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(54) Title
METHOD OF COMBATING ACYCLOVIR-RESISTANT HERPES SIMPLEX VIRAL INFECTIONS

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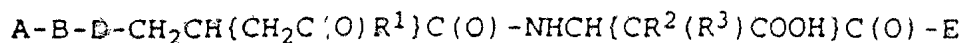
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(56) Prior Art Documents
WO 93/18056
EP 493770

(57) Claim
 1. A method of treating acyclovir-resistant herpes simplex viral infections in a mammal which comprises administering an effective amount of a peptide derivative of formula 1



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wherein A is phenylacetyl, phenylpropionyl, (4-aminophenyl)propionyl, (4-fluorophenyl)propionyl, (4-hydroxyphenyl)propionyl, (4-methoxyphenyl)propionyl, 2-(phenylmethyl)-3-phenylpropionyl, 2-((4-fluorophenyl)methyl)-3-(4-fluorophenyl)propionyl, 2-((4-methoxyphenyl)methyl)-3-(4-methoxyphenyl)propionyl or benzylaminocarbonyl; B is (N-Me)-Val or (N-Me)-Ile; or A and B taken together form a saturated alkylaminocarbonyl selected from the group of butylaminocarbonyl, 1-methylethylaminocarbonyl, 1-methylpropylaminocarbonyl, 1-ethylpropylaminocarbonyl, 1,1-dimethylbutylaminocarbonyl, 1-ethylbutylamino-

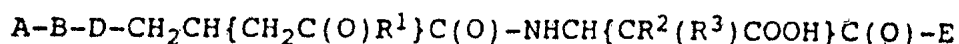
carbonyl, 1-propylbutylaminocarbonyl, 1-ethylpentylaminocarbonyl, 1-butylpentylaminocarbonyl, 1-ethylbutylaminocarbonyl, 2-ethylpentylaminocarbonyl, 1-methyl-1-propylbutylaminocarbonyl, 1-ethyl-1-propylbutylaminocarbonyl, 1,1-dipropylbutylaminocarbonyl, (1-propylcyclopentyl)aminocarbonyl and (1-propylcyclohexyl)aminocarbonyl; D is Val, Ile or Tbg; R^1 is 1-methylethyl, 1,1-dimethylethyl, 1-methylpropyl, 1,1-dimethylpropyl, 2,2-dimethylpropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-methylcyclopentyl, NR^4R^5 wherein R^4 is hydrogen or lower alkyl and R^5 is lower alkyl, or R^4 and R^5 together with the nitrogen atom to which they are attached form a pyrrolidino, piperidino, morpholino or 4-methylpiperazino; R^2 is hydrogen and R^3 is methyl, ethyl, 1-methylethyl, 1,1-dimethylethyl, propyl, 2-propenyl or benzyl, and the carbon atom bearing R^2 and R^3 has the (R)-configuration, or R^2 and R^3 each independently is methyl or ethyl, or R^2 and R^3 together with the carbon atom to which they are attached form a cyclobutyl, cyclopentyl or cyclohexyl; and E is NHR^6 wherein R^6 is 2-methylpropyl, 2,2-dimethylpropyl, 1(R),2,2-trimethylpropyl, 1,1,2,2-tetramethylpropyl, 1(R)-ethyl-2,2-dimethylpropyl, 2-(R,S)-methylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1(R),2,2-trimethylbutyl, 1(R),3,3-trimethylbutyl, 2-ethylbutyl, 2,2-diethylbutyl, 2-ethyl-1(R)-methylbutyl, 2-ethyl-2-methylbutyl, 1(R)-ethyl-3,3-dimethylbutyl, 2,2-dimethylpentyl, *cis*- or *trans*-2-methylcyclohexyl, 2,2-dimethylcyclohexyl or cyclohexylmethyl; or E is $NHCH(R^7)-Z$ wherein the carbon atom bearing R^7 has the (S)-configuration, R^7 is 1,1-dimethylethyl, 1-methylpropyl, 2-methylpropyl, 2,2-dimethylpropyl or cyclohexylmethyl and Z is CH_2OH , $C(O)OH$, $C(O)NH_2$ or $C(O)OR^8$ wherein R^8 is methyl, ethyl or propyl;

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or a therapeutically effective salt thereof.

13. A pharmaceutical composition when used in the treatment of acyclovir-resistant herpes simplex viral infections, comprising as an active ingredient a peptide derivative of formula 1



wherein A is phenylacetyl, phenylpropionyl, (4-aminophenyl)propionyl, (4-fluorophenyl)propionyl, (4-hydroxyphenyl)propionyl, (4-methoxyphenyl)propionyl, 2-(phenylmethyl)-3-phenylpropionyl, 2-((4-fluorophenyl)methyl)-3-(4-fluorophenyl)propionyl, 2-((4-methoxyphenyl)methyl)-3-(4-methoxyphenyl)propionyl or benzylaminocarbonyl; B is (N-Me)-Val or (N-Me)-Ile; or A and B taken together form a saturated alkylaminocarbonyl selected from the group of butylaminocarbonyl, 1-methylethylaminocarbonyl, 1-methylpropylaminocarbonyl, 1-ethylpropylaminocarbonyl, 1,1-dimethylethylaminocarbonyl, 1-ethylbutylaminocarbonyl, 1-propylbutylaminocarbonyl, 1-ethylpentylaminocarbonyl, 1-butylpentylaminocarbonyl, 1-ethylbutylaminocarbonyl, 2-ethylpentylaminocarbonyl, 1-methyl-1-propylbutylaminocarbonyl, 1-ethyl-1-propylbutylaminocarbonyl, 1,1-dipropylbutylaminocarbonyl, (1-propylcyclopentyl)aminocarbonyl and (1-propylcyclohexyl)aminocarbonyl; D is Val, Ile or Tbg; R¹ is 1-methylethyl, 1,1-dimethylethyl, 1-methylpropyl, 1,1-dimethylpropyl, 2,2-dimethylpropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-methylcyclopentyl, NR⁴R⁵ wherein R⁴ is hydrogen or lower alkyl and R⁵ is lower alkyl, or R⁴ and R⁵ together with the nitrogen atom to which they are attached form a pyrrolidino, piperidino, morpholino or 4-methylpiperazino; R² is hydrogen and R³ is methyl, ethyl, 1-methylethyl, 1,1-dimethylethyl, propyl, 2-propenyl

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or benzyl, and the carbon atom bearing R^2 and R^3 has the (R)-configuration, or R^2 and R^3 each independently is methyl or ethyl, or R^2 and R^3 together with the carbon atom to which they are attached form a cyclobutyl, cyclopentyl or cyclohexyl; and E is NHR^6 wherein R^6 is 2-methylpropyl, 2,2-dimethylpropyl, 1(R),2,2-trimethylpropyl, 1,1,2,2-tetramethylpropyl, 1(R)-ethyl-2,2-dimethylpropyl, 2-(R,S)-methylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1(R),2,2-trimethylbutyl, 1(R),3,3-trimethylbutyl, 2-ethylbutyl, 2,2-diethylbutyl, 2-ethyl-1(R)-methylbutyl, 2-ethyl-2-methylbutyl, 1(R)-ethyl-3,3-dimethylbutyl, 2,2-dimethylpentyl, cis- or trans-2-methylcyclohexyl, 2,2-dimethylcyclohexyl or cyclohexylmethyl; or E is $NHCH(R^7)-Z$ wherein the carbon atom bearing R^7 has the (S)-configuration, R^7 is 1,1-dimethylethyl, 1-methylpropyl, 2-methylpropyl, 2,2-dimethylpropyl or cyclohexylmethyl and Z is CH_2OH , $C(O)OH$, $C(O)NH_2$ or $C(O)OR^8$ wherein R^8 is methyl, ethyl or propyl; or a therapeutically acceptable salt thereof;

and a pharmaceutically acceptable carrier therefor.



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(54) Title: METHOD OF COMBATING ACYCLOVIR-RESISTANT HERPES SIMPLEX VIRAL INFECTIONS

(57) Abstract

Disclosed herein is a method for treating acyclovir-resistant herpes infections in a mammal. The method comprises administering a peptide derivative or a combination of the peptide derivative and an antiviral nucleoside analog to the infected mammal. The peptide derivative used for the method is represented by the formula A-B-D-CH₂CH(CH₂C(O)R¹)C(O)-NHCH(CR²(R³)COOH)C(O)-E wherein A is a terminal group, for example an optionally substituted phenylalkanoyl, and B is an N-methyl amino-acid residue; or A and B together form a saturated alkylaminocarbonyl; D is an amino acid residue; R¹ is, for example, an alkyl, cycloalkyl, or a monosubstituted or a disubstituted amino; R² is, for example, hydrogen or alkyl and R³ is alkyl, or R² is hydrogen and R³ is phenylalkyl, or R² and R³ are joined to form a cycloalkyl; and E is a terminal unit, for example, an alkylamino or a monovalent amino acid radical such as NHCH(alkyl)C(O)OH.

Method of Combating Acyclovir-Resistant Herpes Simplex Viral Infections

Field of the Invention

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This invention concerns a method for treating acyclovir-resistant herpes infections in a mammal. The method comprises administering a peptide derivative or a combination of the peptide derivative and an antiviral nucleoside analog.

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Background of the Invention

Acyclovir is the most widely used drug for treating herpes infections. However, the frequency of reports of acyclovir-resistant herpes infections has been increasing in recent years; for example, see P.A. Chatis et al., N. Engl. J. Med., 320, 297 (1989) and S. Safrin, Res. Virol., 143, 125 (1992). Lately, this type of resistant infection is being observed much more often in patients with severe human immunodeficiency virus (HIV) infections. As a result of their immunocompromized condition, the latter patients are very susceptible to infections by herpes simplex virus (HSV), type 1 and especially type 2, infections.

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The biochemical features which characterize acyclovir-resistant HSV isolates have received considerable attention; see the review concerning acyclovir resistant HSV by C.S. Crumpacker, J. Am. Acad. Dermatol., 18, 190 (1988). Briefly, the resistant strains display functional alterations in one or both of the virally encoded enzymes, thymidine kinase (TK) or DNA polymerase (POL).

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In the instance where cells, infected with a wild type HSV, are exposed to acyclovir, viral TK catalyses the monophosphorylation of acyclovir to a much greater extent than cellular enzymes. The resulting monophosphate subsequently is transformed by cellular enzymes to triphosphorylated acyclovir. The latter triphosphate interacts more readily with viral DNA polymerase than with cellular DNA polymerases. Consequently, it is able to reduce viral DNA synthesis by becoming an alternate substrate for the viral enzyme, and by acting as a chain terminator.

Two resistance mechanisms involving viral thymidine kinase have been described: (1) the selection of thymidine kinase-deficient mutants that induce very little enzyme activity after infection, and (b) the selection of mutants possessing a thymidine kinase of altered substrate specificity that is able to phosphorylate thymidine but not acyclovir.

A third resistance mechanism involving DNA polymerase is the result of the selection of mutants encoding an altered enzyme which is resistant to inactivation by acyclovir triphosphate.

Based on the resistance mechanism, acyclovir resistant HSV isolates can be classified as thymidine deficient (TK^D) strains, thymidine altered (TK^A) strains or DNA polymerase altered (POL^A) strains.

Although some success has been reported for the treatment of acyclovir-resistant HSV

infections with the antiviral agent foscarnet, the acyclovir-resistant type of infection is being encountered with increasing frequency. It is now considered to be a wide spread problem in AIDS patients; see K.S. Erlich et al., N. Engl. J. Med., 320, 293 (1989) and S. Safrin, J. Acquired Immun. Defic. Syndr., 5 (Suppl. 1), S29 (1992). Hence, there is a great need for means to manage this manifestation.

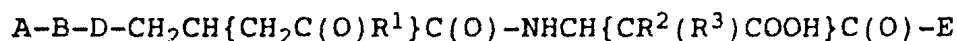
The present invention provides an effective, relatively safe method for the treatment of acyclovir-resistant herpes infections.

The method involves the use of peptide derivatives described by P.L. Beaulieu, R. Déziel, N. Moss and R. Plante in copending patent application PCT/CA/93/0095, filed March 12, 1993 directed to the use of the compounds for treating herpes infections. It is known, however, that antiviral agents effective against herpes infections are not necessarily effective against acyclovir-resistant herpes simplex virus; for example see J.J. O'Brien and D.M. Campoli-Richards, Drugs 37, 233 (1989), pp 252-253. It was therefore surprising and rewarding to find that the present peptide derivatives were effective against acyclovir-resistant herpes simplex virus.

Summary of the Invention

The present invention provides a method for treating acyclovir-resistant herpes simplex viral infections in a mammal. The method comprises administering to the mammal an anti-acyclovir-

resistant herpes effective amount of a peptide derivative of formula 1



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wherein A is phenylacetyl, phenylpropionyl, (4-aminophenyl)propionyl, (4-fluorophenyl)propionyl, (4-hydroxyphenyl)propionyl, (4-methoxyphenyl)propionyl, 2-(phenylmethyl)-3-phenylpropionyl, 2-((4-fluorophenyl)methyl)-3-(4-fluorophenyl)propionyl, 2-((4-methoxyphenyl)methyl)-3-(4-methoxyphenyl)propionyl or benzylaminocarbonyl; B is (N-Me)-Val or (N-Me)-Ile; or A and B taken together form a saturated alkylaminocarbonyl selected from the group of butylaminocarbonyl, 1-methylethylaminocarbonyl, 1-methylpropylaminocarbonyl, 1-ethylpropylaminocarbonyl, 1,1-dimethylethylaminocarbonyl, 1-ethylbutylaminocarbonyl, 1-propylbutylaminocarbonyl, 1-ethylpentylaminocarbonyl, 1-butylpentylaminocarbonyl, 1-ethylbutylaminocarbonyl, 2-ethylpentylaminocarbonyl, 1-methyl-1-propylbutylaminocarbonyl, 1-ethyl-1-propylbutylaminocarbonyl, 1,1-dipropylbutylaminocarbonyl, (1-propylcyclopentyl)aminocarbonyl and (1-propylcyclohexyl)aminocarbonyl; D is Val, Ile or Tbg; R¹ is 1-methylethyl, 1,1-dimethylethyl, 1-methylpropyl, 1,1-dimethylpropyl, 2,2-dimethylpropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-methylcyclopentyl, NR⁴R⁵ wherein R⁴ is hydrogen or lower alkyl and R⁵ is lower alkyl, or R⁴ and R⁵ together with the nitrogen atom to which they are attached form a pyrrolidino, piperidino, morpholino or 4-methylpiperazino; R² is hydrogen and R³ is methyl, ethyl, 1-methylethyl, 1,1-dimethylethyl, propyl, 2-propenyl or benzyl, and the carbon atom bearing R² and R³

has the (R)-configuration, or R^2 and R^3 each independently is methyl or ethyl, or R^2 and R^3 together with the carbon atom to which they are attached form a cyclobutyl, cyclopentyl or cyclohexyl; and E is NHR^6 wherein R^6 is 2-methylpropyl, 2,2-dimethylpropyl, 1(R),2,2-trimethylpropyl, 1,1,2,2-tetramethylpropyl, 1(R)-ethyl-2,2-dimethylpropyl, 2-(R,S)-methylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1(R),2,2-trimethylbutyl, 1(R),3,3-trimethylbutyl, 2-ethylbutyl, 2,2-diethylbutyl, 2-ethyl-1(R)-methylbutyl, 2-ethyl-2-methylbutyl, 1(R)-ethyl-3,3-dimethylbutyl, 2,2-dimethylpentyl, *cis*- or *trans*-2-methylcyclohexyl, 2,2-dimethylcyclohexyl or cyclohexylmethyl; or E is $NHCH(R^7)-Z$ wherein the carbon atom bearing R^7 has the (S)-configuration, R^7 is 1,1-dimethylethyl, 1-methylpropyl, 2-methylpropyl, 2,2-dimethylpropyl or cyclohexylmethyl and Z is CH_2OH , $C(O)OH$, $C(O)NH_2$ or $C(O)OR^8$ wherein R^8 is methyl, ethyl or propyl; or a therapeutically acceptable salt thereof.

