LACTAM COMPOUND

Field of the Invention

The present invention relates to the field of pharmaceutical and organic chemistry and is concerned with a compound which inhibits β-amyloid peptide release and/or its synthesis.

Background of the Invention

Certain lactams, which inhibit β-amyloid peptide release and/or its synthesis, and accordingly, are useful for treating Alzheimer's disease, are described in PCT Application No. PCT/US97/22986.

The present invention provides novel crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate, compositions thereof, methods of using the same, and processes for making the same, and processes for making intermediates thereof. The crystalline dihydrate of the present invention is useful for inhibiting β-amyloid peptide release and/or its synthesis, and, accordingly, is useful in treating Alzheimer's disease.

Since (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one is useful for treating Alzheimer's disease, there is the need to produce it as pure, stable, and crystalline form in order to fulfill exacting pharmaceutical requirements and specifications. The novel crystalline dihydrate of this invention has suitable properties to be conveniently formulated on a commercial scale in, for example, tablets for oral administration.

The process by which the (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3- methyl-2,3,4,5, tetrahydro- 1H-3-benzazepin-2-one is produced also needs to

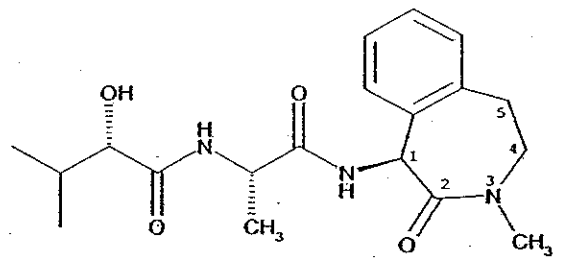
be one which is convenient to operate on a plant scale. In addition, the product should be in a form that is readily filtered, easily dried, and conveniently stored. Furthermore, the present crystalline dihydrate has suitable processing and storage properties.

It has been discovered that (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 tetrahydro- 1H-3-benzazepin-2-one can be prepared in a dihydrate form having advantageous properties and the manufacturing process for the new form fulfills the desirable features described above.

Summary of the Invention

This invention provides (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 tetrahydro- 1H-3- benzazepin-2-one dihydrate.

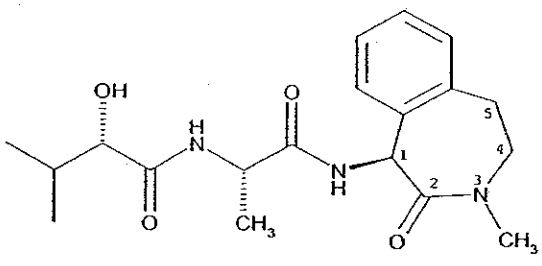
In a first aspect of the present invention, there is provided (N)-((S)-2-Hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate:



dihydrate.

In a second aspect of the present invention, there is provided a crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of the first aspect characterized by the X-ray powder diffraction pattern comprising a peak at 8.36, 12.43, 15.34, 19.22, 20.50, or 20.63 ($2\theta \pm 0.2°$).

In a third aspect of the present invention, there is provided a pharmaceutical composition comprising (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate:



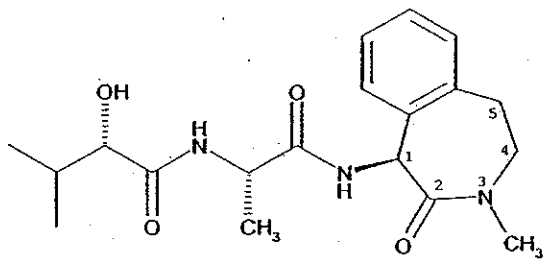
dihydrate

and a pharmaceutically acceptable diluent.

In another embodiment, the present invention provides a pharmaceutical composition comprising (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro- 1H-3-benzazepin-2-one dihydrate and a pharmaceutically acceptable diluent.

In one of its method aspects, this invention is directed to a method for inhibiting β-amyloid peptide release and/or its synthesis comprising administering to a patient in need thereof with an effective amount of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate.

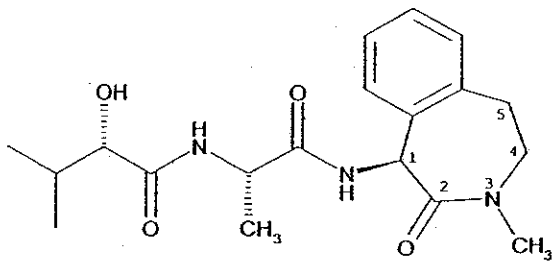
In a fourth aspect of the present invention, there is provided the use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate:



dihydrate

for the preparation of a medicament for inhibiting β-amyloid peptide release or its synthesis.

In a fifth aspect of the present invention, there is provided the use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate



dihydrate

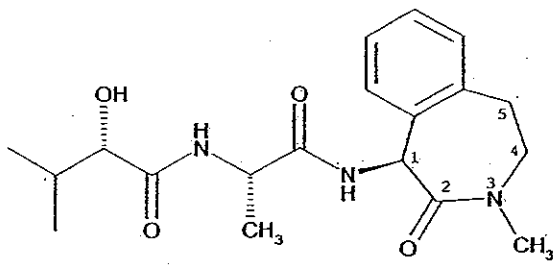
for the preparation of a medicament for the treatment of Alzheimer's Disease.

In a particular method embodiment, the present invention provides a method for treating Alzheimer's disease comprising administering to a patient in need thereof with an effective amount of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate. The present invention also provides a method for preventing or inhibiting the

progression of Alzheimer's disease comprising administering to a patient in need thereof with an effective amount of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino- 3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate.

That is, the present invention provides for the use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate for the manufacture of a medicament for inhibiting β-amyloid peptide release and/or its synthesis, including the treating of Alzheimer's disease.

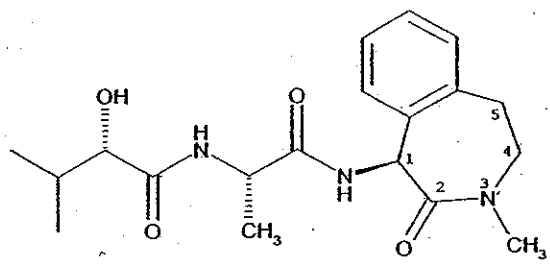
In a sixth aspect of the present invention, there is provided the use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate:



dihydrate

for the preparation of medicament for the prevention of Alzheimer's Disease.

In a seventh aspect of the present invention, there is provided the use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate:

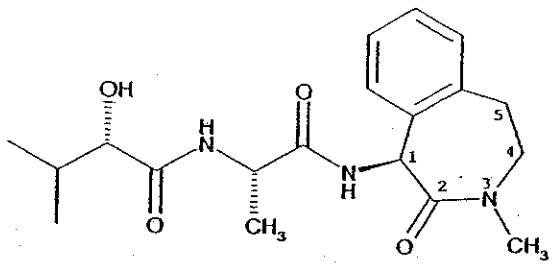


dihydrate

for the manufacture of a medicament for use in the inhibition of the progression of Alzheimer's Disease.

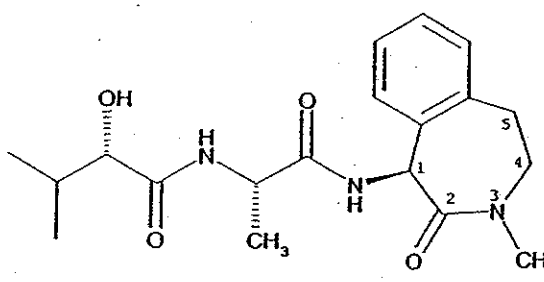
In another embodiment this invention provides a process for making (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate.

In an eighth aspect of the present invention, there is provided a process for preparing the crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate as defined above, comprising crystallizing (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one



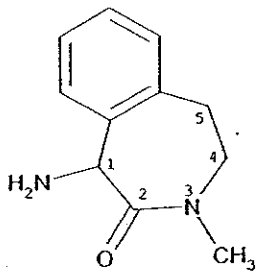
from an aqueous solvent.

In a ninth aspect of the present invention, there is provided a process for preparing (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one:

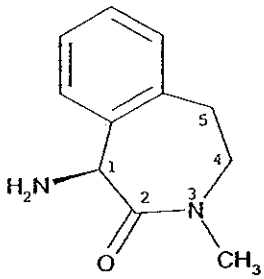


, comprising:

crystallizing 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one

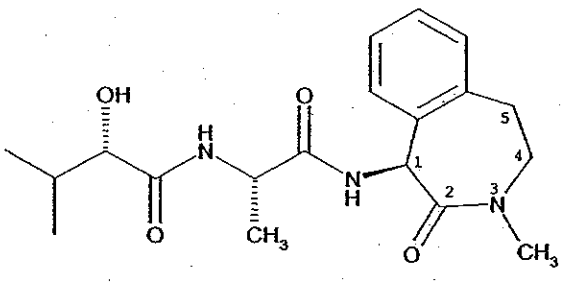


as an acid addition salt of an acid selected from the group consisting of di-p-tolyl-L-tartaric acid, (R)-(-)-l0-camphorsulfonic acid, and (D)-(-)-mandelic acid; coupling the (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one:



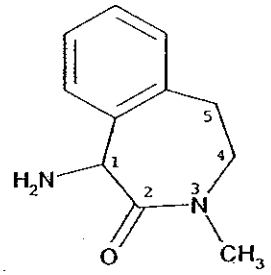
to an amino-protected L-alanine residue, to give an amino-protected 1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one; deprotecting to give 1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one; and coupling with (S)-2-hydroxy-3-methyl-butyric acid.

In a tenth aspect of the present invention, there is provided a process for preparing (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one:

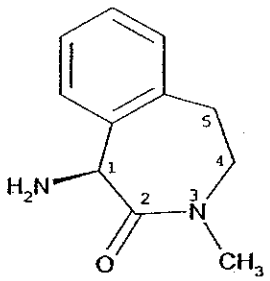


comprising:

crystallizing 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one:



as an acid addition salt of an acid selected from the group consisting of di-p-tolyl-L-tartaric acid, (R)-(-)-l0-camphorsulfonic acid, and (D)-(-)-mandelic acid; coupling the (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one:



to a (S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl) residue.

In still another embodiment this invention provides a processes for making lactams, including (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one and processes for making intermediates therefor.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the terms below have the meanings indicated:

The term "ee" or "enantiomeric excess" refers to the percent by which one enantiomer, E_1, is in excess in a mixture of both enantiomers (E_1 + E_2), as calculated by the equation ((E_1 - E_2) ÷ (E_1 + E_2)) x 100% = ee. As is well known in the art, enatiomeric excess can be determined by capillary electrophoresis and by chiral HPLC of the compounds or derivatives thereof.

Herein, the Cahn-Prelog-Ingold designations of (R)- and (S)- and the designations of L- and D- for stereochemistry

relative to the isomers of glyceraldehyde are used to refer to specific isomers.

The present invention provides (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro- 1H-3-benzazepin-2-one dihydrate, and in particular, a crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl -2,3,4,5-tetrahydro- 1H-3-benzazepin-2-one dihydrate.

A number of methods are available to characterize crystalline forms of organic compounds. For example methods include differential scanning calorimetry, solid state NMR spectrometry, infra-red spectroscopy, and X-ray powder diffraction. Among these X-ray powder diffraction and solid state NMR spectroscopy are very useful for identifying and distinguishing between crystalline forms.

X-ray powder diffraction analysis were performed as follows. Either with or without lightly grinding the sample with an agate mortar and pestle, the sample is loaded into a sample holder for the X-ray powder diffraction measurement. The X-ray powder diffraction patterns were measured using a Siemens D5000 X-ray powder diffractometer equipped with a CuK_α source (λ = 1.54056Å) operated at 50 kV and 40 mA using divergence slit size of 1 mm, receiving slit of 1 mm, and detector slit of 0.1 mm. Each sample was scanned between 4° and 35° (2θ) with a step size of 0.02° and a maximum scan rate of 3 sec/step. Data is collected using a Kevex solid-state silicon lithium detector. Optimally, a silicon standard is run routinely to check the instrument alignment.

It is well known in the crystallography art that, for any given crystal form, the relative intensities and peak widths of the diffraction peaks may vary due to a number of factors, including the effects of preferred orientation and/or particle size. Where the effects of preferred orientation and/or particle size are present, peak

intensities may be altered, but the characteristic peak positions of the polymorph are unchanged. See, e.g., The United States Pharmacopoeia #24, National Formulary #19, pages 1843-1844, 2000.

Grinding was used to minimize intensity variations for the peak intensities of some of the diffractogram disclosed herein. However, if grinding significantly altered the diffractogram or alters the crystalline state of the sample, then the diffractogram of the unground sample should be used. Grinding was done in a small agate mortar and pestle. The mortar was held during the grinding and light pressure was applied to the pestle.

