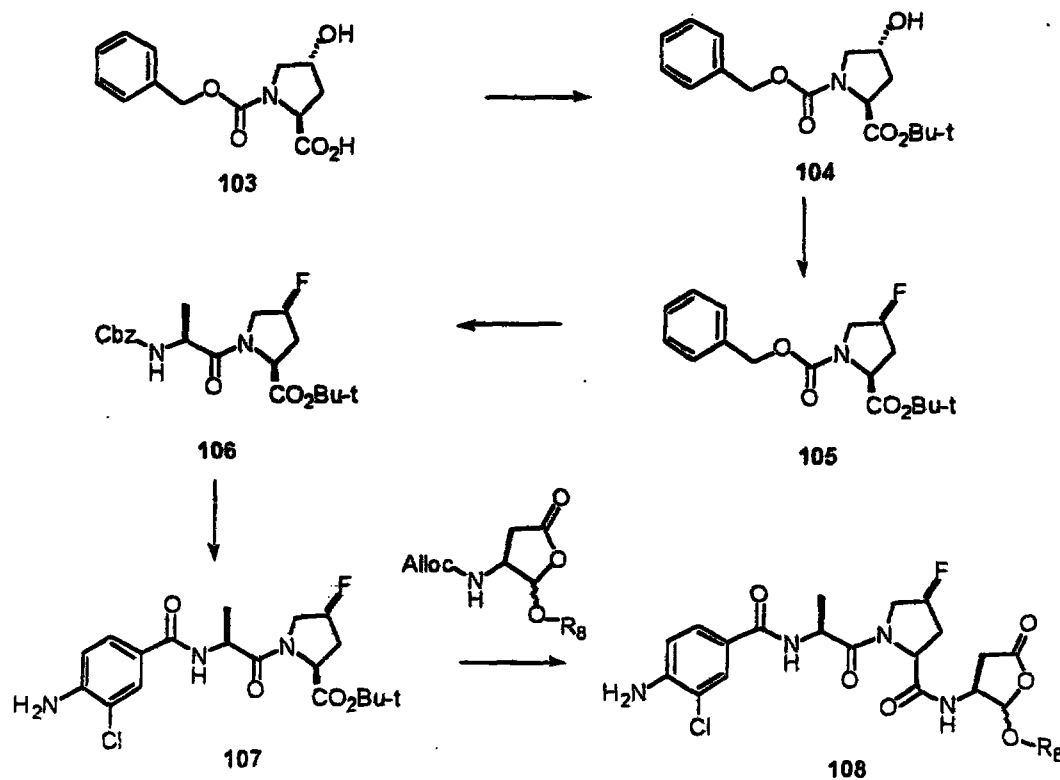
7.52-8.12 (m, 4H). Analytical HPLC 8.30min. LC-MS (ES⁺)
m/e=585.3 (MH⁺).

5 1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-
piperidine-2-carboxylic acid (2-benzyloxy-5-oxo-
tetrahydro-furan-3-yl)-amide (102b).

Prepared as above for 102a to give the title compound
(0.06g, 27% yield) as a yellow solid. ¹H-NMR (500MHz,
CDCl₃) δ 1.2-1.8 (m, 7H), 2.1-2.6 (m, 2H), 2.7-3.2 (m,
10 4H), 3.6-4.0 (m, 1H), 4.3-4.9 (m, 7H), 5.0-5.8 (m, 2H),
6.5-7.0 (m, 2H), 7.2-7.8 (m, 8H) ppm. Analytical HPLC
(cyano column) 14.559 (39.6%), 15.198 (60.4%). LC-MS
(ES⁺): m/e=543 (M+H)

15

Scheme XIX



- 204 -

4-Hydroxy-pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-tert-butyl ester (104).

Compound 104 was prepared according to the procedure
5 used to prepare compound 95.

A suspension of Cbz-Hyp-OH (4.854g, 18mmol) in DMA
(135ml), benzyltriethylammonium chloride (4.105g,
18mmol), K₂CO₃ (64g, 46mmol) and 2-bromo-2-
10 methylpropane (99ml, 859mmol) was stirred at 55°C for
18hours. The mixture was diluted with iced water and
extracted with EtOAc (3x). The organic phase washed
with water, 0.5N NaHSO₄ solution and brine dried and
the solvent in vacuo to yield the title compound as a
15 yellow oil (5.368g, 98% yield). ¹H-NMR (500MHz, CDCl₃)
δ 1.33 (s, 5H), 1.47 (s, 4H), 2.01-2.14 (m, 1H), 2.22-
2.38 (m, 1H), 3.50-3.72 (m, 2H), 4.34-4.45 (m, 1H),
4.45-4.53 (m, 1H), 5.04-5.20 (m, 2H), 7.22-7.42 (m,
5H). Analytical HPLC 10.14min. LC-MS (ES⁺) m/e=322.2
20 (MH⁺).

4-Fluoro-pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-tert-butyl ester (105).

A solution of 104 (4.262, 13.96mmol) in CH₂Cl₂ (100ml)
25 at -78°C was treated with DAST (1.80ml, 13.6mmol),
stirred for 10min then warmed to room temperature and
stirred for 60h under N₂. The mixture was poured into
iced NaHCO₃ (10% solution, 350ml) and extracted with
CH₂Cl₂ (2x). The organic phase was washed with water,
30 brine, dried over anhydrous Na₂SO₄ and concentrated to
give a brown oil (4.299g) which was purified by flash
column chromatography on silica gel using hexanes/EtOAc
(90/10 to 80/20%). The title compound was obtained as a

- 205 -

yellow oil (2.805g, 64% yield). $^1\text{H-NMR}$ (500MHz, CDCl_3)
 δ 1.37 (s, 4.5H), 1.45 (s, 4.5H), 2.20-2.55 (m, 2H),
3.61-3.93 (m, 2H), 4.41 (d, 0.5H), 4.49 (d, 0.5H),
5.03-5.21 (m, 3H), 7.23-7.44 (m, 5H). Analytical HPLC
5 12.15min. LC-MS (ES^+) $m/e=324.2$ (MH^+).

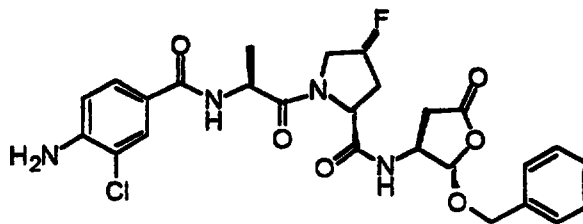
1-(2-Benzylloxycarbonylamino-propionyl)-4-fluoro-
pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-
tert-butyl ester (106).

10 A solution of 105 (2.72g, 8.42mmol) in MeOH (50ml) and
10% Pd/C (1.27g) was stirred under H_2 for 2hours then
filtered through celite and the solvent evaporated to
give a yellow oil (1.526g). This oil was dissolved in
 CH_2Cl_2 (30ml) and treated with DIEA (1.5ml, 8.6mmol),
15 Cbz-ala-OH (2.34g, 10.5mmol) and EDC (2.32g, 12mmol) at
 0°C . The mixture was stirred an additional 10min at
 0°C then allowed to warm to room temperature and stir
for 18hours. The solvent was concentrated in vacuo and
the residue dissolved in EtOAc then washed with 0.5N
20 NaHSO_4 (2x), saturated NaHCO_3 (2x) and brine. The
organic layer was dried over anhydrous Na_2SO_4 and
evaporated to give a white solid which was purified by
flash column chromatography, eluting using
hexanes/EtOAc (80/20 to 60/40%). The title compound
25 was isolated as a white solid (286g, 86% from 4-fluoro-
pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-
tert-butyl ester). $^1\text{H-NMR}$ (500MHz, CD_3OD) δ 1.26-1.59
(m, 12H), 2.20-2.67 (m, 2H), 3.45-4.13 (m, 2H), 4.25-
4.47 (m, 1H), 4.58-4.71 (m, 1H), 4.96-5.17 (m, 2H),
30 5.19-5.45 (m, 1H), 7.23-7.48 (m, 5H). Analytical HPLC
16.36min. LC-MS (ES^+) $m/e=395.3$ (MH^+).

- 206 -

1- [2- (4-Amino-3-chloro-benzoylamino)-propionyl]-4-fluoro-pyrrolidine-2-carboxylic acid-tert-butyl ester (107).

5 A suspension of 106 (2.65g, 6.72mmol) in MeOH (40ml) and 10% Pd/C (1.32g) was stirred under an atmosphere of H₂ for 1.5hours, filtered through celite and the concentrated to give a waxy solid (1.694g). The solid was dissolved in CH₂Cl₂ (25ml) and treated with DIEA (3.4ml, 19.5mmol), 4-amino-3-chlorobenzoic acid (1.362g, 7.9mmol), HOBT (1.164g, 8.62mmol) and EDC (1.645g, 8.57mmol) at 0°C under N₂. The mixture was allowed to warm to room temperature and stirred for 18hours. The solvent was concentrated in vacuo. The residue was dissolved in EtOAc, washed with water (4x), 0.5N NaHSO₄ (2x), saturated NaHCO₃ (2x) and brine. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to give a white solid which was purified by flash column chromatography, using CH₂Cl₂/MeOH (99/1 to 98/2%). The product obtained as a white solid (2.705g, 97%). ¹H-NMR (500MHz, CD₃OD) δ 1.33 (s, 9H), 1.48 (d, 3H), 2.31-2.55 (m, 2H), 3.93 (d,d, 1H), 4.02-4.21 (m, 1H), 4.59-4.76 (m, 1H), 5.31 (br s, 0.5H), 5.41 (br s, 0.5H), 6.78 (d, 1H), 7.57 (d,d, 1H), 7.78 (s, 1H), 8.31 (d, 1H). Analytical HPLC 14.14min. LC-MS (ES⁺) m/e=414.2 (MH⁺).

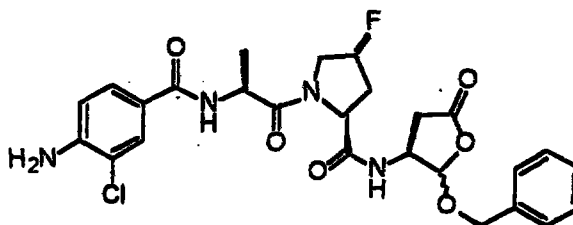


108a

- 207 -

1- [2- (4-Amino-3-chloro-benzoylamino)-propionyl]-4-fluoro-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (108a).

Prepared from syn-(2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester and 107a following the method used for the synthesis of 98a. The title compound was isolated as a white solid (41mg, 15% yield). ¹H-NMR (500MHz, CD₃OD) δ 0.94 (d, 0.3H), 1.07 (d, 1H), 1.40 (m, 1.7H), 2.21-2.65 (m, 2.2H), 2.70-2.85 (m, 1.4H), 2.96-3.08 (m, 1.4H), 2.96-3.08 (dd, 0.4H), 3.57-4.24 (m, 3H), 4.41-4.93 (m, 4H), 5.14-5.45 (m, 1H), 5.60-5.67 (m, 0.6H), 5.77 (d, 0.4H), 6.77 (dd, 1H), 7.15-7.41 (m, 5H), 7.51-7.62 (m, 1H), 7.77 (dd, 1H). Analytical HPLC 12.83min. LC-MS (ES⁺) m/e= 547.1 (MH⁺).



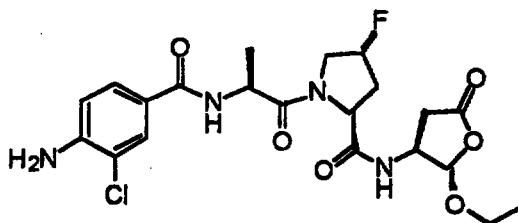
108b

1- [2- (4-Amino-3-chloro-benzoylamino)-propionyl]-4-fluoro-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (108b).

Prepared from (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester 107a following the method used for the synthesis of 98a. The title compound was isolated as a white solid (654mg, 54% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.07 (d, 0.5H), 1.25-1.56 (m, 2.5H), 2.21-2.65 (m, 2.3H), 2.68-2.89 (m, 1H), 2.91-3.10 (m, 0.7H), 3.57-4.23 (m, 2H), 4.32-4.95 (m, 5H), 5.16-5.52 (m, 1H), 5.45-5.50 (m, 0.3H), 5.54-5.58 (m, 0.2H), 5.61-5.67 (m, 0.3H), 5.77 (d, 0.2H), 6.72-6.84 (m, 1H),

- 208 -

7.16-7.41 (m, 5H), 7.50-7.65 (m, 1H), 7.71-7.87 (m, 1H). Analytical HPLC 12.83min. LC-MS (ES⁺) m/e=547.1 (MH⁺).



108c

5

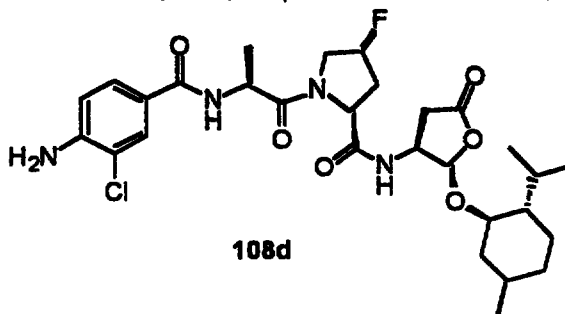
1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-4-fluoro-pyrrolidine-2-carboxylic acid (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (108c).

10 Prepared from *syn*-(2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester and 107a following the method used for the synthesis of 98a to give the title compound (100.3mg, 38% yield). ¹H-NMR (500MHz, CD₃OD) δ

1.09 (t, 1.2H), 1.25 (t, 1.8H), 1.40 (d, 1H), 1.49 (d, 2H), 2.33-2.61 (m, 2H), 2.65-2.95 (m, 2H), 3.44-4.30

15 (m, 4H), 4.47-4.79 (m, 3H), 5.18-5.25 (m, 0.2H), 5.27-5.36 (m, 0.5H), 5.39-5.46 (m, 0.3H), 5.56 (m, 1H), 6.72-6.94 (m, 0.8H), 7.54-7.69 (m, 0.8H), 7.79 (d, 0.55H), 8.06 (d, 0.55H), 9.00 (d, 0.3H). Analytical

20 HPLC 8.46min. LC-MS (ES⁺) m/e= 485.2 (MH⁺).



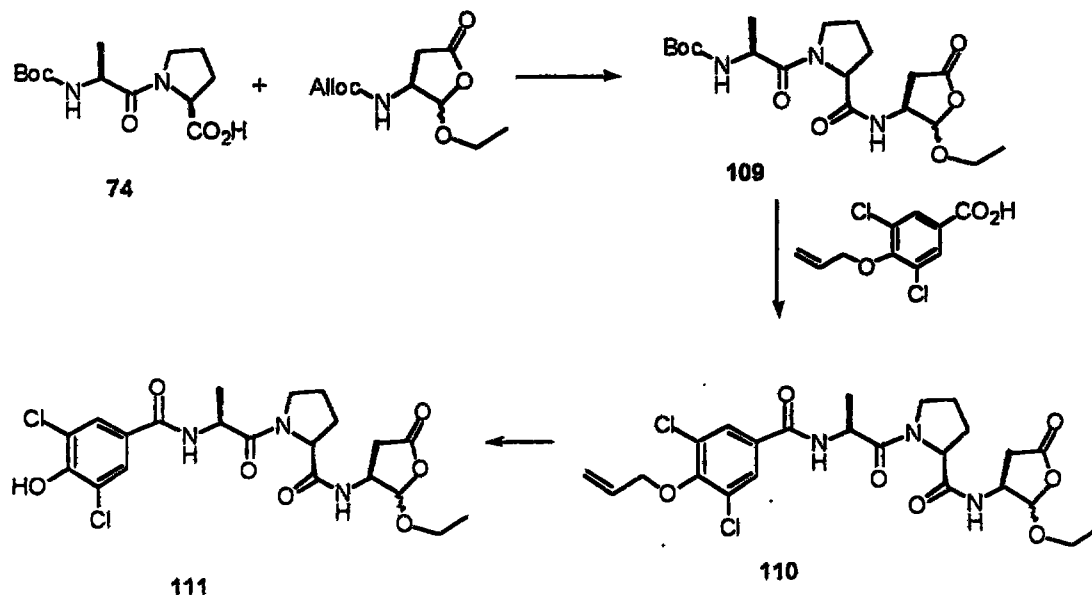
108d

- 209 -

1- [2- (4-Amino-3-chloro-benzoylamino) -propionyl] -4-fluoro-pyrrolidine-2-carboxylic acid [2- (2-isopropyl-5-methyl-cyclohexyl) -5-oxo-tetrahydro-furan-3-yl] -amide (108d) .

- 5 Prepared from {2- [1R- (2S-Isopropyl-5R-methyl-cyclohexyloxy)] -5-oxo-tetrahydro-furan-3-yl} -carbamic acid allyl ester and 107a following the method used for the synthesis of 98a to give the title compound (95mg, 31% yield). ¹H-NMR (500MHz, CD₃OD) δ 0.42 (d, 2H),
 10 0.57 (d, 2H), 0.60-1.10 (m, 10H), 1.22-1.76 (m, 6H), 1.96-2.17 (m, 1H), 2.29-2.60 (m, 2H), 2.61-2.88 (m, 1.5H), 3.02-3.23 (dd, 0.5H), 3.37-3.47 (m, 0.5H), 3.50-3.61 (m, 0.5H), 3.63-4.24 (m, 2H), 4.48-4.62 (m, 3H),
 15 5.18-5.48 (m, 1H), 5.72 (d, 0.4H), 5.82 (d, 0.6H), 6.77-6.84 (m, 1H), 7.53-7.67 (m, 1H), 7.78 (d, 0.4H), 7.84 (d, 1H) Analytical HPLC 8.34min. LC-MS (ES+) m/e= 595 (MH⁺) .

Scheme XX



20

- 210 -

{2-[2-(2-Ethoxy-5-oxo-tetrahydro-furan-3-yl)carbamoyl]-pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl}carbamic acid tert-butyl ester (109).

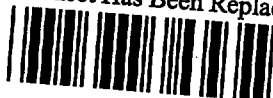
Prepared from (2-ethoxy-5-oxo-tetrahydro-furan-3-yl) carbamic acid allyl ester 74 following the method used in the synthesis of 75 to give the title compound as a pale yellow solid (660mg, 73% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.14-1.36 (m, 6H), 1.42 (s, 9H), 1.75-2.29 (m, 4H), 2.48 (dd, 0.5H), 2.58 (dd, 0.5H), 2.72-2.85 (m, 0.5H), 2.99 (dd, 0.5H), 3.43-3.91 (m, 4H), 4.07-4.52 (m, 2.5H), 4.53-4.72 (m, 0.5H), 5.37 (s, 0.5H), 5.57 (d, 0.5H). Analytical HPLC (mixture of 2 diastereomers) 7.92, 8.14min. LC-MS (ES+) m/e=414.3 (MH+).

1-[2-(4-Allyloxy-3,5-dichloro-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (110).

Prepared from 109 and 4-allyloxy-3,5-dichloro-benzoic acid following the method used in the synthesis of 82 to give the title compound as a white solid (228mg, 65%). ¹H-NMR (500MHz, CD₃OD) δ 1.10-1.30 (m, 4H), 1.32-1.52 (m, 3H), 1.63-2.31 (m, 4H), 2.41-2.50 (d, 0.5H), 2.52-2.61 (dd, 0.5H), 2.67-2.81 (m, 0.5H), 2.94-3.05 (dd, 0.5H), 3.47-3.96 (m, 4H), 4.21-4.81 (m, 5H), 5.22-5.32 (m, 1H), 5.35-5.49 (m, 1.5H), 5.55-5.63 (m, 0.5H), 6.06-6.21 (m, 1H), 7.90 (s, 2H). Analytical HPLC (mixture of 2 diastereomers) 12.56min. LC-MS (ES+) m/e=542.3 (MH+).