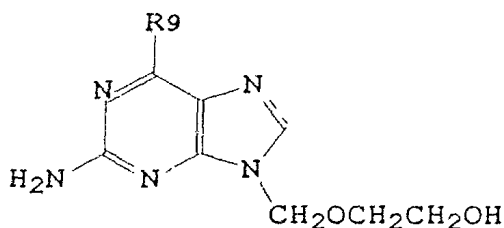
A preferred method of this invention for treating acyclovir-resistant herpes simplex infections comprises administering a peptide of formula 1 wherein A is phenylpropionyl, 2-(phenylmethyl)-3-phenylpropionyl or benzylamino-carbonyl; B is (R-Me)Val; D is Tbg; R^1 is 1-methylethyl, 1,1-dimethylethyl, 1-methylpropyl, 1,1-dimethylpropyl, 2,2-dimethylpropyl, cyclobutyl, cyclopentyl, cyclohexyl or 1-methylcyclopentyl; R^2 is hydrogen and R^3 is methyl, ethyl, 1-methylethyl, propyl or benzyl, and the carbon atom bearing R^2 and R^3 has the (R)-configuration, or R^2 and R^3 each independently is

methyl or ethyl, or R^2 and R^3 together with the carbon atom to which they are attached form a cyclobutyl, cyclopentyl or cyclohexyl; and E is NHR^6 wherein R^6 is 2,2-dimethylpropyl, 1(R),2,2-trimethylpropyl, 1(R)-ethyl-2,2-dimethylpropyl, 2,2-dimethylbutyl or 1(R)-ethyl-3,3-dimethylbutyl or E is $NHCH(R^7)-Z$ wherein the carbon atom bearing R^7 has the (S)-configuration, R^7 is 2,2-dimethylpropyl and Z is CH_2OH , $C(O)OH$, $C(O)NH_2$ or $C(O)OR^8$ wherein R^8 is methyl, ethyl or propyl; or a therapeutically acceptable salt thereof.

Another preferred method for treating acyclovir-resistant herpes simplex infections comprises administering a peptide derivative of formula 1 wherein A and B together form a saturated alkylaminocarbonyl selected from the group consisting of 1-ethylpropylaminocarbonyl, 1-ethylbutylaminocarbonyl, 1-propylbutylaminocarbonyl, 2-ethylpentylaminocarbonyl, 1-methyl-1-propylbutylaminocarbonyl, 1-ethyl-1-propylbutylaminocarbonyl, 1,1-dipropylbutylaminocarbonyl and (1-propylcyclopentyl)aminocarbonyl; and D, R^1 , R^2 , R^3 and E are as defined in the last instance; or a therapeutically acceptable salt thereof.

Another aspect of this invention involves a method of treating an acyclovir-resistant herpes viral infection in a mammal by administering thereto an anti-acyclovir-resistant herpes effective amount of a combination of the peptide derivative of formula 1, or a therapeutically acceptable salt thereof, and an antiviral nucleoside analog, or a therapeutically acceptable salt thereof.

The antiviral nucleoside analog employed in the combination is one which is enzymatically convertible (in vivo) to a viral DNA polymerase inhibitor of, and/or an alternative substrate for, a herpes DNA polymerase. The antiviral nucleoside analog can be selected from known nucleoside analogs. Preferred nucleoside analogs of the invention include acyclovir and its analogs; for example, the compounds of formula 2



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wherein R⁹ is hydrogen, hydroxy or amino, or a therapeutically acceptable salt thereof. (Formula 2 wherein R⁹ is hydroxy represents acyclovir.)

Other preferred antiviral nucleoside analogs for use according to the present invention include vidarabine, idoxuridine, trifluridine, ganciclovir, edoxudine, brovavir, fiacitabine, penciclovir, famciclovir and rociclovir.

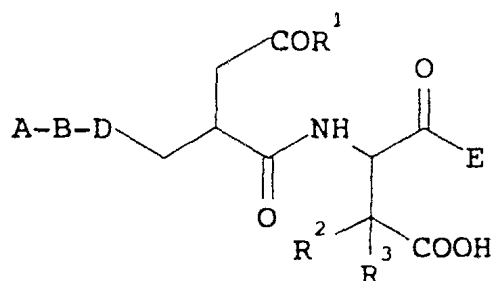
Description of the Drawings

Figures 1 and 2 are graphic representations of results obtained by applying the isobole method for demonstrating synergy in a study involving the activity of combinations of acyclovir and a peptide derivative of formula 1 against acyclovir-resistant herpes simplex viruses.



Details of the InventionGENERAL

Alternatively, formula 1 can be illustrated
5 as:



The term "residue" with reference to an amino
10 acid or amino acid derivative means a radical
derived from the corresponding α -amino acid by
eliminating the hydroxyl of the carboxy group and
one hydrogen of the α -amino group.

15 In general, the abbreviations used herein for
designating the amino acids and the protective
groups are based on recommendations of the IUPAC-
IUB Commision of Biochemical Nomenclature, see
European Journal of Biochemistry 138, 9 (1984).
20 For instance, Val, Ile, Asp, and Leu represent the
residues of L-valine, L-isoleucine, L-aspartic
acid and L-leucine, respectively.

The asymmetric carbon atoms residing in the
25 principal linear axis (i.e. the backbone) of the
peptide derivatives of formula 1, exclusive of the
terminal groups A and Z (of E) but including the
carbon atom bearing "R⁷" when E is NHCH(R⁷)-Z as
defined herein, have an S configuration. An
30 exception occurs, however, for the carbon atom

bearing the $\text{CH}_2\text{C}(\text{O})\text{R}^1$ side chain wherein R^1 is a lower alkyl or lower cycloalkyl as defined herein. For the latter exception, the carbon atom has the R configuration.

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Asymmetric carbon atoms residing in the side chain of an amino acid or derived amino acid residue, in the terminal group A, and in the terminal group E when E represents NHR^6 as defined
10 herein, may have the S or R configuration.

The symbols "Me", "Et", "Pr" and "Bu" represent the alkyl radicals methyl, ethyl, propyl and butyl, respectively.

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The symbols " MeEt_2C " and " EtPr_2C " for example represent the radicals 1-ethyl-1-methylpropyl and 1-ethyl-1-propylbutyl, respectively.

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The symbol "Tbg" represents the amino acid residue of (S)-2-amino-3,3-dimethylbutanoic acid. " γMeLeu " represents the amino acid residue of (S)-2-amino-4,4-dimethylpentanoic acid. The symbol " $\gamma\text{MeLeucinol}$ " represents (S)-2-amino-4,4-
25 dimethylpentanol with one hydrogen removed from the α -amino group.

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Other symbols used herein are: (N-Me)Val for the residue of (S)-3-methyl-2-(methylamino)-butanoic acid; (N-Me)Ile for the residue of (S)-3-methyl-2-(methylamino)pentanoic acid; (N-Me)₃Tbg for the residue of (S)-2-(methylamino)-3,3-dimethyl butanoic acid; Asp(cyBu) for the residue of (S)- α -amino-1-carboxycyclobutaneacetic acid; and Asp(cyPn) for the residue of (S)- α -amino-1-
35 carboxycyclopentaneacetic acid.

The term "lower alkyl" as used herein, either alone or in combination with another radical, means straight chain alkyl radicals containing one to six carbon atoms and branched chain alkyl radicals containing three to six carbon atoms and includes methyl, ethyl, propyl, butyl, hexyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl and 1,1-dimethylethyl.

The term "1-(lower alkyl)-(lower cycloalkyl)" as used herein means a lower cycloalkyl radical bearing a lower alkyl substituent at position 1; for example, 1-ethylcyclopropyl, 1-propylcyclopentyl and 1-propylcyclohexyl.

The term "lower cycloalkyl" as used herein, either alone or in combination with another radical, means saturated cyclic hydrocarbon radicals containing from three to six carbon atoms and includes cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The term "pharmaceutically acceptable carrier" as use herein means a non-toxic, generally inert vehicle for the active ingredient which does not adversely affect the ingredient.

The term "physiologically acceptable carrier" as used herein means an acceptable cosmetic vehicle of one or more non-toxic excipients which do not react with or reduce the effectiveness of the active ingredient contained therein.

The term "effective amount" means a predetermined antiviral amount of the antiviral

agent, i.e. an amount of the agent sufficient to be effective against the viral organisms in vivo.

5 The term "coupling agent" as used herein means an agent capable of effecting the dehydrative coupling of an amino acid or peptide free carboxy group with a free amino group of another amino acid or peptide to form an amide bond between the reactants. Similarly, such
10 agents can effect the coupling of an acid and an alcohol to form corresponding esters. The agents promote or facilitate the dehydrative coupling by activating the carboxy group. Descriptions of such coupling agents and activated groups are
15 included in general text books of peptide chemistry; for instance, E. Schröder and K.L. Lübke, "The Peptides", Vol. 1, Academic Press, New York, N.Y., 1965, pp 2-128, and K.D. Kopple, "Peptides and Amino acids", W.A. Benjamin, Inc.,
20 New York, N.Y., 1966, pp 33-51. Examples of coupling agents are diphenylphosphoryl azide, 1,1'-carbonyldiimidazole, dicyclohexylcarbodiimide, N-hydroxysuccinimide, or 1-hydroxybenzotriazole in the presence of dicyclohexylcarbodiimide. A very practical and useful
25 coupling agent is (benzotriazol-1-yloxy)-tris-(dimethylamino)phosphonium hexafluorophosphate, described by B. Castro et al., Tetrahedron Letters, 1219 (1975), see also D. Hudson, J. Org.
30 Chem., 53, 617 (1988), either by itself or in the presence of 1-hydroxybenzotriazole. Still another very practical and useful coupling agent is the commercially available 2-(1H-benzotriazol-1-yl)-N, N, N', N'-tetramethyluronium tetrafluoroborate.

The antiviral nucleoside analogs, and their therapeutically acceptable salts, for use according to the present invention are a well known class of compounds. As noted above, the members of this class are characterized by the manner in which they mediate an antiviral effect against herpes viruses, i.e. by in vivo inhibition of viral DNA polymerase. Important members of this class are acyclovir and its analogs which are described by H.J. Schaeffer in US patent 4,199,574, issued April 22, 1980; see also H.J. Schaeffer et al., Nature (London), 272, 583 (1978) and T.A. Krenitsk et al., Proc. Natl. Acad. Sci. USA, 81, 3209 (1984). The compound of formula 2 wherein R⁹ is hydroxy is "acyclovir", also known by its chemical name, 9-[(2-hydroxyethoxy)methyl]guanine. The compound of formula 2 wherein R⁹ is hydrogen has the names 6-deoxyacyclovir and 2-amino-9-[(2-hydroxyethoxy)methyl]adenine; and the compound of formula 2 wherein R⁹ is amino has the chemical name, 2,6-diamino-9-[(2-hydroxyethoxy)methyl]purine.

It is to be understood that the compound of formula 2 in which R⁹ is hydroxy can exist in its tautomeric form, i.e. 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one, and that the compound can be a mixture of the two tautomeric forms, the percentage of each tautomer in the mixture being dependent on the physical environment of the compound. Tautomeric forms also are possible for the other antiviral nucleoside analogs having an enolizable carbonyl.

Other antiviral nucleosides contemplated for use according to the present invention include vidarabine (9- β -D-arabinofuranosyladenine monohydrate), see R.J. Whitley et al., N. Engl. J. Med., 307, 971 (1982); idoxudine (2'-deoxy-5-iodouridine), see W.H. Prusoff, Biochim. Biophys. Acta, 32, 295 (1959); trifluridine [2'-deoxy-5-(trifluoromethyl)uridine], see C. Heidelberger, US patent 3,201,387, issued August 17, 1965; ganciclovir 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine, see J.P. Verheyden and J.C. Martin, US patent 4,355,032, issued October 19, 1982; edoxudine (5-ethyl-2'-deoxyuridine), see K.K. Gauri, US patent 3,553,192, issued January 5, 1971; brovavir [(E)-5-(2-bromovinyl)-2'-deoxyuridine], see Y. Benoît et al., Eur. J. Pediatrics, 143, 198 (1985); fiacitabine (2'-fluoro-deoxy-5-iodouridine), see B. Leyland-Jones et al., J. Infect. Dis., 154, 430 (1986), penciclovir (9-[4-hydroxy-3-(hydroxy-methyl)-butyl]guanine, see S.E. Fowler et al., Br. J. Clin. Pharmacol., 28, 236P (1989); famciclovir (9-[4-acetoxy-3-(acetoxymethyl)butyl]adenine, see R.A.V. Hodge et al., Antimicrob. Agents Chemotherap., 33, 1765 (1989); and rociclovir (9-[(1,3-diisopropoxy-2-propoxy)methyl]adenine, see E. Winklemann et al., Arzneim.-Forsch., 38, 1545 (1988).