Peak position was obtained in 2θ values and peak intensities for the most prominent features (relative intensities greater than 20%) were measured using a double-derivative peak picking method.

Accordingly, the present invention is directed to crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl -2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate characterized by the X-ray powder diffraction patterns in Table 1, which lists the 2θ values and relative intensities (I_o/I_{100}) greater than 20%, measured for an unground sample and for a samples after 5 and 10 minutes of grinding and using the methodology described above with CuK_α radiation:

Table 1

unground		5 min grinding		10 min grinding	
2θ (°)	I_o/I_{100} (%)	2θ (°)	I_o/I_{100} (%)	2θ (°)	I_o/I_{100} (%)
8.361	100	8.349	87.6	8.367	88.7
12.433	51.4	12.429	78.5	12.424	91.6
		13.24	27.9	13.254	30.4
15.344	49.8	15.336	100	15.352	100
		16.858	28.5	16.88	28.3
19.224	27.9	19.233	47.8	19.24	52
20.495	27.5	20.48	44.4		
20.63	38.8	20.644	43	20.601	48.9
		22.254	19.5	22.045	21.6
		22.63	27.6	22.654	34.9
		24.388	26.4	23.388	19.7
				24.387	28.2
				27.744	19
				22.237	21.2

The intensities of the samples ground for 5 and 10 minutes are more representative of the diffraction pattern where attempts were made to minimize the effects of preferred orientation and/or particle size. It should also be noted that the computer-generated, unrounded numbers are listed in this table.

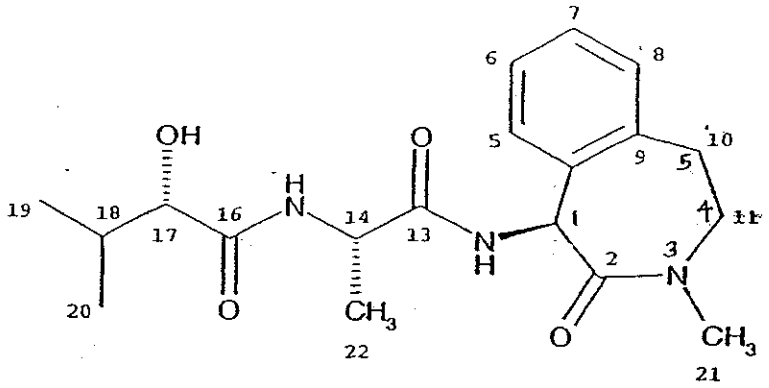
Thus, a properly prepared sample crystalline of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H- 3-benzazepin-2-one dihydrate may be characterized by X-ray diffraction pattern in 2θ values using CuK_α radiation having peaks as described in Table 1, and in particular having a peak at 8.36, 12.43, 15.34, 19.22, 20.50, or 20.63; more particularly having a peak at 8.36, 12.43, or 15.34; peaks at 8.36 and 12.43; 8.36 and 15.34; 8.36, 12.43, and 15.34; or at 8.36, 12.43, 15.34, 19.22, 20.50, and 20.63.

Crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate may also be characterized by solid state NMR spectroscopy. Solid state ^{13}C chemical

shifts reflect not only the molecular structure of but also the electronic environment of the molecule in the crystal.

Solid state NMR (^{13}C) analysis can be carried out using ^{13}C Cross polarization/magic angle spinning (CP/MAS). NMR (solid-state NMR or SSNMR) spectra were obtained using a Varian Unity 400 MHz spectrometer operating at a carbon frequency of 100.580 MHz, equipped with a complete solids accessory and Varian 7 mm VT CP/MAS probe. Acquisition parameters were are follows: 90° proton r.f. pulse width 4.0 μs, contact time 1.0 ms, pulse repetition time 5 s, MAS frequency 7.0 kHz, spectral width 50 kHz, and acquisition time 50 ms. Chemical shifts were referenced to the methyl group of external hexamethylbenzene (δ = 17.3 ppm), that is, by sample replacement with hexamethylbenzene.

Chemical shift data for (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-4,5,6,7-tetrahydro-2H-3-benzazepin-2-one dihydrate (based on the peak assignments, below) are shown in Table 2.



Site	Solution in DMSO	Dihydrate (solid)
2, 13, 16	169.27, 171.73, 172.89	169.9*, 174.1*, 176.7*
3, 11, 14	51.45, 47.05, 47.72	46.9, 51.3
4	134.14	134.8*
5, 6	124.18, 125.99	124.0
7	127.19	127.9, 128.9
8	130.38	132.1
9	135.32	136.9*
10, 18	30.73, 31.29	31.1
17	75.01	75.6
19, 20, 22	15.99, 18.66, 19.13	16.6*, 21.4*
21	34.18	35.3*

Asterisks (*) denote peaks appearing in the interrupted decoupling spectrum.

Thus, crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate may be characterized by solid state ^{13}C nuclear magnetic resonance having chemical shift (ppm) of 16.6; 176.7, 174.1, 169.9, 136.9, 134.8, 132.1, 127.9, 128.9, 124.0, 75.6, 51.3, 46.9, 35.3, 31.1, 21.4, or 16.6; more particularly, 75.6, 35.3, 21.4, or 16.6; any two of 75.6, 35.3, 21.4, and 16.6; 75.6, 35.3, 21.4, and 16.6; or at 176.7, 174.1, 169.9, 136.9, 134.8, 132.1, 127.9, 128.9, 124.0, 75.6, 51.3, 46.9, 35.3, 31.1, 21.4, and 16.6.

In another embodiment this invention provides a process for making (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate comprising crystallizing (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-

3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one from aqueous solvents under conditions which yield (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate.

The precise conditions under which (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate is formed may be empirically determined and it is only possible to give a number of methods which have been found to be suitable in practice.

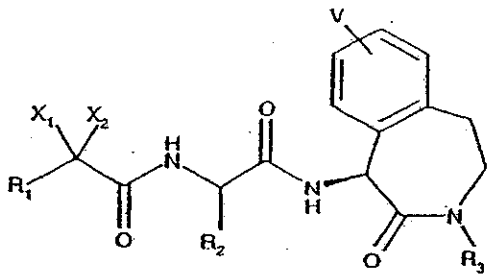
Thus, for example, (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate may be prepared by crystallization under controlled conditions. Crystallization from a solution and slurrying techniques are contemplated to be within the scope of the present process. In particular, the dihydrate of the present invention can be prepared by crystallization from an aqueous solvent. An suitable solvent is one that is capable of containing sufficient water, at the concentrations used, to form the present dihydrate. Preferred solvents are those that are water miscible, such as acetone, lower alcohols (like methanol, ethanol, and isopropanol), acetic acid, and acetonitrile. In practice, it has been found that aqueous acetone is preferred. For a given aqueous solvent the amount of water used will depend on the relative solubility of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one the solvent compared to water and whether a crystallization or slurrying technique is used.

A crystallization is generally carried out by dissolving, (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one in an aqueous solvent and then allowing the

solution to cool, with or without the addition of more water, to give a solid. Typically, the crystallization is carried out at initial temperatures of about 40°C to reflux temperature of the selected aqueous solvent. The mixture is then cooled to give the crystalline dihydrate. Seeding may be advantageous. Preferably the crystallization solution is cooled slowly. The crystallization is most conveniently cooled to temperatures of ambient temperature to about -20°C.

In still another embodiment this invention provides a processes for making lactams, including (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl- 2,3,4,5- -tetrahydro- 1H-3-benzazepin-2-one and processes for making intermediates therefor.

More specifically, in one process embodiment, the present invention provides a process for making lactams of formula I



formula I

wherein

R_1 is alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic;

R_2 is alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, aryl, heteroaryl and heterocyclic;

R_3 is alkyl,

X_1 is hydrogen, hydroxy or fluoro,

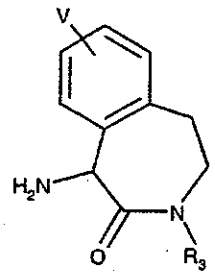
X_2 is hydrogen, hydroxy or fluoro, or

X_1 and X_2 together form an oxo group; and

V is from 1 to 3 groups independently selected from the group consisting of hydrogen, hydroxy, acyl, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, amino, aminoacyl, alkaryl, aryl, aryloxy, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, thioalkoxy, substituted thioalkoxy, and trihalomethyl;

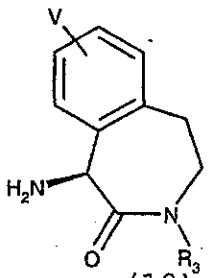
comprising:

(a1) resolution of a lactam of formula (4)



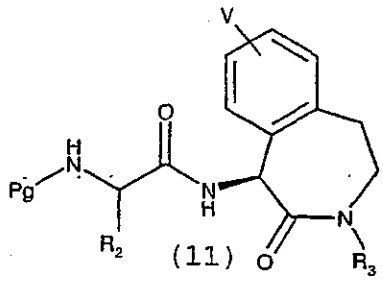
(4)

wherein R_3 and V are as described for the compound of formula I, by fractional crystallization of its by fractional crystallization of its dibenzoyltartrate, (R)-(-)-10-camphorsulfonic acid, and (D)-(-)-mandelic acid salts to give a compound of formula (10)

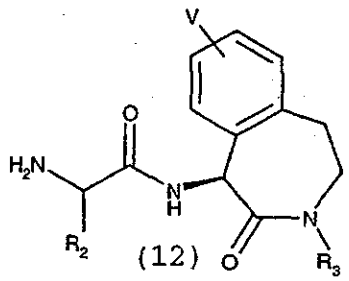


(10)

(b) coupling with an appropriate amino-protected amino acid of formula the PgNH-CHR_2-C(O)-A wherein R_2 is as defined for the compound of formula I and A is an activating group, for example -OH, -Br, or -Cl, and Pg is an amine protecting group to give a compound of formula (11)



(c) deprotection of a compound of formula (11) to give a compound of formula (12); and



(d) coupling a compound of formula (12) with an appropriate compound of formula $R_1CX_1X_2$-C(O)A_1 wherein R_1, X_1, and X_2 are as described for the compound of formula I and A_1 is an activating group, for example -OH, -Br, or -Cl; or

(a2) coupling a compound of formula (10) with a compound of the formula $R_1CX_1X_2$-C(O)NH-CHR_2-C(O)-A wherein R_1, R_2, X_1, and X_2 are as defined for the compound of formula I and A is an activating group, for example -OH, -Br, or -Cl.

As used herein, the terms below have the meanings indicated:

"Alkyl" refers to monovalent alkyl groups preferably having from 1 to 10 carbon atoms and more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, n-hexyl, and the like.

The term "C_1-C_4 alkyl" refers to monovalent alkyl groups preferably having from 1 to 4 carbon atoms. This term is exemplified by groups such as methyl, ethyl, n-

propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, and tert-butyl.

"Substituted alkyl" refers to an alkyl group, preferably of from 1 to 10 carbon atoms, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, cyano, halogen, hydroxyl, carboxyl, keto, thioketo, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl,-SO2-heteroaryl, and mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-heteroarylamino, mono- and di-heterocyclic amino, and unsymmetric di-substituted amines having different substituents selected from alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic.

"Alkylene" refers to divalent alkylene groups preferably having from 1 to 10 carbon atoms and more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methylene ($-CH_2-$), ethylene ($-CH_2CH_2-$), the propylene isomers (e.g., $-CH_2CH_2CH_2-$ and $-CH(CH_3)CH_2-$) and the like.

"Substituted alkylene" refers to an alkylene group, preferably of from 1 to 10 carbon atoms, having from 1 to 3 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, cyano, halogen, hydroxyl, carboxyl, keto, thioketo, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, heteroaryl, heterocyclic, nitro, and mono- and di-alkylamino, mono- and di-

(substituted alkyl)amino, mono- and di-arylamino, mono- and di-heteroarylamino, mono- and di-heterocyclic amino, and unsymmetric di-substituted amines having different substituents selected from alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form one or more cycloalkyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group. Preferably such fused cycloalkyl groups contain from 1 to 3 fused ring structures.

"Alkenylene" refers to divalent alkenylene groups preferably having from 2 to 10 carbon atoms and more preferably 2 to 6 carbon atoms. This term is exemplified by groups such as ethenylene (-CH=CH-), the propenylene isomers (e.g., $-CH_2CH=CH-$ and $-C(CH_3)=CH-$) and the like.

"Substituted alkenylene" refers to an alkenylene group, preferably of from 2 to 10 carbon atoms, having from 1 to 3 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, cyano, halogen, hydroxyl, carboxyl, keto, thioketo, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, heteroaryl, heterocyclic, nitro, and mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-heteroarylamino, mono- and di-heterocyclic amino, and unsymmetric di-substituted amines having different substituents selected from alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form one or more cycloalkyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group.