1-[2-(3,5-Dichloro-4-hydroxy-benzoylamino)-propionyl]-pyrrolidine-2-carboxylic acid (2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (111).

This Sheet Has Been Replaced

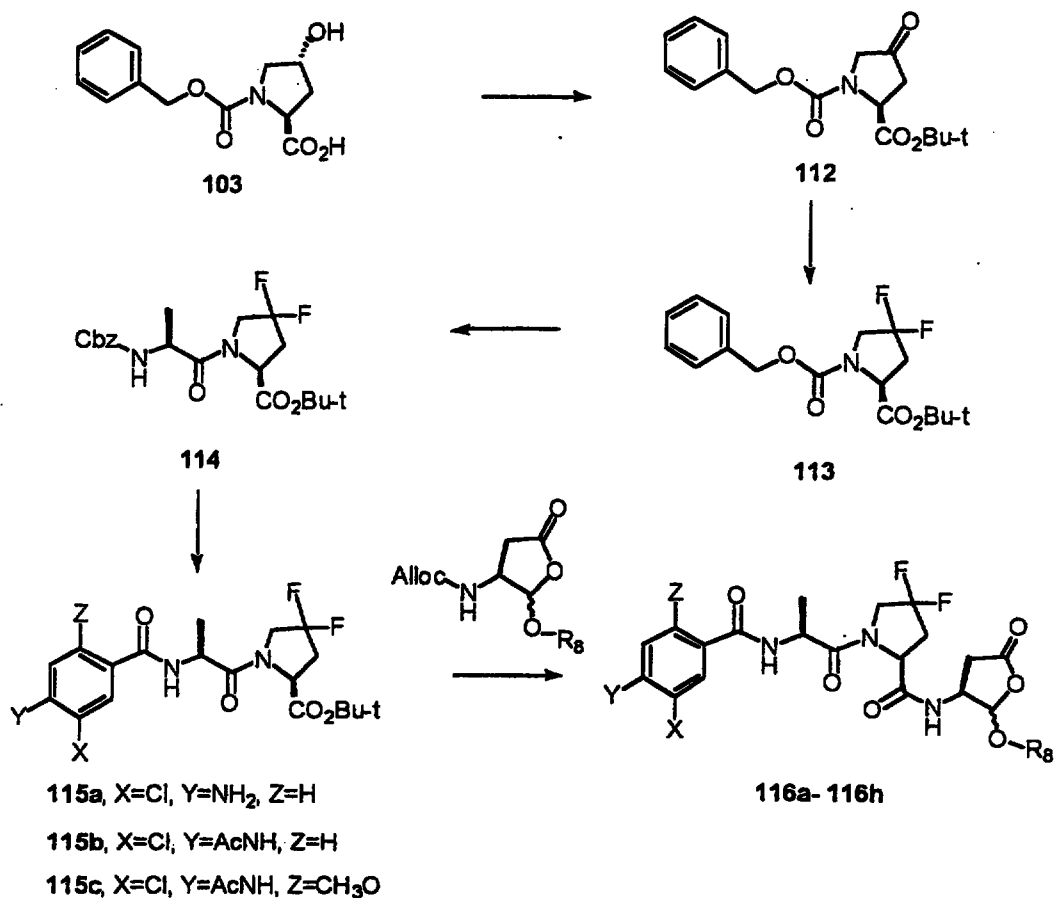


- 211 -

To a solution of 110 (194mg, 0.36mmol) in CH₂Cl₂ (5ml) was added DMBA (70.7mg, 0.45mmol) and Pd(PPh₃)₄ (50.3mg, 0.044mmol) at 0°C. The solution was warmed to room temperature after 15mins, stirred for 2hours, diluted with CH₂Cl₂ then washed with water (2x) and brine. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to give the crude product. Flash chromatography using CH₂Cl₂/MeOH (99/1 to 95/5%) afforded the title compound (138.6mg, 77% yield). ¹H-NMR (500MHz, CD₃OD) δ 1.13-1.31 (m, 3H), 1.35-1.49 (m, 3H), 1.84-2.35 (m, 4H), 2.43-3.05 (m, 2H), 3.48-3.93 (m, 4H), 4.22-4.80 (m, 3H), 5.38 (d, 0.4H), 5.46 (s, 0.1H), 5.55-5.61 (m, 0.5H), 7.76-7.94 (m, 2H). Analytical HPLC 8.70min. LC-MS (ES⁺) m/e=502.2 (MH⁺).

15

Scheme XXI



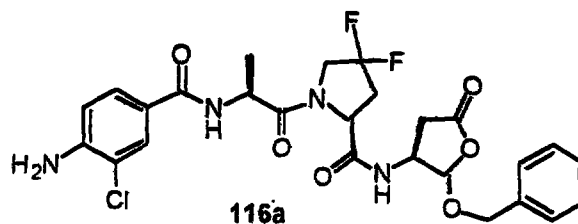
Compounds 116a- 116h were prepared as described above for compounds 98 only substituting 1-(2-benzyloxycarbonylamino-propionyl)-4,4-difluoro-pyrrolidine-2-carboxylic acid tert-butyl ester (114) for 1-(2-benzyloxycarbonylamino-propionyl)-pyrrolidine-2-carboxylic acid tert-butyl ester (95).

Preparation of 1-(2-benzyloxycarbonylamino-propionyl)-4,4-difluoro-pyrrolidine-2-carboxylic acid tert-butyl ester (114).

A solution of 4,4-difluoro-pyrrolidine-1,2-dicarboxylic acid-1-benzyl ester-2-tert-butyl ester (113)

- 213 -

(Karanewsky, et.al., J. Med. Chem. 33, pp. 1459-1469 (1990)) (0.42 g, 1.23 mmol) and 10% palladium on carbon (0.22g) in methanol (6 mL) was stirred at 1 atm hydrogen pressure for 3h. The mixture was filtered
5 through Celite and evaporated. The residue was dissolved in CH₂Cl₂ (4 mL) and DMF (2 mL) and cooled to 0°C. 2-Benzyloxycarbonylamino-propionic acid (0.30 g, 1.35 mmol), EDC (0.30, 1.54 mmol), DIEA (0.65 mL) and HOBT (0.17 g, 1.23 mmol) was added and the reaction was
10 stirred 0.5h at 0°C, then 16h at room temperature under nitrogen. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate, then was washed with 10% sodium bisulfate, saturated sodium bicarbonate, water and brine, was dried over sodium
15 sulfate and was evaporated. Purification by flash chromatography on silica, eluted with 25:75 ethyl acetate: hexanes provided 1-(2-benzyloxycarbonylamino-propionyl)-4,4-difluoro-pyrrolidine-2-carboxylic acid tert-butyl ester (0.39 g, 77% yield) as a colorless
20 oil.
¹H-NMR (500 MHz, CDCl₃) δ 1.3-1.6 (m, 12H), 2.5 (m, 0.8H), 2.7 (m, 1.2H), 3.9 (m, 1H), 4.1 (m, 1H), 4.4 (m, 1H), 4.7 (m, 1H), 5.1 (m, 2H), 5.59 (br d, J=7.7Hz, 0.8H), 5.7 (br d, J=7.7Hz, 0.2H), 7.35 (m, 5H) ppm.
25 Analytical HPLC (cyano column) 17.069 min. LC-MS (ES+): m/e=413 (M+H), 357 (M+H-tert-butyl), 313 [M+H-(CO₂tert-butyl)].

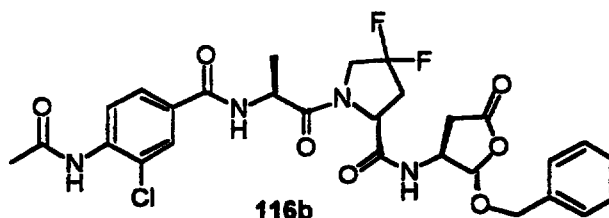


- 214 -

1- [2- (4-Amino-3-chloro-benzoylamino) -propionyl] -4,4-difluoro-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl) -amide (116a).

5 Prepared from 115a and syn-40 to afford the title compound as an off-white solid (0.14g, 73% yield). ¹H-NMR (500 MHz, CD₃OD) δ 1.0-1.5 (m, 3H), 2.0-3.5 (m, 4H+CH₃OH), 3.5-5.5 (M, 6H+H₂O), 5.6-5.8 (m, 1H), 6.7-6.8 (m, 1H), 7.1-7.8 (m, 8H), 8.2-8.6 (m, 1H) ppm.

10 Analytical HPLC (cyano column) 13.744 min. LC-MS (ES⁺): m/e=565 (M+H).

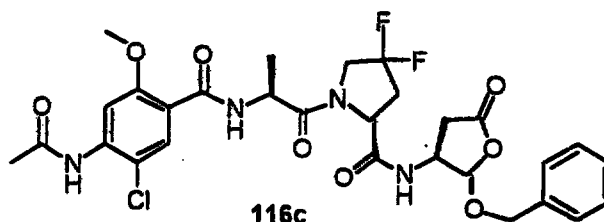


15 1- [2- (4-Acetylamino-3-chloro-benzoylamino) -propionyl] -4,4-difluoro-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl) -amide (116b).

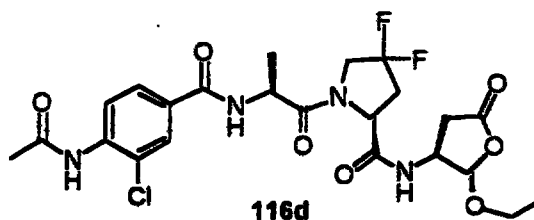
Prepared from 115b and syn-40 to afford the title compound as an off-white solid (0.08 g, 38% yield).

20 ¹H-NMR (500 MHz, CDCl₃) δ 1.03 (d, J=6.9Hz, 0.4H), 1.30 (d, J=6.9Hz, 0.6H), 2.25 (d, J=2.9Hz, 3H), 2.4-3.2 (m, 4H), 3.6-4.4 (m, 4H), 4.6-4.9 (m 3H), 5.52 (d, J=5.2Hz, 0.6H), 5.78 (d, J=5.2Hz, 0.4H), 6.6 (br s, 1H), 6.9-7.9 (m, 8H), 8.39 (d, J=8.1 Hz, 0.4H), 8.44 (d, J= 8.3Hz, 0.6H), 8.74 (d, J=6.8Hz, 1H) ppm. Analytical HPLC
25 (cyano column) 11.830 min. LC-MS (ES⁺): m/e= 607 (M+H).

- 215 -



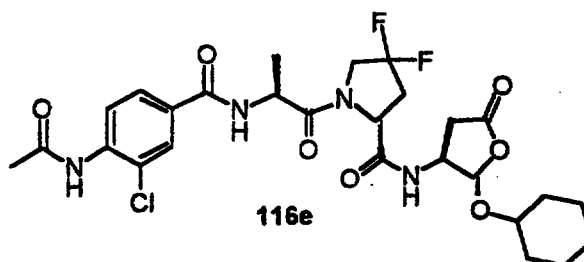
1- [2- (4-Acetylamino-5-chloro-2-methoxy-benzoylamino) -
 propionyl] -4,4-difluoro-pyrrolidine-2-carboxylic acid
 5 (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (116c).
 Prepared from 115c and syn-40 to afford the title
 compound as an off-white (0.07g, 29% yield). ¹H-NMR
 (500 MHz, CDCl₃) δ 0.99 (d, J=6.9Hz, 1.35H), 1.32 (d,
 J=6.9Hz, 1.65H), 2.25 (s, 1.5H), 2.26 (s, 1.5H), 2.3-
 10 3.2 (m, 4H), 3.95 (s, 0.55H), 3.98 (s, 0.45H), 3.7-4.1
 (m, 2.5H), 4.2-4.5 (m, 1.5H), 4.6-4.9 (m, 3H), 5.52 (d,
 J=5.3Hz, 0.55H), 5.80 (d, J=5.3Hz, 0.45H), 7.0-7.4 (m,
 4H), 7.7-7.9 (m, 2H), 8.0-8.4 (m, 2H), 8.49 (d,
 J=6.5Hz, 1H), 8.93 (d, J=6.7Hz, 1H) ppm. Analytical
 15 HPLC (cyano column) 12.959 min. LC-MS (ES⁺): m/e=637
 (M+H).



20 1- [2- (4-Acetylamino-3-chloro-benzoylamino) -propionyl] -
 4,4-difluoro-pyrrolidine-2-carboxylic acid (2-ethoxy-5-
 oxo-tetrahydro-furan-3-yl)-amide (116d).
 Prepared from 115b and syn-(2-ethoxy-5-oxo-tetrahydro-
 furan-3-yl)-carbamic acid allyl ester to afford the
 25 title compound as a 92:8 mixture of epimers. Off-white
 solid (0.27g, 66% yield). ¹H-NMR (500 MHz, CDCl₃) δ 1.0-

- 216 -

1.5 (m, 6H), 2.25 (s, 1.8H), 2.26 (s, 1.2H), 2.3-3.1 (m, 4H), 3.3-4.3 (m, 4H), 4.5-4.9 (m, 3H), 5.45 (d, J=5.3Hz, 0.75H), 5.59 (d, J=5.2Hz, 0.25H), 6.7-7.1 (m, 2H), 7.62 (dd, J=8.7, 2.0Hz, 1H), 7.76 (m, 1H), 7.85 (d, J=2.0Hz, 1H), 8.48 (m, 1H) ppm. Analytical HPLC (C18 column) 13.300 (91.8%), 14.046 (8.2%) min. LC-MS (ES+): m/e 545 (M+H).



10

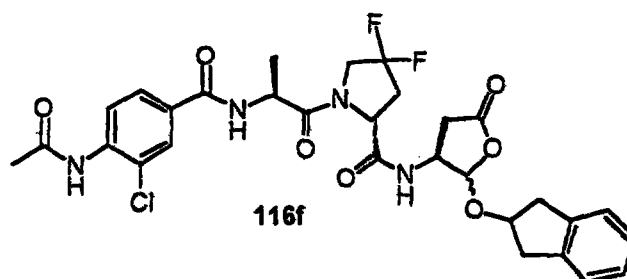
1- [2- (4-Acetylamino-3-chloro-benzoylamino)-propionyl] - 4,4-difluoro-pyrrolidine-2-carboxylic acid (2-cyclohexyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (116e).

15

Prepared from 115b and syn-(2-cyclohexyloxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester to afford the title compound as a 93:7 mixture of epimers.

¹H-NMR (500 MHz, CDCl₃) δ 1.0-2.0 (m, 13H), 2.25 (s, 2H), 2.26 (s, 1H), 2.40 (dd, J=17.3, 10.1Hz, 1H), 2.84 (dd, J=17.3, 8.5Hz, 1H), 2.5-3.0 (m, 2H), 3.5-4.3 (m, 3.5H), 4.5-4.9 (m, 2.5H), 5.59 (d, J=5.3Hz, 0.75H), 5.76 (d, J=5.2Hz, 0.25 H), 6.74 (br d, J= 5.7Hz, 0.25H), 6.93 (br d, J=7.1Hz, 1H), 7.06 (br d, J=7.8Hz, 0.75H), 7.62 (dd, J=8.6, 2.0Hz, 1H), 7.78 (m, 1H), 7.85 (d, J=2.0Hz, 1H), 8.35 (br d, J=6.6Hz, 0.25H), 8.50 (br d, J=8.2Hz, 0.75H) ppm. Analytical HPLC (C18 column) 17.112 (93%), 17.433 (7%) min. LC-MS (ES+): m/e=599 (M+H).

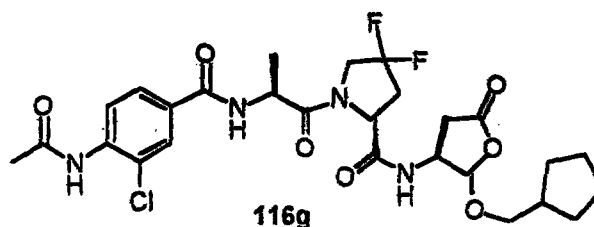
- 217 -



1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-
4,4-difluoro-pyrrolidine-2-carboxylic acid [2-(indanol-
5 2-yl)oxy-5-oxo-tetrahydro-furan-3-yl]-amide (116f).

Prepared from 115b and [2-(indanol-2-yl)oxy-5-oxo-
tetrahydro-furan-3-yl]-carbamic acid allyl ester to
afford the title compound as a 62:38 mixture of
epimers. Off-white solid (0.34g, 71% yield). ¹H-NMR
10 (500 MHz, CDCl₃) δ 1.09 (d, J=6.9Hz, 0.6H), 1.21 (d,
J=6.9Hz, 0.9H), 1.33 (d, J=6.9Hz, 0.9H), 1.42 (d,
J=6.9Hz, 0.6H), 2.28 (s, 2H), 2.29 (s, 1H), 2.40 (dd,
J=17.4, 10.3Hz, 1H), 2.4-3.3 (m, 7H), 3.6-4.2 (m, 2H),
4.5-4.8 (m, 4H), 5.66 (m, 0.6H), 5.84 (d, J=4.3Hz,
15 0.2H), 6.22 (m, 0.2H), 6.7-7.0 (m, 2H), 7.2-7.3 (m,
4H), 7.5-7.7 (m, 1H), 7.8-8.0 (m, 2H), 8.52 (m, 0.6H),
8.62 (br d, J=6.5Hz, 0.4H) ppm. Analytical HPLC (C18
column) 16.556 (62.0%), 16.824 (38.0%) min. LC-MS
(ES⁺): m/e=633 (M+H).

20

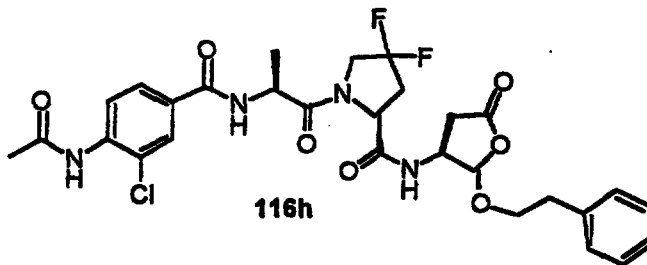


1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-
4,4-difluoro-pyrrolidine-2-carboxylic acid (2-
4-cyclopentylmethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (116g).

- 218 -

cyclopentylmethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (116g).

- Prepared from 115b and *syn*-(2-cyclopentylmethoxy-5-oxo-tetrahydro-furan-3-yl)-carbamic acid allyl ester to afford the title compound as an off-white solid (0.20g, 44% yield). ¹H-NMR (500 MHz, CDCl₃) δ 1.0-1.8 (m, 11H), 1.9-3.0 (m, 5H), 2.26 (s, 3H), 3.29 (m, 0.25H), 3.47 (m, 0.75H), 3.58 (m, 0.25H), 3.74 (m, 0.75H), 3.8 (m, 0.75H), 4.1 (m, 0.25H), 4.25 (m, 1H), 4.4-4.8 (m, 3H), 5.44 (d, J=5.2Hz, 0.75H), 5.62 (d, J=5.2Hz, 0.25H), 6.7 (br, 0.25H), 6.91 (d, J=7.1Hz, 1H), 7.1 (m, 0.75H), 7.59 (d, J=8.5Hz, 0.25H), 7.63 (dd, J=8.5, 2.5Hz, 0.75H), 7.75 (m, 1H), 7.86 (d, J=1.8Hz, 1H), 8.33 (br d, J=6.5Hz, 0.25H), 8.49 (br d, J=8.4Hz, 0.75H) ppm.
- Analytical HPLC (C18 column) 17.705 min. LC-MS (ES+): m/e=599 (M+H).