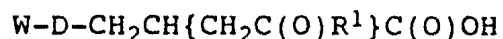
Process for Preparing the Peptide Derivatives of Formula 1

The peptide derivatives of formula 1 can be prepared by processes which incorporate therein methods commonly used in peptide synthesis such as the classical solution coupling of amino acid

residues and/or peptide fragments. Such methods are described, for example, by E. Schröder and K. Lübke, cited above, in the textbook series, "The Peptides: Analysis, Synthesis, Biology", E. Gross et al., Eds., Academic Press, New York, N.Y., 1979-1987, Volumes 1 to 8, and by J.M. Stewart and J.D. Young in "Solid Phase Peptide Synthesis", 2nd ed., Pierce Chem. Co., Rockford, IL, USA, 1984.

A common feature of the aforementioned processes for the peptide derivatives is the protection of the reactive side chain groups of the various amino acid residues or derived amino acid residues (or, if required, non-peptidic fragments of the peptide derivative) with suitable protective groups which will prevent a chemical reaction from occurring at that site until the protective group is ultimately removed. Also common is the protection of an α -amino group on an amino acid or a fragment while that entity reacts at the carboxy group, followed by the selective removal of the α -amino protective group to allow subsequent reaction to take place at that location. Another common feature is the initial protection of the C-terminal carboxyl of the amino acid residue or peptide fragment, if present, which is to become the C-terminal function of the peptide derivative, with a suitable protective group which will prevent a chemical reaction from occurring at that site until the protective group is removed after the desired sequence of the peptide derivative has been assembled.

A key intermediate for the peptides of formula 1 is the intermediate of formula 2



2

wherein W is an α -aminoprotective group, e.g. tert-butyloxycarbonyl (Boc), benzyloxycarbonyl (Z)
5 or fluoren-9-ylmethoxycarbonyl (Fmoc), and D and R^1 are as defined herein. The compound can be prepared by a Michael addition of an allyl ester of the formula $W-D-CH_2C(O)OCH_2CH=CH_2$ to a fumaroyl derivative of the formula (lower alkyl)-
10 $OC(O)CH=CHC(O)R^1$ to give the Michael adduct of formula $W-D-CH\{C(O)OCH_2CH=CH_2\}CH\{CH_2C(O)R^1\}C(O)O-$ (lower alkyl).

Thereafter, treatment of the latter compound
15 with tetrakis(triphenylphosphine) palladium and triphenylphosphine in the presence of pyrrolidine, according to the method of R. Déziel, Tetrahedron Letters, 28, 4371 (1987), effects hydrolysis and subsequent decarboxylation of the allyl ester to
20 give the corresponding alkyl ester of the key intermediate. Hydrolysis of the latter ester in the presence of a base, e.g. sodium hydroxide or lithium hydroxide, gives the key intermediate as a diastereoisomeric mixture.

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Alternatively, the key intermediate of formula 2 wherein W and D are as defined in the last instance and R^1 is NR^4R^5 wherein R^4 and R^5 each is lower alkyl or R^4 and R^5 together with the
30 nitrogen atom to which they are attached form a pyrrolidino, piperidino, morpholino or 4-methylpiperazino can be prepared as follows: The previously mentioned allyl ester of formula $W-D-CH_2C(O)OCH_2CH=CH_2$ wherein W and D are as defined
35 herein is reacted according to the conditions of the Michael reaction with a fumaroyl derivative of

formula $\text{BzlOC(O)CH=CHC(O)OBzl}$ {(E)-2-butenedioic acid dibenzyl ester} to give the corresponding Michael adduct of formula $\text{W-D-(RS)-CH}\{\text{CH}\{\text{C(O)OBzl}\}\text{CH}_2\text{C(O)OBzl}\}\text{C(O)-OCH}_2\text{CH=CH}_2$.

- 5 Treatment of the latter adduct with tetrakis(triphenylphosphine) palladium and triphenylphosphine in the presence of pyrrolidine (Déziel, vide supra) gives the corresponding dibenzyl ester of formula $\text{W-D-CH}_2\text{-(R,S)-CH}\{\text{CH}_2\text{C(O)OBzl}\}\text{C(O)OBzl}$.
- 10 Subsequent hydrogenolysis in the presence of palladium hydroxide on carbon gives the corresponding dicarboxylic acid which is transformed into the corresponding anhydride by heating with excess acetic anhydride. Reaction of
- 15 the anhydride with the appropriate secondary amine in the presence of pyridine gives a mixture of regioisomers with a preponderance of the desired isomer of formula $\text{W-D-CH}_2\text{-(RS)-CH}\{\text{CH}_2\text{C(O)NR}^4\text{R}^5\}\text{C(O)-OH}$ in which W, D and NR^4R^5 are as defined
- 20 herein. The latter product is converted to its benzyl ester and the resulting mixture of regioisomers is separated by high performance liquid chromatography to give the desired isomer as the preponderant product. Subsequent
- 25 hydrogenation of the latter product in the presence of palladium hydroxide on carbon yields the key intermediate of formula 2 wherein W and D are as defined herein and R^1 is NR^4R^5 as defined herein.

- 30 In general, therefore, a peptide of formula 1 can be prepared by the stepwise coupling, in the order of the sequence of the peptide, of the appropriate amino acid or derived amino acid residues, and non-peptidic fragments of the
- 35 peptide (such as the key intermediates), which if required are suitably protected, and eliminating

all protecting groups, if present, at the completion of the stepwise coupling to obtain the peptide of formula 1. More specific processes are illustrated in the examples hereinafter.

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The peptide of formula 1 of this invention can be obtained in the form of a therapeutically acceptable salt. In the instance where a particular peptide has a residue which functions as a base, examples of such salts of the base are those with organic acids, e.g. acetic, lactic, succinic, methanesulfonic or p-toluenesulfonic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and also salts with inorganic acids such as hydrohalic acids, e.g. hydrochloric acid, or sulfuric acid, or phosphoric acid. If desired, a particular acid addition salt is converted into another acid addition salt, such as a non-toxic, pharmaceutically acceptable salt, by treatment with the appropriate ion exchange resin in the manner described by R.A. Boissonnas et al., *Helv. Chim. Acta*, 43, 1849 (1960).

In the instance where a particular peptide has one or more free carboxy groups, examples of such salts of the carboxy group are those with the sodium, potassium or calcium cations, or with organic bases, for example, triethylamine or N-methylmorpholine.

Antiherpes Activity

The antiviral activity of the peptide derivatives of formula 1 can be demonstrated by biochemical, microbiological and biological

procedures showing the inhibitory effect of the compounds on the replication of the acyclovir-resistant herpes simplex viruses, types 1 and 2 (HSV-1 and HSV-2).

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A method for demonstrating the inhibitory effect of the peptide derivatives of formula 1 on viral replication is the cell culture technique; see, for example, T. Spector et al., Proc. Natl. Acad. Sci. USA, 82, 4254 (1985).

10

The therapeutic effect of the peptide derivatives can be demonstrated in laboratory animals, for instance, by using an assay based on the murine model of herpes simplex virus-induced ocular disease for antiviral drug testing, described by C.R. Brandt et al., J. Virol. Meth., 36, 209 (1992).

15

When a peptide derivative of this invention, or one of its therapeutically acceptable salts, is employed as an antiviral agent, it is administered topically or systemically to warm-blooded animals, e.g. humans, pigs or horses, in a vehicle comprising one or more pharmaceutically acceptable carriers, the proportion of which is determined by the solubility and chemical nature of the peptide derivative, chosen route of administration and standard biological practice. For topical administration, the peptide derivative can be formulated in pharmaceutically accepted vehicles containing 0.1 to 5 percent, preferably 0.5 to 2 percent, of the active agent. Such formulations can be in the form of a solution, cream or lotion.

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For systemic administration, the peptide derivative of formula 1 is administered orally or by either intravenous, subcutaneous or intramuscular injection, in compositions with pharmaceutically acceptable vehicles or carriers. For administration by injection, it is preferred to use the peptide in solution in a sterile aqueous vehicle which may also contain other solutes such as buffers or preservatives as well as sufficient quantities of pharmaceutically acceptable salts or of glucose to make the solution isotonic.

Suitable vehicles or carriers for the above noted formulations are described in standard pharmaceutical texts, e.g. in "Remington's Pharmaceutical Sciences", 18th ed, Mack Publishing Company, Easton, Penn., 1990.

The dosage of the peptide derivative will vary with the form of administration and the particular active agent chosen. Furthermore, it will vary with the particular host under treatment. Generally, treatment is initiated with small increments until the optimum effect under the circumstances is reached. In general, the peptide derivative is most desirably administered at a concentration level that will generally afford antivirally effective results without causing any harmful or deleterious side effects.

With reference to topical application, the peptide derivative is administered directly in a suitable topical formulation to the infected area of the body e.g. the skin, the eye, or part of the oral or genital cavity, in an amount sufficient to cover the infected area. The treatment should be

repeated, for example, every four to six hours until lesions heal.

5 With reference to systemic administration, the peptide derivative of formula 1 is administered at a dosage of 1.0 mg to 10 mg per kilogram of body weight per day, although the aforementioned variations will occur. However, a dosage level that is in the range of from about
10 1.0 mg to 5 mg per kilogram of body weight per day is most desirably employed in order to achieve effective results.

15 Although the formulation disclosed hereinabove are indicated to be effective and relatively safe medications for treating herpes viral infections, the possible concurrent administration of these formulations with other
20 antiviral medications or agents to obtain beneficial results is not excluded. Such antiviral medications or agents include the previously noted antiviral nucleosides, antiviral surface active agents or antiviral interferons such as those disclosed by S.S. Asculai and F.
25 Rapp in U.S. patent 4,507,281, March 26, 1985.

30 More specifically with respect to treating acyclovir-resistant herpes viral infections by concurrent administration, it has been found that the antiherpes activity of an antiviral nucleoside
35 analogs can be enhanced synergistically, without the concomitant enhancement of toxic effects, by combining the same with a peptide derivative of formula 1. Accordingly, there is provided herewith a pharmaceutical composition for treating
acyclovir-resistant herpes infections in a mammal

comprising a pharmaceutically or veterinarily acceptable carrier, and an effective amount of the combination of an antiviral nucleoside analog or a therapeutically acceptable salt thereof, and a peptide derivative of formula 1 or a therapeutically acceptable salt thereof.

The term "synergistic effect" when used in relation to the antiviral or antiherpes activity of the above defined combination of the nucleoside analog and peptide derivative of formula 1 means an antiviral or antiherpes effect which is greater than the predictive additive effect of the two individual components of the combination.

When utilizing the combination of this invention for treating herpes acyclovir-resistant infections, the combination is administered to warm blooded animals, e.g. humans, pigs or horses, in a vehicle comprising one or more pharmaceutically acceptable carriers, the proportion of which is determined by the solubility and chemical nature of the nucleoside analog and the peptide derivative of formula 1, chosen route of administration, standard biological practice, and by the relative amounts of the two active ingredients to provide a synergistic antiviral effect. In a preferred manner, the combination is administered topically. For example, the two active agents (i.e. the antiviral nucleoside analog and the peptide derivative of formula 1, or their therapeutically acceptable salts) can be formulated in the form of solutions, emulsions, creams, or lotions in pharmaceutically acceptable vehicles. Such formulation can contain 0.01 to 1.0 percent by

weight of the nucleoside analog, or a therapeutically acceptable salt thereof, and about 0.05 to 1 percent by weight of the peptide derivative of formula 1, or a therapeutically acceptable salt thereof.

In any event, the two active agents are present in the pharmaceutical composition in amounts to provide a synergistic antiherpes effect.

The following examples illustrate further this invention. Temperatures are given in degrees Celsius. Solution percentages or ratios express a volume to volume relationship, unless stated otherwise. Nuclear magnetic resonance spectra were recorded on a Bruker 200 MHz or 400 MHz spectrometer (a 400 MHz spectrum being noted in the preamble); the chemical shifts (δ) are reported in parts per million. Abbreviations used in the examples include Boc: tert-butyloxycarbonyl; Bu: butyl; Bzl: benzyl; DMF: dimethylformamide; Et: ethyl; EtOH: ethanol; EtOAc: ethyl acetate; Et₂O: diethyl ether; Me: methyl; MeOH: methanol; Pr: propyl; TLC: thin layer chromatography; THF: tetrahydrofuran.

Example 1

General Procedure for Coupling Reactions

{See also R. Knorr et al., Tetrahedron Letters, 30, 1927 (1989).}

The first reactant, i.e. a free amine (or its hydrochloride salt), is dissolved in CH₂Cl₂ or

acetonitrile and the solution is cooled to 4°. Under a nitrogen atmosphere, four equivalents of N-methylmorpholine is added to the stirred solution. After 20 min., one equivalent of the second reactant, i.e. a free carboxylic acid, and 1.05 equivalent of the coupling agent are added. (Practical and efficient coupling reagents for this purpose are (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate or preferably 2-(1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate. The reaction is monitored by TLC. After completion of the reaction, the CH₂Cl₂ (or acetonitrile) is evaporated under reduced pressure. The residue is dissolved in EtOAc. The solution is washed successively with 1N aqueous citric acid, 10% aqueous Na₂CO₃ and brine. The organic phase is dried (MgSO₄), filtered and concentrated to dryness under reduced pressure. The residue is purified on silica gel (SiO₂) according to Still's flash chromatography technique {W.C. Still et al., J. Org. Chem., 43, 2923 (1978)}.

Example 2

Preparation of the Intermediate H-Asp(cyPn)(Bzl)-NH-(S)-CH(CH₂CMe₃)CH₂OBzl

(a) (S)-α-Azido-1-{(phenylmethoxy)carbonyl}-cyclopentaneacetic acid: This compound was prepared from 2-oxospiro[4.4]nonane-1,3-dione, described by M.N. Aboul-Enein et al., Pharm. Acta Helv., 55, 50 (1980), according to the asymmetric azidation method utilizing the Evan's auxiliary, see D.A. Evans et al., J. Amer. Chem. Soc., 112, 4011 (1990).

More explicitly, a 1.6 M hexane solution of butyllithium (469 ml, 750 mmol) was added dropwise under an argon atmosphere to a solution of the chiral auxiliary, 4(S)-(1-methylethyl)-2-oxazolidinone, {96.8 g, 750 mmol, described by L. N. Pridgen and J. Prol., J. Org. Chem., 54, 3231 (1989)} in dry THF at -40°. The mixture was stirred at -40° for 30 min and then cooled to -78°. 2-Oxospiro[4.4]nonane-1,3-dione was added dropwise to the cooled mixture. The mixture then was stirred at 0° for 1 h. Thereafter, a 20% (w/v) aqueous solution of citric acid (600 mL) was added to the mixture. The organic phase was separated and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with brine, dried (MgSO₄) and concentrated under reduced pressure to give 3-{2-(1-carboxycyclopentyl)-1-oxoethyl}-4(S)-(1-methylethyl)-2-oxazolidinone as a pink solid (300 g).