"Alkaryl" refers to -alkylene-aryl groups preferably having from 1 to 8 carbon atoms in the alkylene moiety and

from 6 to 10 carbon atoms in the aryl moiety. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

"Alkoxy" refers to the group "alkyl-O-". Preferred alkoxy groups include, by way of example, methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, 1,2-dimethylbutoxy, and the like.

"Substituted alkoxy" refers to the group "substituted alkyl-O-" where substituted alkyl is as defined above.

"Alkylalkoxy" refers to the group "-alkylene-O-alkyl" which includes by way of example, methylenemethoxy ($-CH_2OCH_3$), ethylenemethoxy ($-CH_2CH_2OCH_3$), n-propylene-iso-propoxy ($-CH_2CH_2CH_2OCH(CH_3)_2$), methylene-t-butoxy ($-CH_2-O-C(CH_3)_3$) and the like.

"Alkylthioalkoxy" refers to the group "-alkylene-S-alkyl" which includes by way of example, methylenethiomethoxy ($-CH_2SCH_3$), ethylenethiomethoxy ($-CH_2CH_2SCH_3$), n-propylene-thio-iso-propoxy ($-CH_2CH_2CH_2SCH(CH_3)_2$), methylenethio-t-butoxy ($-CH_2SC(CH_3)_3$) and the like.

"Alkenyl" refers to alkenyl groups preferably having from 2 to 10 carbon atoms and more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-2 sites of alkenyl unsaturation. Preferred alkenyl groups include ethenyl ($-CH=CH_2$), n-propenyl ($-CH_2CH=CH_2$), iso-propenyl ($-C(CH_3)=CH_2$), and the like.

"Substituted alkenyl" refers to an alkenyl group as defined above having from 1 to 3 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, cyano, halogen, hydroxyl, carboxyl, keto, thioketo, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, heteroaryl, heterocyclic, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl,

$-SO_2$-aryl, $-SO_2$-heteroaryl, and mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-heteroarylamino, mono- and di-heterocyclic amino, and unsymmetric di-substituted amines having different substituents selected from alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic.

"Alkynyl" refers to alkynyl groups preferably having from 2 to 10 carbon atoms and more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-2 sites of alkynyl unsaturation. Preferred alkynyl groups include ethynyl ($-CH{\equiv}CH_2$), propargyl ($-CH_2C{\equiv}CH$) and the like.

"Substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 3 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, cyano, halogen, hydroxyl, carboxyl, keto, thioketo, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, heteroaryl, heterocyclic, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl, and mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-heteroarylamino, mono- and di-heterocyclic amino, and unsymmetric di-substituted amines having different substituents selected from alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic.

"Acyl" refers to the groups alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclic-C(O)- where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl and heterocyclic are as defined herein.

"Acylamino" refers to the group -C(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted

alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

"Aminoacyl" refers to the group -NRC(O)R where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

"Aminoacyloxy" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

"Acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclic-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, aryl, heteroaryl, and heterocyclic are as defined herein.

"Aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents selected from the group consisting of acyloxy, 1 to 5 and preferably 1 to 3 substituents selected from the group consisting of hydroxy, acyl, alkyl, alkoxy, alkenyl, alkynyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heterocyclic, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-

aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, -SO_2-heteroaryl, trihalomethyl, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-heteroarylamino, mono- and di-heterocyclic amino, and unsymmetric di-substituted amines having different substituents selected from alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic, and the like. Preferred substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy.

"Aryloxy" refers to the group aryl-O- wherein the aryl group is as defined above including optionally substituted aryl groups as also defined above.

"Carboxyalkyl" refers to the group "-C(O)Oalkyl" where alkyl is as defined above.

"Cycloalkyl" refers to cyclic alkyl groups of from 3 to 12 carbon atoms having a single cyclic ring or multiple condensed rings, including fused, bridged and spiro bicyclic or multicyclic compounds. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

"Substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 (preferably 1 to 3) substituents selected from the group consisting of hydroxy, acyl, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, amino, aminoacyl, alkaryl, aryl, aryloxy, keto, thioketo, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, thioalkoxy, substituted thioalkoxy, trihalomethyl and the like.

"Cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 8 carbon atoms having a single cyclic ring and at least one point of internal unsaturation. Examples of suitable

cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

"Substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents selected from the group consisting of hydroxy, acyl, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, amino, aminoacyl, alkaryl, aryl, aryloxy, carboxyl, keto, thioketo, carboxylalkyl, cyano, halo, nitro, heteroaryl, thioalkoxy, substituted thioalkoxy, trihalomethyl and the like.

"Halo" or "halogen" refers to fluoro, chloro, bromo and iodo and preferably is either fluoro or chloro.

"Heteroaryl" refers to an aromatic carbocyclic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents selected from the group consisting of alkyl, substituted alkyl, alkoxy, substituted alkoxy, aryl, aryloxy, halo, nitro, heteroaryl, thiol, thioalkoxy, substituted thioalkoxy, thioaryloxy, trihalomethyl and the like. Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl), including fused, bridged and spiro bicyclic or multicyclic compounds. Preferred heteroaryls include pyridyl, pyrrolyl and furyl.

"Heterocycle" or "heterocyclic" refers to a monovalent saturated or unsaturated group having a single ring or multiple condensed rings, including fused, bridged and spiro bicyclic or multicyclic compounds, having from 1 to 15 carbon atoms and from 1 to 4 hetero atoms selected from nitrogen, sulfur or oxygen within the ring.

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5 substituents selected from the group consisting of alkyl, substituted alkyl, alkoxy, substituted alkoxy, aryl, aryloxy, halo, nitro, heteroaryl, thiol, thioalkoxy, substituted thioalkoxy, thioaryloxy, trihalomethyl, and the like. Such heterocyclic groups can have a single ring or multiple condensed rings. Preferred heterocyclics include morpholino, piperidinyl, and the like.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

"Oxyacylamino" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

"Thiol" refers to the group -SH.

"Thioalkoxy" refers to the group -S-alkyl.

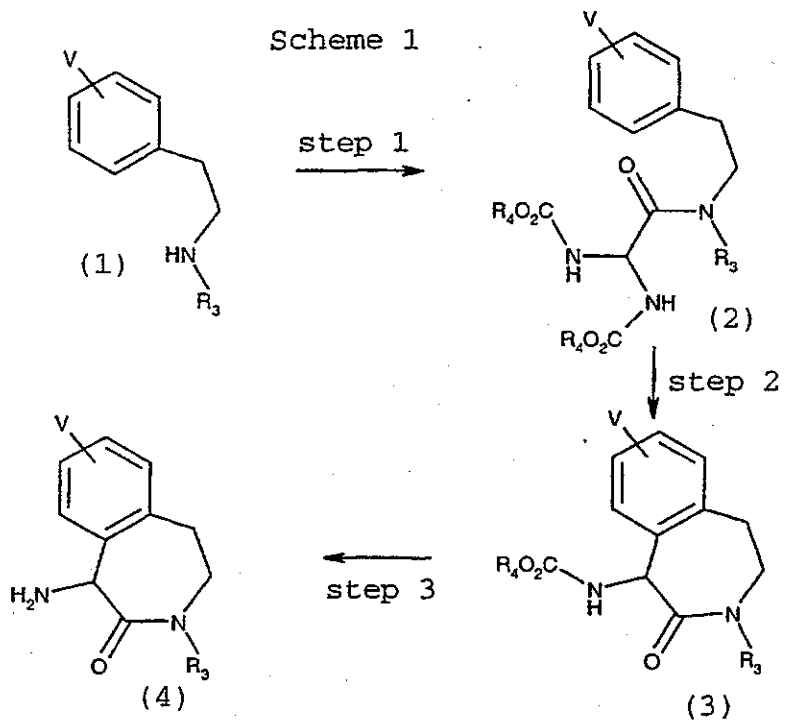
"Substituted thioalkoxy" refers to the group -S-substituted alkyl.

"Thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

"Heteroaryloxy" refers to the group heteroaryl-o- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

In particular process embodiments the present inventions provide methods for making a compound of formula (4) as described in Schemes 1 and 2. In the Schemes below, all substituents, unless otherwise indicated, are as previously defined and all reagents are well known and appreciated in the art.

Scheme 1



In Scheme 1, step 1, an appropriate N-alkylphenethylamine of formula (1) is acylated with a suitable bisalkoxycarbonylacetate transfer reagent to give a compound of formula (2). An appropriate N-alkylphenethylamine of formula (1) is one in which V and R_3 are as desired in the final product of formula I. Such N-alkylphenethylamines are readily prepared by the reaction of a 2-bromo or 2-chloroethylbenzene, under conditions well known and appreciated in the art, with an amine of the formula H_2N-R_3. A suitable bisalkoxycarbonylacetate

transfer reagent is one in which R_4 is C_1-C_4 alkyl and transfers a bisalkoxycarbonylacetyl group to the compound of formula (1), such as, bisalkoxycarbonylacetic acids and bisalkoxycarbonylacetyl chlorides. (See Ben-Ishai, Tetrahedron, 43, 439-450 (1987)).

For example, an appropriate N-alkylphenethylamine of formula (1) is contacted with a suitable bisalkoxycarbonylacetic acid to give a compound of formula (2). Such coupling reactions are common in peptide synthesis and synthetic methods used therein can be employed. For example, well known coupling reagents such as carbodiimides with or without the use of well known additives such as N-hydroxysuccinimide, 1-hydroxybenzotriazole, etc. can be used to facilitate this acylation. Such coupling reactions often use a suitable base to scavenge the acid generated during the reaction. Suitable bases include, by way of example, triethylamine, N,N-diisopropylethylamine, N-methylmorpholine and the like. The reaction is conventionally conducted in an inert aprotic polar diluent such as dimethylformamide, methylene chloride, chloroform, acetonitrile, tetrahydrofuran and the like. Typically the reaction is carried out at temperatures of from about 0°C to about 60°C and typically require from about 1 to about 24 hours. Upon reaction completion, the product of formula (2) is recovered by conventional methods including extraction, precipitation, chromatography, filtration, trituration, crystallization and the like.

Alternatively, for example, an appropriate N-alkylphenethylamine of formula (1) is contacted with a suitable bisalkoxycarbonylacetyl chloride to give a compound of formula (2). Such acid chlorides are readily prepared from the corresponding acids by methods well known in the art, such as by the action of phosphorous trichloride, phosphorous oxychloride, phosphorous pentachloride, thionyl chloride, or oxalyl chloride, with or without a small amount

of dimethylformamide, in an inert solvent such as, toluene, methylene chloride, or chloroform; at temperatures of from about 0-80°C. The reaction is typically carried out for a period of time ranging from 1 hour to 24 hours. The acid chloride can be isolated and purified or can often be used directly, that is, with or without isolation and/or purification. Such acylation reactions generally use a suitable base to scavenge the acid generated during the reaction. Suitable bases include, by way of example, pyridine, triethylamine, N,N-diisopropylethylamine, N-methylmorpholine and the like. The reaction is conventionally conducted in an inert aprotic polar diluent such as methylene chloride, chloroform, tetrahydrofuran and the like. Typically the reaction is carried out at temperatures of from about -20°C to about 80°C and typically require from about 1 to about 24 hours. Upon reaction completion, the product of formula (2) is recovered by conventional methods including extraction, precipitation, chromatography, filtration, trituration, crystallization and the like.

