

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
31 March 2005 (31.03.2005)

PCT

(10) International Publication Number  
**WO 2005/027975 A1**

(51) International Patent Classification<sup>7</sup>: **A61K 45/06**,  
31/196, 31/197, 31/445, 31/473, 31/27, 31/407, 31/55,  
31/662, 31/454

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(21) International Application Number:  
PCT/IB2004/002981

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(22) International Filing Date:  
8 September 2004 (08.09.2004)

(81) Designated States (*unless otherwise indicated, for every  
kind of national protection available*): AE, AG, AL, AM,  
AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,  
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,  
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,  
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,  
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,  
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,  
ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
0322140.5 22 September 2003 (22.09.2003) GB

(84) Designated States (*unless otherwise indicated, for every  
kind of regional protection available*): ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,  
SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,  
GW, ML, MR, NE, SN, TD, TG).

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Published:

— with international search report

*For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.*

(54) Title: COMBINATIONS OF ALPHA-2-DELTA LIGANDS AND ACETYLCHOLINESTERASE INHIBITORS

(57) Abstract: The instant invention relates to a combination of an alpha-2-delta ligand and an AChE inhibitor for use in therapy, particularly in the treatment of pain, particularly neuropathic pain. Particularly preferred alpha-2-delta ligands are gabapentin and pregabalin. Particularly preferred AChE inhibitors are donepezil (Aricept<sup>®</sup>), tacrine (cognex<sup>®</sup>), rivastigmine (Exelon<sup>®</sup>), physostigmine (Synapton<sup>®</sup>), galantamine (Reminyl), metrifonate (Promem), neostigmine (Prostigmin) and icopezil.

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COMBINATIONS OF ALPHA-2-DELTA LIGANDS AND ACETYLCHOLINESTERASE INHIBITORS

FIELD OF THE INVENTION

5 This invention relates to a combination of an alpha-2-delta ligand and an acetylcholine esterase inhibitor, particularly for the treatment of pain and related disorders. It also relates to a method for treating pain and related disorders through the use of effective amounts of alpha-2-delta ligand and acetylcholine esterase inhibitor combinations.

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BACKGROUND TO THE INVENTION

An alpha-2-delta receptor ligand is any molecule which binds to any sub-type of the human calcium channel alpha-2-delta subunit. The calcium channel alpha-2-delta subunit  
15 comprises a number of sub-types which have been described in the literature:  
e.g. N. S. Gee, J. P. Brown, V. U. Dissanayake, J. Offord, R. Thurlow, and G. N. Woodruff, *J-Biol-Chem* 271 (10):5768-76, 1996, (type 1);  
Gong, J. Hang, W. Kohler, Z. Li, and T-Z. Su, *J.Membr.Biol.* 184 (1):35-43, 2001, (types  
2 and 3);  
20 E. Marais, N. Klugbauer, and F. Hofmann, *Mol.Pharmacol.* 59 (5):1243-1248, 2001.  
(types 2 and 3); and  
N. Qin, S. Yagel, M. L. Momplaisir, E. E. Codd, and M. R. D'Andrea. *Mol.Pharmacol.* 62  
(3):485-496, 2002, (type 4). They may also be known as GABA analogs.

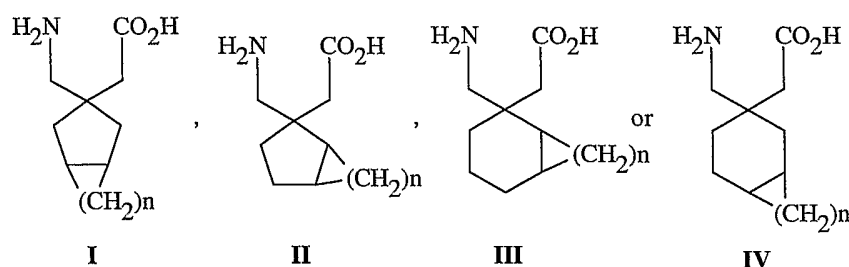
25 Alpha-2-delta ligands have been described for a number of indications. The best known alpha-2-delta ligand, gabapentin (Neurontin®), 1-(aminomethyl)-cyclohexylacetic acid, was first described in the patent literature in the patent family comprising US4024175. The compound is approved for the treatment of epilepsy and neuropathic pain.

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A second alpha-2-delta ligand, pregabalin, (S)-(+)-4-amino-3-(2-methylpropyl)butanoic acid, is described in European patent application publication

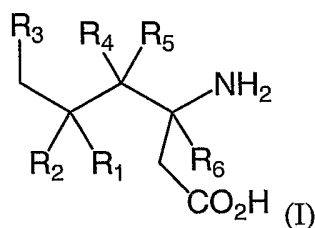
number EP641330 as an anti-convulsant treatment useful in the treatment of epilepsy and in EP0934061 for the treatment of pain.