The latter solid (ca 750 mmol) was dissolved in acetonitrile (1 L). Benzyl bromide (128.3 g, 89.2 mL, 750 mmol) and 1,8-diazabicyclo[5.4.0]-undec-7-ene (114 g, 112 mL, 750 mmol) were added to the solution. The mixture was stirred under argon for 16 h. The volatiles were removed under reduced pressure. The residue was dissolved in H₂O/EtOAc. The organic phase was separated, washed with a 10% (w/v) aqueous solution of citric acid, brine, dried (MgSO₄) and concentrated to dryness under reduced pressure to give an oil. Crystallization of the oil from hexane/EtOAc gave the corresponding benzyl ester as a white solid (204 g, 73%).

A solution of the latter compound (70 g, 187 mmol) in dry THF (200 mL) was cooled to -78° . A 0.66 M THF solution of potassium 1,1,1,3,3,3-hexamethyldisilazane (286 mL, 189 mmol) containing 6% (w/v) cumene was added over a period of 15 min to the cooled solution. The mixture was stirred at -78° for 45 min. A solution of 2,4,6-triisopropyl-benzenesulfonyl azide (67 g, 216 mmol) in dry THF (100 mL) was added in one portion to the cold mixture, followed two minutes later by the addition of glacial acetic acid (50 mL, 860 mmol). The mixture was warmed and stirred at $35-45^{\circ}$ for 1 h. The volatiles were removed under reduced pressure. The yellow residue was triturated with hexane/EtOH (4:1, 1.7 L). The resulting white solid was collected on a filter. The filtrate was mixed with SiO_2 (230-240 mesh). Volatiles were removed under reduced pressure and the residual solid was dried at 35° under reduced pressure to remove cumene. The residual solid then was placed on a column of SiO_2 . Elution of residual solid and SiO_2 with hexane-EtOAc, 9:1 and concentration of the eluent gave 3-{{2(S)-azido-1-oxo-2-{1-((phenylmethoxy)carbonyl)cyclopentyl}-ethyl}-4(S)-(1-methylethyl)-2-oxazolidinone (66 g, 86%).

A solution of the latter compound (13.42 g, 32.4 mmol) in THF/ H_2O (3:1, 608 mL) was cooled to 0° . Hydrogen peroxide/ H_2O (3:7, 16.3 mL, 518 mmol of H_2O_2) was added to the cooled solution; followed by the addition of $\text{LiOH}\cdot\text{H}_2\text{O}$ (2.86 g, 68.2 mmol). The mixture was stirred at 0° for 45 min and then quenched with a 10% (w/v) aqueous solution of sodium sulfite (400 mL). After NaHCO_3 (1.93 g) had been added, the mixture was

concentrated under reduced pressure. The chiral auxiliary was recovered by continuous extraction (aqueous NaHCO₃/chloroform) for 20 h. Thereafter, the aqueous phase was cooled to 0° rendered acidic by the addition of concentrated HCl and then extracted with EtOAc. The extract was washed with brine, dried (MgSO₄) and concentrated under reduced pressure to give the desired compound as a white solid (8.2 g, 84%). The ¹H NMR (CDCl₃) of the compound showed: δ 1.6-1.8 (m, 5H), 1.95-2.05 (m, 2H), 2.20-2.30 (m, 1H), 4.55 (s, 1H), 5.12 (s, 2H) and 7.4 (m, 5H).

The compound is used in section (c) of this example.

(b) NH₂-(S)-CH(CH₂CMe₃)CH₂OBzl: H-γMeLeu-OH was reduced with LiBH₄/Me₃SiCl according to the method of A. Giannis and K. Sandhoff, Angew. Chem. Int. Ed. Engl., 28, 218 (1989) to give the aminoalcohol NH₂-(S)-CH(CH₂CMe₃)CH₂OH. A mixture of the latter compound (812 mg, 6.2 mmol), triethylamine (659 mg, 6.51 mmol) and di-tert-butyl dicarbonate (1.42 g, 6.51 mmol) in dry THF (15 mL) was stirred under a nitrogen atmosphere at 4° for 15 min and then at room temperature for 4 h. The THF was evaporated under reduced pressure. The residue was dissolved in EtOAc. The solution was washed with 10% aqueous citric acid, 5% aqueous NaHCO₃ and brine. The organic phase was dried (MgSO₄) and concentrated to dryness under reduced pressure. The residue was purified by flash chromatography (SiO₂, eluent: hexane-EtOAc, 2:1) to give Boc-NH-(S)-CH(CH₂CMe₃)-CH₂OH (1.23 g, 86%).

Tetrabutylammonium bisulfate (106 mg) and 50% aqueous NaOH (3 mL) were added successively to a

solution of Boc-NH-(S)-CH(CH₂CMe₃)CH₂OH (1.23 g, 5.35 mmol) in benzyl chloride (13 mL). The resulting mixture was stirred at 35-40° for 90 min, diluted with EtOAc, and washed with H₂O and brine. The organic phase was dried (MgSO₄) and concentrated to dryness under reduced pressure. The residue was dissolved in hexane. The solution was poured onto a column of SiO₂. The column was eluted with hexane to remove benzyl chloride, and then with hexane-EtOAc (2:1) to give Boc-NH-(S)-CH(CH₂CMe₃)CH₂OBzl. The ¹H NMR (CDCl₃) of the latter compound showed δ 0.95 (s, 9H), 1.42 (s, 9H), 1.30-1.55 (m, 2H), 3.42 (d, J = 4 Hz, 2H), 3.88 (broad, 1H), 4.54 (m, 3H), 7.23-7.4 (m, 5H). The latter compound (1.28 g, 3.99 mmol) was dissolved in 6 N HCl/dioxane (10 mL). The solution was stirred under a nitrogen atmosphere at 4° for 45 min. Evaporation of the solvent gave the hydrogen chloride salt of the desired compound (1.05 g). The compound is used without further purification in the next section of this example.

(c) The title compound of this example: By following the coupling procedure of example 1 and using the hydrogen chloride salt of NH₂-(S)-CH(CH₂CMe₃)CH₂OBzl of the preceding section as the first reactant and (S)-α-azido-1-((phenylmethoxy)-carbonyl)cyclopentaneacetic acid of section (a) of this example as the second reactant, N-((S)-1-benzyloxymethyl-3,3-dimethylbutyl)-(S)-α-azido-1-((phenylmethoxy)carbonyl) cyclopentaneacetamide was obtained. Reduction of the latter compound with tin(II) chloride in MeOH according to the method of N. Maiti et al., Tetrahedron Letters, 27, 1423 (1986) gave the title compound of this example. The ¹H NMR (CDCl₃) of the compound showed δ 0.98 (s, 9H), 1.22-2.25 (m, 12H), 3.4 (d, J = 4

Hz, 2H), 3.64 (s, 1H), 4.18 (broad m, 1H), 4.52 (s, 2H), 5.12 (s, 2H), 7.18 (d, J = 7 Hz, 1H), 7.22-7.38 (broad m, 10H).

5

Example 3Preparation of the Intermediate Boc-Tbg-CH₂-(RS)-CH(CH₂C(O)CMe₃)C(O)OH

10 (a) Magnesium salt of monoallyl malonate: A solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (100 g, 0.69 mol) and allyl alcohol (47 mL, 0.69 mol in benzene (800 mL) was heated at reflux for 24 h. The solvent was evaporated under reduced
15 pressure. Distillation of the residue under reduced pressure gave monoallyl malonate (71 g, 71%, bp 123-127°/2.7 mm Hg). The latter ester (71 g, 0.48 mol) was dissolved in dry THF (300 mL). Magnesium ethoxide (28.5 g, 0.245 mol) was added
20 to the solution. The mixture was stirred under argon for 4 h at room temperature (20-22°). The solvent was evaporated under reduced pressure and the residue was triturated with Et₂O to give a tan solid. The solid was ground into fine particles
25 and dried under reduced pressure to give the desired magnesium salt (56 g, 73%). The salt is used in the next section of this example.

(b) Boc-Tbg-CH₂C(O)OCH₂=CH₂: 1,1-Carbonyldiimidazole (12.6 g, 78 mmol) was added to
30 a solution of Boc-Tbg-OH (15 g, 64 mmol) in dry acetonitrile (150 mL). The mixture was stirred under argon for 2 h at room temperature. The magnesium salt of monoallyl malonate (24 g, 78 mmol) and 4-(N,N-dimethylamino)pyridine (100 mg)
35 were added to the mixture. The mixture was heated at reflux for 1 h and then stirred at room

temperature for 18 h. Thereafter, the mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (300 mL). The solution was washed with 10% aqueous citric acid (2 x 100 mL) and brine (2 x 100 mL), dried (MgSO₄) and evaporated to dryness. The residue was purified by flash chromatography (SiO₂, eluent: hexane-EtOAc, 9:1) to give the desired allyl ester (19.4 g, 96%) as a brown oil which crystallized on standing. ¹H NMR (CDCl₃), note that this compound exists as a mixture of keto-enol tautomers in a 3:1 ratio in chloroform, δ 0.96 (s, 9H, enol form), 1.04 (s, 9H), 1.46 (s, 9H), 3.65 (s, 2H), 3.90 (d, J = 8.5 Hz, 1H, enol form), 4.20 (d, J = 7.5 Hz, 1H), 4.65 (m, 2H), 5.10 (broad d, J = 7.5 Hz, 1H), 5.20-5.40 (m, 2H), 5.80-6.05 (m, 1H), 12 (s, 1H, enol form). The allyl ester is used in section (d) of this example.

(c) (E)-5,5-Dimethyl-4-oxo-2-hexenoic acid ethyl ester: This ethyl ester was prepared according to the method of S. Manfredini et al., Tetrahedron Letters, 29, 3997 (1988). The oily crude product was purified by flash chromatography (SiO₂, eluent: hexane) to give the desired ethyl ester as a yellow oil. ¹H NMR (CDCl₃) δ 1.21 (s, 9H), 1.33 (t, J = 7.5 Hz, 3H), 4.28 (q, J = 7.5 Hz, 2H), 6.78 (d, J = 15.5 Hz, 1H), 7.51 (d, J = 15.5 Hz, 1H). The ethyl ester is used in the next section of this example.

(d) The title compound of this example: Boc-Tbg-CH₂C(O)OCH₂=CH₂ (0.67 g, 2.1 mmol), described in section (b) of this example, was dissolved in anhydrous THF (25 mL) under an argon atmosphere. Sodium hydride (60% oil dispersion, 0.095 g, 2.4 mmol) was added to the solution. The mixture was stirred at room temperature for 30 min. (E)-5,5-

dimethyl-4-oxo-2-hexenoic acid ethyl ester (0.435 g, 2.36 mmol), described in section (c) of this example, was added to the mixture. The reaction mixture was stirred until the reaction was complete as judged by TLC (about 6 h). Thereafter, the mixture was quenched with 10% aqueous citric acid. THF was removed under reduced pressure and the resulting concentrate was extracted with EtOAc (3 x 25 mL). The extract was washed with H₂O, dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO₂, eluent: hexane-EtOAc, 9:1) to give the corresponding Michael reaction adduct (1.06 g, 100%).

The Michael adduct was transformed to Boc-Tbg-CH₂-(RS)-CH(CH₂C(O)CMe₃)C(O)OH as follows: Tetrakis(triphenylphosphine) palladium(0) (0.20 g, 0.18 mmol) and triphenylphosphine (0.060 g, 0.23 mmol) were dissolved in CH₂Cl₂ (10 mL) under an argon atmosphere. Acetonitrile (20 mL) was added and the solution was cooled to 0°. Pyrrolidine (0.28 mL, 2.7 mmol) and then the Michael adduct (1.06 g, 2.1 mmol) were added to the solution. The mixture was allowed to come to room temperature over 1 h and then stirred for 20 h. Thereafter, the reaction mixture was heated at reflux for 1 h under argon to complete the reaction. The solvent was evaporated and the residue was purified by flash chromatography (SiO₂, eluent: hexane-EtOAc, 9:1) to give Boc-Tbg-CH₂-(RS)-CH(CH₂C(O)CMe₃)C(O)OEt (0.77 g, 83%). Thereafter, the latter compound (0.77 g, 1.7 mmol) was dissolved in ethyleneglycol dimethyl ether - H₂O (1:1, 10 mL). Lithium hydroxide monohydrate (0.31 g, 7.4 mmol) was added to the solution. The

5 mixture was stirred at room temperature for 4 h,
rendered acidic with 10% aqueous citric acid (20
mL) and extracted with EtOAc (3x25 mL). The
extract was dried (MgSO₄) and concentrated under
10 reduced pressure to give the title compound of
this example as a tan solid (0.69 g, 80% yield
overall from Boc-Tbg-CH₂C(O)O-CH₂CH=CH₂). The
product was a 55:45 mixture of diastereoisomers
(shown by NMR). ¹H NMR (CDCl₃) δ 0.97 (s, 9H,
10 minor isomer), 0.99 (s, 9H, major isomer), 1.14
(s, 18H), 1.48 (s, 18H), 2.68-3.12 (m, 8H), 3.30
(m, 2H), 4.08 (d, J = 9Hz, 1H, minor isomer), 4.10
(d, J = 9Hz, 1H, major isomer), 5.11 (d, J = 9Hz,
2H).

15

Example 4

Preparation of the Intermediate H-Tbg-
CH₂CH(CH₂C(O)CMe₃)C(O)-Asp(cyPn)(Bzl)-NH-(S)-
20 CH(CH₂C(O)Me₃)CH₂OBzl

By following the coupling procedure of
example 1 and using the title compound of example
2(3.42 g, 7.13 mmol) as the first reactant and the
25 title compound of example 3 (2.50 g, 6.48 mmol) as
the second reactant, flash chromatography (SiO₂,
eluent: hexane-EtOAc, 4:1) of the crude product
gave the corresponding N-Boc derivative of the
title compound (4.64 g, 84%; R_f = 0.21, hexane-
30 EtOAc, 7:3). A solution of the latter derivative
(4.64 g, 5.44 mmol) in 6 N HCl/dioxane (50 mL) was
stirred at room temperature for 1 h and then
concentrated under reduced pressure. The residue
was dissolved in Et₂O. The latter solution was
35 washed with saturated aqueous solution of NaHCO₃
and brine, dried (MgSO₄) and concentrated to give

a yellow oil consisting of two diastereoisomers. The two isomers were separated by flash chromatography (SiO₂, eluent: hexane-EtOAc-MeOH, 5:4.5:0.5). The desired isomer (i.e. the more polar; R_f = 0.18, EtOAc-hexane-MeOH, 7:3:0.5) was obtained as a colourless oil (2.52 g, 52%). The isomer, the title compound of this example, is used without further purification in the next example.