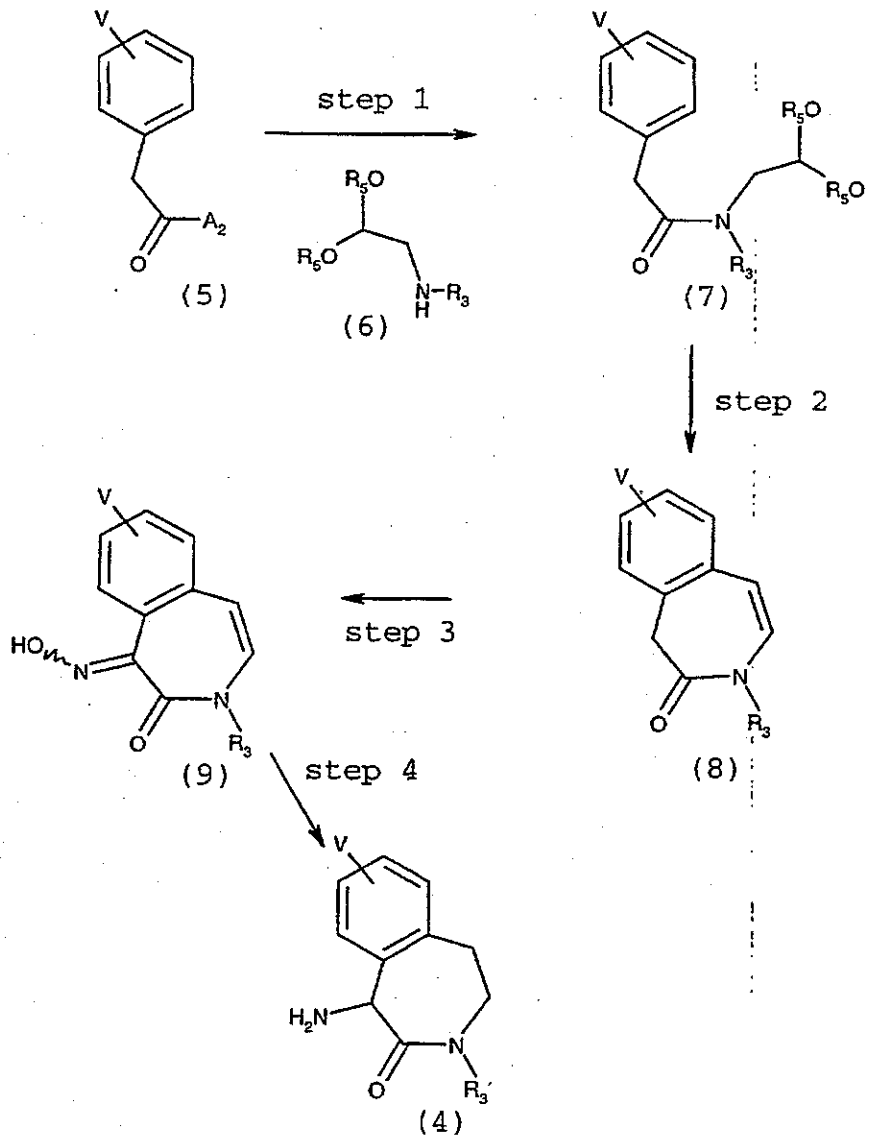
In Scheme 1, step 2, a compound of formula (2) is cyclized to give a compound of formula (3).

For example, a compound of formula (2) is contacted with a acid, such as trifluoromethanesulfonic acid or sulfuric acid. The reaction is typically carried out using the selected acid as a solvent. Typically the reactants are initially mixed at temperatures of from about -20°C to about 0°C and then allowed to warm to temperatures of about ambient temperature to about 60°C. The cyclization reaction typically require from about 12 to about 72 hours. Upon reaction completion, the product of formula (2) is recovered by conventional methods including extraction, precipitation, chromatography, filtration, trituration, crystallization and the like.

In Scheme 1, step 3, a compound of formula (3) is deprotected to give a compound of formula (4).

The removal of such alkoxycarbonyl amine protecting groups is well known and appreciated in the art. For example see, Protecting Groups in Organic Synthesis, Theodora Greene (1st and 2nd Editions, Wiley-Interscience) and Ben-Ishai, Tetrahedron, 43, 439-450 (1987).

Scheme 2



In Scheme 2, step 1, an appropriate phenyl acetic acid derivative of formula (5) is coupled with an appropriate acetal of formula (6) to give a compound of formula (7). An

appropriate phenyl acetic acid derivative of formula (5) is one in which V is as desired in the final product of formula I and A_2 is an activated group, for example, -OH, -Cl, or -Br. An appropriate acetal of formula (6) is one in which R_3 is as desired in the final product of formula I and R_5 is a C_1-C_4 alkyl. Such coupling reactions are common in peptide synthesis and synthetic methods used therein can be employed as are described in Scheme 1, step 1.

Also, the coupling depicted in Scheme 2, step 2, can be carried out under Schotten-Baumann conditions using an acid halide of appropriate phenyl acetic acid of formula (5) and an appropriate acetal of formula (6) in a mixed solvent, such as, methyl t-butyl ether, ethyl acetate, tetrahydrofuran, acetone, or diethyl ether and water. Such reaction are carried out using a suitable base, such as sodium hydroxide, potassium hydroxide, sodium carbonate, potassium carbonate, sodium bicarbonate, or potassium bicarbonate. Typically the reaction is stirred or agitated vigorously and is carried out at temperatures of from about -20°C to about 80°C and typically require from about 1 to about 24 hours. Upon reaction completion, the product of formula (7) is recovered by conventional methods including extraction, precipitation, chromatography, filtration, trituration, crystallization and the like.

In Scheme 2, step 2, a compound of formula (7) is cyclized to give a compound of formula (8). Such cyclization reactions are carried out in a acid, such as sulfuric acid. Typically the acid is used as the solvent. In general, the reaction is carried out at temperatures of from about -20°C to about 150°C and typically require from about 1 to about 24 hours. Upon reaction completion, the product of formula (8) is recovered by conventional methods including extraction, precipitation, chromatography, filtration, trituration, crystallization and the like.

In Scheme 2, step 3, a compound of formula (8) undergoes an amine transfer reaction to give a compound of formula (9). In Scheme 2 an oximation is depicted. Such oximation are accomplished by contacting the enolate of a compound of formula (8) with an oxime transfer reagents, such as an alkyl nitrite ester. The enolate of a compound of formula (8) can be prepared by reacting the compound of formula (8) with a suitable base, such as potassium t-butoxide, lithium diisopropylamide, lithium hexamethyldisilazide, sodium hexamethyldisilazide, potassium hexamethyldisilazide, and the like. Such oximinations are exemplified by Wheeler, et al., Organic Syntheses, Coll. Vol. VI, p. 840 which describes the reaction of isoamyl nitrite with a ketone to prepare the desired oxime. The reaction is typically carried out in a solvent, such as tetrahydrofuran. In general, the reaction is carried out at temperatures of from about -20°C to about 50°C and typically require from about 1 to about 24 hours. Upon reaction completion, the product of formula (8) is recovered by conventional methods including extraction, precipitation, chromatography, filtration, trituration, crystallization and the like.

Alternately, such an amine transfer reaction can be accomplished through the azide. An azide can be formed by the reaction of the enolate of a compound of formula (8) with an azide transfer reagent, such as toluenesulfonyl azide and triisopropylbenzenesulfonyl azide. Such reaction are exemplified in Evans, et al., J. Am. Chem. Soc., 112:4011-4030 (1990)41. The reaction is typically carried out in a solvent, such as tetrahydrofuran. In general, the reaction is carried out at temperatures of from about -20°C to about 50°C and typically require from about 1 to about 24 hours. Upon reaction completion, the product of formula (8) having an azide instead of an oxime is recovered by conventional methods including extraction, precipitation,

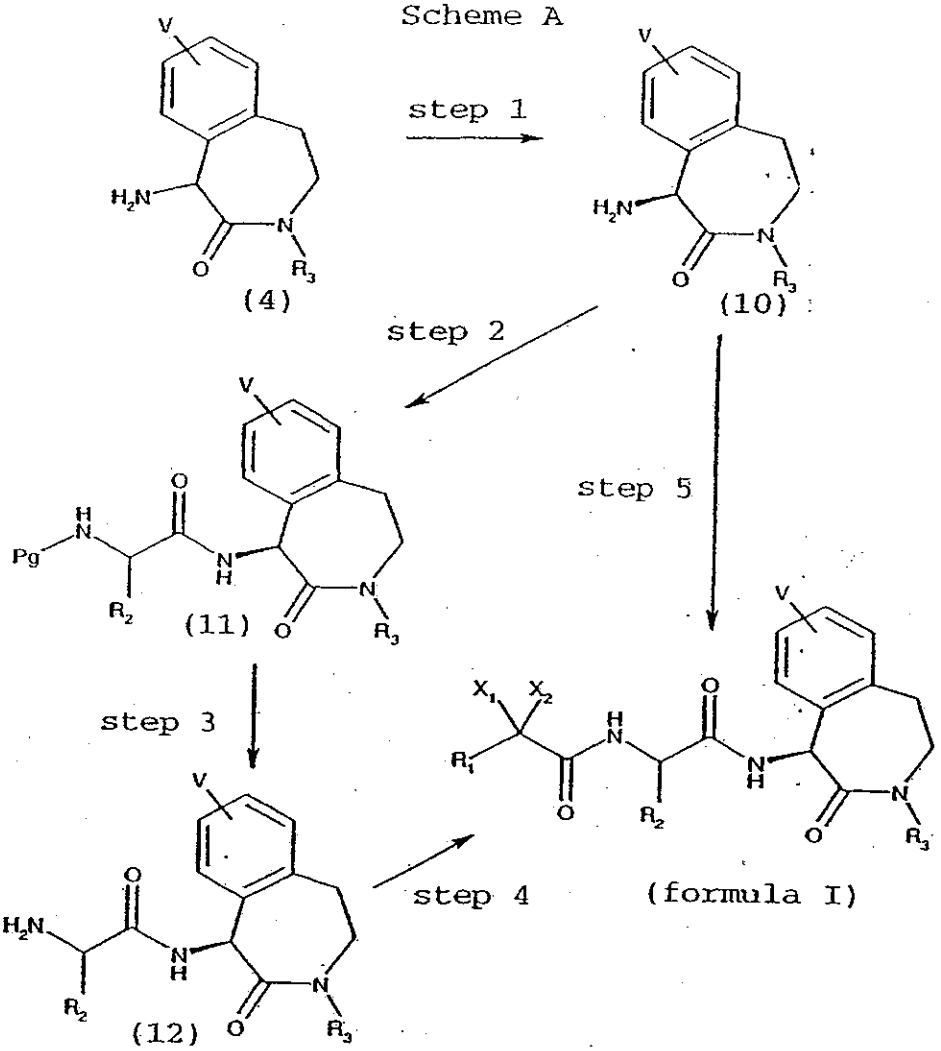
chromatography, filtration, trituration, crystallization and the like.

As depicted in Scheme 2, step 4, an oxime is reduced to the compound of formula (4). Such reductions are accomplished by treatment with hydrogen and a suitable catalyst, such as Raney-nickel or palladium catalysts, such as palladium-on-carbon. The reaction is typically carried out in a solvent, such as tetrahydrofuran, ethyl acetate, or lower alcohols, such as methanol, ethanol, and isopropanol, acetic acid, water, aqueous ammonia, and the like, and mixtures thereof. The reaction generally carried out at hydrogen pressures ranging from atmospheric pressure to about 600 psi (4137 kPa). In general, the reaction is carried out at temperatures of from about 20°C to about 100°C and typically require from about 1 to about 24 hours. Upon reaction completion, the product of formula (4) is recovered by conventional methods including extraction, precipitation, chromatography, filtration, trituration, crystallization and the like.

Alternately, where the amine is transferred via an azide, the azido group is reduced. Such reductions are carried out by hydrogenation as described above.

In a further embodiment, the present invention provides stereo specific processes for making lactams, compounds of formula I, including (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one, and processes of making chiral intermediates thereof. Such processes are described in Scheme A.

Scheme A



Scheme A, step 1, depicts the stereochemical resolution of an appropriate lactam of formula (4) to give a lactam of formula (10). As will be appreciated by the skilled artisan, the present processes are not necessarily limited to the preparation of a single isomer. Rather the present processes are capable of preparing either of the specific enantiomers of the lactams and is particularly suited to preparing the isomers of 1-amino-3-alkyl-4,5,6,7-tetrahydro-2H-3-benzazepin-2-ones.

Because of the greater biological activity associated with the (S)-isomer at the 2,3,4,5 -tetrahydro-1H-3-benzazepin-2-one moiety, the present invention is most useful as a preparation of a substantially pure (S)-isomer.

As used herein the term "substantially pure" refers to enantiomeric purity of (R)- or (S)-lactam, and particularly (R)- and (S)-1-amino-3- methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one. Accordingly to the present invention substantially pure (S)-1-amino-3-alkyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one can be prepared comprising the (S)-enantiomer which is greater than 80%, preferably greater than 90%, more preferably greater than 95%, most preferably greater than 97%.

For example, the individual isomers of the compound of formula (4) can be resolved by fractional crystallization of dibenzoyltartrate, (R)-(-)-10-camphorsulfonic acid, and (D)-(-)-mandelic acid salts. It is expected that a wide variety of dibenzolytartarates are suitable for this purpose. In particular, the dibenzoyl esters having a para substituent selected from the group consisting of hydrogen, halogen, C_1-C_4 alkyl, and C_1-C_4 alkoxy are preferred with di-p-toluoyl-tartrate being most preferred. Di-p-toluoyl-L-tartrate is used to obtain the (S)-isomer.

In a preferred embodiment of Scheme A, step 1, the compound of formula (4) is one in which V is hydrogen and R_3 is C_1-C_4alkyl, including methyl, ethyl, propyl, iso-propyl, butyl, iso-butyl, and sec-butyl; and most preferred is the use of compounds of formula (4) in which V is hydrogen and R_3 is methyl.

According to the present process, the compound of formula (4) is contacted with the selected acid. Generally, from about 0.4 molar equivalents to a large excess of the selected acid can be used with about 0.4 to 1.5 molar equivalents being preferred and with about 0.5 to 1.1 molar equivalents being more preferred.