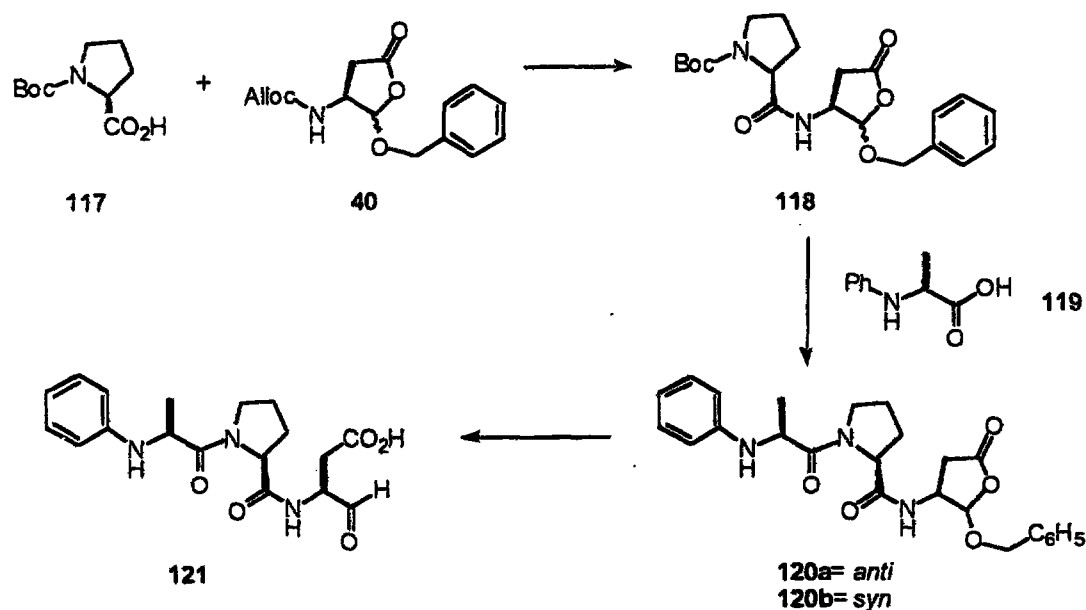
- 1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-4,4-difluoro-pyrrolidine-2-carboxylic acid (2-phenylethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (116h).**

- Was prepared from 115b and *syn*-(5-oxo-2-phenethyloxy-tetrahydro-furan-3-yl)-carbamic acid allyl ester to afford the title compound as an off-white solid (0.15g, 24% yield). ¹H-NMR (500MHz, CDCl₃) δ 1.29 (d, J=6.9Hz, 0.75H), 1.40 (d, J=6.9Hz, 2.25H), 2.25 (s, 2.25H), 2.26 (s, 0.75H), 2.3-3.0 (m, 6H), 3.7-4.8 (m, 7H), 5.38 (d, J=5.3Hz, 0.75H), 5.67 (d, J=5.1Hz, 0.25H), 6.65 (m,

- 219 -

1H), 6.90 (d, J=7.0Hz, 0.75H), 7.06 (d, J=7.6Hz, 0.25H), 7.1-7.3 (m, 5H), 7.57 (d, J=8.6Hz, 0.25H), 7.63 (d, J=8.6Hz, 0.75H), 7.75 (m, 1H), 7.86 (d, J=1.8Hz, 1H), 8.35 (d, J=6.2 Hz, 0.25H), 8.49 (d, J=8.3Hz, 0.75H) ppm. Analytical HPLC (C18 column) 17.265 min. LC-MS (ES+): m/e=621 (M+H).

Scheme XXII



2-(2-Benzoyloxy-5-oxo-tetrahydro-furan-3-ylcarbamoyl)-pyrrolidine-1-carboxylic acid tert-butyl ester (118). Prepared from 40 (1.16 g, 4.0 mmol) and Boc-Pro-OH according to the procedure used to prepare 100 (Scheme XVIII) to afford 1.53 g (94% yield) of the title compound as a white solid. ¹H-NMR (500 MHz, CDCl₃): δ 1.61 (br, 9H), 1.88 (br, 2H), 2.00-2.50 (m, 3H), 2.80-3.10 (m, H), 3.20-3.60 (m, 2H), 4.05-4.45 (m, 1.5H), 4.58-4.80 (m, 1.5H), 4.83-4.98 (m, H), 5.43-5.53 (m, H), 7.26-7.45 (m, 5H), 7.60-7.80 (d, H); Analytical HPLC: 11.32 min; LC-MS: m/e = 405 (M+H⁺).

- 220 -

2-Phenylaminopropionic acid (119).

A mixture of alanine (356 mg, 4.0 mmol), iodobenzene (816 mg, 4.0 mmol), trans-dichlorobis(tri-o-tolylphosphine) palladium (II) {Pd[P(o-Tol)₃]₂Cl₂} (160 mg, 0.2 mmol), copper (I) iodide (40 mg, 0.2 mmol), K₂CO₃ (552 mg, 4.0 mmol), benzyltriethylammonium chloride (160 mg, 0.8 mmol), triethylamine (1.6 mL) and water (0.8 mL) in DMF (8 mL) was stirred under nitrogen atmosphere at 100°C for 20 hours. The mixture was cooled to room temperature, diluted with ethyl acetate (50 mL) and water (50 mL), acidified with 6N HCl to the pH = 2 to 3. The aqueous layer was extracted with ethyl acetate (50 mL x 4). The combined organic layers were washed with water, brine, dried over anhydrous Na₂SO₄, filtered and evaporated in vacuo to give a red oil. Flash chromatography using hexane/ethyl acetate/acetic acid (95/5/0.5 to 80/20/0.5) to afford 300 mg (45% yield) of the title compound as a pink solid. ¹H-NMR (500 MHz, CDCl₃/CD₃OD = 0.5 ml/3 drops): δ 1.45 (d, 3H), 4.02-4.15 (m, H), 6.57-6.70 (m, 3H), 7.11-7.25 (m, 2H); Analytical HPLC: 6.10 min. LC-MS: m/e = 166 (M+H⁺).

1-(2-Phenylamino-propionyl)-pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide (120a and 120b).

A solution of 118 (405 mg, 1.0 mmol) was treated with TFA (2 mL) in CH₂Cl₂ (2 mL) for one hour. The reaction solution was evaporated in vacuo and azeotrapped with CH₂Cl₂ four times to give pyrrolidine-2-carboxylic acid (2-benzyloxy-5-oxo-tetrahydro-furan-3-yl)-amide as a pale yellow solid. ¹H-NMR (500 MHz, CDCl₃): δ 1.87-2.15 (m, 4H), 2.30-2.70 (m, 2H), 2.80-3.08 (m, H), 3.45 (br,

- 221 -

2H), 4.35-4.98 (m, 3H), 5.30-5.56 (m, H), 7.10-7.60 (m, 5H); Analytical HPLC: 7.78 / 8.20 min.; LC-MS: m/e = 305 (M+H⁺).

- 5 2-Phenylaminopropionic acid (119) (300 mg, 1.8 mmol) in CH₂Cl₂ (10 mL) was treated with HOBT (270 mg, 2.0 mmol) and EDC (2.1 g, 11 mmol) at 0°C for 10 min. Diisopropylethylamine (2 mL) was added followed by a solution of pyrrolidine-2-carboxylic acid (2-benzyloxy-
10 5-oxo-tetrahydro-furan-3-yl)-amide in CH₂Cl₂ (10 mL). The mixture was stirred at room temperature for 4 hours, diluted with CH₂Cl₂ (40 mL), washed with water then brine. The organic layer was dried over anhydrous Na₂SO₄, filtered and evaporated in vacuo to give a pale
15 yellow solid. Flash chromatography using CH₂Cl₂ /methanol (99/1 to 98/2) afforded 151 mg (33% yield) of anti diastereomer of the title compound (120a) and 129 mg (29% yield) of syn diastereomer (120b) as a white solid. ¹H-NMR (500 MHz, CDCl₃) for the anti
20 diastereomer: δ 1.37-1.41 (m, 3H), 1.50-2.45 (m, 4H), 2.60-2.70 (m, 0.3H), 2.89-2.94 (m, 0.7H), 3.40-3.80 (m, 2H), 4.10-4.50 (m, 3H), 4.50-4.90 (m, 3H), 5.26 (s, 0.3H), 5.38 (s, 0.7H), 6.45-6.60 (m, 2.3H), 6.65-6.80 (m, H), 7.10-7.20 (m, 2.5H), 7.25-7.50 (m, 4.5H), 7.53-
25 7.70 (m, 0.7H), 7.82 (d, H). For the syn diastereomer: δ 0.86-0.89 (m, H), 1.20-1.40 (m, 4H), 1.80-2.45 (m, 4H), 2.80-2.86 (m, H), 3.58-3.65 (m, 2H), 4.20-4.40 (m, H), 4.50-4.75 (m, 2H), 4.90 (d, H), 5.52 (d, H), 6.45-6.70 (m, 3H), 6.75-6.85 (m, H), 7.10-7.20 (m, 2.3H),
30 7.30-7.50 (m, 5.7H); Analytical HPLC: 10.55 min for anti diastereomer and 10.62 min for syn diastereomer; LC/MS: m/e = 452 (M+H⁺) for both diastereomers.

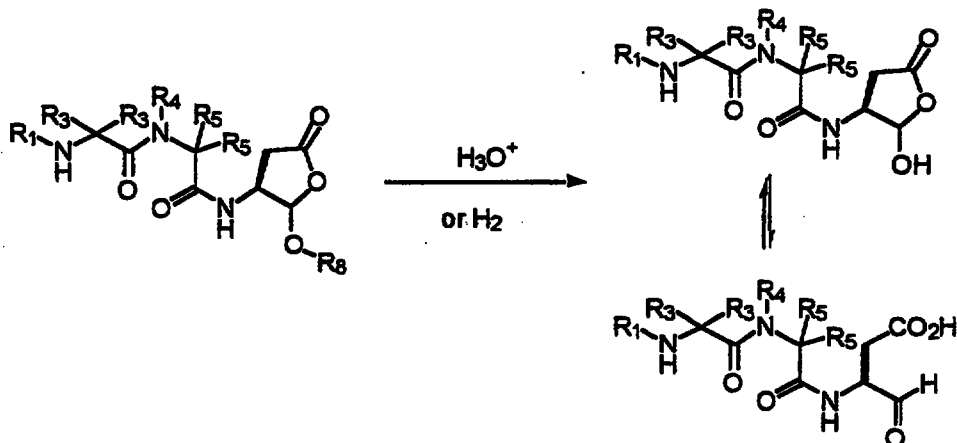
- 222 -

4-Oxo-3-{[1-(2-phenylamino-propionyl)-pyrrolidine-2-carbonyl]-amino}-butyric acid (121).

Prepared from 120 (151 mg, 0.33 mmol) using hydrolysis method A to afford 101 mg (83% yield) of the title compound as a white solid. $^1\text{H-NMR}$ (500 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ = 1/1): δ 1.20-1.65 (m, 2H), 1.65-2.35 (m, 3H), 2.40-3.00 (m, H), 3.20-3.80 (m, 2H), 3.90-4.90 (m, 7H), 7.25-7.80 (m, 5H); Analytical HPLC: 6.38 min.; LC-MS: $m/e = 362$ ($\text{M}+\text{H}^+$).

GENERAL PROCEDURES FOR THE PREPARATION OF COMPOUNDS OF EMBODIMENT C FORMULA I (SCHEMES XXIII-XXV)

Scheme XXIII



Hydrolysis Method A:

A 0.005-50 mmole sample of the alkylhemiacetal was dissolved in 2.5 N $\text{HCl}/\text{CH}_3\text{CN}$ (10/ 1) and stirred at room temperature until the reaction was complete. The resulting aqueous layer was washed with diethyl ether (2 x 20 mL) and lyophilized to afford the product.

Hydrolysis Method B:

- 223 -

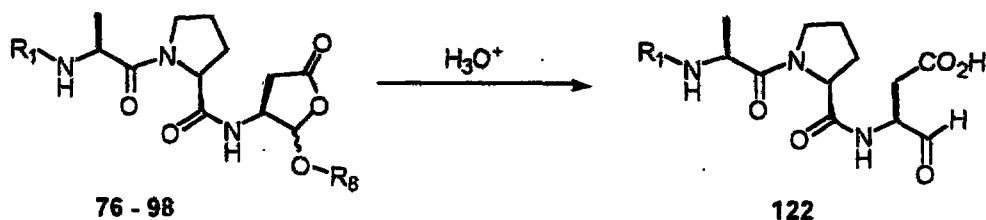
A 0.005-50 mmole sample of alkylhemiacetal was taken into neat formic acid and stirred overnight at room temperature. The mixture was triturated with a 3:1 mixture of hexane/diethyl ether to give a precipitate. The solvent was decanted and the precipitate washed with diethyl ether to afford the product.

Hydrolysis Method C:

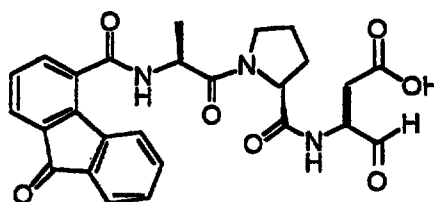
A 0.005-50 mmole sample of the alkylhemiacetal was dissolved in CH_3OH and 20% $\text{Pd}(\text{OH})_2/\text{C}$ and stirred under H_2 until the reaction was complete. The resulting suspension was filtered and the solution concentrated in vacuo, then triturated with a 3:1 mixture of hexane/diethyl ether to give a precipitate. The solvent was decanted and the precipitate washed with diethyl ether to afford the product.

Hydrolysis Method D:

A 0.005-50 mmole sample of the alkylhemiacetal in $\text{CH}_3\text{CN}/\text{water}$ (1/2) was shaken with acidic resin (Dowex 50w x 2, H^+ type) until the reaction was complete. The solution was filtered and the resin washed with $\text{CH}_3\text{CN}/\text{water}$ (1/4). The resulting water layer was washed with diethyl ether, concentrated to a smaller volume in vacuo then lyophilized to afford the product.

Scheme XXIV

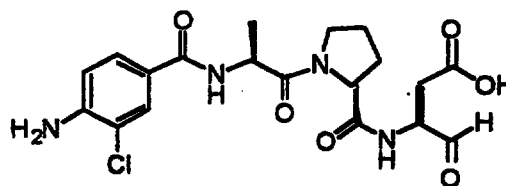
- 224 -



122a

4-Oxo-3-[(1-{2-[9-oxo-9H-fluorene-4-carbonyl]-amino}-propionyl)-pyrrolidine-2-carbonyl]-amino]-butyric acid (122a).

A 109.0mg (0.19mmol) sample of 91 was hydrolyzed according to method A to afford 88mg (96% yield) of the title compound: Analytical HPLC 7.15min. LC-MS (ES⁺) m/e=492.2 (M+H).

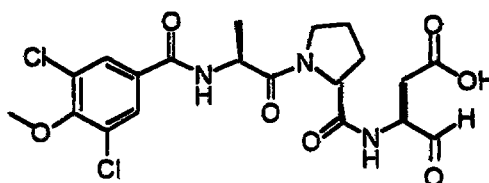


122b

3-({1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butylric acid (122b).

A 51.0mg (0.096mmole) sample of 76 was hydrolyzed according to method A to afford 43.0mg (100% yield) of the title compound: ¹H-NMR (500 MHz, CD₃OD/D₂O: 0.5 mL/10 drops): δ 1.37-1.52 (m, 3H), 1.80-2.20 (m, 3H), 2.20-2.37 (m, H), 2.49-2.60 (m, H), 2.60-2.75 (m, H), 3.70-3.80 (m, H), 3.80-3.95 (m, H), 4.20-4.35 (m, H), 4.40-4.50 (m, H), 4.50-4.70 (m, H), 4.70-4.85 (m, H), 6.85-6.87 (d, H), 7.58-7.60 (m, H), 7.77 (s, H); retention time on analytical HPLC: 6.54 min; LC-MS: m/z = 439 (M+H⁺).

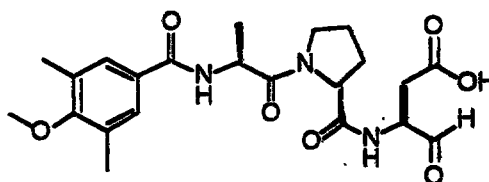
- 225 -



122c

3-({1-[2-(3,5-Dichloro-4-methoxy-benzoylamino)-
propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric
acid (122c).

A 51.0mg (0.088mmole) sample of 92 was hydrolyzed
according to method A to afford 24.0mg (56% yield) of
the title compound: Analytical HPLC 6.41min. LC-MS
(ES⁺) m/e=488.3 (M+H).

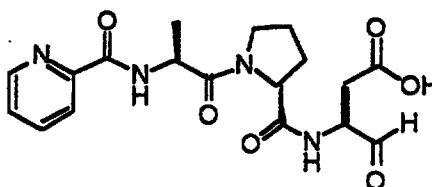


122d

3-({1-[2-(4-Methoxy-3,5-dimethyl-benzoylamino)-
propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric
acid (122d).

A 55.0mg (0.102mmole) sample of 77 was hydrolyzed
according to method A to afford 44.0mg (96% yield) of
the title compound: Analytical HPLC (C18) 8.70min, ¹H-
NMR (CDCl₃, 500Mhz): δ 1.23-1.70 (m, 3H), 1.80-2.70 (m,
10H), 2.70-3.15 (m, 2H), 3.58-4.20 (m, 5H), 4.32-5.50
(m, 3H), 5.60-6.00 (m, H), 6.80-7.90 (m, 4H); LC-MS
(ES⁺) m/e=448.2 (M+H).

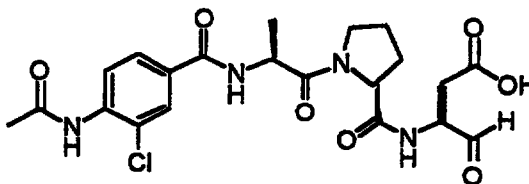
- 226 -



122e

4-Oxo-3-[(1-{2-[pyridine-2-carbonyl]-amino}-propionyl)-pyrrolidine-2-carbonyl]-amino-butyric acid (122e).

5 A 55.0mg (0.114mmole) sample of 88 was hydrolyzed according to method A to afford 30.0mg (67% yield) of the title compound: Analytical HPLC 4.60min. LC-MS (ES⁺) m/e=391.3 (M+H).



122f

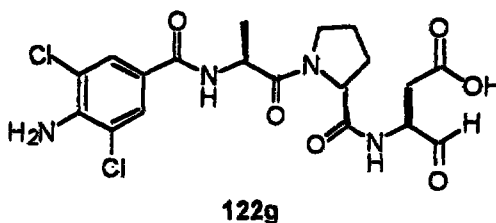
10

3-({1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (122f).