Example 5

Preparation of Boc-(N-Me)Val-Tbq-CH₂(R)-CH(CH₂C(O)CMe₃)C(O)-Asp(cyPn)(Bzl)-NH-(S)-CH(CH₂CMe₃)CH₂OBzl

By following the coupling procedure of example 1 and using the title compound of example 4 (4.45 g, 5.95 mmol) as the first reactant and N-methylvaline (4.41 g, 17.9 mmol) as the second reactant, flash chromatography (SiO₂, eluent: hexane-EtOAc, 7:3) of the crude product gave the title compound of this example (4.02 g, 72% yield). ¹H NMR (CDCl₃) δ 0.87 (d, J = 7 Hz, 6H), 0.90 (s, 18H), 1.10 (s, 9H), 1.48 (s, 9H), 1.5 - 2.0 (m, 10H), 2.30 (m, 1H), 2.5 - 3.1 (m, 5H), 2.80 (s, 3H), 3.30 (m, 2H), 4.0 (d, J = 12.5 Hz, 1H), 4.20 (m, 1H), 4.32 (d, J = 8.5 Hz, 1H), 4.49 (d, J = 4.5 Hz, 2H), 4.64 (d, J = 11 Hz, 1H), 5.18 (d, J = 5 Hz, 2H), 6.78 (broad d, J = 8.5 Hz, 1H), 7.11 (broad d, J = 8.5 Hz, 1H), 7.18 (broad d, J = 11 Hz, 1H), 7.2-7.45 (m, 10H).

Example 6Preparation of PhCH₂CH₂C(O)-(N-Me)Val-Tbg-CH₂-(R)-CH(CH₂C(O)CMe₃)C(O)-Asp(cyPn)-γMeLeucinol

5

A solution of the title compound of example 5 (4.02 g, 4.25 mmol) in 6N HCl/dioxane (30 mL) was stirred at room temperature for 1 h and then concentrated under reduced pressure to give the free N-terminal amino derivative of the title compound of example 5 in the form of its hydrochloride salt. Thereafter, by following the coupling procedure of example 1 and using the latter amino derivative as the first reactant and benzenepropionic acid (2.00 g, 13.3 mmol) as the second reactant, the purification of the crude product by flash chromatography (SiO₂, eluent: hexane-EtOAc, 3:2) gave PhCH₂CH₂C(O)-N-Me-Val-Tbg-CH₂-(R)-CH(CH₂C(O)CMe₃)C(O)-Asp-(cyPn)(OBzl)-NH-(S)-CH(CH₂CMe₃)CH₂OBzl as a white foam (4.00 g, 94%; R_f = 0.35, hexane-EtOAc, 1:1).

The latter compound (4.00 g, 4.03 mmol) was subjected to hydrogenolysis {20% Pd(OH)₂/C (200 mg), 1 atmosphere of H₂, EtOH, 5 h}. After completion of the reaction, the catalyst was removed from the reaction mixture by filtration through a 45 μm membrane. The filtrate was concentrated under reduced pressure to give a clear oil. The oil was dissolved in Et₂O (100 mL). The solution was evaporated to dryness under reduced pressure. The dissolving and evaporating process was repeated whereby a white solid was obtained. The solid was triturated with hexane, filtered and dried under reduced pressure to give the title compound (3.12 g, 95%) ¹H NMR (d₆-DMSO),

400 MHz; note: the compound exists in DMSO as a 50:50 mixture of two rotamers δ 0.71-0.92 (m, 24H), 1.05 (s, 4.5H), 1.06 (s, 4.5H), 1.20-1.78 (m, 10H), 1.93-2.16 (m, 2H), 2.48-2.83 (m, 6H), 2.84 (s, 1.5H), 2.92 (s, 1.5H), 2.96-3.06 (m, 1H), 3.10-3.23 (m, 2H), 3.72-3.81 (m, 1H), 4.09-4.14 (m, 1H), 4.22 (d, 8 Hz, 0.5H), 4.54-4.62 (broad m, 1H), 4.73-4.81 (m, 1.5H), 7.12-7.29 (m, 6H), 7.94 (d, $J = 10$ Hz, 1H), 8.04 (d, $J = 8$ Hz, 0.5H), 8.32 (d, $J = 8.5$ Hz, 0.5H).

By following the procedure of example 6 but replacing benzenepropionic acid with 2-(phenylmethyl)-3-phenylpropionic acid (dibenzylacetic acid), (PhCH₂)₂CHC(O)-(N-Me)Val-Tbg-CH₂-(R)-CH(CH₂C(O)-CMe₃)C(O)-Asp(cyPn)- γ MeLeucinol is obtained.

Example 7

20 Preparation of Et₂CHNHC(O)-Tbg-CH₂-(R)-CH(CH₂C(O)-CMe₃)CO-Asp(cyPn)- γ MeLeucinol

1-Ethylpropyl isocyanate (28 mg, 0.248 mmol) was added to a solution of the title compound of example 4 (23 mg, 0.030 mmol) and triethylamine (6 mg, 0.057 mmol) in anhydrous CH₂Cl₂. The reaction mixture was stirred under an argon atmosphere at 0° for 1 h and then at room temperature for 18 h. TLC (EtOAc-hexane, 1:1) indicated the completion of the reaction. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (SiO₂, eluent: hexane-EtOAc, 6:4) to give the corresponding dibenzyl derivative of the title compound of this example (16 mg). ¹H NMR (400 MHz, CDCl₃) δ 0.9 (t, $J = 7$ Hz, 3H), 0.92 (t, $J = 7$ Hz, 3H), 0.94 (s, 9H), 0.95 (s, 9H),

1.10 (s, 9H), 1.25-1.90 (m, 14H), 2.52 (m, 1H),
2.68 (m, 1H), 2.81 (m, 1H), 2.94-3.07 (m, 2H),
3.27 (dd, J = 7.2, 9 Hz, 1H), 3.36 (dd, J = 5.5,
9 Hz, 1H), 3.46 (m, 1H), 4.07 (d, J = 9 Hz, 1H),
5 4.23 (m, 1H), 4.28 (d, J = 9 Hz, 1H), 4.48 (dd, J
= 10 Hz, 2H), 4.66 (d, J = 10 Hz, 1H), 4.74 (d, J
= 9 Hz, 1H), 5.17 (dd, J = 14 Hz, 2H), 7.10 (d, J
= 8.5 Hz, 1H), 7.2-7.45 (m, 11H).

10 The latter dibenzyl derivative was subjected
to hydrogenolysis (10% palladium on carbon, 1
atmosphere, EtOH) to give the title compound.
Mass spectrum: 703 (M + Na)⁺.

15 Example 8

Preparation of Other Representative Intermediates
for the Elaboration of the C-Terminus of Peptides
of Formula 1.

20 (a) NH₂-(R)-CH(Et)CMe₃: To a cooled solution
(0°) of 4,4-dimethyl-3-pentanone (106 g, 0.92
mmol) and (R)-α-methylbenzylamine (111 g, 0.92
mmol) in benzene (1 L), a solution of TiCl₄ (50.5
25 mL, 0.46 mmol) in benzene (200 mL) was added at a
rate that kept the temperature of the mixture
below 10°. Thereafter, the mixture was stirred
mechanically for 3 h at 40°, cooled to room
temperature and filtered through diatomaceous
30 earth. The diatomaceous earth was washed with
Et₂O. The combined filtrate and wash was
concentrated. The residue was dissolved in dry
MeOH (2 L). The solution was cooled to 0° and
NaBH₄ (20 g, 0.53 mmol) was added portionwise
35 while maintaining the temperature of the mixture
below 5°. The methanol was evaporated. The

residue was dissolved in Et₂O. The solution was washed with brine, dried (MgSO₄) and evaporated to dryness to give a reddish oil (a 18:1 mixture of diastereoisomers as indicated by NMR). The oil was purified by flash chromatography (SiO₂, eluent: EtOAc/hexane, 7:93) to afford N-(1(R)-phenylethyl)-1(R)-ethyl-2,2-dimethylpropyl-amine as a liquid (110 g, 54%). This material was dissolved in hexane (1.5 L). 6N HCl in dioxane (90 mL) was added to the solution over a period of 15 min. The resulting white solid was collected on a filter and then washed with hexane to provide N-(1(R)-phenylethyl)-1(R)-ethyl-2,2-dimethylpropyl hydrochloride (125 g, 97%). ¹H NMR(CDCl₃) δ 0.55 (t, J = 7.5 Hz, 3H), 1.14 (s, 9H), 1.54-1.95 (m, 2H), 2.23 (d, J = 6.5 Hz, 3H), 2.36-2.44 (m, 1H), 4.31-4.49 (m, 1H), 7.30-7.48 (m, 3H), 7.74-7.79 (m, 2H).

A solution of the latter compound (41.5 g) in MeOH (120 mL) was mixed with 10% (w/w) Pd/C and the mixture was shaken under 50 psi of hydrogen on a Parr hydrogenator at room temperature for 48 h. The mixture was filtered and the filtrate was concentrated to give the desired NH₂-(R)-CH(Et)C-Me₃ in the form of its hydrochloric acid addition salt, as a white solid (25 g, 100%). ¹H NMR(CDCl₃) δ 1.10 (s, 9H), 1.22 (t, J = 7 Hz, 2H), 1.58-1.90 (m, 2H), 2.70-2.85 (m, 1H), 8.10-8.40 (broad s, 3H).

In the same manner but replacing 4,4-dimethyl-3-pentanone with 3,3-dimethyl-2-butanone in the preceding procedure, NH₂-(R)-CH(Me)CMe₃.HCl is obtained.

Example 9

Preparation of Other Representative Intermediates
for Elaborating the N-Terminus of Peptide
5 Derivatives of Formula 1 According to the
Procedure of Example 7.

(a) 1-Propylbutyl isocyanate: This intermediate
was prepared from commercially available 4-
10 aminoheptane by the procedure of V.S. Goldesmidt
and M. Wick, Liebigs Ann. Chem., 575, 217 (1952).

(b) 1-Ethyl-1-(2-propenyl)-3-butenyl isocyanate:
A solution of propionitrile (14.5 g, 264 mmol) in
15 dry Et₂O (40 mL) was added dropwise to 1.0 M allyl
magnesium bromide/Et₂O (880 mL). The reaction
mixture was mechanically stirred at reflux for 2
h, after which time it was cooled to 0°. A
saturated aqueous solution of NH₄Cl (320 mL) was
20 added cautiously to the cooled reaction mixture.
The organic phase was separated, dried (MgSO₄),
cooled to 0° and then mixed at the same
temperature with 1 M HCl/Et₂O (200 mL). The
resulting solid was collected and dried under
25 reduced pressure (ca 27 g). The latter material
dissolved in CH₂Cl₂ (200 mL). The solution was
washed with a 10% (w/v) aqueous solution of Na₂CO₃
(2 X) and then brine, dried (MgSO₄) and
concentrated to dryness to afford a yellow oil.
30 The oil was distilled (82-85°/20 Torr) to give 1-
ethyl-1-(2-propenyl)-3-butenylamine as a colorless
liquid (11.6 g, 34%); ¹H NMR(CDCl₃), 400 MHz) δ
0.89 (t, J = 7 Hz, 3H), 1.39 (q, J = 7 Hz, 2H),
2.11 (d, J = 7 Hz, 2H), 5.06-5.14 (m, 4H), 5.80-
35 5.89 (m, 2H).

The latter compound was converted to 1-ethyl-1-(2-propenyl)-3-butenyl isocyanate by the procedure of V.S. Goldesmidt and M. Wick, *supra*.

5 The first step of the process of preceding section b, i.e. the preparation of 1-ethyl-1-(2-propenyl)-3-butenylamine, is based on a general method described by G. Alvernhe and A. Laurent, *Tetrahedron Lett.*, 1057 (1973). The overall
10 process, with the appropriate choice of reactants, can be used to prepare other requisite isocyanate intermediates for the eventual preparation of peptide derivatives having unsaturation at the N-terminus, i.e. an unsaturated alkylaminocarbonyl
15 such as 1-methyl-1-(2-propenyl)-3-butenyl. Note, however, that when the requisite isocyanate intermediates are applied according to the procedure of example 7, then the ultimate product will be the corresponding peptide derivative of
20 formula 1 in which the N-terminus is saturated. Moreover, the latter procedure represents a practical process for preparing such corresponding peptides; for example, $\text{Pr}_2\text{CHNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-Asp(cyPn)-NH-(R)-CH(Et)CMe}_3$,
25 {FAB/mass spectrum (m/z): 693 $[\text{M} + \text{H}]^+$, the title peptide derivative noted in example 10, hereinafter.

30 Thus, by using the appropriate intermediates, the serial coupling and the deprotection procedures of examples 1 to 7 can be used to prepare other compounds of formula 1, such as those exemplified in the table of the following
35 example. In some cases, precipitation of the final product does not afford pure material. In those instances, the product can be purified by

semi preparative HPLC on a C-18 reversed-phase column using a gradient of acetonitrile and water, each containing 0.06% TFA. To this end, the crude product was dissolved in 0.1 M aqueous NH_4OH and the pH of the solution was brought back to about 7 using 0.1 M aqueous AcOH , prior to purification. When applicable, diastereoisomeric mixtures were separated in this fashion.

Some examples of other compound of formula 1 that can be prepared thus are $(\text{PhCH}_2)_2\text{CHC}(\text{O})-(\text{N-Me})\text{Val-Tbg-CH}_2-(\text{R})-\text{CH}(\text{CH}_2\text{C}(\text{O})\text{CMe}_3)\text{C}(\text{O})-\text{Asp}(\text{cyPn})-\text{NHCH}_2\text{CMe}_3$ and $(\text{PhCH}_2)_2\text{CHC}(\text{O})-(\text{N-Me})\text{Val-Tbg-CH}_2-(\text{R})-\text{CH}(\text{CH}_2\text{C}(\text{O})\text{CMe}_3)\text{C}(\text{O})-\text{Asp}(\text{cyPn})-\text{NH}-(\text{R})-\text{CH}(\text{Me})\text{CMe}_3$.