Further, International Patent Application Publication No. WO0128978, describes a series of novel bicyclic amino acids, their pharmaceutically acceptable salts, and their prodrugs of formula:



wherein  $n$  is an integer of from 1 to 4, where there are stereocentres, each center may be independently R or S, preferred compounds being those of Formulae I-IV above in which  $n$  is an integer of from 2 to 4.

International Patent application No. PCT/IB03/00976, unpublished at the filing date of the present invention, describes compounds of the formula I, below:



wherein  $R_1$  is hydrogen or  $(C_1-C_6)$ alkyl optionally substituted with from one to five fluorine atoms;

$R_2$  is hydrogen or  $(C_1-C_6)$ alkyl optionally substituted with from one to five fluorine atoms; or

$R_1$  and  $R_2$ , together with the carbon to which they are attached, form a three to six membered cycloalkyl ring;

$R_3$  is  $(C_1-C_6)$ alkyl,  $(C_3-C_6)$ cycloalkyl,  $(C_3-C_6)$ cycloalkyl- $(C_1-C_3)$ alkyl, phenyl, phenyl- $(C_1-C_3)$ alkyl, pyridyl, pyridyl- $(C_1-C_3)$ alkyl, phenyl-N(H)-, or pyridyl-N(H)-, wherein each of the foregoing alkyl moieties can be optionally substituted with from one to five fluorine atoms, preferably with from zero to three fluorine atoms, and wherein said

phenyl and said pyridyl and the phenyl and pyridyl moieties of said phenyl-(C<sub>1</sub>-C<sub>3</sub>)alkyl and said pyridyl-(C<sub>1</sub>-C<sub>3</sub>)alkyl, respectively, can be optionally substituted with from one to three substituents, preferably with from zero to two substituents, independently selected from chloro, fluoro, amino, nitro, cyano, (C<sub>1</sub>-C<sub>3</sub>)alkylamino, (C<sub>1</sub>-C<sub>3</sub>)alkyl optionally substituted with from one to three fluorine atoms and (C<sub>1</sub>-C<sub>3</sub>)alkoxy optionally substituted with from one to three fluorine atoms;

R<sub>4</sub> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>)alkyl optionally substituted with from one to five fluorine atoms;

R<sub>5</sub> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>)alkyl optionally substituted with from one to five fluorine atoms; and

R<sub>6</sub> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>)alkyl;

and the pharmaceutically acceptable salts of such compounds.

Acetylcholine esterase inhibitors ('AChE inhibitors') have been indicated for the treatment of cognitive disorders such as the mild to moderate dementia of the Alzheimer's type. In particular, donepezil hydrochloride, (±)-2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one hydrochloride (ARICEPT®) has been marketed for the treatment of Alzheimer's disease.

20

### SUMMARY OF THE INVENTION

It has now been found that combination therapy with an alpha-2-delta ligand and an AChE inhibitor results in improvement in the treatment of pain. Furthermore, when administered simultaneously, sequentially or separately, the alpha-2-delta ligand and AChE inhibitor may interact in a synergistic manner to control pain. This synergy allows a reduction in the dose required of each compound, leading to a reduction in the side effects and enhancement of the clinical utility of the compounds.

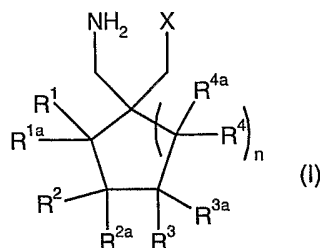
Accordingly, the invention provides, as a first aspect, a combination product comprising an alpha-2-delta ligand and an AChE inhibitor. Preferably, the compounds are combined in a ratio to provide a synergistic interaction.

As an alternative or further aspect, the invention provides a pharmaceutical composition for the curative, prophylactic or palliative treatment of pain, particularly neuropathic pain, comprising a combination of an alpha-2-delta ligand and an AChE inhibitor. Preferably, the compounds are combined in a ratio to provide a synergistic interaction.