15 A 52mg (0.091mmole) sample of 78 was hydrolyzed according to method A to afford 40mg (91% yield) of the title compound: ¹H NMR (500MHz, CD₃OD) δ 1.08-1.61 (m, 3H), 1.77-2.41 (m, 3H), 2.21 (s, 3H), 2.41-2.77 (m, 2H), 3.43-3.63 (m, 0.3H), 3.65-3.76 (m, 1H), 3.81-3.94 (m, 1H), 4.18-4.34 (m, 1H), 4.42-4.64 (m, 1.7H), 4.77 (q, 1H), 7.79 (dd, 1H); Analytical HPLC 4.97min. LC-MS (ES⁺) m/e=481.3 (M+H).

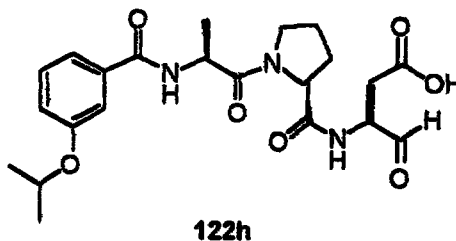
20

- 227 -



3-({1-[2-(4-Amino-3,5-dichloro-benzoylamino)-
propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyrlic
acid (122g).

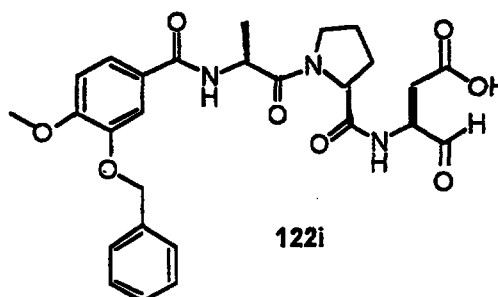
A 44.3mg (0.079mmole) sample of 89 was hydrolyzed
according to method A to afford 30mg (81% yield) of the
title compound: Analytical HPLC 5.40min. LC-MS (ES⁺)
m/e=473.2 (M+H).



3-({1-[2-(3-Isopropoxy-benzoylamino)-propionyl]-
pyrrolidine-2-carbonyl}-amino)-4-oxo-butyrlic acid
(122h).

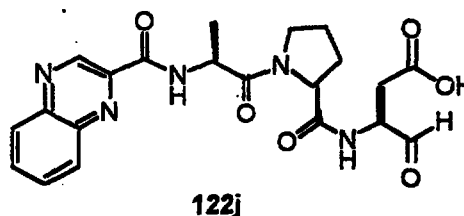
A 52.0mg (0.097mmole) sample of 79 was hydrolyzed
according to method A to afford 30.0mg (69% yield) of
the title compound: Analytical HPLC 8.92min. LC-MS
(ES⁺) m/e=448.3 (M+H).

- 228 -



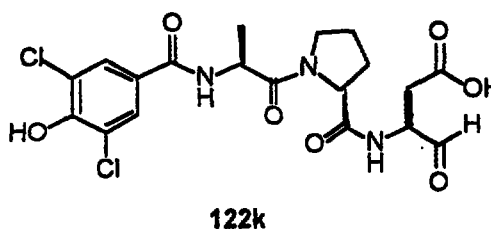
3-({1-[2-(3-benzyloxy-4-methoxy-benzoylamino)-propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyrlic acid (122i).

A 50.8mg (0.082mmole) sample of 81 was hydrolyzed according to method A to afford 22.4mg (52% yield) of the title compound: Analytical HPLC 6.72min. LC-MS (ES⁺) m/e=526.3 (M+H).



4-Oxo-3-[(1-{2-[(quinoxaline-2-carbonyl)-amino]-propionyl}-pyrrolidine-2-carbonyl)-amino]-butyric acid (122j).

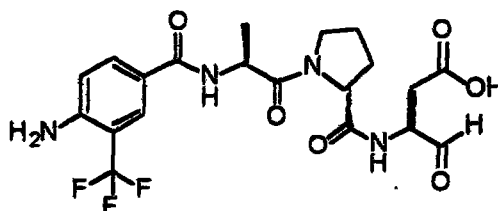
A 38.0mg (0.072mmole) sample of 80 was hydrolyzed according to method A to afford 32.0mg (100% yield) of the title compound: Analytical HPLC 5.95min. LC-MS (ES⁺) m/e=442.3 (M+H).



- 229 -

3-({1-[2-(3,5-Dichloro-4-hydroxy-benzoylamino)-propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (122k).

5 A 35mg (0.060mmole) sample of 83 was hydrolyzed according to method A to afford 29.4mg (75% yield) of the title compound: Analytical HPLC 7.91min. ¹H-NMR (500 MHz, CD₃OD) δ 1.47 (m, 3H), 1.8-2.3 (m, 4H), 2.49 (m, 1H), 2.61 (m, 1H), 3.5 (br m, 0.2H), 3.69 (br m, 0.9H), 3.84 (br m, 0.9H), 4.27 (m, 1H), 4.46 (m, 1H), 4.57 (m, 1H), 4.73 (m, 1H), 7.83 (m, 2H) ppm, LC-MS (ES⁺) m/e=474.1 (M+H).

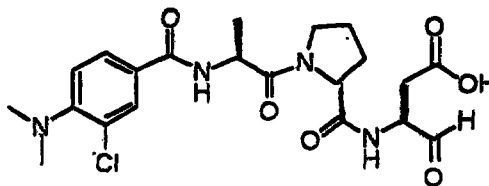


122i

15

3-({1-[2-(4-Amino-3-trifluoromethyl-benzoylamino)-propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (122l).

20 A 10mg (0.021mmole) sample of 98w was hydrolyzed according to method A to afford 7.9mg (94% yield) of the title compound: Analytical HPLC 6.64min. LC-MS (ES⁺) m/e=473.3 (M+H).



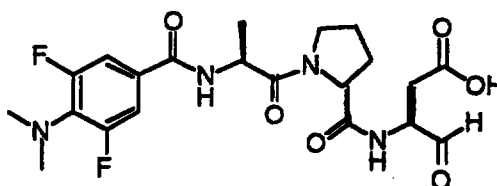
122m

25

- 230 -

3 - ({1 - [2 - (3-Chloro-4-dimethylamino-benzoylamino) - propionyl] - pyrrolidine-2-carbonyl} - amino) - 4-oxo-butyric acid (122m).

5 A 10.0mg (0.021mmole) sample of 98x was hydrolyzed according to method A to afford 7.0mg (84% yield) of the title compound: Analytical HPLC 5.15min. LC-MS (ES⁺) m/e=467.3 (M+H).

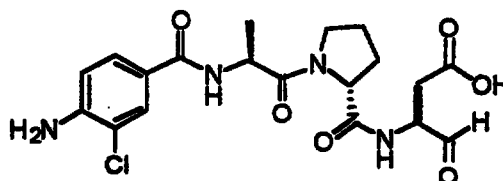


122n

10

3 - ({1 - [2 - (4-Dimethylamino-3,5-difluoro-benzoylamino) - propionyl] - pyrrolidine-2-carbonyl} - amino) - 4-oxo-butyric acid (122n).

15 A 20.0mg (0.043mmole) sample of 98y was hydrolyzed according to method A to afford 16.8mg (100% yield) of the title compound: Analytical HPLC 5.86min. LC-MS (ES⁺) m/e=469.3 (M+H).



122o

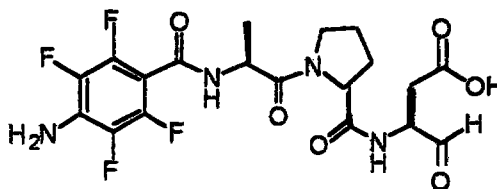
20

3 - ({1 - [2 - (4-Amino-3-chloro-benzoylamino) - propionyl] - pyrrolidine-2-carbonyl} - amino) - 4-oxo-butyric acid (122o).

25 A 20.0mg (0.046mmole) sample of 98m was hydrolyzed according to method A to afford 16.7mg (100% yield) of

- 231 -

the title compound: Analytical HPLC 8.47min. LC-MS
(ES⁺) m/e=439.2 (M+H).

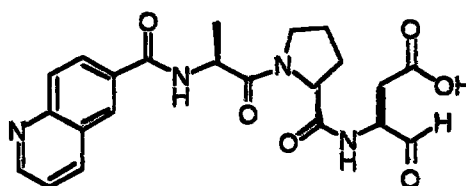


122p

5

3-({1-[2-(4-Amino-2,3,5,6-tetrafluoro-benzoylamino)-
propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric
acid (122p).

A 20.0mg (0.042mmole) sample of 98z was hydrolyzed
according to method A to afford 15.3mg (91% yield) of
the title compound: Analytical HPLC 7.90min. LC-MS
(ES⁺) m/e=477.2 (M+H).



122q

15

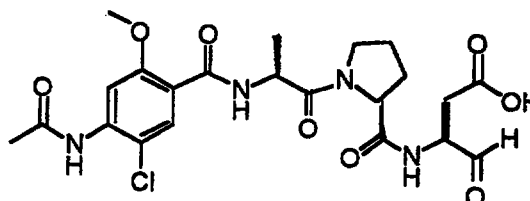
4-Oxo-3-[(1-{2-[(quinoline-6-carbonyl)-amino]-
propionyl}-pyrrolidine-2-carbonyl)-amino]-butyric acid
(122q).

A 44mg (0.080mmole) sample of 93 was hydrolyzed
according to method A to afford 41mg (100% yield) of
the title compound: ¹H NMR (500MHz, CD₃OD) δ 1.24-1.69
(m, 3H), 1.75-2.37 (m, 4H), 2.39-2.87 (m, 2H), 3.46-
4.04 (m, 2H), 4.11-4.77 (m, 3H), 8.19 (dd, 1H), 8.33
(d, 1H), 8.56-8.58 (m, 1H); 8.85 (s, 1H), 9.27-9.39 (m,

20

- 232 -

2H); Analytical HPLC 4.91min. LC-MS (ES⁺) m/e=441.2 (M+H).

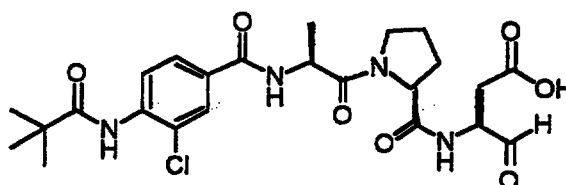


122r

5

3-({1-[2-(4-Acetylamino-5-chloro-2-methoxy-benzoylamino)-propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (122r).

10 A 44.5mg (0.074mmole) sample of 87 was hydrolyzed according to method A to afford 34.5mg (91% yield) of the title compound: Analytical HPLC 6.88min. LC-MS (ES⁺) m/e=511.2 (M+H).



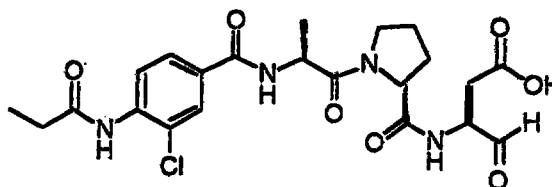
122s

15

3-[(1-{2-[3-Chloro-4-(2,2-dimethyl-propionylamino)-benzoylamino]-propionyl}-pyrrolidine-2-carbonyl)-amino]-4-oxo-butyric acid (122s).

20 A 19.0mg (0.036mmole) sample of 98aa was hydrolyzed according to method A to afford 14.5mg (90% yield) of the title compound: Analytical HPLC 7.28min. LC-MS (ES⁺) m/e=523.3 (M+H).

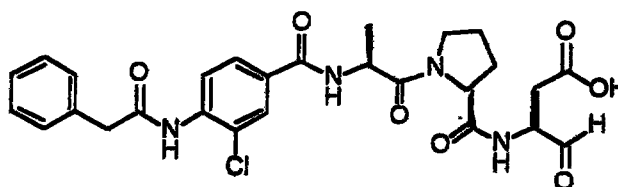
- 233 -



122t

3-({1-[2-(3-Chloro-4-propionylamino-benzoylamino)-
propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyrac
acid (122t).

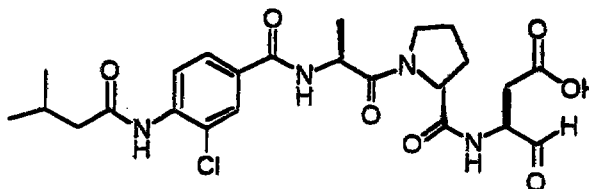
A 21.0mg (0.042mmole) sample of 98ab was hydrolyzed
according to method A to afford 17.5mg (97% yield) of
the title compound: Analytical HPLC 5.72min. LC-MS
(ES⁺) m/e=495.2 (M+H).



122u

3-({1-[2-(3-Chloro-4-phenylacetyl-amino-benzoylamino)-
propionyl]-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyrac
acid (122u).

A 10.0mg (0.017mmole) sample of 98ac was hydrolyzed
according to method A to afford 7.9mg (85% yield) of
the title compound: Analytical HPLC 7.52min. LC-MS
(ES⁺) m/e=557.2 (M+H).



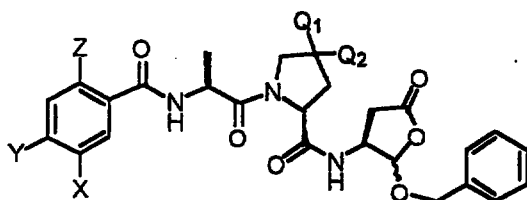
122v

- 234 -

3 - [(1-{2-[3-Chloro-4-(3-methyl-butyrylamino)-benzoylamino]-propionyl}-pyrrolidine-2-carbonyl)-amino]-4-oxo-butyric acid (122v).

- 5 An 8.0mg (0.015mmole) sample of 98ad was hydrolyzed according to method A to afford 6.5mg (96% yield) of the title compound: Analytical HPLC 6.92min. LC-MS (ES⁺) m/e=523.2 (M+H).

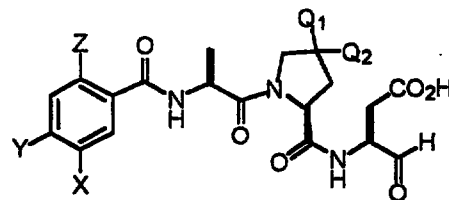
Scheme XXV



108a, X=Cl, Y=NH₂, Z=H, Q₁=F, Q₂=H

116b, X=Cl, Y=AcNH, Z=H, Q₁=Q₂=F

10 116c, X=Cl, Y=AcNH, Z=CH₃O, Q₁=Q₂=F



123a, X=Cl, Y=NH₂, Z=H, Q₁=F, Q₂=H

123b, X=Cl, Y=AcNH, Z=H, Q₁=Q₂=F

123c, X=Cl, Y=AcNH, Z=CH₃O, Q₁=Q₂=F

3 - ({1-[2-(4-Amino-3-chloro-benzoylamino)-propionyl]-4-fluoro-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (123a).

- 15 A 12.4mg (0.022mmole) sample of 108b was hydrolyzed according to method A to afford 9.6mg (93% yield) of the title compound: Analytical HPLC 6.99min. LC-MS (ES⁺) m/e=473.2 (M+H).

20 3 - ({1-[2-(4-Acetylamino-3-chloro-benzoylamino)-propionyl]-4,4-difluoro-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (123b).

- A 26.2mg (0.043mmole) sample of 116b was hydrolyzed according to method A to afford 10.8mg (49% yield) of the title compound: Analytical HPLC 9.89min. LC-MS (ES⁺) m/e=517.2
- 25

- 235 -

3-({1-[2-(4-Acetylamino-3-chloro-2-methoxy-benzoylamino)-propionyl]-4,4-difluoro-pyrrolidine-2-carbonyl}-amino)-4-oxo-butyric acid (123c).

- 5 A 23.1mg (0.036mmole) sample of 116c was hydrolyzed according to method A to afford 1.8mg (9% yield) of the title compound: Analytical HPLC 11.87min. LC-MS (ES⁺) m/e=547.1 (M+H).

10 BIOLOGICAL METHODS

We obtained *in vitro*, *ex vivo*, and *in vivo* data for selected compounds of this invention using the methods described below. The results are shown in the Tables 2-8. The designation "ND" indicates that the compound was not tested in the described assay.

15 In the ICE Caspase assays, category "A" indicates <10 nM inhibition. Category "B" indicates 10-1000 nM inhibition. Category "C" indicates >1000 nM inhibition. See Tables 2 and 3.

20 In the PBMC assay, category "A" indicates <500 nM inhibition. Category "B" indicates 500-1000 nM inhibition. Category "C" indicates 1001-2000 nM inhibition. Category "D" indicates >2000 nM inhibition. See Table Table 4.

25 In the whole blood assay, category "A" indicates <2500 nM inhibition. Category "B" indicates 2500-7500 nM inhibition. Category "C" indicates >7500 nM. See Table 5

30 In the *in situ* metabolism assay, values of [f(g) X f(h)] are disclosed as follows: category "A" indicates <0.25. Category "B" indicates 0.25-0.49. Category "C" indicates 0.5-0.75. Category "D" indicates >0.75. In the biliary excretion measurement,

- 236 -

category "A" indicates <5%. Category "B" indicates 5-10%. Category "C" indicates >10%. See Table 6.

In the i.v. clearance assay, values are reported as follows: category "A" indicates <50.
5 Category "B" indicates 50-80. Category "C" indicates >80. See Table 7.

In the bioavailability assay, the Cmax values ($\mu\text{g/ml}$) are disclosed as follows: category "A" indicates <2.5. Category "B" indicates 2.5-5.0.
10 Category "C" indicates >5.0. The AUC values ($\mu\text{g} \times \text{hr/ml}$) are disclosed as follows: category "A" indicates <2.5. Category "B" indicates 2.5-5.0. Category "C" indicates >5.0. Half-life (hrs) ranges are disclosed as follows: category "A" indicates <1.5.
15 Category "B" indicates 1.5-2.0. Category "C" indicates >2.0. The F values (%) are disclosed as follows: category "A" indicates <33. Category "B" indicates 33-67. Category "C" indicates >67. See Table 8.

In Vitro Assays

20 Enzyme Inhibition

Ki values for test compounds with the various caspases were obtained by the method of Margolin et al. (J. Biol. Chem., 272 pp. 7223-7228 (1997)). Assays
were performed in 10 mM Tris (Sigma Corp, St Louis MO)
25 pH 7.5, 1 mM Dithiothreitol (DTT, Research Organic INC, Cleveland, OH) and 0.1% CHAPS (Pierce, Rockford IL) at 37 °C. For caspase-3, a solution of 8% glycerol was added to the assay buffer to improve enzyme stability. A 65 μL aliquot of the assay buffer and 5 μL aliquot of
30 the appropriate dilutions of inhibitor in DMSO where pipetted into a 96 well plate, treated with 10 μL of caspase, then diluted in assay buffer (0.5-40 nM active protein by active site titration). A control

- 237 -

containing DMSO but no compound was included for each determination. The plates were then incubated for 15 minutes at 37 °C, before addition of the appropriate substrate (20 µL, final concentration 1-4 X K_M , final assay volume 100 µL) to initiate the reaction.

Reaction rates were measured at 37°C either by following the time dependant increase in absorbance at 405 nM (for the pNA substrates) or in fluorescence (Ex 390, Em 460) (for the AMC substrates). The rates obtained were plotted against inhibitor concentration and the data fit to the Morrison tight-binding equation for competitive inhibitors (Morrison, J.F., Biochem. Biophys. Acta, 185 pp. 269-286 (1969)). The substrates used for the individual assays were as follows:

Caspase-1 Suc-YVAD-pNA (Bachem, King of Prussia, PA) (final concentration in the assay 80 µM),

Caspase-3 Ac-DEVD-pNA (Bachem, King of Prussia, PA) (final concentration in assay, 60 µM)

Caspase-4 Ac-WEHD-AMC (Synpep, Dublin, CA) (final concentration in Assay 20 µM),

Caspase-7 Ac-DEVD-AMC (Bachem, King of Prussia, PA) (final concentration in assay 50 µM),

Caspase-8 Ac-DEVD-pNA (Bachem, King of Prussia, PA) (final concentration in assay 80 µM).