The process is typically carried out by crystallizing the acid addition salt from a solution. In particular, solvents such as lower alcohols, including methanol,

ethanol, n-propanol, isopropanol, butanol, sec-butanol, iso-butanol, t-butanol, amyl alcohol, iso-amyl alcohol, t-amyl alcohol, hexanol, cyclopentanol, and cyclohexanol are suitable, with methanol, ethanol, and isopropanol being preferred. The use of an anti-solvent may be advantageous. As used herein, the term "anti-solvent" refers to a solvent in which the salt is significantly less soluble compared to solvent. Preferably, when an anti-solvent is used it is miscible with the selected solvent. Suitable anti-solvents include ethers, such as diethyl ether, methyl t-butyl ether, and the like, and lower alkyl acetates, such as methyl acetate, ethyl acetate, iso-propyl acetate, propyl acetate, iso-butyl acetate, sec-butyl acetate, butyl acetate, amyl acetate, iso-amyl acetate, and the like, and alkanes, such as pentane, hexane, heptane, cyclohexane, and the like. When the present process is carried out by crystallizing the acid addition salt from the racemic mixture, care must be taken in using an anti-solvent to avoid crystallization of the salt of the undesired diastereomeric salt.

Typically, the crystallization is carried out at initial temperatures of about 40°C to reflux temperature of the selected solvent(s) and at initial concentrations of from about 0.05 molar to about 0.25 molar. The mixture is then cooled to give the salt. Seeding may be advantageous. Stirring of the initial precipitate for from about 4 to 48 hours may be advantageous. Preferably the crystallization solution is cooled slowly. The crystallization is most conveniently cooled to temperatures of ambient temperature to about -20°C. The salt can be collected using techniques that are well known in the art, including filtration, decanting, centrifuging, evaporation, drying, and the like. The compound of formula (10) can be used directly as the acid addition salt of the selected acid. Alternately, before use the compound of formula (10) can be isolated as

another acid addition salt after acid exchange or can by isolated as the base by extraction under basic conditions as is well known and appreciated in the art.

In a preferred embodiment the present invention provides a dynamic resolution of (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one of substantial enantiomeric purity comprising crystallizing 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one as its acid addition salt of an acid selected from the group consisting of di-p-tolyl-L-tartaric acid, (R)-(-)-10-camphorsulfonic acid, and (D)-(-)-mandelic acid as a dynamic process in the presence of an aromatic aldehyde. The dynamic process has the advantage that the 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one undergoes conversion to a single isomer during the crystallization, thus, improving the yield and avoiding a waste stream which includes an undesired isomer.

It is expected that a wide variety of aldehydes are suitable for the present process, we have found that a number of aldehydes are particularly suitable in practice. Specifically, we have found that salicylic acids are preferred and salicylaldehyde, 5-nitrosalicylaldehyde, and 3,5-dichlorosalicylaldehyde are more preferred in the present dynamic resolution process.

Accordingly, when the present process is carried out as a dynamic resolution, 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one is contacted with the selected acid in the presence of an aldehyde. Generally, for the dynamic resolution from about 0.9 to 1.2 molar equivalents of acid are used, with about 1 molar equivalents being preferred. The aldehyde is generally used in a catalytic amount. Typically, about 0.5 to 0.001 molar equivalents of aldehyde are used, with about 0.1 to about 0.01 molar equivalents being preferred.

The dynamic process is typically carried out in a solvent or a solvent without an anti-solvent as described above. The mixture of 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one, the selected acid, and aldehyde are stirred to allow conversion to the desired isomer. Generally this conversion is carried out at temperatures of from ambient temperature to the refluxing temperature of the solvent. Generally conversion requires 6 to 48 hours.

As will be appreciated by the skilled artisan, when the present process is carried out as a dynamic resolution, use of the acid addition salt of (S)-1-amino-3-methyl-2,3,4,5 tetrahydro- 1H-3-benzazepin-2-one can be complicated by the presence of a small amount of aldehyde in the isolated product. Thus, after dynamic resolution it is preferred that (S)-1-amino-3-methyl-2,3,4,5-tetrahydro- 1H-3-benzazepin-2-one be isolated by salt exchange, preferably as the hydrochloride salt, before its use or formation of base.

As will be appreciated by those skilled in the art, the compounds of formula (10) can be used in a variety of processes to prepare compounds useful for the treatment of Alzheimer's disease. These processes are described in PCT Application No. PCT/US97/22986, filed 22 December 1997 and are described below. The processes of the present invention are characterized in that they produce the compounds of formula I by taking advantage of the resolution depicted in Scheme A, step 1.

Scheme A, step 2, depicts the coupling reaction of an appropriate amino-protected amino acid of formula the $PgNH-CHR_2-C(O)-A$ and an appropriate lactam of formula (10). Appropriate amino-protected amino acids are ones in which Pg is an amine protecting group, R_2 is as desired in the final product of formula I, and A is an activating group, for example -OH or -Cl, capable of coupling with the amino group of the compound of formula (10). Such amino-protected amino

acids are readily available to the person skilled in the art.

Preferred amino-protected amino acid of formula the $PgNH-CHR_2-C(O)-A$ are those in which Pg is t-butoxycarbonyl and benzyloxycarbonyl, R_2 is methyl, and those having the stereochemistry of an L-amino acid.

The coupling reaction depicted in Reaction Scheme A, step 2, involves a reaction which is conventionally conducted for peptide synthesis and synthetic methods used therein can also be employed. Such methods are described in detail in Scheme 1, step 1.

Reaction Scheme A, step 3, depicts the deprotection of a compound of formula (11) to give a compound of formula (12). Such deprotections of amino protecting groups is well known and appreciated in the art.

Reaction Scheme A, step 4, depicts the coupling reaction of an appropriate compound of formula (13), $R_1CX_1X_2-C(O)A_1$ and a compound of formula (12) to give a compound of formula I. Appropriate compounds of formula (13) are compounds in which R_1, X_1, and X_2 are as desired in the final product of formula I and are well known in the art, including PCT Application No. PCT/US97/22986, filed 22 December 1997, and as described herein. An appropriate compound of formula (13) may also have the stereochemistry that is desired in the final compound of formula I. The coupling reaction depicted in step 3 is carried out using the acid of formula (13) (compounds in which A_1 is -OH) or the acid halide derived therefrom (compounds in which A_1 is -Cl or -Br), in a manner similar to those taught in Scheme 1, step 1.

An alternative method for preparing the compounds of formula I is depicted in Scheme A, step 5, which shows the coupling reaction of an appropriate compound of formula (10) and an appropriate compound of formula (14),

$R_1CX_1X_2-C(O)-NH-CHR_2-C(O)A_2$, to directly give a compound or formula I. An appropriate compound of formula (10) is as described in step 2. An appropriate compound of formula (14) is one in which R_1, X_1, X_2, and R_2 are as desired in the final product of formula I. An appropriate compound of formula (14) is also one in which the stereochemistry is as desired in the final product of formula I.

Compounds of formula (14) are readily prepared by coupling carboxy-protected amino acids, $H_2N-CHR_2-C(O)OPg_1$, with compounds of formula (13) as described above. Again such coupling reactions are well known in the art and afford a product, which after deprotection, provides a compound of formula (14).

The present invention is further illustrated by the following examples and preparations. These examples and preparations are illustrative only and are not intended to limit the invention in any way.

The terms used in the examples and preparations have their normal meanings unless otherwise designated. For example "°C" refers to degrees Celsius; "mmol" refers to millimole or millimoles; "g" refers to gram or grams; "mL" refers milliliter or milliliters; "brine" refers to a saturated aqueous sodium chloride solution; "THF" refers to tetrahydrofuran; "HPLC" refers to high pressure liquid chromatography; etc.

Example 1

Synthesis of 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one

To a slurry of sodium hydride (1.1 eq) in 15 mL of dry DMF was added 2,3,4,5-tetrahydro-1H-3-benzazepin-2-one (0.0042 moles) as a solution in 10 mL of DMF. Methyl iodide (about 2 eq.) was then added. When complete by TLC, the reaction mixture was poured over ice and extracted into ethyl acetate. The organic layer was washed with water,

followed by brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by HPLC (LC 2000), eluting with an ethyl acetate/hexane system to give 3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one

3-Methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one eq.) was dissolved in THF and isoamylnitrite (1.2 eq.) was added. The mixture was cooled to 0°C in an ice bath. NaHMDS (1.1 eq., 1M in THF) was added dropwise. After stirring for 1 hour or until the reaction was complete, the mixture was concentrated then acidified with 1N aqueous hydrochloric acid solution and extracted with ethyl acetate. The organic portion was dried and concentrated to yield a crude product which was purified by silica gel chromatography to give 1-hydroxyimino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one one: Mass spectroscopy $(M+H)^+$, 205.1.

1-Hydroxyimino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one was dissolved in EtOH/NH_3 (20:1) and hydrogenated in a bomb using Raney nickel and hydrogen (500 psi/3447kPa) at 100°C for 10 hours. The resulting mixture was filtered and concentrated to provide an oil which was purified by silica gel chromatography to yield the title compound.

Example 2

Synthesis of 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one

To a 20 L Morton flask was added MTBE (5.52 L, 7 volumes) and (N-methylamino)-acetaldehyde dimethyl acetal (614 g, 5 mol) to form a solution at room temperature. A solution of sodium bicarbonate prepared by the addition of sodium bicarbonate (546g, 6.5 mol) and water (6.31 L, 8 volume) was added to the Morton reaction flask. The mixture was cooled to less than 10°C and a MTBE (789 mL) solution of phenylacetyl chloride (789 g, 5 mol) was added dropwise to

the cooled reaction mixture over a 1 h period. After addition, the reaction mixture was stirred at room temperature for 1 h. At this stage an HPLC analysis indicated that the reaction was completed. Extractive workup with MTBE (4 volumes), anhydrous magnesium sulfate drying followed by concentration on the rotary evaporator provided 1.187 kg (98%) of N-methyl-N-(2,2-dimethoxyethyl)phenylacetamide as a liquid, $(M+H)^+$ = 237.9. To a 5 L Morton flask under a strong nitrogen atmosphere was added H_2SO_4, (1.42 L) and N-methyl-N-(2,2-dimethoxyethyl)phenylacetamide (712 g, 3 mol) was added dropwise to the reaction flask which caused an exotherm (22 to 78°C). The resulting reaction was then heated to 110°C for 3 h then cooled to room temperature and transferred to a 20 L Morton flask. At less than 10°C, the reaction mixture was quenched with aqueous sodium hydroxide (9.18 L, 5 N). Extractive workup with ethyl acetate (2 X 2.85 L), drying with sodium sulfate followed by concentrating to a solid, provided 520 g (73.5%) of 3-methyl-6,7-dihydro-2H-3-benzazepin-2-one as a solid. This material may be recrystallized from MTBE for added purity to give a solid, mp = 81-82°C; $(M+H)^+$ = 174.2.

A THF (0.5 L) solution of 3-methyl-6,7-dihydro-2H-3-benzazepin-2-one (113.8 g, 0.657 mol) was cooled to 0°C and isoamyl nitrite (100.75 g, 0.86 mol) was added dropwise. To the resulting mixture was added LiHMDS (1 N THF solution, 854 mL, 0.854 mol) at a rate such that the temperature remained below 10°C. After addition, the reaction was allowed to stir at room temperature for 2-3 h while monitoring for the reaction progress by HPLC. Upon completion of the reaction, the mixture was cooled to 0°C, and the pH adjusted from 12 to 2-3 using aqueous HCl (2N). The resulting precipitate was stirred for 12-16 h before

isolation by filtration and drying to provide 86.3g (64.9 %) of 1-hydroxyimino-3-methyl-6,7-dihydro-2H-3-benzazepin-2-one; mp = 225-226°C; $(M+H)^+$ = 203.0.

An ethanol (525 mL) solution of 1-hydroxyimino-3-methyl-6,7-dihydro-2H-3-benzazepin-2-one (35 g, 0.173 mol) was added to an autoclave along with palladium on carbon (10%, 3.5 g) as a dilute HCl (concentrated aqueous, 17.5 g in 17 mL water) slurry. The resulting mixture was hydrogenated at 50°C and 250 psi (1723 kPa) until the reaction was completed. The reaction mixture was filtered over a pad of celite using ethanol as solvent and the filtrate concentrated to 90 mL. Water (350 mL) was added to the concentrate and the resulting solution further concentrated to about 200 mL. Dichloromethane (350 mL) was added to the aqueous solution before adjusting the pH to 11-11.5 with aqueous sodium hydroxide (1 N). The organic portion was separated and the aqueous portion extracted with dichloromethane (175 mL). The combined extracts were concentrated to a residue that crystallized upon standing to give the title compound: mp = 69-81°C; (M+H)+ = 191.0.