Still some other examples of compounds of formula 1 are:

$\text{Pr}_2\text{CHNHC}(\text{O})-\text{Tbg-CH}_2-(\text{R})-\text{CH}(\text{CH}_2\text{C}(\text{O})\text{CMe}_3)\text{C}(\text{O})-\text{Asp}(\text{cyPn})-\text{NHCH}_2\text{CMe}_3$, FAB/mass spectrum (m/z): 665 $[\text{M} + \text{H}]^+$;

$\text{EtPr}_2\text{CNHC}(\text{O})-\text{Tbg-CH}_2-(\text{R})-\text{CH}(\text{CH}_2\text{C}(\text{O})\text{CMe}_3)\text{C}(\text{O})-\text{Asp}(\text{cyPn})-\text{NH}-(\text{R})-\text{CH}(\text{Et})\text{CMe}_3$, FAB/mass spectrum (m/z): 722 $[\text{M} + \text{H}]^+$; and

$\text{EtPr}_2\text{CNHC}(\text{O})-\text{Tbg-CH}_2-(\text{R})-\text{CH}(\text{CH}_2\text{C}(\text{O})\text{CMe}_3)\text{C}(\text{O})-\text{Asp}(\text{cyPn})-\text{NHCH}_2\text{CMe}_3$, FAB/mass spectrum (m/z): 693 $[\text{M} + \text{H}]^+$.

Example 10

Study Showing The Effect Of The Peptide Derivative, $\text{Pr}_2\text{CHNHC}(\text{O})-\text{Tbg-CH}_2-(\text{R})-\text{CH}(\text{CH}_2\text{C}(\text{O})\text{CMe}_3)-\text{C}(\text{O})-\text{Asp}(\text{cyPn})-\text{NH}-(\text{R})-\text{CH}(\text{Et})\text{CMe}_3$, And

Combination Thereof With Acyclovir Against A Wild Type HSV-1 Clinical Isolate And Two Acyclovir Resistant HSV-1 Clinical Isolates.

5 Viruses: The clinical isolates have been described by S.L. Sacks et al., Annals of Internal Medicine, 111, 893 (1989). They were obtained from a 25-year-old woman with acute myelogenous leukemia in first relapse from an allogeneic bone marrow transplant. The initial cultures for herpes simplex virus from day 8 after the bone graft were found to be positive (isolate 294, defined as wild type HSV-1). Acyclovir treatment was then initiated, and viral cultures were still 15 positive on day 36 (isolate 615 representing resistant HSV-1). One sub-isolate 615.9 (ACV^rTK clinical isolate) was characterized to be acyclovir-resistant, forcarnet-sensitive and to have a diminished thymidine kinase (TK) activity. 20 615.9 was subsequently confirmed to be a TK deficient mutant. In contrast, subisolate 615.8 (ACV^rPOL clinical isolate) was characterized to be acyclovir and forcarnet resistant and had thymidine kinase activities near to that observed 25 in the pretreatment isolate 294. 615.8 was subsequently confirmed to be a DNA polymerase mutant.

Assay:

30

BHK-21/C13 cells (ATCC CCL 10) are incubated for two days in 150 cm² T-flasks (1.5 x 10⁶ cells/flask) with alpha-MEM medium (Gibco Canada Inc., Burlington, Ontario, Canada) supplemented 35 with 8% (v/v) fetal bovine serum (FBS, Gibco Canada Inc.). The cells are trypsinized and then

transferred to fresh media in a 24 well plate to give 2.5×10^5 cells in 750 μL of media per well. The cells are incubated at 37° for a period of 6 h to allow them to adhere to the plate. Thereafter,

5 the cells are washed once with 500 μL of alpha-MEM supplemented with 0.5% (v/v) FBS and then incubated with 750 μL of the same media (low serum) for 3 days. After this period of serum starvation, the low serum medium is removed and

10 the cells are incubated in 500 μL of BBMT for 2 to 3 hours. [BBMT medium is described by P. Brazeau et al., Proc. Natl. Acad. Sci. USA, 79, 7909 (1982).] Thereafter, the cells are infected with HSV-2 (multiplicity of infection = 0.02 PFU/cell)

15 in 100 μL of BBMT medium. (Note: The HSV-2 used was strain HG-52, see Y. Langelier and G. Buttin, J. Gen. Virol., 57, 21 (1981); the virus was stored at -80° .) Following 1 h of virus adsorption at 37° , the media is removed and the

20 cells are washed with BBMT (3 X 250 μL). The cells in each well are incubated with or without (control) appropriate concentrations of the test agent dissolved in 200 μL of BBMT medium. After 29 h of incubation at 37° , the infected cells are

25 harvested by first freezing the plate at -80° , followed by thawing. The cells in each well are scraped off the surface of the well with the help of the melting ice fragments. After complete thawing, the cell suspensions are collected and

30 each well is rinsed with 150 μL of BBMT medium. The viral sample (suspension plus washing) is sonicated gently for 4 min at 4° . Cell debris are removed by centrifugation (1000 times gravity for 10 minutes at 4°). The supernatant is collected

35 and stored at -80° until determination of viral titer.

Viral titration was performed by a modification of the colorimetric assay method of M. Langlois et al., Journal of Biological Standardization, 14, 201 (1986).

More specifically, in a similar manner as described above, BHK-21/C13 cells are trypsinized and transferred to fresh media in a 96 well microtiter plate to give 20,000 cells in 100 μ L of media per well. The cells in the prepared plate are incubated at 37° for 2 h. During that time, the viral sample is thawed and sonicated gently for 15 seconds, and log dilutions of the sample are prepared (1/5 sequential: 50 μ L of the sample plus 200 μ L of BBMT medium, sequential dilutions being done with a multichannel pipette.

On completion of the above 2 hour incubation of the BHK-21/C13 cells, the media is replaced with alpha-MEM medium supplemented with 3% (v/v) FBS. The cells are now ready to be infected with the various sample dilutions of virus. Aliquots (50 μ L) of the various dilutions are transferred into the appropriate wells of the plate. The resulting infected cells are incubated for 2 days at 37°. Then 50 μ L of a 0.15% (v/v) solution of neutral red dye in Hank's Balanced Salt Solution (pH 7.3, Gibco Canada Inc.) is added to each well. The prepared plate is incubated for 45 min at 37°. Medium from each well is then aspirated and the cells are washed once with 200 μ L of Hank's Balanced Salt Solution. After the wash, the dye is released from the cells by the addition of 100 μ L of a 1:1 mixture of 0.1 M Sorensen's citrate buffer (pH 4.2) and ethanol. [Sorensen's citrate

buffer is prepared as follows: Firstly, a 0.1 M disodium citrate solution is prepared by dissolving citric acid monohydrate (21 g) in 1 N aqueous NaOH (200 mL) and adding sufficient filtered H₂O to make 1 L. Secondly, the 0.1 M disodium citrate solution (61.2 mL) is mixed with 0.1 N aqueous HCl (38.8 mL) and the pH of the resulting solution is adjusted to 4.2 if necessary.] The mixture in the wells is subjected to a gentle vortex action to ensure proper mixing. The plate wells are scanned by a spectrophotometer plate reader at 540 nm to assess the number of viable cells. In this manner, the percentage of virus growth inhibition can be determined for the various concentrations of the test agent, and the concentration of the test agent causing a 50% inhibition of virus replication, i.e. the IC₅₀ can be calculated.

The approach taken was to evaluate acyclovir and the peptide derivative, each alone and then in various combinations in the preceding assay. Thus, the comparative effectiveness of the two compounds against the isolates was demonstrated. Furthermore, a synergistic effect between the two compounds was evaluated and confirmed by applying the isobole method to the results obtained in these studies; see J. Sühnel, J. Antiviral Research, 13, 23 (1990) for a description of the isobole method.

More explicitly with reference to the isobole method, this method requires experimental data generated for the two test compounds, each alone and in different dose combinations at equi-effective levels. In this way selected

concentrations of the title peptide derivative (IC₅, IC₁₀, IC₂₀ and IC₃₀) were added in combination with various doses of acyclovir and the IC₅₀'s were evaluated. For this experiment, the IC₅, IC₁₀, IC₂₀ and IC₃₀ of the title peptide derivative as well as the doses of acyclovir were derived from curves previously obtained. An isobologram is generated using a value termed FIC₆₀(acyclovir) (which is the ratio of the concentration of acyclovir required to inhibit HSV replication by 60% in the presence of a fixed concentration of the title peptide derivative to the concentration required in the absence of the peptide derivative). This is plotted against a term representing the ratio of the fixed concentration of the peptide derivative to the concentration of the peptide derivative that reduced 60% inhibition of HSV replication in the absence of acyclovir.

equations:

X axis :

$$= \frac{[\text{the fixed concentration of the peptide derivative added}]}{\text{IC}_{60} \text{ of the peptide derivative alone}}$$

Y axis :

$$\text{FIC}_{60}(\text{acyclovir}) = \frac{\text{IC}_{60}(\text{acyclovir} + X \mu\text{M of the peptide derivative})}{\text{IC}_{60}(\text{acyclovir alone})}$$

Results:

TABLE I lists the results obtained when acyclovir and the title peptide of this example

were assayed alone in the preceding cell culture assay with the wild type HSV-1 clinical isolate and the two acyclovir-resistant HSV-1 clinical isolates.

5

TABLE I

Compounds	Isolate 294	Isolate 615.8	Isolate 615.9
	IC ₅₀ (μ M)	IC ₅₀ (μ M)	IC ₅₀ (μ M)
Acyclovir	1.1	19	55
Title Peptide Derivative	2.0	2.0	6.2

10

The results from TABLE I demonstrate that compounds of formula 1 are active against wild type HSV-1, and that they exhibit similar efficacy against acyclovir-resistant HSV-1, such as POL-mutants and TK-deficient mutants; whereas acyclovir loses much of its efficacy (being above 20 to 40 times less effective) against the mutant strains.

15

20

The following TABLES II, III and IV are illustrative of the results obtained when combinations of acyclovir and the title peptide derivative of this example were evaluated according to the preceding cell culture assay with the wild type HSV-1 clinical isolate and the two acyclovir-resistant HSV-1 clinical isolates.

TABLE II

ANTIVIRAL ACTIVITY SYNERGY STUDY	
Isolate 294 (Pretreatment clinical isolate)	
Fixed concentration of the Title Peptide Derivative (μM)	Acyclovir IC_{50} ⁽¹⁾ in combination with Title Peptide Derivative (μM)
0.3	0.85
0.5	0.70
0.7	0.35
0.9	0.30
1.0	0.30
1.2	0.22
1.4	0.10
1.6	0.13

TABLE III

ANTIVIRAL ACTIVITY SYNERGY STUDY	
Isolate 615.8 (ACV ^I POL treatment isolate)	
Fixed concentration of the Title Peptide Derivative (μM)	Acyclovir IC_{50} ⁽²⁾ in combination with Title Peptide Derivative (μM)
0.3	15.0
0.5	13.0
0.7	7.0
0.9	8.0
1.0	5.3
1.2	5.0
1.4	1.7
1.6	2.4

TABLE IV
ANTIVIRAL ACTIVITY
SYNERGY STUDY

Isolate 615.9 (ACV ^{TK} treatment isolate)	
Fixed concentration of the Title Peptide Derivative (μM)	Acyclovir IC ₅₀ ⁽²⁾ in combination with Title Peptide Derivative (μM)
1.0	33.0
2.0	17.0
3.0	5.5
4.0	5.6
5.0	2.0
6.0	0.9
7.0	< 0.02
8.0	< 0.02

Notes respecting the preceding four tables:

- IC₅₀ values are not corrected for solubility.
- 5 • All stock solutions were ultracentrifuged at 100,000 x g for 30 minutes at 4° prior to use.
- The solubility of the title peptide derivative at 20 μM is 85%.
- The cytotoxicity of the title peptide derivative is 89 μM (value not corrected).
- 10 (1) IC₅₀ obtained from a dose response curve of acyclovir ranging from 7.6×10^{-4} to 1.5×10^1 μM in the presence of the corresponding fixed concentration of the title peptide derivative as shown in the column to the left.
- 15 (2) IC₅₀ obtained from a dose response curve of acyclovir ranging from 1.5×10^{-4} to 1.5×10^2 μM in the presence of the corresponding fixed concentration of the title peptide derivative as shown in the column to the left.
- 20

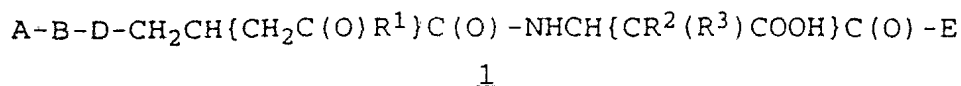
TABLES II, III and IV demonstrate that the peptides of formula 1 are able to potentiate the activity of acyclovir against wild type HSV-1 and against acyclovir-resistant HSV-1 mutants, when the two agents are used in combination. The results show a proportional lowering of the IC^{50} of acyclovir as the ratio of the concentrations of the peptide to acyclovir is increased.

Accompanying Figure 1 is a graphic illustration of the positive results (synergism) obtained in the application of the isobole method in a study with acyclovir and the title peptide of this example using the subisolate 615.8 (ACV^rPOL clinical isolate).

Similarly, accompanying Figure 2 is a graphic illustration of the positive results in a study with acyclovir and the peptide of this example using the subisolate 615.9 (ACV^rTK).