Examples of alpha-2-delta ligands for use with the present invention are those compounds generally or specifically disclosed in US4024175, particularly gabapentin, EP641330, particularly pregabalin, US5563175, WO9733858, WO9733859, WO9931057, WO9931074, WO9729101, WO02085839, particularly [(1R,5R,6S)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid, WO9931075, particularly 3-(1-Aminomethyl-cyclohexylmethyl)-4H-[1,2,4]oxadiazol-5-one and C-[1-(1H-Tetrazol-5-ylmethyl)-cycloheptyl]-methylamine, WO9921824, particularly (3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, WO0190052, WO0128978, particularly (1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ )(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid, EP0641330, WO9817627, WO0076958, particularly (3S,5R)-3-aminomethyl-5-methyl-octanoic acid, PCT/IB03/00976, particularly (3S,5R)-3-amino-5-methyl-heptanoic acid, (3S,5R)-3-amino-5-methyl-nonanoic acid and (3S,5R)-3-Amino-5-methyl-octanoic acid, EP1178034, EP1201240, WO9931074, WO03000642, WO0222568, WO0230871, WO0230881, WO02100392, WO02100347, WO0242414, WO0232736 and WO0228881 and pharmaceutically acceptable salts and solvates thereof, all of which are incorporated herein by reference.

Preferred alpha-2-delta ligands of the present invention include: gabapentin, pregabalin, [(1R,5R,6S)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid, 3-(1-Aminomethyl-cyclohexylmethyl)-4H-[1,2,4]oxadiazol-5-one, C-[1-(1H-Tetrazol-5-ylmethyl)-cycloheptyl]-methylamine, (3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, (1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ )(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid, (3S,5R)-3-Aminomethyl-5-methyl-octanoic acid, (3S,5R)-3-amino-5-methyl-heptanoic acid, (3S,5R)-3-amino-5-methyl-nonanoic acid and (3S,5R)-3-Amino-5-methyl-octanoic acid.

Useful cyclic alpha-2-delta ligands of the present invention may be depicted by the following formula (I):



5 wherein X is a carboxylic acid or carboxylic acid bioisostere;

n is 0, 1 or 2; and

$R^1$ ,  $R^{1a}$ ,  $R^2$ ,  $R^{2a}$ ,  $R^3$ ,  $R^{3a}$ ,  $R^4$  and  $R^{4a}$  are independently selected from H and  $C_1$ - $C_6$  alkyl, or

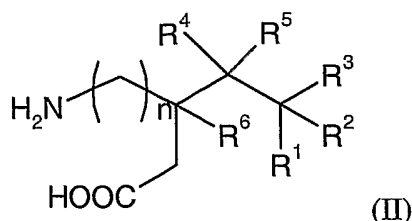
10  $R^1$  and  $R^2$  or  $R^2$  and  $R^3$  are taken together to form a  $C_3$ - $C_7$  cycloalkyl ring, which is optionally substituted with one or two substituents selected from  $C_1$ - $C_6$  alkyl, or a pharmaceutically acceptable salt or solvate thereof.

In formula (I), suitably,  $R^1$ ,  $R^{1a}$ ,  $R^{2a}$ ,  $R^{3a}$ ,  $R^4$  and  $R^{4a}$  are H and  $R^2$  and  $R^3$  are independently selected from H and methyl, or  $R^{1a}$ ,  $R^{2a}$ ,  $R^{3a}$  and  $R^{4a}$  are H and  $R^1$  and  $R^2$  or  
 15  $R^2$  and  $R^3$  are taken together to form a  $C_3$ - $C_7$  cycloalkyl ring, which is optionally substituted with one or two methyl substituents. A suitable carboxylic acid bioisostere is selected from tetrazolyl and oxadiazolonyl. X is preferably a carboxylic acid.

In formula (I), preferably,  $R^1$ ,  $R^{1a}$ ,  $R^{2a}$ ,  $R^{3a}$ ,  $R^4$  and  $R^{4a}$  are H and  $R^2$  and  $R^3$  are  
 20 independently selected from H and methyl, or  $R^{1a}$ ,  $R^{2a}$ ,  $R^{3a}$  and  $R^{4a}$  are H and  $R^1$  and  $R^2$  or  $R^2$  and  $R^3$  are taken together to form a  $C_4$ - $C_5$  cycloalkyl ring, or, when n is 0,  $R^1$ ,  $R^{1a}$ ,  $R^{2a}$ ,  $R^{3a}$ ,  $R^4$  and  $R^{4a}$  are H and  $R^2$  and  $R^3$  form a cyclopentyl ring, or, when n is 1,  $R^1$ ,  $R^{1a}$ ,  $R^{2a}$ ,  $R^{3a}$ ,  $R^4$  and  $R^{4a}$  are H and  $R^2$  and  $R^3$  are both methyl or  $R^1$ ,  $R^{1a}$ ,  $R^{2a}$ ,  $R^{3a}$ ,  $R^4$  and  $R^{4a}$  are H and  $R^2$  and  $R^3$  form a cyclobutyl ring, or, when n is 2,  $R^1$ ,  $R^{1a}$ ,  $R^2$ ,  $R^{2a}$ ,  $R^3$ ,  $R^{3a}$ ,  $R^4$  and  $R^{4a}$   
 25 are H, or, n is 0,  $R^1$ ,  $R^{1a}$ ,  $R^{2a}$ ,  $R^{3a}$ ,  $R^4$  and  $R^{4a}$  are H and  $R^2$  and  $R^3$  form a cyclopentyl ring.