Example 3

Synthesis of 1-amino-3-methyl-4,5,6,7-tetrahydro-2H-3-benzazepin-2-one

To a 22 L Morton flask was added dichloromethane (4.73 L, 8 volumes), N-methylphenethylamine (591 g, 4.33 mol), and aqueous sodium bicarbonate (436.7 g, 5.2 mol in 4.73 L of water). The mixture was cooled to less than 5°C and dichloromethane (887 mL) solution of chloroacetyl chloride (513.7 g, 4.55 mol) was added dropwise to the cooled reaction mixture over a 70 min period. After addition, an HPLC analysis indicated that the reaction was completed. The layers were separated and the aqueous layer was

extracted with dichloromethane. Combined organic layers were dried over anhydrous magnesium sulfate and concentrated on the rotary evaporator to provide 915.7 g (99.8%) of N-methyl-N-(2-phenylethyl)-1-chloroacetamide: (M+H) = 212.1.

To a 12 L flask under a nitrogen atmosphere was added N-methyl-N-(2-phenylethyl)-1-chloroacetamide (883.3 g, 4.17 mol) and ortho-dichlorobenzene (6.18 L). Add aluminum chloride (1319 g, 10.13 mol) which caused an exotherm (22 to 50°C). The resulting reaction was then heated to 165°C for 2.5 h then cooled to room temperature over about 14 hours. The reaction mixture was cooled to about 0°C, and was added to cold water (8.86 L, about 5°C) in four portions in order to keep exotherm to about 40°C. The layers were separated and aqueous layer was extracted with dichloromethane (7.07 L) and the layers separated. The organic layers were combined and extracted with aqueous hydrochloric acid (8.83 L, 1N) and then a saturated aqueous sodium bicarbonate solution (7.07 L), dried over magnesium sulfate, combined with silica gel (883 g) and applied to a column of silica gel (3.53 kg, in a sintered glass funnel, packed as a slurry in dichloromethane). The column was eluted with dichloromethane until 25 L were collected and then with ethyl acetate to provide the product. The product containing fraction were evaporated to 3-methyl-4,5,6,7-tetrahydro-2H-3-benzazepin-2-one as a tan solid, 608 g (83%).

In a 22 L flask, under nitrogen, was 3-methyl-4,5,6,7-tetrahydro-2H-3-benzazepin-2-one (606 g, 3.46 mol) and isoamyl nitrite (543 g, 4.5 mol) in THF (7.88 L). The mixture was cooled to about 0°C before LiHMDS (1 N THF solution, 4.5 L, 04.5 mol) was added at a rate such that the temperature remained below about 7°C. After addition, the reaction was allowed to stir at room temperature for about 2

h while monitoring for the reaction progress by HPLC. Upon completion of the reaction, the mixture was cooled to about 0°C, and the pH adjusted from 12 to about 2-1 using aqueous HCl (2N). The resulting precipitate was stirred for about 6 h before isolation by filtration and drying to provide 1-hydroxyimino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one 604.7 g (85.6%).

1-Hydroxyimino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one (625 g, 3.06 mol) and 3A ethanol (15.6 L), as a dilute HCl (concentrated aqueous hydrochloric acid (312 g in 320 mL water), slurry was added to an autoclave along with palladium on carbon (10%, 120 g). The resulting mixture was hydrogenated at 50°C and 250 psi (1723 kPa) with vigorous agitation until the reaction was completed (about 4 hours). The reaction mixture was filtered over a pad of celite using ethanol as solvent and the filtrate concentrated give a solid. The solid was treated with dichloromethane (6 L) and 1N aqueous sodium hydroxide solution was added until the pH to of the aqueous layer was between 11-11.5. The mixture was agitated, the layers were separated, and the aqueous layer was extracted with dichloromethane (2 L). The organic layers were dried over magnesium sulfate, filtered, and evaporated in a rotary evaporator to give the title compound 477 g (81.9%).

Example 4

Synthesis of (S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one

1-Amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one (1.544g, 8.12 mmol) was heated gently in 15 mL methanol to form a solution. In another flask, di-p-toluoyl-l-tartaric acid (3.12 g, 8.08 mmol) was dissolved in 15 mL methanol and added via pipette to the warm amine solution. The mixture was heated as solids precipitated. An additional

30 mL of methanol was added to achieve a solution, which was refluxed for 30-40 minutes and then slowly cooled to ambient temperature to give a solid. After stirring for about 18 hours, the solid was collected by filtration and rinsed with a small amount of cold methanol to give 2.24 g of (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one p-toluoyl-L-tartaric acid salt (96% yield, 94.7% ee).

(S)-1-Amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one di-p-toluoyl-L-tartaric acid salt (11.83 g, 20.5 mmol) was dissolved in 45 mL of aqueous 1.0 N sodium hydroxide solution and extracted with methylene chloride (3 X 25mL). The combined methylene chloride layers were washed with 35 mL aqueous 1.0 N sodium hydroxide solution, then brine solution, and dried over anhydrous $MgSO_4$. Removal of solvent under vacuum gave the title compound (3.38 g) as a colorless oil (87% yield, 93.2% ee).

Example 5

Synthesis of (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one

1-Amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one (6.0 g, 31.5 mmol) was heated gently in 75 mL methanol to form a solution and combined with a solution of di-p-toluoyl-L-tartaric acid (12.2 g, 31.5 mmol) in 75 mL of warm methanol. The solution was seeded and a solid formed. An additional 100 mL of methanol was added and the mixture was allowed to stir. After stirring for about 18 hours, the solid was collected by filtration and rinsed with a small amount of cold methanol to give 6.7 g of a solid. The solid was combined with methanol (200 mL), and stirred. After 18 hours, the solid was collected to give (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one di-p-toluoyl-L-tartaric acid salt (4.4 g). Isolation of the base by the

procedure described in Example 4 gave the title compound (96% ee).

Example 6

Synthesis of (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one

In a 22 L vessel, under nitrogen, 1-amino-3-methyl-4,5,6,7-tetrahydro-2H-3-benzazepin-2-one (438 g, 2.3 mol) was heated (about 40°C) to provide a solution in methanol (4.38 mL). In another flask, di-p-toluoyl-l-tartaric acid (889.7 g, 2.3 mol) was dissolved in 4.38 L of methanol and heated to about 40°C before the solution of 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one was added. The heating was continued and an additional 6.13 L of methanol was added before the mixture was refluxed for about 45 minutes and then slowly cooled to ambient temperature to give a solid. After stirring for about 18 hours, the solid was collected by filtration and rinsed with a small amount of mother liquors, and after air drying, with about 2 L of ethyl acetate to give 561.6 g of (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one di-p-toluoyl-L-tartaric acid salt. Combine (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one di-p-toluoyl-L-tartaric acid salt, dichloromethane (6.57 L) and 1N aqueous sodium hydroxide solution (6.57 L) and agitate. Separate the layers and extract the organic layer twice with and 1N aqueous sodium hydroxide solution (3.28 L), once with brine (2.46 L) before drying over magnesium sulfate, filtering, and evaporating on a rotary evaporator to give the title compound 250 g (57.4%, 94.1% ee).

Example 7

Synthesis of (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one hydrochloric acid salt

1-Amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one (31.9g, 168 mmol) was slurried in about 300 mL isopropyl acetate and heated to 45°C. In a separate flask, (R)-(-)-D-mandelic acid (25.0g, 164 mmol) was heated in about 130 mL of isopropyl alcohol until a solution formed and was added to the 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one/isopropyl acetate slurry obtained above to give a solution from which a precipitate quickly formed. The mixture was stirred at 45°C for about 3 hours. 5-Nitrosalicylaldehyde (2-hydroxy-5-nitrobenzaldehyde) (1.40 g, 8.38 mmol, 5 mol%) was added to the warm solution and the mixture was stirred at 45°C. After about 14 hours, the slurry was cooled to ambient temperature and stirred for 2 hours before the solids were collected by filtration and rinsed with 70 mL of cold isopropyl acetate, and dried in the vacuum oven at 40°C to obtain 46.62 g of (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one (R)-mandelic acid salt (82.9% yield, 98.4% ee).

(S)-1-Amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one (R)-mandelic acid salt (2.42g, 7.06 mmol, 98.4% ee) was slurried in 25 mL ethyl acetate at ambient temperature. Concentrated aqueous hydrochloric acid (1.1 mL, about 11.2 mmol) was added and the mixture was heated to 50°C with vigorous stirring for 3.5 hours. The slurry was cooled to ambient temperature and filtered, rinsed with the methyl t-butyl ether (about 10 mL) to give 1.48g of the title compound (92.5% yield, 97.9% ee).

Example 8

Synthesis of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one

A round bottom flask was charged with N-t-Boc-L-alanine (1.0 eq.), hydroxybenzotriazole hydrate (about 1.1 eq.) and (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one (1.0 eq.) in THF under nitrogen atmosphere. Hunig's base (N,N-diisopropylethylamine, 1.1 eq.) was added to the well stirred mixture followed by EDC (1.1 eq.). After stirring from 4 to 17 hours at ambient temperature the solvent was removed at reduced pressure, the residue taken up in ethyl acetate and water, washed with saturated aqueous sodium bicarbonate solution, 1 N aqueous HCl, brine, dried over anhydrous sodium sulfate, filtered, and the solvent removed at reduced pressure to provide 1-(N-t-Boc-L-alaninyl)amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one: mass spectroscopy $(M+H)^+$, 362.3.

A stream of anhydrous HCl gas was passed through a stirred solution of 1-(N-t-Boc-L-alaninyl)amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one in 1,4-dioxane(0.03-0.09 M), chilled in a ice bath to about 10°C under NZ, for 10-15 minutes. The solution was capped, then the cooling bath removed, and the solution was allowed to warm to ambient temperature with stirring for 2-8 hours, monitoring 1.0 by TLC for the consumption of starting material. The solution was concentrated to give 1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one which was used without further purification.

1-(L-Alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepin-2-one(1.0 eq.), hydroxybenzotriazole hydrate (1.1 eq.) and (S)-2-hydroxy-3-methyl-butyric acid (1.0 eq.) in THF under nitrogen atmosphere. Hunig's base

(N,N-diisopropylethylamine, 1.1 eq.) was added to the well stirred mixture followed by EDC (1.1 eq.). After stirring from 4 to 17 hours at ambient temperature the solvent was removed at reduced pressure, the residue taken up in ethyl acetate (or similar solvent) and water, washed with saturated aqueous sodium bicarbonate solution, 1 N HCl, brine, dried over anhydrous sodium sulfate and the solvent removed at reduced pressure to provide the title compound.

Example 9

Synthesis of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one

A round bottom flask was charged with N-t-Boc-L-alanine (249.5 g, 1.32 mol), hydroxybenzotriazole hydrate (232.2 g, 1.52 mol), and (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one (250.8 g, 1.32 mol) in THF (3.76 L) under nitrogen atmosphere. The mixture was cooled to less than 5°C before adding Hunig's base (N,N-diisopropylethylamine, 188.4 g, 1.45 mol) followed by EDC (283.7 g, 1.45 mol). After stirring 6 hours the reaction mixture was warmed to ambient temperature and stirred for about 14 hours. The solvent was removed at reduced pressure, the residue taken up in ethyl acetate (3.76 L) and water (1.76 L), the layers were separated, the organic layer extracted with water (1.76 L), the aqueous layers combined and extracted with ethyl acetate (1.76 L). The organic layers were combined, extracted with saturated aqueous sodium bicarbonate solution (1.76 L), dried over anhydrous sodium sulfate, filtered, and evaporated in on a rotary evaporator to provide 1-(N-t-Boc-L-alaninyl)amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one 463 g (97.2%).

An ethyl acetate solution of HCl was prepared by passing anhydrous HCl gas, using a subsurface dispersion

tube, through ethyl acetate (1.76 L) cooled to about 0°C. The ethyl acetate solution of HCl prepared above was added to a vigorously stirred slurry of 1-(N-t-Boc-L-alaninyl)amino-3- methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one (462 g, 1.28 mol) in ethyl acetate (3.7 L). An additional amount of ethyl acetate (1 L) was added and the reaction mixture was allowed to warm to room temperature and stirred for 22 h. The reaction mixture was filtered to give a solid. The solid was slurryed with acetonitrile (5 L), heated to reflux and then cooled to about 60°C before filtering and drying to give 1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one 389.8 g (94.7%).

1-(L-Alaninyl)-(S)-amino-3-methyl-2,3,4,5 -tetrahydro-3-methyl- 1H-3 -benzazepin-2-one (369.5 g, 1.18 mol), hydroxybenzotriazole hydrate (207.6 g, 1.36 mol), Hunig's base (N,N-diisopropylethylamine, 352.2 g, 2.71 mol), and (S)-2-hydroxy-3-methyl-butyric acid (140.6 g, 1.18 mol) in THF (4.8 L) were combined under a nitrogen atmosphere and cooled to less than 5°C. EDC (253.7 g, 1.3 mol) was added and the reaction mixture was allowed to warm to ambient temperature and to stir. After about 25 hours the reaction mixture was diluted with dichloromethane (5.54 L) and extracted with water (2.22 L). The organic layer was extracted with water (2.22 L), the aqueous layers were combined and extracted with dichloromethane (5.54 L). The organic layers were combined, extracted twice with water (2.22 L), with saturated aqueous sodium bicarbonate solution (2.22 L), dried over anhydrous sodium sulfate, filtered, and evaporated in on a rotary evaporator to provide the title compound 428 g (100%).