Table 2. Caspase-1 Inhibition Data

Example	Caspase-1 Ki (nM)
5a	A
5b	A
5c	A
5d	A
5e	B
5f	B

- 238 -

Example	Caspase-1 Ki (nM)
5g	B
5h	A
5i	A
5j	A
5k	A
5l	B
5m	A
5n	A
5o	B
5p	B
5q	B
5r	B
5s	B
5t	C
5u	B
5v	B
5w	B
5x	A
5y	A
5z	A
5aa	A
5ab	B
5ac	A
5ad	A
5ae	B
5af	B
5ag	A
5ah	B
5ai	A
5aj	B
5ak	B

- 239 -

Example	Caspase-1 Ki (nM)
5a1	A
5am	A
5an	B
5ao	B
5ap	B
5aq	B
5ar	A
5as	A
5at	B
5au	B
5av	B
5aw	A
5ax	A
5ay	A
5az	A
5ba	A
5bb	A
5bc	B
5bd	A
7a	A
7b	B
7c	A
7d	A
7e	B
7f	B
7g	A
7h	B
7i	B
7j	C
7k	B
7l	B

- 240 -

Example	Caspase-1 Ki (nM)
7m	B
7n	B
7o	A
7p	A
7q	B
7r	B
7s	B
7t	B
7u	B
7v	B
7w	A
7x	B
7y	B
7z	B
7aa	A
7ab	B
7ac	B
7ad	B
7ae	B
7af	B
7ag	B
7ah	A
7ai	A
7aj	A
7ak	A
7al	B
7am	A
7an	A
7ao	B
7ap	B
7aq	B

- 241 -

Example	Caspase-1 Ki (nM)
7ar	A
7as	A
7at	A
9a	B
9b	A
9c	A
9d	A
9e	A
9f	A
9g	A
15a	A
15b	A
15c	A
15d	B
15e	B
15f	B
16a	B
16b	A
17a	B
17b	B
17c	A
17d	B
17e	B
18a	B
18b	A
18c	B
18d	B
18e	A
18f	B
20a	A
20b	A

- 242 -

Example	Caspase-1 Ki (nM)
20c	A
20d	B
20e	A
20f	A
20g	A
20h	A
20i	B
20j	B
20k	A
20l	A
20m	A
20n	A
20o	A
20p	A
20q	B
20r	B
20s	A
20t	B
23a	A
23b	B
23c	A
23d	A
23e	A
23f	B
23g	A
23h	A
23i	B
24a	A
24b	C
24c	A
24d	B

- 243 -

Example	Caspase-1 Ki (nM)
24e	B
25a	A
25b	A
25c	A
25d	A
25e	A
26a	A
26b	A
26c	A
26d	A
26e	A
26f	A
26g	A
26h	A
27a	B
27b	B
27c	B
27d	A
27e	B
27f	B
27g	A
27h	B
27i	B
27j	B
27k	B
27l	B
27m	B
27n	B
28a	A
28b	A
28c	A

- 244 -

Example	Caspase-1 Ki (nM)
29a	A
29b	A
29c	A
29d	A
29e	A
29f	A
29g	A
29h	A
29i	A
29j	A
29k	A
29l	B
29m	A
29n	B
29o	B
29p	A
29q	A
29r	A
29s	B
32a	C
32b	C
32c	C
32d	C
32e	B
34	C
G1	C
G2	B
42	B
46b	A
46a	C
57	A

- 245 -

Example	Caspase-1 Ki (nM)
65	A
61	A
69	A
73	A
121	C
122a	A
122b	A
122c	A
122d	A
122e	B
122f	A
122g	A
122h	B
122i	A
122j	B
122k	A
122l	B
122m	B
122n	B
122o	C
122p	A
122q	B
122r	B
122s	B
122t	B
122u	A
122v	B
123a	B
123b	B
123c	B

- 246 -

**Table 3. Caspase-3, Caspase-4, and Caspase-8
Inhibition Data**

Example	Caspase-3 Ki (nM)	Caspase-4 Ki (nM)	Caspase-8 Ki (nM)
7c	C	ND	C
7d	C	ND	B
7f	C	ND	C
24a	C	ND	ND
29a	C	ND	ND
29b	C	ND	ND
32d	B	ND	ND
46b	B	ND	ND
69	C	ND	B
122b	C	A	B
122d	C	A	C
122f	C	ND	B
122k	C	ND	B

5 PBMC Cell Assay

**IL-1 β Assay with a Mixed Population of Human Peripheral
Blood Mononuclear Cells (PBMC) or Enriched Adherent
Mononuclear Cells**

Processing of pre-IL-1 β by ICE can be
 10 measured in cell culture using a variety of cell
 sources. Human PBMC obtained from healthy donors
 provides a mixed population of lymphocyte subtypes and
 mononuclear cells that produce a spectrum of
 interleukins and cytokines in response to many classes
 15 of physiological stimulators. Adherent mononuclear
 cells from PBMC provides an enriched source of normal
 monocytes for selective studies of cytokine production
 by activated cells.

- 247 -

Experimental Procedure:

5 An initial dilution series of test compound
in DMSO or ethanol is prepared, with a subsequent
dilution into RPMI-10% FBS media (containing 2 mM L-
glutamine, 10 mM HEPES, 50 U and 50 ug/ml pen/strep)
respectively to yield drugs at 4x the final test
concentration containing 0.4% DMSO or 0.4% ethanol.
The final concentration of DMSO is 0.1% for all drug
10 dilutions. A concentration titration which brackets
the apparent K_i for a test compound determined in an
ICE inhibition assay is generally used for the primary
compound screen.

15 Generally 5-6 compound dilutions are tested
and the cellular component of the assay is performed in
duplicate, with duplicate ELISA determinations on each
cell culture supernatant.

PBMC Isolation and IL-1 Assay:

20 Buffy coat cells isolated from one pint human
blood (yielding 40-45 ml final volume plasma plus
cells) are diluted with media to 80 ml and LeukoPREP
separation tubes (Becton Dickinson) are each overlaid
with 10 ml of cell suspension. After 15 min
25 centrifugation at 1500-1800 xg, the plasma/media layer
is aspirated and then the mononuclear cell layer is
collected with a Pasteur pipette and transferred to a
15 ml conical centrifuge tube (Corning). Media is
added to bring the volume to 15 ml, gently mix the
30 cells by inversion and centrifuge at 300 xg for 15 min.
The PBMC pellet is resuspended in a small volume of
media, the cells are counted and adjusted to 6×10^6
cells/ml.

- 248 -

For the cellular assay, 1.0 ml of the cell suspension is added to each well of a 24-well flat bottom tissue culture plate (Corning), 0.5 ml test compound dilution and 0.5 ml LPS solution (Sigma #L-3012; 20 ng/ml solution prepared in complete RPMI media; final LPS concentration 5 ng/ml). The 0.5 ml additions of test compound and LPS are usually sufficient to mix the contents of the wells. Three control mixtures are run per experiment, with either LPS alone, solvent vehicle control, and/or additional media to adjust the final culture volume to 2.0 ml. The cell cultures are incubated for 16-18 hr at 37 °C in the presence of 5% CO₂.

At the end of the incubation period, cells are harvested and transferred to 15 ml conical centrifuge tubes. After centrifugation for 10 min at 200 xg, supernatants are harvested and transferred to 1.5 ml Eppendorf tubes. It may be noted that the cell pellet may be utilized for a biochemical evaluation of pre-IL-1 β and/or mature IL-1 β content in cytosol extracts by Western blotting or ELISA with pre-IL-1 β specific antisera.

Isolation of Adherent Mononuclear cells:

PBMC are isolated and prepared as described above. Media (1.0 ml) is first added to wells followed by 0.5 ml of the PBMC suspension. After a one hour incubation, plates are gently shaken and nonadherent cells aspirated from each well. Wells are then gently washed three times with 1.0 ml of media and final resuspended in 1.0 ml media. The enrichment for adherent cells generally yields 2.5-3.0 x 10⁵ cells per well. The addition of test compounds, LPS, cell

- 249 -

incubation conditions and processing of supernatants proceeds as described above.

ELISA:

5 Quantikine kits (R&D Systems) may be used for the measurement of mature IL-1 β . Assays are performed according to the manufacturer's directions. Mature IL-1 β levels of about 1-3 ng/ml in both PBMC and adherent mononuclear cell positive controls are observed. ELISA
10 assays are performed on 1:5, 1:10 and 1:20 dilutions of supernatants from LPS-positive controls to select the optimal dilution for supernatants in the test panel.

 The inhibitory potency of the compounds can be represented by an IC₅₀ value, which is the
15 concentration of inhibitor at which 50% of mature IL-1 β is detected in the supernatant as compared to the positive controls.

 The skilled practitioner realizes that values obtained in cell assays may depend on multiple factors.
20 The values may not necessarily represent fine quantitative results.

Table 4. PBMC Cell Assay Data

Example	PBMC IC ₅₀ (nM)
5a	D
5b	B
5c	B
5d	B
5e	B
5f	C
5g	C
5h	A

- 250 -

Example	PBMC IC50 (nM)
5i	C
5j	D
5k	D
5l	A
5m	A
5n	B
5r	D
5s	B
5u	C
5v	D
5w	B
5x	B
5y	B
5z	B
5aa	B
5ab	B
5ac	A
5ad	A
5ag	B
5ai	A
5aj	B
5ak	B
5al	D
5am	D
5ao	D
5aq	D
5ar	D
5as	D
5at	D
5au	D
5av	D

- 251 -

Example	PBMC IC50 (nM)
5aw	C
5ax	B
5ay	B
5az	B
5ba	C
5bb	B
5bd	C
7a	D
7b	B
7c	A
7d	A
7e	D
7f	D
7g	A
7h	B
7k	B
7l	B
7m	B
7n	B
7o	A
7p	D
7q	D
7s	B
7t	D
7u	D
7v	D
7w	C
7x	D
7y	D
7z	C
9a	B

- 252 -

Example	PBMC IC50 (nM)
9b	B
9c	A
9d	B
9e	C
9f	B
9g	C
15a	D
15b	C
15c	B
15d	C
15e	D
15f	D
16a	A
16b	C
17b	C
17c	D
17d	D
17e	B
18a	B
18b	B
18c	B
18d	B
18e	C
18f	D
20a	B
20b	B
20c	C
20d	D
20e	C
20f	B
20g	A

- 253 -

Example	PBMC IC ₅₀ (nM)
20h	A
20i	B
20j	D
20k	A
20l	B
20m	B
20n	B
20o	A
20p	A
20q	B
20r	B
20s	C
20t	B
23a	C
23b	B
23c	C
23d	B
23e	B
23f	C
23g	C
23h	C
23i	A
24a	B
24b	D
24c	A
24d	B
24e	C
25a	A
25b	B
25c	B
26a	C

- 254 -

Example	PBMC IC50 (nM)
26b	B
26c	B
26d	B
26e	A
26f	A
26g	A
26h	A
27a	A
28a	B
28b	B
28c	B
29a	A
29b	C
29c	B
29d	B
29e	A
29g	B
29h	A
29i	A
29j	B
29k	A
29l	B
29m	B
42	D
46b	D
57	B
65	B
61	B
69	A
73	B
122a	D

- 255 -

Example	PBMC IC ₅₀ (nM)
122b	B
122c	D
122d	B
122e	C
122f	B
122g	B
122h	C
122i	C
122j	D
122k	A
122l	B

Whole Blood Assay for IL-1 β Production

Whole blood assay IC₅₀ values for compounds
5 of this invention were obtained using the method
described below:

Purpose:

The whole blood assay is a simple method for
10 measuring the production of IL-1 β (or other cytokines)
and the activity of potential inhibitors. The
complexity of this assay system, with its full
complement of lymphoid and inflammatory cell types,
spectrum of plasma proteins and red blood cells is an
15 ideal *in vitro* representation of human *in vivo*
physiologic conditions.

Materials:

Pyrogen-free syringes (~ 30 cc)
20 Pyrogen-free sterile vacuum tubes containing
lyophilized Na₂EDTA (4.5 mg/10 ml tube)

- 256 -

- Human whole blood sample (~ 30-50 cc)
1.5 ml Eppendorf tubes
Test compound stock solutions (~ 25mM in DMSO or other solvent)
- 5 Endotoxin -free sodium chloride solution (0.9%) and HBSS
Lipopolysaccharide (Sigma; Cat.# L-3012) stock solution at 1mg/ml in HBSS
IL-1 β ELISA Kit (R & D Systems; Cat # DLB50)
- 10 TNF α ELISA Kit (R & D Systems; Cat # DTA50)
Water bath or incubator

Whole Blood Assay Experimental Procedure:

Set incubator or water bath at 30 °C.

- 15 Aliquot 0.25ml of blood into 1.5 ml eppendorf tubes.
Note: be sure to invert the whole blood sample tubes after every two aliquots. Differences in replicates may result if the cells sediment and are not uniformly suspended. Use of a positive displacement pipette will
- 20 also minimize differences between replicate aliquots.

- Prepare drug dilutions in sterile pyrogen-free saline by serial dilution. A dilution series which brackets the apparent K_i for a test compound determined in an ICE inhibition assay is generally used
- 25 for the primary compound screen. For extremely hydrophobic compounds, prepare compound dilutions in fresh plasma obtained from the same blood donor or in PBS-containing 5% DMSO to enhance solubility.

- Add 25 μ l test compound dilution or vehicle
- 30 control and gently mix the sample. Then add 5.0 μ l LPS solution (250 ng/ml stocked prepared fresh: 5.0 ng/ml final concentration LPS), and mix again. Incubate the tubes at 30 °C in a water bath for 16-18 hr with occasional mixing. Alternatively, the tubes can be

- 257 -

placed in a rotator set at 4 rpm for the same incubation period. This assay should be set up in duplicate or triplicate with the following controls: negative control - no LPS; positive control - no test inhibitor; vehicle control - the highest concentration of DMSO or compound solvent used in the experiment. Additional saline is added to all control tubes to normalize volumes for both control and experimental whole blood test samples.

After the incubation period, whole blood samples are centrifuged for 10 minutes at ~ 2000 rpm in the microfuge, plasma is transferred to a fresh microfuge tube and centrifuged at 1000 x g to pellet residual platelets if necessary. Plasma samples may be stored frozen at -70 °C prior to assay for cytokine levels by ELISA.

ELISA:

R & D Systems (614 McKinley Place N.E. Minneapolis, MN 55413) Quantikine kits may be used for measurement of IL-1 β and TNF- α . The assays are performed according to the manufacturer's directions. IL-1 β levels of ~ 1-5 ng/ml in positive controls among a range of individuals may be observed. A 1:200 dilution of plasma for all samples is usually sufficient for experiments for ELISA results to fall on the linear range of the ELISA standard curves. It may be necessary to optimize standard dilutions if you observe differences in the whole blood assay. Nerad, J.L. et al., J. Leukocyte Biol., 52, pp. 687-692 (1992).

Table 5. Whole Blood Assay Data

Example	Whole Blood IC50 (nM)
5a	A
5b	A
5c	A
5d	B
5e	A
5f	B
5h	A
5i	C
5j	C
5k	B
5l	C
5m	A
5n	B
5r	C
5s	C
5u	A
5w	A
5x	A
5y	B
5z	C
5aa	A
5ab	B
5ac	A
5ad	A
5ag	B
5ai	C
5aj	C
5ak	C
5ax	B
5ay	B

- 259 -

Example	Whole Blood IC50 (nM)
5az	A
5bb	B
7a	B
7b	A
7c	A
7d	B
7e	C
7f	B
7g	B
7h	A
7k	A
7l	A
7m	B
7n	A
7o	A
7p	B
7q	A
7s	B
7t	B
7u	B
7v	A
7w	A
7x	C
7y	C
7z	A
7aa	B
7ab	A
7ac	B
7ad	B
7ah	B
7ai	B

- 260 -

Example	Whole Blood IC50 (nM)
7aj	C
7ak	B
7am	B
7an	B
7ao	B
7ap	C
9a	B
9b	B
9c	A
9d	A
9e	B
9f	A
9g	B
15a	B
15b	B
15c	A
16b	A
18a	A
18b	A
18c	A
18d	A
18e	A
18f	B
20a	A
20b	C
20c	B
20d	B
20e	B
20f	A
20g	A
20h	A

- 261 -

Example	Whole Blood IC50 (nM)
20i	A
20k	A
20l	A
20m	A
20n	A
20o	A
20p	A
20q	C
20r	B
20s	A
20t	A
23a	B
23b	B
23c	B
23d	A
23e	B
23f	A
23g	A
23h	A
23i	B
24a	B
24c	B
24d	B
24e	B
25a	B
25b	B
25c	B
25d	A
25e	C
26c	B
26d	A

- 262 -

Example	Whole Blood IC50 (nM)
26e	A
26f	B
26g	B
26h	A
27a	A
27b	A
27d	A
27e	A
27f	B
27g	B
27h	B
27i	B
27l	B
27m	B
27n	A
28a	B
28b	B
28c	C
29a	A
29c	A
29d	A
29e	A
29g	A
29h	A
29i	A
29j	A
29k	A
29l	A
29n	B
29o	A
29p	A

- 263 -

Example	Whole Blood IC50 (nM)
29q	A
29r	A
29s	A
G2	B
42	B
46b	B
57	C
65	A
61	A
69	A
73	A
122a	A
122b	A
122c	B
122d	A
122e	B
122f	A
122g	A
122h	A
122i	A
122j	B
122k	A
122l	A
122m	B
122p	B
122q	B
122r	B
122s	B
123a	A
123b	B

- 264 -

Ex Vivo AssaysMetabolism and Excretion

Single pass perfusion studies in rat were performed to assess gastrointestinal (GI) wall metabolism (f(g)), liver metabolism (f(h)), and biliary excretion. The method used has been described in Pang, C.S., J. Pharmacol. Exp. Therapeutics, 333, pp. 788-798 (1984).

10 Table 6. Metabolism and Excretion Data

Example	f(g)Xf(h)	Biliary Excretion
5c	A	C
5k	B	A
5m	B	C
7d	D	A
7f	C	A
7ac	C	C
18f	D	A
20f	B	C
20g	B	A
23b	B	B
24a	C	A
24c	A	C
24e	A	C
25d	C	C
25e	C	B
26c	A	C
26e	B	B
26f	A	C
27a	C	A
27b	B	A
29b	A	B

- 265 -

Example	f(g)Xf(h)	Biliary Excretion
29g	B	B
29n	C	A
29o	C	A
29p	B	C
29q	C	A
29r	C	B
46b	B	C
57	B	B
65	B	C
69	C	A
122a	B	A
122b	C	A
122c	C	B
122d	C	A
122r	B	A

In Vivo Assays

In vivo Rat Clearance Assay - Clearance Rates

5 The rate of clearance in the rat (ml/min/kg)
for compounds of this invention may be obtained using
the method described below:

Representative Procedure

10 Cannulations of the jugular and carotid
vessels of rats under anesthesia are performed one day
prior to the pharmacokinetic study. M.J. Free, R.A.
Jaffee; 'Cannulation techniques for the collection
blood and other bodily fluids'; in: Animal Models;
p. 480-495; N.J. Alexander, Ed.; Academic Press;
(1978). Drug (10mg/mL) is administered via the jugular
15 vein in a vehicle usually consisting of: propylene
glycol/saline, containing 100mM sodium bicarbonate in a
1:1 ratio. Animals are dosed with 10-20 mg drug/kg and

- 266 -

blood samples are drawn at 0, 2, 5, 7, 10, 15, 20, 30, 60, and 90 minutes from an indwelling carotid catheter. The blood is centrifuged to plasma and stored at -20 °C until analysis. Pharmacokinetic analysis of data is performed by non-linear regression using standard software such as RStrip (MicroMath Software, UT) and/or Pcnnonlin (SCI Software, NC) to obtain clearance values.