CLAIMS:

1. A method of treating acyclovir-resistant herpes simplex viral infections in a mammal which comprises
 5 administering an effective amount of a peptide derivative of formula 1



- 10 wherein A is phenylacetyl, phenylpropionyl, (4-aminophenyl)propionyl, (4-fluorophenyl)propionyl, (4-hydroxyphenyl)propionyl, (4-methoxyphenyl)propionyl, 2-(phenylmethyl)-3-phenylpropionyl, 2-
 15 { (4-fluorophenyl)methyl }-3-(4-fluorophenyl)propionyl, 2- { (4-methoxyphenyl)methyl }-3-(4-methoxyphenyl)propionyl or benzylaminocarbonyl; B is (N-Me)-Val or (N-Me)-Ile; or A and B taken together form a saturated alkylaminocarbonyl selected from the group of butylaminocarbonyl, 1-
 20 methylethylaminocarbonyl, 1-methylpropylaminocarbonyl, 1-ethylpropylaminocarbonyl, 1,1-dimethylbutylaminocarbonyl, 1-ethylbutylaminocarbonyl, 1-propylbutylaminocarbonyl, 1-ethylpentylaminocarbonyl, 1-butylpentylaminocarbonyl, 1-ethylbutylaminocarbonyl, 2-ethylpentylaminocarbonyl, 1-methyl-1-propylbutylaminocarbonyl, 1-ethyl-1-propylbutylaminocarbonyl, 1,1-dipropylbutylaminocarbonyl, (1-propylcyclopentyl)aminocarbonyl and (1-propylcyclohexyl)aminocarbonyl; D is Val, Ile or Tbg; R¹ is 1-methylethyl, 1,1-dimethylethyl, 1-methylpropyl, 1,1-dimethylpropyl, 2,2-dimethylpropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-methylcyclopentyl, NR⁴R⁵ wherein R⁴ is hydrogen or lower alkyl and R⁵ is lower alkyl, or R⁴ and R⁵ together with the nitrogen atom to
 35 which they are attached form a pyrrolidino,



piperidino, morpholino or 4-methylpiperazino; R^2
 is hydrogen and R^3 is methyl, ethyl, 1-
 methylethyl, 1,1-dimethylethyl, propyl, 2-propenyl
 or benzyl, and the carbon atom bearing R^2 and R^3
 5 has the (R)-configuration, or R^2 and R^3 each
 independently is methyl or ethyl, or R^2 and R^3
 together with the carbon atom to which they are
 attached form a cyclobutyl, cyclopentyl or
 cyclohexyl; and E is NHR^6 wherein R^6 is 2-
 10 methylpropyl, 2,2-dimethylpropyl, 1(R),2,2-
 trimethylpropyl, 1,1,2,2-tetramethylpropyl, 1(R)-
 ethyl-2,2-dimethylpropyl, 2-(R,S)-methylbutyl,
 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1(R),2,2-
 trimethylbutyl, 1(R),3,3-trimethylbutyl, 2-
 15 ethylbutyl, 2,2-diethylbutyl, 2-ethyl-1(R)-
 methylbutyl, 2-ethyl-2-methylbutyl, 1(R)-ethyl-
 3,3-dimethylbutyl, 2,2-dimethylpentyl, *cis*- or
trans-2-methylcyclohexyl, 2,2-dimethylcyclohexyl
 or cyclohexylmethyl; or E is $NHCH(R^7)-Z$ wherein
 20 the carbon atom bearing R^7 has the (S)-
 configuration, R^7 is 1,1-dimethylethyl, 1-
 methylpropyl, 2-methylpropyl, 2,2-dimethylpropyl
 or cyclohexylmethyl and Z is CH_2OH , $C(O)OH$,
 $C(O)NH_2$ or $C(O)OR^8$ wherein R^8 is methyl, ethyl or
 25 propyl;
 or a therapeutically effective salt thereof.

2. A method as claimed in
 claim 1 wherein the peptide derivative is a
 30 compound of formula 1 wherein A is
 phenylpropionyl, 2-(phenylmethyl)-3-phenylpropion-
 yl or benzylaminocarbonyl; B is (N-Me)Val; D is
 Tbg; R^1 is 1-methylethyl, 1,1-dimethylethyl, 1-
 methylpropyl, 1,1-dimethylpropyl, 2,2-dimethyl-
 35 propyl, cyclobutyl, cyclopentyl, cyclohexyl or 1-
 methylcyclopentyl; R^2 is hydrogen and R^3 is



methyl, ethyl, 1-methylethyl, propyl or benzyl,
 and the carbon atom bearing R^2 and R^3 has the (R)-
 configuration, or R^2 and R^3 each independently is
 methyl or ethyl, or R^2 and R^3 together with the
 5 carbon atom to which they are attached form a
 cyclobutyl, cyclopentyl or cyclohexyl; and E is
 NHR^6 wherein R^6 is 2,2-dimethylpropyl, 1(R),2,2-
 trimethylpropyl, 1(R)-ethyl-2,2-dimethylpropyl,
 2,2-dimethylbutyl or 1(R)-ethyl-3,3-dimethylbutyl
 10 or E is $NHCH(R^7)-Z$ wherein the carbon atom bearing
 R^7 has the (S)-configuration, R^7 is 2,2-
 dimethylpropyl and Z is CH_2OH , $C(O)OH$, $C(O)NH_2$ or
 $C(O)OR^8$ wherein R^8 is methyl, ethyl or propyl; or
 a therapeutically acceptable salt thereof.

15

3. A method as claimed in
 claim 1 wherein the peptide derivative is a
 compound of formula 1 wherein A and B together
 form a saturated alkylaminocarbonyl selected from
 20 the group of 1-ethylpropylaminocarbonyl, 1-ethyl-
 butylaminocarbonyl, 1-propylbutylaminocarbonyl, 2-
 ethylpentylaminocarbonyl, 1-methyl-1-propylbutyl-
 aminocarbonyl, 1-ethyl-1-propylbutylaminocarbonyl,
 1,1-dipropylbutylaminocarbonyl and (1-propylcyclo-
 25 pentyl)aminocarbonyl; D is Tbg; R^1 is 1-
 methylethyl, 1,1-dimethylethyl, 1-methylpropyl,
 1,1-dimethylpropyl, 2,2-dimethylpropyl, cyclobut-
 yl, cyclopentyl, cyclohexyl or 1-methylcyclo-
 30 pentyl; R^2 is hydrogen and R^3 is methyl, ethyl, 1-
 methylethyl, propyl or benzyl, and the carbon atom
 bearing R^2 and R^3 has the (R)-configuration, or R^2
 and R^3 each independently is methyl or ethyl, or
 R^2 and R^3 together with the carbon atom to which
 they are attached form a cyclobutyl, cyclopentyl
 35 or cyclohexyl; and E is NHR^6 wherein R^6 is 2,2-
 dimethylpropyl, 1(R),2,2-trimethylpropyl, 1(R)-



ethyl-2,2-dimethylpropyl, 2,2-dimethylbutyl or
 1(R)-ethyl-3,3-dimethylbutyl or E is $\text{NHCH(R}^7\text{)-Z}$
 wherein the carbon atom bearing R^7 has the (S)-
 configuration, R^7 is 2,2-dimethylpropyl and Z is
 5 CH_2OH , C(O)OH , C(O)NH_2 or C(O)OR^8 wherein R^8 is
 methyl, ethyl or propyl; or a therapeutically
 acceptable salt thereof.

4. A method as claimed in
 10 claim 1 wherein the peptide derivative is selected
 from the group consisting of:

$\text{PhCH}_2\text{CH}_2\text{C(O)-(N-Me)Val-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)-}$
 $\text{CMe}_3\text{)C(O)-Asp(cyPn)-}\gamma\text{MeLeucinol,}$

15 $\text{Et}_2\text{CHNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-}\gamma\text{MeLeucinol,}$

$\text{Pr}_2\text{CHNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 20 $\text{Asp(cyPn)-NH-(R)-CH(Et)CMe}_3\text{,}$

$\text{Pr}_2\text{CHNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-NHCH}_2\text{CMe}_3\text{,}$

25 $\text{EtPr}_2\text{CNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-NH-(R)-CH(Et)CMe}_3\text{, and}$

$\text{EtPr}_2\text{CNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 30 $\text{Asp(cyPn)-NHCH}_2\text{CMe}_3\text{.}$

5. A method as claimed in any one of the preceding
 claims wherein said peptide derivative is
 employed in combination with an antiviral
 nucleoside analog, or a therapeutically acceptable
 35 salt thereof .



6. A method as claimed in claim 5
 wherein the peptide derivative is a compound of
 formula 1 wherein A is phenylpropionyl, 2-
 (phenylmethyl)-3-phenylpropionyl or benzylamino-
 carbonyl; B is (N-Me)Val; D is Tbg; R¹ is 1-
 methylethyl, 1,1-dimethylethyl, 1-methylpropyl,
 1,1-dimethylpropyl, 2,2-dimethylpropyl, cyclobut-
 yl, cyclopentyl, cyclohexyl or 1-methylcyclo-
 pentyl; R² is hydrogen and R³ is methyl, ethyl, 1-
 methylethyl, propyl or benzyl, and the carbon atom
 bearing R² and R³ has the (R)-configuration, or R²
 and R³ each independently is methyl or ethyl, or
 R² and R³ together with the carbon atom to which
 they are attached form a cyclobutyl, cyclopentyl
 or cyclohexyl; and E is NHR⁶ wherein R⁶ is 2,2-
 dimethylpropyl, 1(R),2,2-trimethylpropyl, 1(R)-
 ethyl-2,2-dimethylpropyl, 2,2-dimethylbutyl or
 1(R)-ethyl-3,3-dimethylbutyl or E is NHCH(R⁷)-Z
 wherein the carbon atom bearing R⁷ has the (S)-
 configuration, R⁷ is 2,2-dimethylpropyl and Z is
 CH₂OH, C(O)OH, C(O)NH₂ or C(O)OR⁸ wherein R⁸ is
 methyl, ethyl or propyl; or a therapeutically
 acceptable salt thereof.

7. A method as claimed in claim 5
 wherein the peptide derivative is a compound of
 formula 1 wherein A and B together form a
 saturated alkylaminocarbonyl selected from the
 group of 1-ethylpropylaminocarbonyl, 1-ethyl-
 butylaminocarbonyl, 1-propylbutylaminocarbonyl, 2-
 ethylpentylaminocarbonyl, 1-methyl-1-propylbutyl-
 aminocarbonyl, 1-ethyl-1-propylaminocarbonyl, 1,1-
 dipropylbutylaminocarbonyl and (1-propylcyclo-
 pentyl)aminocarbonyl; D is Tbg; R¹ is 1-
 methylethyl, 1,1-dimethylethyl, 1-methylpropyl,
 1,1-dimethylpropyl, 2,2-dimethylpropyl, cyclobut-



yl, cyclopentyl, cyclohexyl or 1-methylcyclopentyl; R^2 is hydrogen and R^3 is methyl, ethyl, 1-methylethyl, propyl or benzyl, and the carbon atom bearing R^2 and R^3 has the (R)-configuration, or R^2 and R^3 each independently is methyl or ethyl, or R^2 and R^3 together with the carbon atom to which they are attached form a cyclobutyl, cyclopentyl or cyclohexyl; and E is NHR^6 wherein R^6 is 2,2-dimethylpropyl, 1(R),2,2-trimethylpropyl, 1(R)-ethyl-2,2-dimethylpropyl, 2,2-dimethylbutyl or 1(R)-ethyl-3,3-dimethylbutyl or E is $NHCH(R^7)-Z$ wherein the carbon atom bearing R^7 has the (S)-configuration, R^7 is 2,2-dimethylpropyl and Z is CH_2OH , $C(O)OH$, $C(O)NH_2$ or $C(O)OR^8$ wherein R^8 is methyl, ethyl or propyl; or a therapeutically acceptable salt thereof.

8. A method as claimed in claim 5 wherein the peptide derivative is selected from the group consisting of:

$PhCH_2CH_2C(O)-(N-Me)Val-Tbg-CH_2-(R)-CH(CH_2C(O)-CMe_3)C(O)-Asp(cyPn)-\gamma MeLeucinol,$

$Et_2CHNHC(O)-Tbg-CH_2-(R)-CH(CH_2C(O)CMe_3)C(O)-Asp(cyPn)-\gamma MeLeucinol,$

$Pr_2CHNHC(O)-Tbg-CH_2-(R)-CH(CH_2C(O)CMe_3)C(O)-Asp(cyPn)-NH-(R)-CH(Et)CMe_3,$

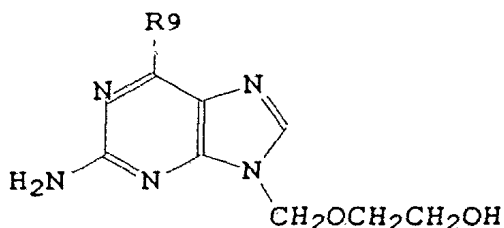
$Pr_2CHNHC(O)-Tbg-CH_2-(R)-CH(CH_2C(O)CMe_3)C(O)-Asp(cyPn)-NHCH_2CMe_3,$

$EtPr_2CNHC(O)-Tbg-CH_2-(R)-CH(CH_2C(O)CMe_3)C(O)-Asp(cyPn)-NH-(R)-CH(Et)CMe_3,$ and



$\text{EtPr}_2\text{CNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-NHCH}_2\text{CMe}_3\text{.}$

9. A method as claimed in any one of claims 5 to 8
 5 wherein the nucleoside analog is a compound of
 formula 2



2

- 10 wherein R^9 is hydrogen, hydroxy or amino, or a
 therapeutically acceptable salt thereof.

10. A method as claimed in any one of claims 5 to 8
 wherein the nucleoside analog is selected from the
 15 group consisting of vidarabine, idoxuridine,
 trifluridine, ganciclovir, edoxudine, broavir,
 fiacitabine, penciclovir, famciclovir and
 rosiglovir.

- 20 11. A method as claimed in any one of claims 5 to 8
 wherein the peptide derivative is selected from
 the group consisting of:

25 $\text{PhCH}_2\text{CH}_2\text{C(O)-(N-Me)Val-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)-}$
 $\text{CMe}_3\text{)C(O)-Asp(cyPn)-}\gamma\text{MeLeucinol,}$

$\text{Et}_2\text{CHNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-}\gamma\text{MeLeucinol,}$



$\text{Pr}_2\text{CHNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-NH-(R)-CH(Et)CMe}_3\text{,}$

5 $\text{Pr}_2\text{CHNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-NHCH}_2\text{CMe}_3\text{,}$

$\text{EtPr}_2\text{CNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-NH-(R)-CH(Et)CMe}_3\text{, and}$
 10

$\text{EtPr}_2\text{CNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-NHCH}_2\text{CMe}_3\text{; and}$

15 wherein the nucleoside analog is acyclovir.

12. A method as claimed in any one of claims 1 to 11
 preceded by the step of noting or determining that said
 infection is resistant to acyclovir.