Example 10

Synthesis of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5~~,6,7~~-tetrahydro-~~2H~~1H-3-benzazepin-2-one dihydrate

(N)-((S)-2-Hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one solid was taken up in acetone (3.42 L) and water (0.856 L) with slight warming (40°C). The solution was split into ~2 L portions and to each was added water (7.19 L) while warming the hazy solution to 50°C. Upon complete addition of water the hazy solution was allowed to cool to ambient to give a solid which was stirred as a slurry at ambient temperature for about 14 hours before filtering and drying to give the title compound 310.6 g (66.2%).

When employed as a pharmaceutical the present invention is usually administered in the form of a pharmaceutical composition. Thus, in another embodiment, the present invention provides pharmaceutical compositions comprising an effective amount of crystalline N-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate and a pharmaceutically acceptable diluent. Such compositions are used for inhibiting β-amyloid peptide release and/or its synthesis, including the treatment of Alzheimer' disease. Thus, the present invention encompasses the use of crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate for the manufacture of a medicament for inhibiting β-amyloid peptide release and/or its synthesis, and specifically including, treating Alzheimer's disease.

Crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate can be administered by a variety

of routes. The present compound can be administered in any form or mode which makes the compound bioavailable in an effective amount, including oral and parenteral routes. For example, the present compound can be administered orally, by inhalation, subcutaneously, intramuscularly, intravenously, transdermally, intranasally, rectally, occularly, topically, sublingually, buccally, and the like.

In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. The compound of the present invention can be administered alone or in the form of a pharmaceutical composition, that is, combined with pharmaceutically acceptable diluents, such as carriers or excipients, the proportion and nature of which are determined by the solubility and chemical properties of the present compound, the chosen route of administration, and standard pharmaceutical practice. (*Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co. (1990)).

The present pharmaceutical compositions are prepared in a manner well known in the pharmaceutical art. The carrier or excipient may be a solid, semi-solid, or liquid material which can serve as a vehicle or medium for the active ingredient. Suitable carriers or excipients are well known in the art. The pharmaceutical composition may be adapted for oral, inhalation, parenteral, or topical use and may be administered to the patient in the form of tablets, capsules, aerosols, inhalants, suppositories, solution, suspensions, or the like.

For the purpose of oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gums and the like.

These preparations should contain at least 4% of the compound of the present invention, the active ingredient, but may be varied depending upon the particular form and may conveniently be between 2% to about 90% of the weight of the unit. The amount of the compound present in compositions is such that a suitable dosage will be obtained. Preferred compositions and preparations according to the present invention may be determined by a person skilled in the art.

The tablets, pills, capsules, troches, and the like may also contain one or more of the following adjuvants: binders such as microcrystalline cellulose, gum tragacanth or gelatin; excipients such as starch or lactose, disintegrants such as alginic acid, Primogel, corn starch and the like; lubricants such as magnesium stearate, silicon oil, or Sterotex; glidants such as colloidal silicon dioxide; and sweetening agents such as sucrose or saccharin may be added or a flavoring agent such as peppermint, methyl salicylate or orange flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or a fatty oil. Other dosage unit forms may contain other various materials which modify the physical form of the dosage unit, for example, as coatings. Thus, tablets or pills may be coated with sugar, shellac, or other coating agents. A syrup may contain, in addition to the present compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors. Materials used in preparing these various compositions should be pharmaceutically pure and non-toxic in the amounts used.

For the purpose of parenteral administration, the compound of the present invention may be incorporated into a solution or suspension. These preparations typically contain at least 0.1% of the compound of the invention, but may be varied to be between 0.1 and about 90% of the weight

thereof. The amount of the compound present in such compositions is such that a suitable dosage will be obtained. The solutions or suspensions may also include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylene diaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Preferred compositions and preparations are able to be determined by one skilled in the art.

The compound of the present invention may also be administered topically, and when done so the carrier may suitably comprise a solution, ointment, or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bees wax, mineral oil, diluents such as water and alcohol, and emulsifiers, and stabilizers. Topical formulations may contain a concentration of the formula I or its pharmaceutical salt from about 0.1 to about 10% w/v (weight per unit volume).

Another preferred formulation of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compound of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by

reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

In order to more fully illustrate the operation of this invention, typical pharmaceutical compositions are described below. The examples are illustrative only, and are not intended to limit the scope of the invention in any way.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity(mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity(mg/tablet)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5
Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity(mg/tablet)
Active Ingredient	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg
Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinyl-pyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50° to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg
Total	150.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

Ingredient	Amount
Active Ingredient	25 mg
Saturated fatty acid glycerides to	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 ml dose are made as follows:

Ingredient	Amount
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%)	
Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 ml

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

Capsules, each containing 15 mg of medicament are made as follows:

Ingredient	Quantity(mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 560 mg quantities.

Formulation Example 9

A subcutaneous formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1.0 mg
corn oil	1 ml

Depending on the solubility of the active ingredient in corn oil, up to about 5.0 mg or more of the active ingredient may be employed in this formulation, if desired).

Formulation Example 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

In one of its method aspects, this invention is directed to a method for inhibiting β-amyloid peptide release and/or its synthesis comprising administering to a patient in need thereof with an effective amount of crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate. In a particular method embodiment, the present invention provides a method for treating Alzheimer's disease comprising administering to a patient in need thereof with an effective amount of crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5 -tetrahydro- 1H-3-benzazepin-2-one dihydrate.

It is also recognized that one skilled in the art may affect the Alzheimer's disease by treating a patient presently afflicted with the disease or by prophylactically treating a patient at risk to develop the disease. Thus, the terms "treatment" and "treating" are intended to refer to all processes wherein there may be a slowing, interrupting, arresting, controlling, or stopping of the progression of Alzheimer's disease, but does not necessarily indicate a total elimination of all symptoms. As such, the present methods include for preventing the onset of Alzheimer's disease in a patient at risk for developing Alzheimer's disease, inhibiting the progression of Alzheimer's disease, and treatment of advanced Alzheimer's disease.

As used herein, the term "patient" refers to a warm blooded animal, such as a mammal, which is afflicted with a disorder associated with increase β-amyloid peptide release and/or its synthesis, including Alzheimer's disease. It is understood that guinea pigs, dogs, cats, rats, mice, horses, cattle, sheep, and humans are examples of animals within the scope of the meaning of the term. Patients in need of such treatment are readily diagnosed.

As used herein, the term "effective amount" of a compound of formula I refers to an amount which is effective in inhibiting β-amyloid peptide release and/or its synthesis, and specifically, in treating Alzheimer's disease.

An effective amount can be readily determined by the attending diagnostician, as one skilled in the art, by the use of conventional techniques and by observing results obtained under analogous circumstances. In determining an effective amount, the dose of crystalline N-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-4,5,6,7-tetrahydro-2H-3-benzazepin-2-one dihydrate, a number

of factors are considered by the attending diagnostician, including, but not limited to: the potency and characteristics of N-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one; the species of patient; its size, age, and general health; the degree of involvement or the severity of the disease; the response of the individual patient; the mode of administration; the bioavailability characteristics of the preparation administered; the dose regimen selected; the use of other concomitant medication; and other relevant circumstances.

An effective amount of crystalline N-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate is expected to vary from about 0.1 milligram per kilogram of body weight per day (mg/kg/day) to about 100 mg/kg/day. Preferred amounts are able to be determined by one skilled in the art.

The N-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of the present invention can be tested in various biological systems including the following.

Example A

Cellular Screen for the Detection of Inhibitors of β-Amyloid Production

Numerous compounds of formula I above were assayed for their ability to inhibit β-amyloid production in a cell line possessing the Swedish mutation. This screening assay employed cells (K293 = human kidney cell line) which were stably transfected with the gene for amyloid precursor protein 751 (APP751) containing the double mutation $Lys_{651}Met_{652}$ to $Asn_{651}Leu_{652}$ (APP751 numbering) in the manner described in International Patent Application Publication No. 94/10569[8] and Citron et al.[12]. This mutation is commonly called the Swedish mutation and the cells, designated as "293 751 SWE", were plated in Corning 96-well plates at 2-4

x 10^4 cells per well in Dulbecco's minimal essential media (Sigma, St. Louis, MO) plus 10% fetal bovine serum. Cell number is important in order to achieve β-amyloid ELISA results within the linear range of the assay (~0.2 to 2.5 ng per mL).

Following overnight incubation at 37°C in an incubator equilibrated with 10% carbon dioxide, media were removed and replaced with 200 □L of a compound of formula I (drug) containing media per well for a two hour pretreatment period and cells were incubated as above. Drug stocks were prepared in 100% dimethyl sulfoxide such that at the final drug concentration used in the treatment, the concentration of dimethyl sulfoxide did not exceed 0.5% and, in fact, usually equaled 0.1%.

At the end of the pretreatment period, the media were again removed and replaced with fresh drug containing media as above and cells were incubated for an additional two hours. After treatment, plates were centrifuged in a Beckman GPR at 1200 rpm for five minutes at room temperature to pellet cellular debris from the conditioned media. From each well, 100 μL of conditioned media or appropriate dilutions thereof were transferred into an ELISA plate precoated with antibody 266 [P. Seubert, *Nature* (1992) **359**:325-327] against amino acids 13-28 of β-amyloid peptide as described in International Patent Application Publication No. 94/10569[8] and stored at 4°C overnight. An ELISA assay employing labeled antibody 3D6 [P. Seubert, *Nature* (1992) **359**:325-327] against amino acids 1-5 of β-amyloid peptide was run the next day to measure the amount of β-amyloid peptide produced.

Cytotoxic effects of the compounds were measured by a modification of the method of Hansen, et al. To the cells remaining in the tissue culture plate was added 25 μL of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO) stock solution (5 mg/mL) to a final concentration of 1 mg/mL. Cells were incubated at 37□C for one hour, and cellular activity was stopped by the

addition of an equal volume of MTT lysis buffer (20% w/v sodium dodecylsulfate in 50% dimethylformamide, pH 4.7). Complete extraction was achieved by overnight shaking at room temperature. The difference in the OD_{562nm} and the OD_{650nm} was measured in a Molecular Device's UV_{max} microplate reader as an indicator of the cellular viability.

The results of the β-amyloid peptide ELISA were fit to a standard curve and expressed as ng/mL β-amyloid peptide. In order to normalize for cytotoxicity, these results were divided by the MTT results and expressed as a percentage of the results from a drug free control. All results are the mean and standard deviation of at least six replicate assays.

Example B

In Vivo Suppression of β-Amyloid Release and/or Synthesis

This example illustrates how the compounds of this invention could be tested for *in vivo* suppression of β-amyloid release and/or synthesis. For these experiments, 3 to 4 month old PDAPP mice are used [Games et al., (1995) *Nature* 373:523-527]. Depending upon which compound is being tested, the compound is usually formulated at between 1 and 10 mg/mL. Because of the low solubility factors of the compounds, they may be formulated with various vehicles, such as corn oil (Safeway, South San Francisco, CA); 10% ethanol in corn oil; 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals International, Natick MA); and carboxy-methyl-cellulose (Sigma Chemical Co., St. Louis MO).

The mice are dosed subcutaneously with a 26 gauge needle and 3 hours later the animals are euthanized via CO_2 narcosis and blood is taken by cardiac puncture using a 1 cc 25G 5/8" tuberculin syringe/needle coated with solution of 0.5 M EDTA, pH 8.0. The blood is placed in a Becton-Dickinson vacutainer tube containing EDTA and spun down for 15 minutes at 1500 xg at 5°C. The brains of the mice are then removed and the cortex and hippocampus are dissected out and placed on ice.

1. Brain Assay

To prepare hippocampal and cortical tissue for enzyme-linked immunosorbent assays (ELISAs) each brain region is homogenized in 10 volumes of ice cold guanidine buffer (5.0 M guanidine-HCl, 50 mM Tris-HCl, pH 8.0) using a Kontes motorized pestle (Fisher, Pittsburgh PA). The homogenates are gently rocked on a rotating platform for three to four hours at room temperature and stored at -20°C prior to quantitation of β-amyloid.

The brain homogenates are diluted 1:10 with ice-cold casein buffer [0.25% casein, phosphate buffered saline (PBS), 0.05% sodium azide, 20 μg/ml aprotinin, 5 mM EDTA, pH 8.0, 10 μg/ml leupeptin], thereby reducing the final concentration of guanidine to 0.5 M, before centrifugation at 16,000 xg for 20 minutes at 4°C. Samples are further diluted, if necessary, to achieve an optimal range for the ELISA measurements by the addition of casein buffer with 0.5 M guanidine hydrochloride added. The β-amyloid standards (1-40 or 1-42 amino acids) were prepared such that the final composition equaled 0.5 M guanidine in the presence of 0.1% bovine serum albumin (BSA).