Representative Analytical:

Rat plasma is extracted with an equal volume of acetonitrile (containing 0.1% TFA). Samples are then centrifuged at approximately 1,000 x g and the supernatant analyzed by gradient HPLC. A typical assay procedure is described below.

200 µL of plasma is precipitated with 200 µL of 0.1% trifluoroacetic acid (TFA) in acetonitrile and 10 µL of a 50% aqueous zinc chloride solution, vortexed then centrifuged at ~1000 x g and the supernatant collected and analyzed by HPLC.

20

HPLC procedure:

Column:	Zorbax SB-CN (4.6 x 150 mm) (5µ particle size)
Column temperature:	50 °C
Flow rate:	1.0 mL/min
Injection volume:	75 µL.
Mobile phase:	A=0.1% TFA in water and B=100% acetonitrile
Gradient employed:	100% A to 30% A in 15.5 min 0% A at 16 min 100% A at 19.2 min
Wavelength:	214 nm

30

- 267 -

A standard curve is run at 20, 10, 5, 2 and 1 $\mu\text{g/mL}$ concentrations.

Table 7. Clearance Data

Example	Rat I.V. Clearance (ml/min/kg)
7d	A
7f	B
20h	B
20m	A
65	C
122b	B
122c	C
122d	B
122f	A

5

Bioavailability

Oral pharmacokinetic studies

Male Sprague-Dawley rats (Harlan, Indianapolis, IN, 300-350 g) were anesthetized by an intramuscular injection of ketamine/rompun mixture. A PE-50 cannula was inserted in the right carotid artery for arterial blood sampling. The rats were allowed to recover from surgery overnight (≥ 16 hours) prior to being used in the study. Test compounds were administered orally in 25% Cremophor EL/water (w/w) or 100% propylene glycol (PG) in a dose volume of 10 mL/kg. Blood samples (~0.30 mL) were removed at 0.25, 0.50, 1.0, 1.5, 2, 3, 4, 6, and 8 hours post-dose, plasma separated by centrifugation and stored at -20°C pending analysis. Quantitation of the plasma samples was conducted using a gradient HPLC/MS/MS or enzymatic method detailed below:

- 268 -

HPLC/MS/MS Method for the quantitation of ICE
inhibitors in rat plasma

Sample Preparation

- 5 • 50µl of plasma are aliquotted into Ependorf
centrifuge vials.
- An equal volume of Acetonitrile is added to the
plasma to precipitate plasma proteins.
- Samples are Vortexed for 5 minutes, and centrifuged at
14,000 rpms for 5 minutes.
- 10 • 75µl of the supernatant is loaded into 12mm HPLC
liquid sampler vials.
- 50µl of sample is injected for analysis via the mass
spectrometer.

15 HPLC Instrumental Parameters

HPLC: Hewlett Packard HP1100 Binary Solvent
Delivery System.

HPLC Gradient Conditions

- 20 A = H₂O 0.2% Formic Acid
 B = Acetonitrile 0.2% Formic Acid

Mobile Phase

	Time	%A	%B
25	0	100	0
	2	100	0
	5	0	100
	11	0	100
30	11.5	100	0
	17	100	0

HPLC Analytical Column: Keystone Phenyl -2 Hypersil
2.0x100mm, 5µ 120Å pore pore size, P/N# 105-39-2

- 269 -

Injection Volume: 50 μ l
Flow Rate: 0.20 mL/min.

Mass Spectrometry Instrumental Parameters

5 Instrument: P E Sciex API-365 Tandem Mass Spectrometer
Ionization Technique: Turbo-Ionspray (ESI)
Polarity: Positive
Dwell Time: 300msec
Pause Time: 5msec
10 Scan time: 0.9sec
Scan Mode: MRM (Multiple Reaction Monitoring)

ICE ENZYMATIC ASSAY FOR THE QUANTITATION OF ICE
15 INHIBITORS IN RAT PLASMA
50 μ L of plasma was extracted with 150 μ L
acetonitrile, sonicated, vortexed,
centrifuged at 10,000xg and 180 μ L of the supernatant
dried in a Sorvall vortex evaporator at room
20 temperature. Samples were reconstituted in 100 μ L
buffer (10 mM tris-HCl, pH 7.5 with 0.1%CHAPS, 1 mM
DTT) with sonication. 10 μ L of each sample was mixed
with 10 μ L ICE (1.1 mg/mL) in a microtitre plate with
60 μ L buffer. Samples were incubated for 15 min. at
25 room temperature then 20 μ L Succ YVAD-pNA(400 μ M,
prewarmed to 37°C) added, and the plate monitored at
405 nm for 20 min. at 37°C used a SpectraMax reader.
The data were fitted using a 4 parameter fit with the
the SpectraMax software using an extracted standard
30 curve. The assay was linear from 0.15 to 2.0-3.0 μ g/mL
aldehyde.

- 270 -

Pharmacokinetic Parameters

Pharmacokinetic analysis of these plasma concentration data was conducted using noncompartmental methods. The area under the curve ($AUC_{(0-t)}$) was estimated from time zero to the last measured time point using the linear trapezoidal rule. The rate of elimination (k_e) was estimated by log-linear regression from the terminal phase of the plasma concentration-time curves. Area under the tail of the curve was estimated as the ratio of the last measured concentration to k_e . The area under the curve from time zero to infinity ($AUC_{(0-\infty)}$) was obtained by addition of the area under the tail to $AUC_{(0-t)}$. Elimination half-life was estimated as $0.693/k_e$. The observed values for the peak plasma concentration (C_{max}) were recorded. For prodrug studies: aldehyde availability (bioavailability) was calculated as: $(AUC_{ald}/prodrug\ p.o.) / (AUC_{ald}/ald\ i.v.) \times (dose\ ald, ald\ i.v. / dose\ prodrug, prodrug\ p.o.) \times (MW\ prodrug / MW\ aldehyde)$.

Table 8. Bioavailability Data

Example	C_{max} ($\mu g/mL$)	AUC ($\mu gXh/mL$)	$t_{1/2}$ (hrs)	F (%)
56	A	B		A
45	A	B		
90	C	C	A	C
85	A	B	B	A
68	A	C	B	
76	C	C	C	
77	B	B	A	A
78	A	A	B	A
89	B	C	C	
83	A	C	C	

- 271 -

Example	Cmax ($\mu\text{g/mL}$)	AUC ($\mu\text{gXh/mL}$)	t 1/2 (hrs)	F (%)
98d	A	A	B	
98h	A	C	B	
98e	C	C	B	
98c	B	C	C	
98k	B	C	B	
98ae	A	A	B	
98af	A	A	B	
98b	C	C	B	
111	A	A	C	
98o	A	A	B	
108a	A	A	B	
98ag	C	C	B	C
98a	B	B	C	
98am	A	A	B	
116a	C	C	B	
98an	A	A	B	
116g	A	A	C	
116h	A	A	B	
116e	A	A	B	
108b	A	A	B	

Antiviral Assays

The efficacy of the compounds of this invention at treating or preventing antiviral related diseases, disorders, or conditions may be evaluated in various *in vitro* and *in vivo* assays. For example, assays may be preformed to determine the ability of these compounds to inhibit inflammatory responses associated with viral infections. *In vitro* assays may employ whole cells or isolated cellular components. *In vivo* assays include animal models for viral diseases.

- 272 -

Examples of such animal models include, but are not limited to, rodent models for HBV or HCV infection, the Woodchuck model for HBV infection, and chimpanzee model for HCV infection.

5 Compounds of this invention may also be evaluated in animal models for dietary alcohol-induced disease.

 Other assays that may be used to evaluate the compounds of this invention are disclosed in PCT
10 application PCT/US96/20843, published June 26, 1997, under publication no. WO 97/22619. Such assays include *in vivo* pharmacokinetic studies in the mouse, inhibition of ICE homologs, inhibition of apoptosis, *in vivo* acute assay for anti-inflammatory efficacy,
15 measurement of blood levels of drugs, IGIF assays, mouse carrageenan peritoneal inflammation assay, and type II collagen-induced arthritis.

 Insofar as the compounds of this invention are able to inhibit caspases, particularly ICE, *in*
20 vitro and furthermore, may be delivered orally to mammals, they are of evident clinical utility for the treatment of IL-1-, apoptosis-, IGIF-, and IFN- γ -mediated diseases.

 While we have described a number of
25 embodiments of this invention, it is apparent that our basic constructions may be altered to provide other embodiments which utilize the products and processes of this invention.

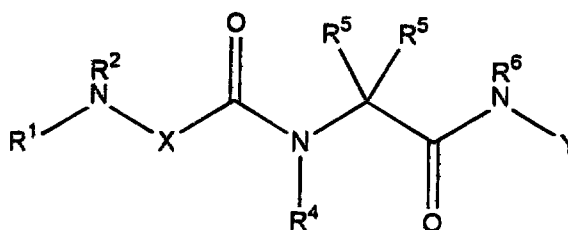


- 273 -

What is claimed is:

1. A compound represented by formula I:

5

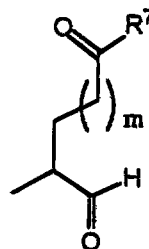


15

wherein:

Y is:

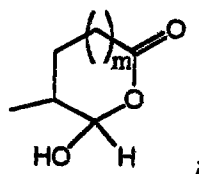
(a)



20

provided that when R^7 is -OH then Y can also be:

(b)



25

X is $-C(R^3)_2-$ or $-N(R^3)-$;

m is 0 or 1;

30

$$\begin{aligned} R^1 \text{ is } H, -R^8, -C(O)R^8, -C(O)C(O)R^8, -S(O)_2R^8, \\ -S(O)R^8, -C(O)OR^8, -C(O)N(H)R^8, -S(O)_2N(H)-R^8, \\ -S(O)N(H)-R^8, -C(O)C(O)N(H)R^8, -C(O)CH=CHR^8, \end{aligned}$$

This Sheet Has Been Replaced





$-\text{C}(\text{O})\text{CH}_2\text{OR}^8$, $-\text{C}(\text{O})\text{CH}_2\text{N}(\text{H})\text{R}^8$, $-\text{C}(\text{O})\text{N}(\text{R}^8)_2$, $-\text{S}(\text{O})_2\text{N}(\text{R}^8)_2$,
 $-\text{S}(\text{O})\text{N}(\text{R}^8)_2$, $-\text{C}(\text{O})\text{C}(\text{O})\text{N}(\text{R}^8)_2$, $-\text{C}(\text{O})\text{CH}_2\text{N}(\text{R}^8)_2$, $-\text{CH}_2\text{R}^8$,
 $-\text{CH}_2\text{-alkenyl-R}^8$, or $-\text{CH}_2\text{-alkynyl-R}^8$;

5 R^2 is $-\text{H}$ and each R^3 is independently $-\text{H}$, an
amino acid side chain, $-\text{R}^8$, alkenyl- R^9 , or alkynyl- R^9 ,
or each R^3 , together with the atom to which they are
bound, form a 3 to 7 membered cyclic or heterocyclic
cyclic ring system, or R^2 and one R^3 together with the
10 atoms to which they are bound, form a 3 to 7 membered
cyclic or heterocyclic ring system, wherein a hydrogen
atom bound to any -alkyl or -cycloalkyl carbon atom is
optionally replaced by $-\text{R}^{10}$, a hydrogen atom bound to
any -aryl or -heteroaryl carbon atom is optionally
15 replaced by $-\text{R}^{11}$, a hydrogen atom bound to any nitrogen
atom of the ring system is optionally replaced by $-\text{R}^1$;

R^4 is $-\text{H}$ and each R^5 is independently $-\text{H}$, an
amino acid side chain, $-\text{R}^8$, -alkenyl- R^9 , or -alkynyl-
20 R^9 , or R^4 and one R^5 together with the atoms to which
they are bound form a 3 to 7 membered cyclic or
heterocyclic ring system, wherein a hydrogen atom bound
to any -alkyl or -cycloalkyl carbon atom is optionally
replaced by R^{10} , a hydrogen atom bound to any -aryl or
25 -heteroaryl carbon atom is optionally replaced by R^{11} ,
and a hydrogen atom bound to any nitrogen atom of the
ring system is optionally replaced with R^1 ;

R^6 is $-\text{H}$;

30

R^7 is $-\text{OH}$, $-\text{OR}^8$, or $-\text{N}(\text{H})\text{OH}$;

This Sheet Has Been Replaced





- 275 -

each R^8 is independently -alkyl, -cycloalkyl, -aryl, -heteroaryl, -heterocyclyl, -alkylcycloalkyl, -alkylaryl, -alkylheteroaryl, or -alkylheterocyclyl, wherein a hydrogen atom bound to any -alkyl or -cycloalkyl carbon atom is optionally replaced by R^{10} , a hydrogen atom bound to any -aryl or -heteroaryl carbon atom is optionally replaced by R^{11} , and a hydrogen atom bound to any nitrogen atom is optionally replaced by R^1 ;

each R^9 is independently -aryl, -heteroaryl, cycloalkyl, or -heterocyclyl, wherein a hydrogen atom bound to any -alkyl or -cycloalkyl carbon atom is optionally replaced by R^{10} , a hydrogen atom bound to any -aryl or -heteroaryl carbon atom is optionally replaced by R^{11} , and a hydrogen atom bound to any nitrogen atom is optionally replaced by R^1 ;

each R^{10} is independently -OH, -SH, -F, -Cl, -Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H, -N(H)C(O)NH₂, -perfluoroalkyl, -O-alkyl, -O-aryl, -O-alkylaryl, -N(H)alkyl, -N(H)aryl, -N(H)-alkylaryl, -N(alkyl)₂, -C(O)N(H)alkyl, -C(O)N(alkyl)₂, -N(H)C(O)alkyl, -N(H)C(O)Oalkyl, -N(H)C(O)Oaryl, -N(H)C(O)Oalkylaryl, -N(H)C(O)Oheteroaryl, -N(H)C(O)Oalkylheteroaryl, -N(H)C(O)Ocycloalkyl, -N(H)C(O)N(H)alkyl, -N(H)C(O)N(alkyl)₂, -N(H)C(O)N(H)aryl, -N(H)C(O)N(H)alkylaryl, -N(H)C(O)N(H)heteroaryl, -N(H)C(O)N(H)alkylheteroaryl, -N(H)C(O)N(H)cycloalkyl, -S-alkyl, -S-aryl, -S-alkylaryl, -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl, -CH₂NH₂, -CH₂N(H)alkyl, or -CH₂N(alkyl)₂, -alkyl,

This Sheet Has Been Replaced



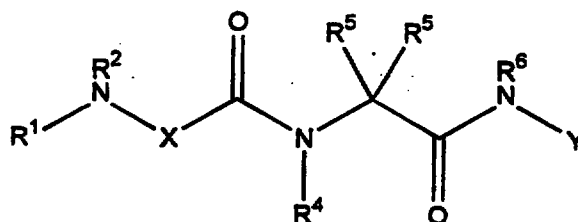


- 276 -

-cycloalkyl, -aryl, -heteroaryl, -heterocyclyl,
 -alkylcycloalkyl -alkylaryl, -alkylheteroaryl, or
 -alkylheterocyclyl, wherein a hydrogen atom bound to
 any -aryl or -heteroaryl carbon atom is optionally
 5 replaced by R^{11} and a hydrogen atom bound to any
 nitrogen atom is optionally replaced by R^1 ; and

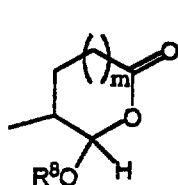
each R^{11} is independently -OH, -SH, -F, -Cl,
 -Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H,
 10 -N(H)C(O)NH₂, -alkyl, -cycloalkyl, -perfluoroalkyl, -O-
 alkyl, -O-aryl, -O-alkylaryl, -N(H)alkyl, -N(H)aryl,
 -N(H)-alkylaryl, -N(alkyl)₂, -C(O)N(H)alkyl,
 -C(O)N(alkyl)₂, -N(H)C(O)alkyl, -N(H)C(O)N(H)alkyl,
 -N(H)C(O)N(alkyl)₂, -S-alkyl, -S-aryl, -S-alkylaryl,
 15 -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl, -CH₂NH₂,
 -CH₂N(H)alkyl, or -CH₂N(alkyl)₂.