13. A pharmaceutical composition when used in the treatment of
 acyclovir-resistant herpes simplex viral
 infections, comprising as an active ingredient a
 peptide derivative of formula 1

$\text{A-B-D-CH}_2\text{CH}\{\text{CH}_2\text{C(O)R}^1\}\text{C(O)-NHCH}\{\text{CR}^2(\text{R}^3)\text{COOH}\}\text{C(O)-E}$

1



wherein A is phenylacetyl, phenylpropionyl, (4-aminophenyl)propionyl, (4-fluorophenyl)propionyl, (4-hydroxyphenyl)propionyl, (4-methoxyphenyl)propionyl, 2-(phenylmethyl)-3-phenylpropionyl, 2-
 5 { (4-fluorophenyl)methyl }-3-(4-fluorophenyl)propionyl, 2- { (4-methoxyphenyl)methyl }-3-(4-methoxyphenyl)propionyl or benzylaminocarbonyl; B is (N-Me)-Val or (N-Me)-Ile; or A and B taken together form a saturated alkylaminocarbonyl selected from
 10 the group of butylaminocarbonyl, 1-methylethylaminocarbonyl, 1-methylpropylaminocarbonyl, 1-ethylpropylaminocarbonyl, 1,1-dimethylethylaminocarbonyl, 1-ethylbutylaminocarbonyl, 1-ethylpentylaminocarbonyl, 1-butylpentylaminocarbonyl, 1-ethylbutylaminocarbonyl, 2-ethylpentylaminocarbonyl, 1-methyl-1-propylbutylaminocarbonyl, 1-ethyl-1-propylbutylaminocarbonyl, 1,1-dipropylbutylaminocarbonyl, (1-propylcyclopentyl)aminocarbonyl and (1-propylcyclohexyl)aminocarbonyl; D is Val, Ile or Tbg; R^1 is 1-methylethyl, 1,1-dimethylethyl, 1-methylpropyl, 1,1-dimethylpropyl, 2,2-dimethylpropyl, cyclobutyl, cyclopentyl, cyclohexyl, 1-methylcyclopentyl, NR^4R^5 wherein R^4
 25 is hydrogen or lower alkyl and R^5 is lower alkyl, or R^4 and R^5 together with the nitrogen atom to which they are attached form a pyrrolidino, piperidino, morpholino or 4-methylpiperazino; R^2 is hydrogen and R^3 is methyl, ethyl, 1-methylethyl, 1,1-dimethylethyl, propyl, 2-propenyl or benzyl, and the carbon atom bearing R^2 and R^3 has the (R)-configuration, or R^2 and R^3 each independently is methyl or ethyl, or R^2 and R^3 together with the carbon atom to which they are attached form a cyclobutyl, cyclopentyl or cyclohexyl; and E is NHR^6 wherein R^6 is 2-
 35



methylpropyl, 2,2-dimethylpropyl, 1(R),2,2-
 trimethylpropyl, 1,1,2,2-tetramethylpropyl, 1(R)-
 ethyl-2,2-dimethylpropyl, 2-(R,S)-methylbutyl,
 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1(R),2,2-
 5 trimethylbutyl, 1(R),3,3-trimethylbutyl, 2-
 ethylbutyl, 2,2-diethylbutyl, 2-ethyl-1(R)-
 methylbutyl, 2-ethyl-2-methylbutyl, 1(R)-ethyl-
 3,3-dimethylbutyl, 2,2-dimethylpentyl, *cis*- or
 10 *trans*-2-methylcyclohexyl, 2,2-dimethylcyclohexyl
 or cyclohexylmethyl; or E is $\text{NHCH(R}^7\text{)-Z}$ wherein
 the carbon atom bearing R^7 has the (S)-
 configuration, R^7 is 1,1-dimethylethyl, 1-
 methylpropyl, 2-methylpropyl, 2,2-dimethylpropyl
 or cyclohexylmethyl and Z is CH_2OH , C(O)OH ,
 15 C(O)NH_2 or C(O)OR^8 wherein R^8 is methyl, ethyl or
 propyl; or a therapeutically acceptable salt
 thereof;
 and a pharmaceutically acceptable carrier
 therefor.

20

14. A pharmaceutical composition as claimed in
 claim 13 wherein the active ingredient comprises a
 combination of a peptide derivative of formula 1
 as defined in claim 13, or a therapeutically
 25 acceptable salt thereof, and an antiviral
 nucleoside analog, or a therapeutically acceptable
 salt thereof, when used in the treatment of acyclovir-resistant
 herpes simplex viral infections.

30

15. A pharmaceutical composition as claimed in
 claim 14 wherein the peptide derivative is
 selected from the group consisting of:

35

$\text{PhCH}_2\text{CH}_2\text{C(O)-(N-Me)Val-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)-}$
 $\text{CMe}_3\text{)C(O)-Asp(cyPn)-}\gamma\text{MeLeucinol,}$



$\text{Et}_2\text{CHNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-}\gamma\text{MeLeucinol,}$

5 $\text{Pr}_2\text{CHNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-NH-(R)-CH(Et)CMe}_3,$

$\text{Pr}_2\text{CHNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-NHCH}_2\text{CMe}_3,$

10 $\text{EtPr}_2\text{CNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-NH-(R)-CH(Et)CMe}_3,$ and

$\text{EtPr}_2\text{CNHC(O)-Tbg-CH}_2\text{-(R)-CH(CH}_2\text{C(O)CMe}_3\text{)C(O)-}$
 $\text{Asp(cyPn)-NHCH}_2\text{CMe}_3;$ and

15

wherein the nucleoside analog is acyclovir.

16. A method according to claim 1 substantially as described herein with reference to the Figures and/or Examples.

DATED this 22ND day of JULY 1997

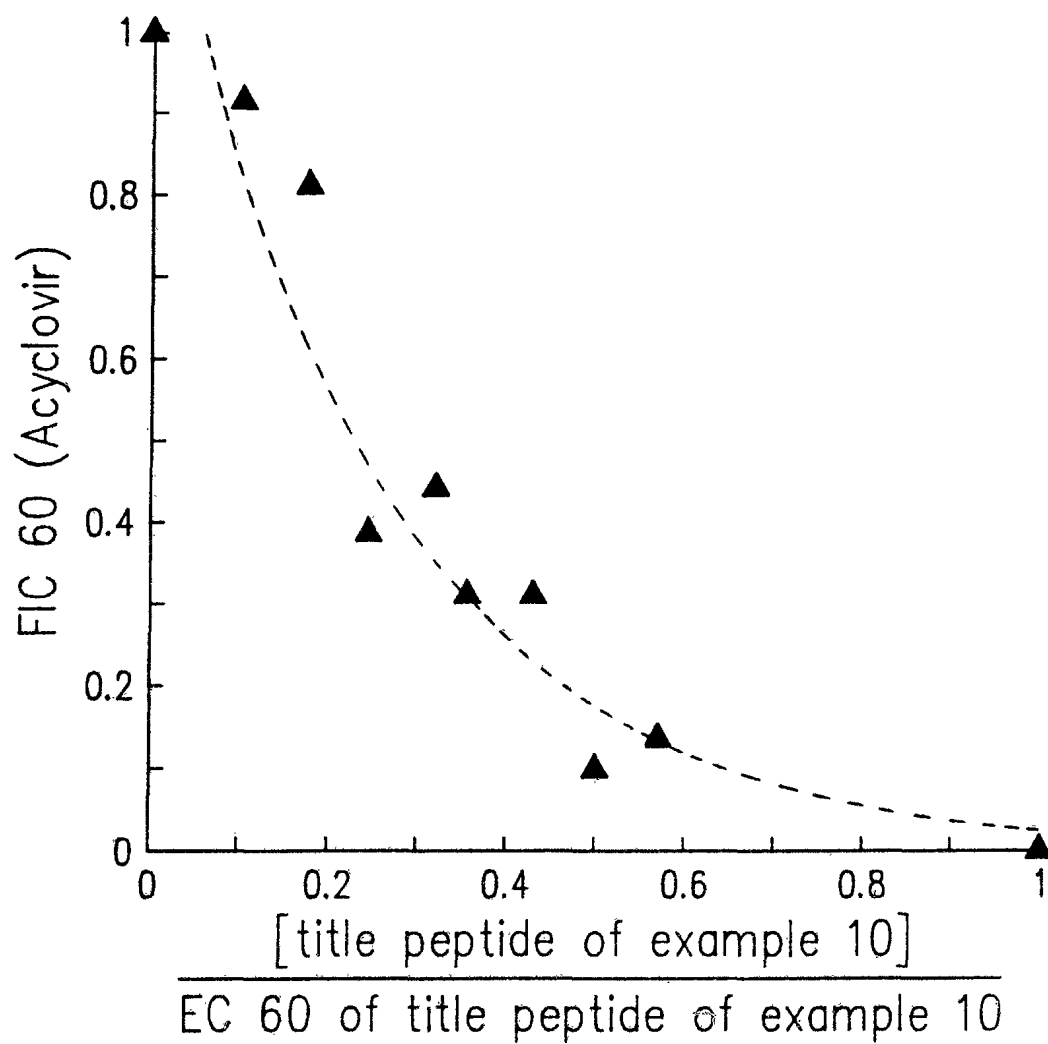
Bio-Mega/Boehringer Ingelheim Research, Inc.

by DAVIES COLLISON CAVE

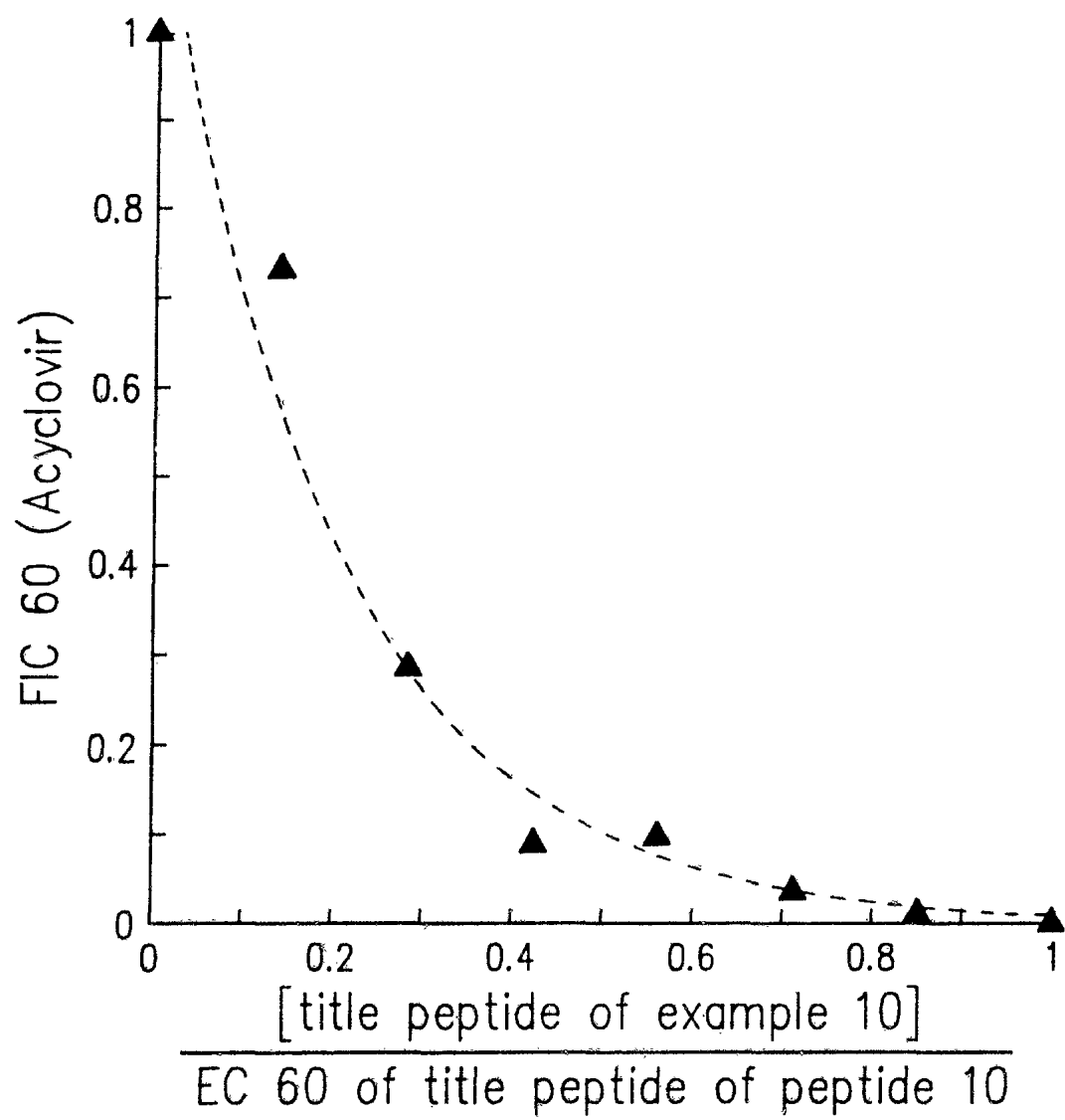
Patent Attorneys for the Applicant(s)



1/2

FIG. 1

2/2

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/CA 94/00242

A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 37/02

According to International Patent Classification (IPC) or to both national classification and IPC ⁵

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	WO, A1, 93/18 056 (BIO-MEGA/BOEHRINGER INGEL- HEIM RESEARCH INC.) 16 September 1993 (16.09.93), claims 1-8, 10, 12-15, 18, 19. --	1-25
X	EP, A2, 0 493 770 (BIO-MEGA INC.) 08 July 1992 (08.07.92), claims 1-8, 13. ----	1, 5- 11, 16- 25

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- * A* document defining the general state of the art which is not considered to be of particular relevance
- * E* earlier document but published on or after the international filing date
- * L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- * O* document referring to an oral disclosure, use, exhibition or other means
- * P* document published prior to the international filing date but later than the priority date claimed

- * T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- * X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- * Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * &* document member of the same patent family

Date of the actual completion of the international search

11 August 1994

Date of mailing of the international search report

14 -09- 1994

Name and mailing address of the ISA

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Authorized officer

BÖHM e.h.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 94/00242

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-11
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-11 are directed to a method of treatment of the human or animal body (Rule 39.1(iv)PCT) the search has been carried out and based on the alleged efforts of the compounds.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

• ANHANG

ANNEX

ANNEXE

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

to the International Search
Report to the International Patent
Application No.

au rapport de recherche inter-
national relatif à la demande de brevet
international n°

PCT/CA 94/00242 SAE 90009

In diesem Anhang sind die Mitglieder
der Patentfamilien der im obenge-
nannten internationalen Recherchenbericht
angeführten Patentdokumente angegeben.
Diese Angaben dienen nur zur Unter-
richtung und erfolgen ohne Gewähr.

This Annex lists the patent family
members relating to the patent documents
cited in the above-mentioned inter-
national search report. The Office is
in no way liable for these particulars
which are given merely for the purpose
of information.

La présente annexe indique les
membres de la famille de brevets
relatifs aux documents de brevets cités
dans le rapport de recherche inter-
national visée ci-dessus. Les renseigne-
ments fournis sont donnés à titre indica-
tif et n'engagent pas la responsabilité
de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
WD A1 9318056	16-09-93	AU A1 38807/93 EP A1 560267 IL A0 105001 JP A2 6025131	05-10-93 15-09-93 08-07-93 01-02-94
EP A2 493770	08-07-92	AU A1 90093/91 AU B2 648855 CA A2 2037448 EP A3 493770 IL A0 100549 JP A2 4275229 NZ A 241121	02-07-92 05-05-94 01-07-92 17-03-93 06-09-92 30-09-92 27-06-94