The total β-amyloid sandwich ELISA, quantitating both β-amyloid (aa 1-40) and β-amyloid (aa 1-42) consists of two monoclonal antibodies (mAb) to β-amyloid. The capture antibody, 266 [P. Seubert, *Nature* (1992) **359**:325-327], is specific to amino acids 13 - 28 of β-amyloid. The antibody 3D6 [Johnson-Wood et al., *PNAS USA* (1997) **94**:1550-1555], which is specific to amino acids 1 - 5 of β-amyloid, is biotinylated and served as the reporter antibody in the assay. The 3D6 biotinylation procedure employs the manufacturer's (Pierce, Rockford IL) protocol for NHS-biotin labeling of immunoglobulins except that 100 mM sodium bicarbonate, pH 8.5 buffer is used. The 3D6 antibody does not recognize secreted amyloid precursor protein (APP) or full-length APP but detects only β-amyloid species with an amino terminal aspartic acid. The assay has a lower limit of sensitivity of ~50 pg/ml (11 pM) and shows no cross-

reactivity to the endogenous murine β-amyloid peptide at concentrations up to 1 ng/ml.

The configuration of the sandwich ELISA quantitating the level of β-amyloid (aa 1-42) employs the mAb 21F12 [Johnson-Wood et al., *PNAS USA* (1997) **94**:1550-1555] (which recognizes amino acids 33-42 of β-amyloid) as the capture antibody. Biotinylated 3D6 is also the reporter antibody in this assay which has a lower limit of sensitivity of ~125 pg/ml (28 pM).

The 266 and 21F12 capture mAbs are coated at 10 μg/ml into 96 well immunoassay plates (Costar, Cambidge MA) overnight at room temperature. The plates are then aspirated and blocked with 0.25% human serum albumin in PBS buffer for at least 1 hour at room temperature, then stored desiccated at 4°C until use. The plates are rehydrated with wash buffer (Tris-buffered saline, 0.05% Tween 20) prior to use. The samples and standards are added to the plates and incubated overnight at 4°C. The plates are washed 3 times with wash buffer between each step of the assay. The biotinylated 3D6, diluted to 0.5 μg/ml in casein incubation buffer (0.25% casein, PBS, 0.05% Tween 20, pH 7.4) is incubated in the well for 1 hour at room temperature. Avidin-HRP (Vector, Burlingame CA) diluted 1:4000 in casein incubation buffer is added to the wells for 1 hour at room temperature. The colormetric substrate, Slow TMB-ELISA (Pierce, Cambridge MA), is added and allowed to react for 15 minutes, after which the enzymatic reaction is stopped with addition of 2 N H_2SO_4. Reaction product is quantified using a Molecular Devices Vmax (Molecular Devices, Menlo Park CA) measuring the difference in absorbance at 450 nm and 650 nm.

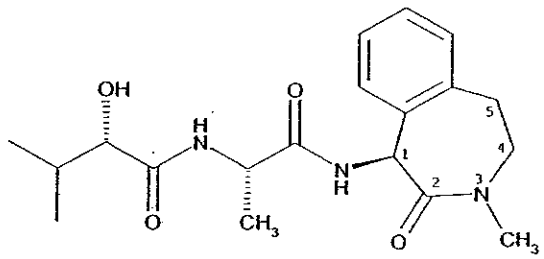
2. Blood Assay

The EDTA plasma is diluted 1:1 in specimen diluent (0.2 gm/l sodium phosphate•H_2O (monobasic), 2.16 gm/l sodium phosphate•$7H_2O$ (dibasic), 0.5gm/l thimerosal, 8.5 gm/l sodium chloride, 0.5 ml Triton X-405, 6.0 g/l globulin-free bovine serum albumin; and water). The samples and standards

in specimen diluent are assayed using the total β-amyloid assay (266 capture/3D6 reporter) described above for the brain assay except the specimen diluent was used instead of the casein diluents described.

From the foregoing description, various modifications and changes in the composition and method will occur to those skilled in the art. All such modifications coming within the scope of the appended claims are intended to be included therein.

1. (N)-((S)-2-Hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate:



dihydrate.

2. A crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of Claim 1 characterized by the X-ray powder diffraction pattern comprising a peak at 8.36, 12.43, 15.34, 19.22, 20.50, or 20.63 (2θ ± 0.2°).

3. The crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of Claim 2 characterized by the X-ray powder diffraction pattern comprising peaks at 8.36 and 15.34 (2θ ± 0.2°).

4. The crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of Claim 2 characterized by the X-ray powder diffraction pattern comprising peaks at 8.36 and 12.43 (2θ ± 0.2°).

5. The crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of Claim 2 characterized by the X-ray powder diffraction pattern comprising peaks at 8.36, 12.43, and 15.34 (2θ ± 0.2°).

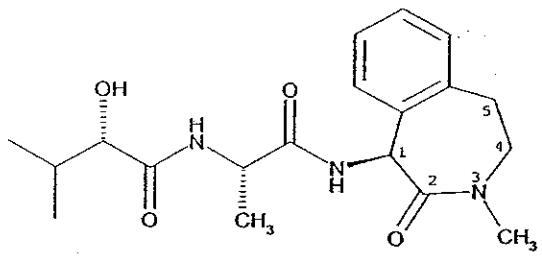
6. The crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of Claim 2 characterized by the X-ray powder diffraction pattern comprising peaks at 8.36, 12.43 15.34, 19.22, 20.50, and 20.63 (2θ ± 0.2°).

7. A crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of Claim 1

characterized by solid state ^{13}C nuclear magnetic resonance having a chemical shift (ppm) of 75.6, 35.3, 21.4, or 16.6.

8. A crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of Claim 1 characterized by solid state ^{13}C nuclear magnetic resonance having a chemical shift (ppm) of 75.6, 35.3, 21.4, and 16.6.

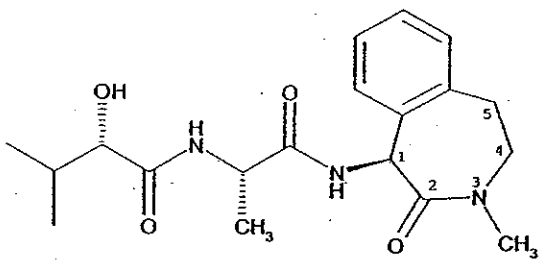
9. A pharmaceutical composition comprising (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate:



dihydrate

and a pharmaceutically acceptable diluent.

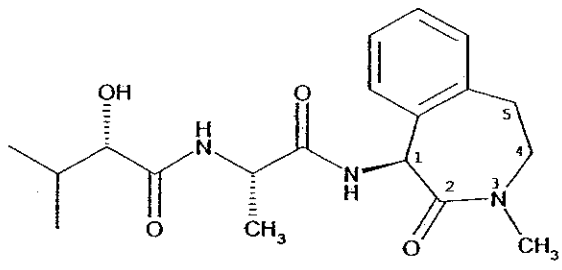
10. The use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate:



dihydrate

for the preparation of a medicament for inhibiting β-amyloid peptide release or its synthesis.

11. The use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate

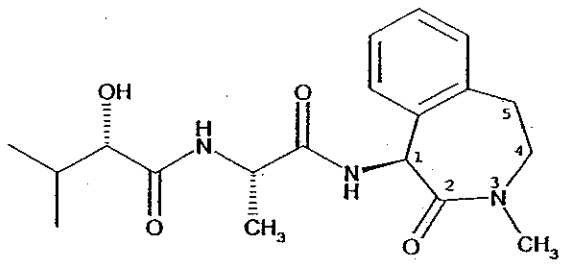


dihydrate

for the preparation of a medicament for the treatment of Alzheimer's Disease.

12. The use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of any one of Claims 2, 3, 4, 5, 6, 7, or 8 for the preparation of a medicament for the treatment of Alzheimer's Disease.

13. The use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate:

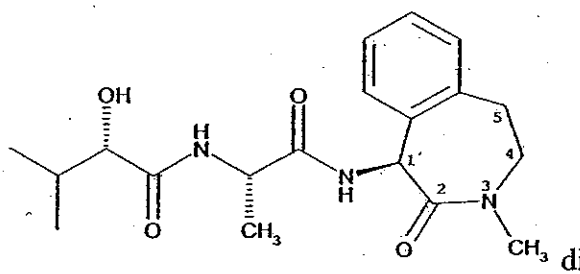


dihydrate

for the preparation of medicament for the prevention of Alzheimer's Disease.

14. The use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of any one of Claims 2, 3, 4, 5, 6, 7, or 8 for the preparation of a medicament for the prevention of Alzheimer's Disease.

15. The use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate:

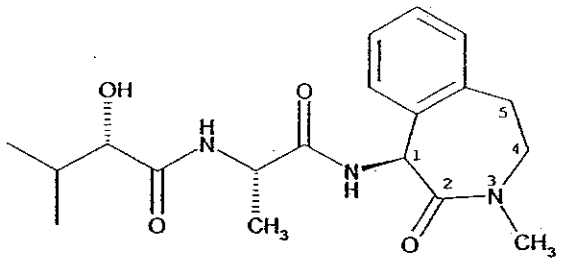


dihydrate

for the manufacture of a medicament for use in the inhibition of the progression of Alzheimer's Disease.

16. The use of (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of any one of Claims 2, 3, 4, 5, 6, 7, or 8 for the manufacture of a medicament for use in the inhibition of the progression of Alzheimer's Disease.

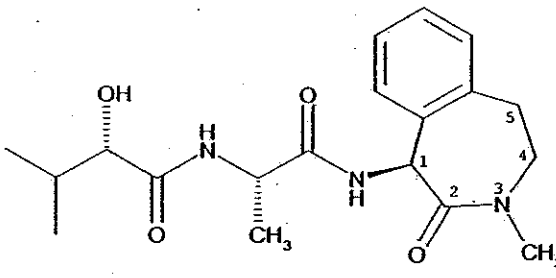
17. A process for preparing the crystalline (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one dihydrate of Claim 2, comprising crystallizing (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one



from an aqueous solvent.

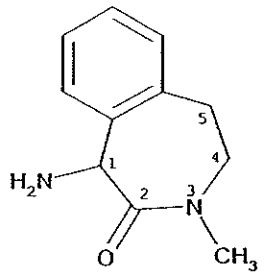
18. A process according to Claim 17 wherein the aqueous solvent comprises acetone and water.

19. A process for preparing (N)-((S)-2-hydroxy-3-methyl-butyryl)-l-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one:

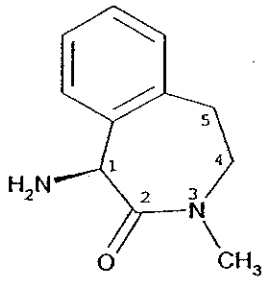


, comprising:

crystallizing 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one

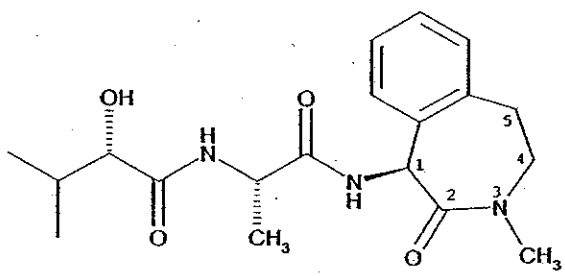


as an acid addition salt of an acid selected from the group consisting of di-p-tolyl-L-tartaric acid, (R)-(-)-l0-camphorsulfonic acid, and (D)-(-)-mandelic acid; coupling the (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one:

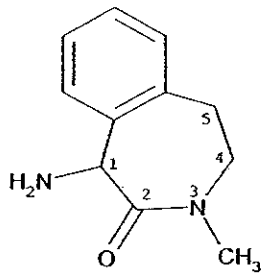


to an amino-protected L-alanine residue, to give an amino-protected 1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one; deprotecting to give 1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one; and coupling with (S)-2-hydroxy-3-methyl-butyric acid.

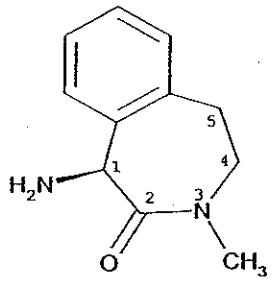
20. A process for preparing (N)-((S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl)-(S)-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one:

 comprising:

crystallizing 1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one:



as an acid addition salt of an acid selected from the group consisting of di-p-tolyl-L-tartaric acid, (R)-(-)-l0-camphorsulfonic acid, and (D)-(-)-mandelic acid; coupling the (S)-1-amino-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one:



to a (S)-2-hydroxy-3-methyl-butyryl)-1-(L-alaninyl) residue.