2. A compound represented by formula I:

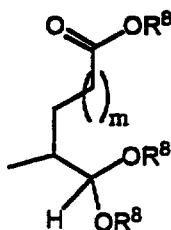


wherein:

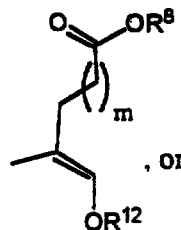
Y is:



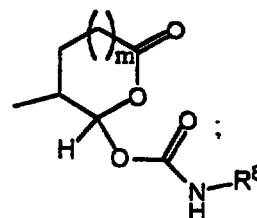
(c)



(d)



(e)



(f)

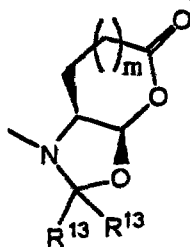
This Sheet Has Been Replaced





- 277 -

provided that when R^6 is not hydrogen, R^6 and Y, together with the nitrogen to which they are bound, form a ring (g):



5

(g) ;

X is $-C(R^3)_2-$ or $-N(R^3)-$;

m is 0 or 1;

10

R^1 is H, $-R^8$, $-C(O)R^8$, $-C(O)C(O)R^8$, $-S(O)_2R^8$, $-S(O)R^8$, $-C(O)OR^8$, $-C(O)N(H)R^8$, $-S(O)_2N(H)-R^8$, $-S(O)N(H)-R^8$, $-C(O)C(O)N(H)R^8$, $-C(O)CH=CHR^8$, $-C(O)CH_2OR^8$, $-C(O)CH_2N(H)R^8$, $-C(O)N(R^8)_2$, $-S(O)_2N(R^8)_2$, $-S(O)N(R^8)_2$, $-C(O)C(O)N(R^8)_2$, $-C(O)CH_2N(R^8)_2$, $-CH_2R^8$, $-CH_2$ -alkenyl- R^8 , or $-CH_2$ -alkynyl- R^8 ;

15

R^2 is -H and each R^3 is independently -H, an amino acid side chain, $-R^8$, alkenyl- R^9 , or alkynyl- R^9 , or each R^3 , together with the atom to which they are bound, form a 3 to 7 membered cyclic or heterocyclic cyclic ring system, or R^2 and one R^3 together with the atoms to which they are bound, form a 3 to 7 membered cyclic or heterocyclic ring system, wherein a hydrogen atom bound to any -alkyl or -cycloalkyl carbon atom is optionally replaced by $-R^{10}$, a hydrogen atom bound to any -aryl or -heteroaryl carbon atom is optionally

25

This Sheet Has Been Replaced





replaced by $-R^{11}$, a hydrogen atom bound to any nitrogen atom of the ring system is optionally replaced by $-R^1$;

5 R^4 is $-H$ and each R^5 is independently $-H$, an amino acid side chain, $-R^8$, $-alkenyl-R^9$, or $-alkynyl-R^9$, or R^4 and one R^5 together with the atoms to which they are bound form a 3 to 7 membered cyclic or heterocyclic ring system, wherein a hydrogen atom bound to any $-alkyl$ or $-cycloalkyl$ carbon atom is optionally
10 replaced by R^{10} , a hydrogen atom bound to any $-aryl$ or $-heteroaryl$ carbon atom is optionally replaced by R^{11} , and a hydrogen atom bound to any nitrogen atom of the ring system is optionally replaced with R^1 ;

15 R^6 is $-H$;

each R^8 is independently $-alkyl$, $-cycloalkyl$, $-aryl$, $-heteroaryl$, $-heterocyclyl$, $-alkylcycloalkyl$, $-alkylaryl$, $-alkylheteroaryl$, or $-alkylheterocyclyl$, wherein a hydrogen atom bound to any $-alkyl$ or
20 $-cycloalkyl$ carbon atom is optionally replaced by R^{10} , a hydrogen atom bound to any $-aryl$ or $-heteroaryl$ carbon atom is optionally replaced by R^{11} , and a hydrogen atom bound to any nitrogen atom is optionally replaced by R^1 ;

25

each R^9 is independently $-aryl$, $-heteroaryl$, $cycloalkyl$, or $-heterocyclyl$, wherein a hydrogen atom bound to any $-alkyl$ or $-cycloalkyl$ carbon atom is optionally replaced by R^{10} , a hydrogen atom bound to
30 any $-aryl$ or $-heteroaryl$ carbon atom is optionally replaced by R^{11} , and a hydrogen atom bound to any nitrogen atom is optionally replaced by R^1 ;

This Sheet Has Been Replaced





- each R¹⁰ is independently -OH, -SH, -F, -Cl, -Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H, -N(H)C(O)NH₂, -perfluoroalkyl, -O-alkyl, -O-aryl, -O-alkylaryl, -N(H)alkyl, -N(H)aryl, -N(H)-alkylaryl, -N(alkyl)₂, -C(O)N(H)alkyl, -C(O)N(alkyl)₂, -N(H)C(O)alkyl, -N(H)C(O)Oalkyl, -N(H)C(O)Oaryl, -N(H)C(O)Oalkylaryl, -N(H)C(O)Oheteroaryl, -N(H)C(O)Oalkylheteroaryl, -N(H)C(O)Ocycloalkyl, -N(H)C(O)N(H)alkyl, -N(H)C(O)N(alkyl)₂, -N(H)C(O)N(H)aryl, -N(H)C(O)N(H)alkylaryl, -N(H)C(O)N(H)heteroaryl, -N(H)C(O)N(H)alkylheteroaryl, -N(H)C(O)N(H)cycloalkyl, -S-alkyl, -S-aryl, -S-alkylaryl, -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl, -CH₂NH₂, -CH₂N(H)alkyl, or -CH₂N(alkyl)₂, -alkyl, -cycloalkyl, -aryl, -heteroaryl, -heterocyclyl, -alkylcycloalkyl -alkylaryl, -alkylheteroaryl, or -alkylheterocyclyl, wherein a hydrogen atom bound to any -aryl or -heteroaryl carbon atom is optionally replaced by R¹¹ and a hydrogen atom bound to any nitrogen atom is optionally replaced by R¹; and

- each R¹¹ is independently -OH, -SH, -F, -Cl, -Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H, -N(H)C(O)NH₂, -alkyl, -cycloalkyl, -perfluoroalkyl, -O-alkyl, -O-aryl, -O-alkylaryl, -N(H)alkyl, -N(H)aryl, -N(H)-alkylaryl, -N(alkyl)₂, -C(O)N(H)alkyl, -C(O)N(alkyl)₂, -N(H)C(O)alkyl, -N(H)C(O)N(H)alkyl, -N(H)C(O)N(alkyl)₂, -S-alkyl, -S-aryl, -S-alkylaryl, -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl, -CH₂NH₂, -CH₂N(H)alkyl, or -CH₂N(alkyl)₂;

R¹² is -C(O)alkyl, -C(O)cycloalkyl, -C(O)alkylenyl, -C(O)alkylaryl, -C(O)alkylheteroaryl, -C(O)heterocyclyl, or -C(O)alkylheterocyclyl; and

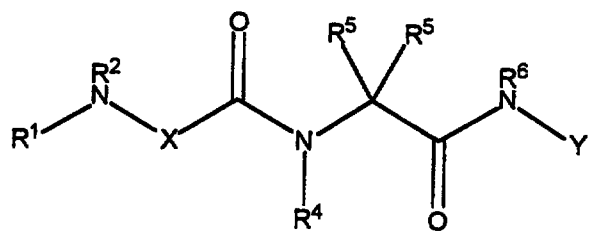




R^{13} is -H, -alkyl, -aryl, -alkylaryl or -alkylheteroaryl.

5

3. A compound represented by formula I:

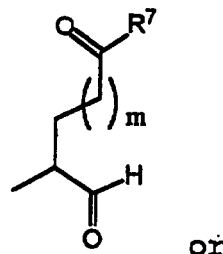


wherein:

Y is:

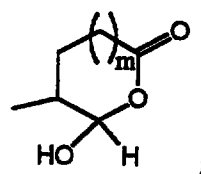
10

(a)



or

(b)



15

m is 0 or 1;

X is $-C(R^3)_2-$

This Sheet Has Been Replaced





- 281 -

R^1 is H, $-R^8$, $-C(O)R^8$, $-C(O)C(O)R^8$, $-S(O)_2R^8$,
 $-S(O)R^8$, $-C(O)OR^8$, $-C(O)N(H)R^8$, $-S(O)_2N(H)-R^8$,
 $-S(O)N(H)-R^8$, $-C(O)C(O)N(H)R^8$, $-C(O)CH=CHR^8$,
 $-C(O)CH_2OR^8$, $-C(O)CH_2N(H)R^8$, $-C(O)N(R^8)_2$, $-S(O)_2N(R^8)_2$,
5 $-S(O)N(R^8)_2$, $-C(O)C(O)N(R^8)_2$, $-C(O)CH_2N(R^8)_2$, $-CH_2R^8$,
 $-CH_2$ -alkenyl- R^8 , or $-CH_2$ -alkynyl- R^8 ;

R^2 is $-H$ and each R^3 is independently $-H$, an
amino acid side chain, $-R^8$, alkenyl- R^9 , or alkynyl- R^9 ,
10 or each R^3 together with the atom to which they are
bound, form a 3 to 7 membered cyclic or heterocyclic
ring system, wherein a hydrogen atom bound to any
-alkyl or -cycloalkyl carbon atom is optionally
replaced by $-R^{10}$, a hydrogen atom bound to any -aryl or
15 -heteroaryl carbon atom is optionally replaced by $-R^{11}$,
a hydrogen atom bound to any nitrogen atom of the ring
system is optionally replaced by $-R^1$;

R^4 is $-H$ and each R^5 is independently $-H$, an
20 amino acid side chain, $-R^8$, -alkenyl- R^9 , or
-alkynyl- R^9 , or R^4 and one R^5 together with the atoms
to which they are bound form a 3 to 7 membered cyclic
or heterocyclic ring system, wherein a hydrogen atom
bound to any -alkyl or -cycloalkyl carbon atom is
25 optionally replaced by R^{10} , a hydrogen atom bound to
any -aryl or -heteroaryl carbon atom is optionally
replaced by R^{11} , and a hydrogen atom bound to any
nitrogen atom of the ring system is optionally replaced
with R^1 ;

30

 R^6 is $-H$;

This Sheet Has Been Replaced





- 282 -

R^7 is -OH, -OR⁸, -N(H)OH, or -N(H)S(O)₂R⁸;

each R⁸ is independently -alkyl, -cycloalkyl,
 5 -aryl, -heteroaryl, -heterocyclyl, -alkylcycloalkyl
 -alkylaryl, -alkylheteroaryl, or -alkylheterocyclyl,
 wherein a hydrogen atom bound to any -alkyl or
 -cycloalkyl carbon atom is optionally replaced by R¹⁰,
 a hydrogen atom bound to any -aryl or -heteroaryl
 10 carbon atom is optionally replaced by R¹¹, and a
 hydrogen atom bound to any nitrogen atom is optionally
 replaced by R¹;

each R⁹ is independently -aryl, -heteroaryl,
 15 cycloalkyl, or -heterocyclyl, wherein a hydrogen atom
 bound to any -alkyl or -cycloalkyl carbon atom is
 optionally replaced by R¹⁰, a hydrogen atom bound to
 any -aryl or -heteroaryl carbon atom is optionally
 replaced by R¹¹, and a hydrogen atom bound to any
 20 nitrogen atom is optionally replaced by R¹;

each R¹⁰ is independently -OH, -SH, -F, -Cl,
 -Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H,
 -N(H)C(O)NH₂, -perfluoroalkyl, -O-alkyl, -O-aryl,
 25 -O-alkylaryl, -N(H)alkyl, -N(H)aryl, -N(H)-alkylaryl,
 -N(alkyl)₂, -C(O)N(H)alkyl, -C(O)N(alkyl)₂,
 -N(H)C(O)alkyl, -N(H)C(O)Oalkyl, -N(H)C(O)Oaryl,
 -N(H)C(O)Oalkylaryl, -N(H)C(O)Oheteroaryl,
 -N(H)C(O)Oalkylheteroaryl, -N(H)C(O)Ocycloalkyl,
 30 -N(H)C(O)N(H)alkyl, -N(H)C(O)N(alkyl)₂,
 -N(H)C(O)N(H)aryl, -N(H)C(O)N(H)alkylaryl,
 -N(H)C(O)N(H)heteroaryl, -N(H)C(O)N(H)alkylheteroaryl,
 -N(H)C(O)N(H)cycloalkyl, -S-alkyl, -S-aryl,
 -S-alkylaryl, -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl,

This Sheet Has Been Replaced





- 283 -

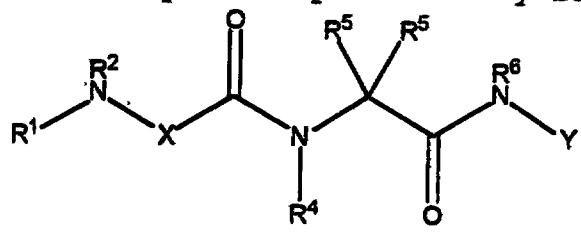
5 -CH₂NH₂, -CH₂N(H)alkyl, or -CH₂N(alkyl)₂, -alkyl, -cycloalkyl, -aryl, -heteroaryl, -heterocyclyl, -alkylcycloalkyl -alkylaryl, -alkylheteroaryl, or -alkylheterocyclyl, wherein a hydrogen atom bound to any -aryl or -heteroaryl carbon atom is optionally replaced by R¹¹ and a hydrogen atom bound to any nitrogen atom is optionally replaced by R¹; and

10 each R¹¹ is independently -OH, -SH, -F, -Cl, -Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H, -N(H)C(O)NH₂, -alkyl, -cycloalkyl, -perfluoroalkyl, -O-alkyl, -O-aryl, -O-alkylaryl, -N(H)alkyl, -N(H)aryl, -N(H)-alkylaryl, -N(alkyl)₂, -C(O)N(H)alkyl, -C(O)N(alkyl)₂, -N(H)C(O)alkyl, -N(H)C(O)N(H)alkyl, 15 -N(H)C(O)N(alkyl)₂, -S-alkyl, -S-aryl, -S-alkylaryl, -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl, -CH₂NH₂, -CH₂N(H)alkyl, or -CH₂N(alkyl)₂;

provided that if one R³ is -H, then the other R³ is not -H.

20

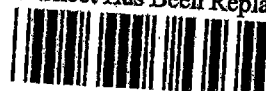
4. A compound represented by formula I:

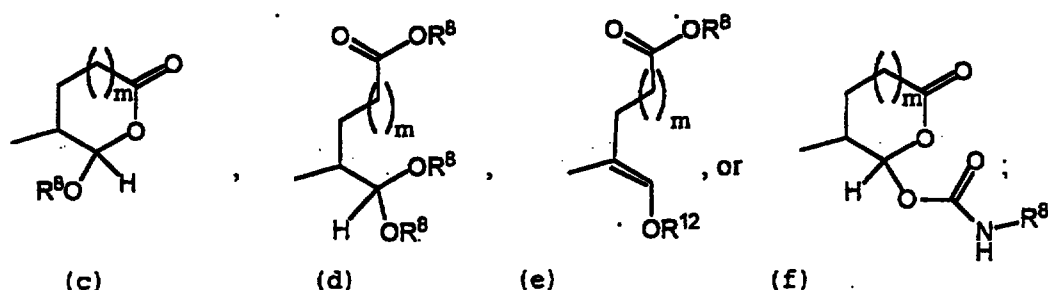


wherein:

25 Y is:

This Sheet Has Been Replaced





m is 0 or 1;

5 X is $-C(R^3)_2-$

R^1 is H, $-R^8$, $-C(O)R^8$, $-C(O)C(O)R^8$, $-S(O)_2R^8$,
 $-S(O)R^8$, $-C(O)OR^8$, $-C(O)N(H)R^8$, $-S(O)_2N(H)-R^8$,
 $-S(O)N(H)-R^8$, $-C(O)C(O)N(H)R^8$, $-C(O)CH=CHR^8$,
 10 $-C(O)CH_2OR^8$, $-C(O)CH_2N(H)R^8$, $-C(O)N(R^8)_2$, $-S(O)_2N(R^8)_2$,
 $-S(O)N(R^8)_2$, $-C(O)C(O)N(R^8)_2$, $-C(O)CH_2N(R^8)_2$, $-CH_2R^8$,
 $-CH_2$ -alkenyl- R^8 , or $-CH_2$ -alkynyl- R^8 ;

R^2 is $-H$ and each R^3 is independently $-H$, an
 15 amino acid side chain, $-R^8$, alkenyl- R^9 , or alkynyl- R^9 ,
 or each R^3 together with the atom to which they are
 bound, form a 3 to 7 membered cyclic or heterocyclic
 ring system, wherein a hydrogen atom bound to any
 $-alkyl$ or $-cycloalkyl$ carbon atom is optionally
 20 replaced by $-R^{10}$, a hydrogen atom bound to any $-aryl$ or
 $-heteroaryl$ carbon atom is optionally replaced by $-R^{11}$,
 a hydrogen atom bound to any nitrogen atom of the ring
 system is optionally replaced by $-R^1$;

25 R^4 is $-H$ and each R^5 is independently $-H$, an
 amino acid side chain, $-R^8$, $-alkenyl-R^9$, or
 $-alkynyl-R^9$, or R^4 and one R^5 together with the atoms

This Sheet Has Been Replaced





- 285 -

to which they are bound form a 3 to 7 membered cyclic or heterocyclic ring system, wherein a hydrogen atom bound to any -alkyl or -cycloalkyl carbon atom is optionally replaced by R^{10} , a hydrogen atom bound to
5 any -aryl or -heteroaryl carbon atom is optionally replaced by R^{11} , and a hydrogen atom bound to any nitrogen atom of the ring system is optionally replaced with R^1 ;

10 R^6 is -H;

each R^8 is independently -alkyl, -cycloalkyl, -aryl, -heteroaryl, -heterocyclyl, -alkylcycloalkyl -alkylaryl, -alkylheteroaryl, or -alkylheterocyclyl,
15 wherein a hydrogen atom bound to any -alkyl or -cycloalkyl carbon atom is optionally replaced by R^{10} , a hydrogen atom bound to any -aryl or -heteroaryl carbon atom is optionally replaced by R^{11} , and a hydrogen atom bound to any nitrogen atom is optionally
20 replaced by R^1 ;

each R^9 is independently -aryl, -heteroaryl, cycloalkyl, or -heterocyclyl, wherein a hydrogen atom bound to any -alkyl or -cycloalkyl carbon atom is
25 optionally replaced by R^{10} , a hydrogen atom bound to any -aryl or -heteroaryl carbon atom is optionally replaced by R^{11} , and a hydrogen atom bound to any nitrogen atom is optionally replaced by R^1 ;

30 each R^{10} is independently -OH, -SH, -F, -Cl, -Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H, -N(H)C(O)NH₂, -perfluoroalkyl, -O-alkyl, -O-aryl, -O-alkylaryl, -N(H)alkyl, -N(H)aryl, -N(H)-alkylaryl,

This Sheet Has Been Replaced





- 286 -

-N(alkyl)₂, -C(O)N(H)alkyl, -C(O)N(alkyl)₂,
 -N(H)C(O)alkyl, -N(H)C(O)Oalkyl, -N(H)C(O)Oaryl,
 -N(H)C(O)Oalkylaryl, -N(H)C(O)Oheteroaryl,
 -N(H)C(O)Oalkylheteroaryl, -N(H)C(O)Ocycloalkyl,
 5 -N(H)C(O)N(H)alkyl, -N(H)C(O)N(alkyl)₂,
 -N(H)C(O)N(H)aryl, -N(H)C(O)N(H)alkylaryl,
 -N(H)C(O)N(H)heteroaryl, -N(H)C(O)N(H)alkylheteroaryl,
 -N(H)C(O)N(H)cycloalkyl, -S-alkyl, -S-aryl,
 -S-alkylaryl, -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl,
 10 -CH₂NH₂, -CH₂N(H)alkyl, or -CH₂N(alkyl)₂, -alkyl,
 -cycloalkyl, -aryl, -heteroaryl, -heterocyclyl,
 -alkylcycloalkyl -alkylaryl, -alkylheteroaryl, or
 -alkylheterocyclyl, wherein a hydrogen atom bound to
 any -aryl or -heteroaryl carbon atom is optionally
 15 replaced by R¹¹ and a hydrogen atom bound to any
 nitrogen atom is optionally replaced by R¹; and

each R¹¹ is independently -OH, -SH, -F, -Cl,
 -Br, -I, -NO₂, -CN, -NH₂, -CO₂H, -C(O)NH₂, -N(H)C(O)H,
 20 -N(H)C(O)NH₂, -alkyl, -cycloalkyl, -perfluoroalkyl, -O-
 alkyl, -O-aryl, -O-alkylaryl, -N(H)alkyl, -N(H)aryl,
 -N(H)-alkylaryl, -N(alkyl)₂, -C(O)N(H)alkyl,
 -C(O)N(alkyl)₂, -N(H)C(O)alkyl, -N(H)C(O)N(H)alkyl,
 -N(H)C(O)N(alkyl)₂, -S-alkyl, -S-aryl, -S-alkylaryl,
 25 -S(O)₂alkyl, -S(O)alkyl, -C(O)alkyl, -CH₂NH₂,
 -CH₂N(H)alkyl, or -CH₂N(alkyl)₂; and

R¹² is -C(O)alkyl, -C(O)cycloalkyl,
 -C(O)alkenyl, -C(O)alkylaryl, -C(O)alkylheteroaryl,
 30 -C(O)heterocyclyl, or -C(O)alkylheterocyclyl.

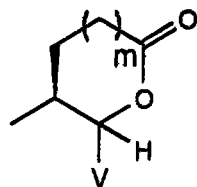
This Sheet Has Been Replaced



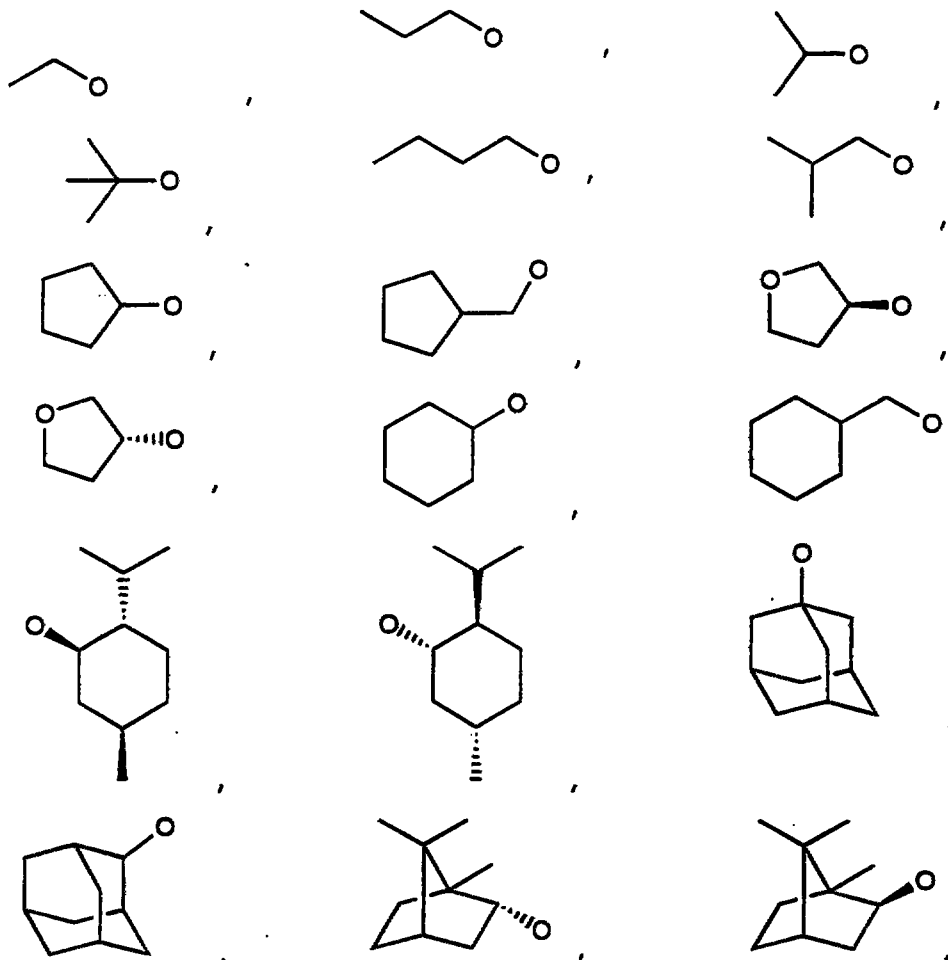


5. The compound according to claim 2 or claim 4 wherein Y is:

10

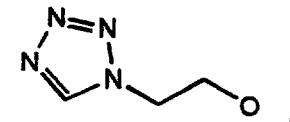
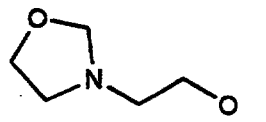
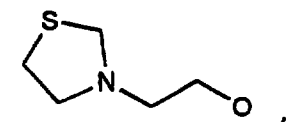
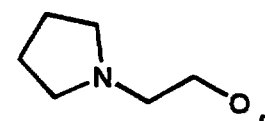
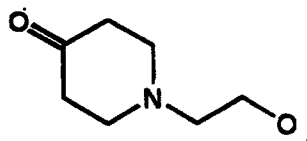
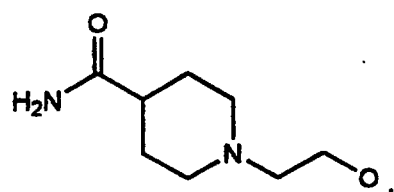
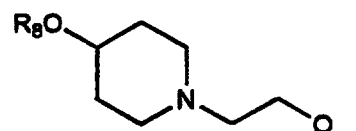
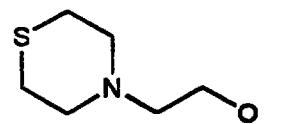
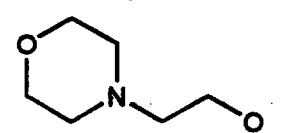
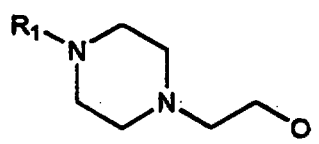
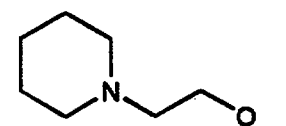
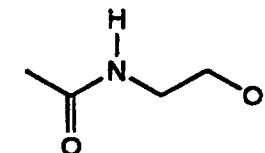
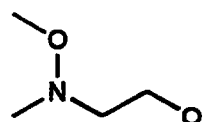
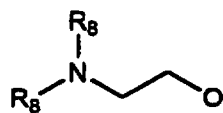
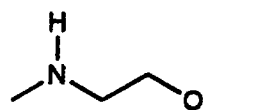
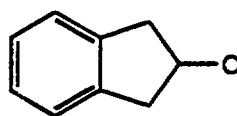
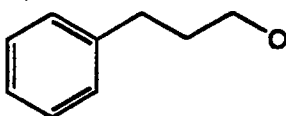
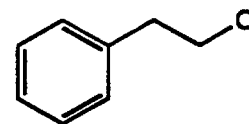
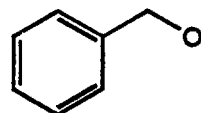
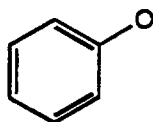
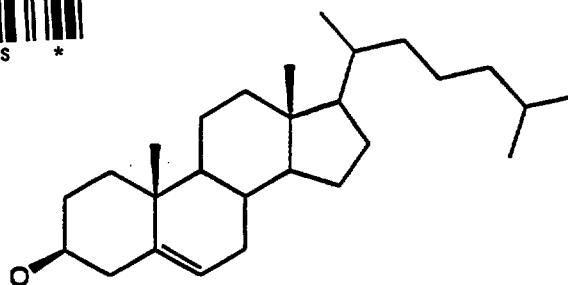


and V is: CH₃O,



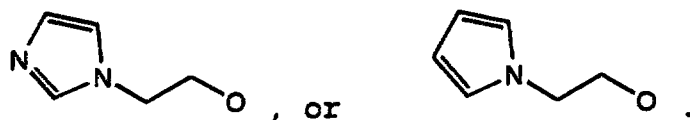
This Sheet Has Been Replaced





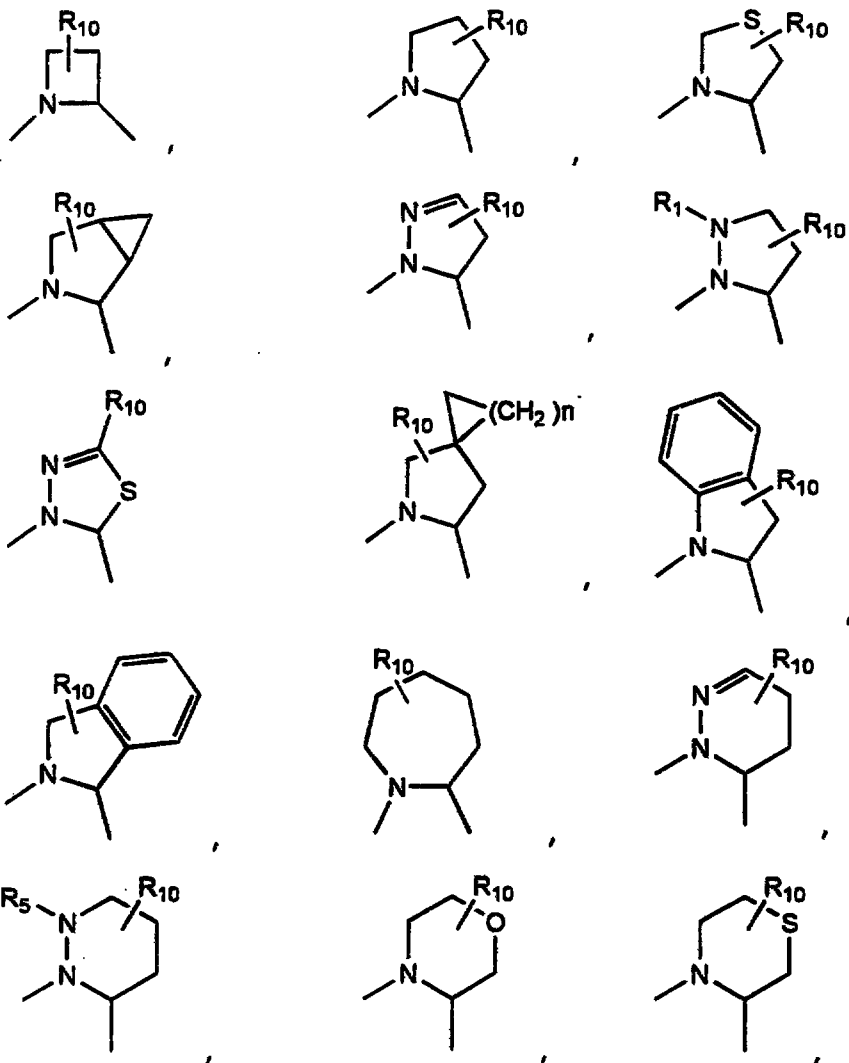
This Sheet Has Been Replaced





6. The compound according to any one of claims 1-5 wherein R^4 and one R^5 together with the atoms to which they are bound form a ring system selected from:

5

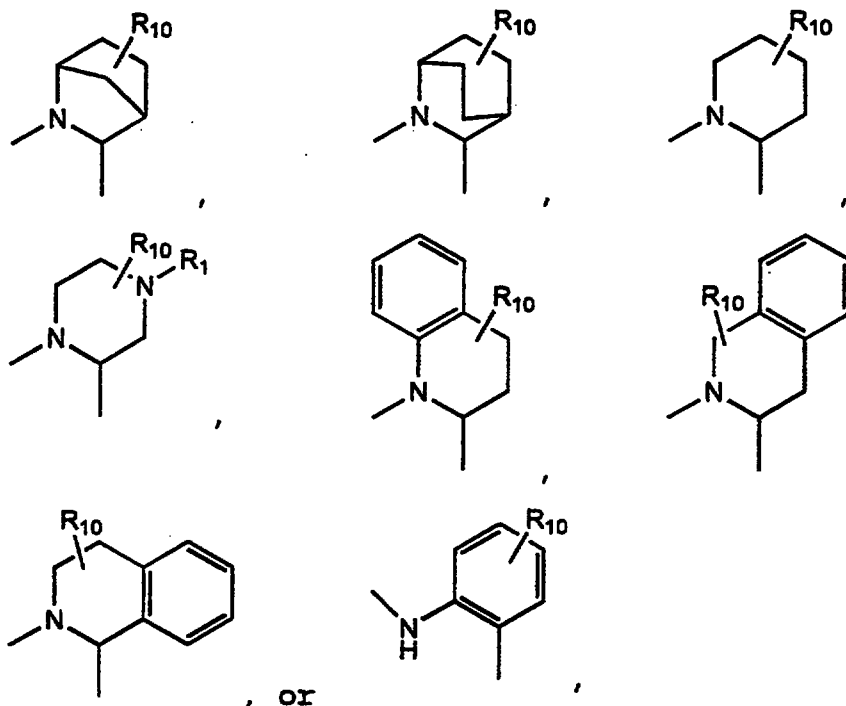


This Sheet Has Been Replaced





- 290 -



7. The compound according to claim 6
wherein:

5 one R^3 is -H and the other R^3 is methyl,
isopropyl, tert-butyl, $-\text{CH}_2\text{SR}^8$, $-\text{CH}_2\text{SO}_2\text{R}^8$, $-\text{CH}_2\text{CH}_2\text{SR}^8$, or
 $-\text{CH}_2\text{CH}_2\text{SO}_2\text{R}^8$.

8. The compound according to claim 7
10 wherein one R^3 is -H and the other R^3 is methyl.

9. The compound according to claim 8
wherein R^1 is $-\text{C}(\text{O})\text{R}^8$ or $-\text{C}(\text{O})\text{C}(\text{O})\text{R}^8$.

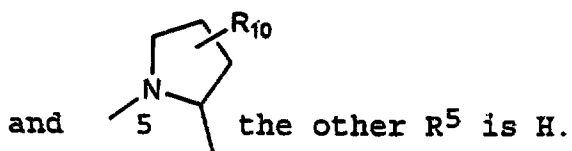
15 10. The compound according to claim 6
wherein:

R^4 and one R^5 together with the atoms to
which they are bound form the ring system:

This Sheet Has Been Replaced



- 291 -



10 11. The compound according to claim 10
wherein one R³ is -H and the other R³ is methyl.

15 12. The compound according to claim 11
wherein R¹ is -C(O)R⁸ or -C(O)C(O)R⁸.

13. The compound according to claim 10
wherein R¹⁰ is 4-fluoro or 4,4-difluoro.

20 14. The compound according to claim 13
wherein one R³ is -H and the other R³ is methyl.

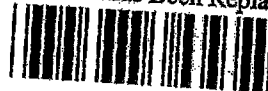
15. The compound according to claim 14
wherein R¹ is -C(O)R⁸ or -C(O)C(O)R⁸.

25 16. The compound according to claim 1 or
claim 3 selected from the group consisting of: 5a-5bd,
7a-7at, 9a-9g, 15a-15f, 16a-16b, 17a-17e, 18a-18f, 20a-
20t, 23a-23i, 24a-24e, 25a-25e, 26a-26h, 27a-27n, 28a-
28c, 29a-29s, 32a-32e, 34, 42, 46, 52, 57, 61, 65, 69,
30 73, 121, 122 a-v, and 123 a-c.

35 17. The compound according to claim 4
selected from the group consisting of: 41, 45, 51, 56,
60, 64, 68, 72, 76-93, 98a-z, aa-az, ba and bb, 101,
102a, 102b, 108a-d, 110, 111, 116a-h, and 120a and b.

18. A pharmaceutical composition comprising:
a) a compound according to any one of claims 1-17; and

This Sheet Has Been Replaced





- 292 -

b) a pharmaceutically acceptable carrier, adjuvant or vehicle.

19. A method for treating or preventing a
5 disease selected from an IL-1 mediated disease, an
apoptosis mediated disease, an inflammatory disease, an
autoimmune disease, a destructive bone disorder, a
proliferative disorder, an infectious disease, a
degenerative disease, a necrotic disease, an excess
10 dietary alcohol intake disease, a viral mediated
disease, inflammatory peritonitis, osteoarthritis,
pancreatitis, asthma, adult respiratory distress
syndrome, glomerulonephritis, rheumatoid arthritis,
systemic lupus erythematosus, scleroderma, chronic
15 thyroiditis, Grave's disease, autoimmune gastritis,
insulin-dependent diabetes mellitus (Type I),
autoimmune hemolytic anemia, autoimmune neutropenia,
thrombocytopenia, chronic active hepatitis, myasthenia
gravis, inflammatory bowel disease, Crohn's disease,
20 psoriasis, atopic dermatitis, graft vs host disease,
osteoporosis, leukemias and related disorders,
myelodysplastic syndrome, multiple myeloma-related bone
disorder, acute myelogenous leukemia, chronic
myelogenous leukemia, metastatic melanoma, Kaposi's
25 sarcoma, multiple myeloma, sepsis, septic shock,
Shigellosis, Alzheimer's disease, Parkinson's disease,
cerebral ischemia, myocardial ischemia, spinal muscular
atrophy, multiple sclerosis, AIDS-related encephalitis,
HIV-related encephalitis, aging, alopecia, neurological
30 damage due to stroke, ulcerative colitis, traumatic
brain injury, organ transplant rejection, hepatitis-B,
hepatitis-C, hepatitis-G, yellow fever, dengue fever,
or Japanese encephalitis, in a patient comprising the
step of administering to said patient a compound

This Sheet Has Been Replaced



- 293 -

according to any one of claims 1-17 or a pharmaceutical composition according to claim 18.

20. The method according to claim 19,
5 wherein the disease is rheumatoid arthritis,
inflammatory bowel disease, Crohn's disease, ulcerative
colitis, inflammatory peritonitis, septic shock,
pancreatitis, traumatic brain injury, organ transplant
10 rejection, osteoarthritis, asthma, psoriasis,
Alzheimer's disease, atopic dermatitis, leukemias and
related disorders, myelodysplastic syndrome, or
multiple myeloma.

21. A method for inhibiting an ICE-mediated
15 function in a patient comprising the step of
administering to said patient a compound according to
any one of claims 1-17 or a pharmaceutical composition
according to claim 18.

22. A method for decreasing IGIF or IFN- γ
20 production in a patient comprising the step of
administering to said patient a compound according to
any one of claims 1-17 or a pharmaceutical composition
according to claim 18.

23. The use of a compound according to any
25 one of claims 1-17 or a pharmaceutical composition
according to claim 18 in the manufacture of a
medicament for treating or preventing a disease
30 selected from an IL-1 mediated disease, an apoptosis
mediated disease, an inflammatory disease, an
autoimmune disease, a destructive bone disorder, a
proliferative disorder, an infectious disease, a
degenerative disease, a necrotic disease, an excess

This Sheet Has Been Replaced





- 294 -

5 dietary alcohol intake disease, a viral mediated
disease, inflammatory peritonitis, osteoarthritis,
pancreatitis, asthma, adult respiratory distress
syndrome, glomerulonephritis, rheumatoid arthritis,
10 systemic lupus erythematosus, scleroderma, chronic
thyroiditis, Grave's disease, autoimmune gastritis,
insulin-dependent diabetes mellitus (Type I),
autoimmune hemolytic anemia, autoimmune neutropenia,
thrombocytopenia, chronic active hepatitis, myasthenia
15 gravis, inflammatory bowel disease, Crohn's disease,
psoriasis, atopic dermatitis, graft vs host disease,
osteoporosis, leukemias and related disorders,
myelodysplastic syndrome, multiple myeloma-related bone
disorder, acute myelogenous leukemia, chronic
20 myelogenous leukemia, metastatic melanoma, Kaposi's
sarcoma, multiple myeloma, sepsis, septic shock,
Shigellosis, Alzheimer's disease, Parkinson's disease,
cerebral ischemia, myocardial ischemia, spinal muscular
atrophy, multiple sclerosis, AIDS-related encephalitis,
25 HIV-related encephalitis, aging, alopecia, neurological
damage due to stroke, ulcerative colitis, traumatic
brain injury, organ transplant rejection, hepatitis-B,
hepatitis-C, hepatitis-G, yellow fever, dengue fever,
or Japanese encephalitis, in a patient.

25

24. The use according to claim 23, wherein
the disease is rheumatoid arthritis, inflammatory bowel
disease, Crohn's disease, ulcerative colitis,
inflammatory peritonitis, septic shock, pancreatitis,
30 traumatic brain injury, organ transplant rejection,
osteoarthritis, asthma, psoriasis, Alzheimer's disease,
atopic dermatitis, leukemias and related disorders,
myelodysplastic syndrome, or multiple myeloma.

This Sheet Has Been Replaced





- 295 -

25. The use of a compound according to any one of claims 1-17 or a pharmaceutical composition according to claim 18 in the manufacture of a medicament for inhibiting an ICE-mediated function in a patient.

26. The use of a compound according to any one of claims 1-17 or a pharmaceutical composition according to claim 18 in the manufacture of a medicament for decreasing IGIF or IFN- γ production in a patient.

This Sheet Has Been Replaced