Useful acyclic alpha-2-delta ligands of the present invention may be depicted by the following formula (II):

6



wherein:

n is 0 or 1, R<sup>1</sup> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>)alkyl; R<sup>2</sup> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>)alkyl; R<sup>3</sup> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>)alkyl; R<sup>4</sup> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>)alkyl; R<sup>5</sup> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>)alkyl and R<sup>6</sup> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>)alkyl, or a pharmaceutically acceptable salt or solvate thereof.

According to formula (II), suitably R<sup>1</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl, R<sup>2</sup> is methyl, R<sup>3</sup> - R<sup>6</sup> are hydrogen and n is 0 or 1. More suitably R<sup>1</sup> is methyl, ethyl, n-propyl or n-butyl, R<sup>2</sup> is methyl, R<sup>3</sup> - R<sup>6</sup> are hydrogen and n is 0 or 1. When R<sup>2</sup> is methyl, R<sup>3</sup> - R<sup>6</sup> are hydrogen and n is 0, R<sup>1</sup> is suitably ethyl, n-propyl or n-butyl. When R<sup>2</sup> is methyl, R<sup>3</sup> - R<sup>6</sup> are hydrogen and n is 1, R<sup>1</sup> is suitably methyl or n-propyl. Compounds of formula (II) are suitably in the 3S,5R configuration.

Examples of AChE inhibitors for use with the invention are donepezil (Aricept®), tacrine (cognex®), rivastigmine (Exelon®), physostigmine (Synapton®), galantamine (Reminyl), metrifonate (Promem), neostigmine (Prostigmin), icopezil, hupazine A, zanapezil (TAK 147), stacofylline, phenserine, (5R,9R)-5-(*r*-chloro-2-hydroxy-3-methoxybenzylidene-amino)-11-ethylidene-7-methyl-1,2,5,6,9,10-hexahydro-5,9-methanocycloocta[*b*]pyridin-2-one (ZT 1), the galantamine derivatives SPH 1371, SPH 1373 and SPH 1375, tolserine, 1-(3-fluorobenzyl)-4-[(2-fluoro-5,6-dimethoxy-1-indanone-2-yl)methyl]piperidine hydrochloride (ER 127528), thiatolserine, (-)-12-amino-3-chloro-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride (huperine X), *N,N*-dimethylcarbamic acid 4-[1(*S*)-(methylamino)-3-(4-nitrophenoxy)propyl] phenyl ester hemifumarate (RS 1259), ipidacrine (Amiridin), velnacrine (Mentane®), eptastigmine (heptylphysostigmine), zifrosilone (2,2,2-trifluoro-1-[3-(trimethylsilyl)phenyl]ethanone), 2-[2-(1-benzylpiperidin-4-yl)ethyl]-2,3-dihydro-9-methoxy-1*H*-pyrrolo[3,4-*b*]quinolin-1-one hemifumerate (T 82), 1,3-dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-*b*]quinazoline (CI 1002), *N*-heptylcarbamic acid

2,4a,9-trimethyl-2,3,4,4a,9,9a-hexahydro-1,2-oxazino[6,5-*b*]indol-6-yl ester-L-tartrate (CHF 2060), 3-(2-[1-(1,3-dioxolan-2-ylmethyl)piperidin-4-yl]ethyl)-3,4-dihydro-2H-1,3-benzoxazine-2,4-dione hydrochloride (E 2030), N-[10-(diethylamino)decyl]carbamic acid (3*aS*,8*aR*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester (MF 247), 5-amino-6-chloro-4-hydroxy-3,4-dihydro-1H-thiopyrano-[3,4-*b*]quinoline (MF 8615), N-[8-(*cis*-2,6-dimethylmorpholin-4-yl)octyl]carbamic acid (3*aS*,8*aR*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester L-bitartrate hydrate (MF 268), (-)N-(3-piperidinopropyl)-N-demethylgalantamine (SPH 1286), N-propargyl-3R-aminoindan-5-yl-ethyl methyl carbamate (TV 3326), and their pharmaceutically acceptable salts.

Preferred AChE inhibitors for use with the invention are donepezil (Aricept®), tacrine (cognex®), rivastigmine (Exelon®), physostigmine (Synapton®), galantamine (Reminyl), metrifonate (Promem), neostigmine (Prostigmin) and icopezil and their pharmaceutically acceptable salts.

The most preferred AChE inhibitor for use with the invention is donepezil.

The suitability of any particular AChE inhibitor can be readily determined by evaluation of its potency and selectivity using literature methods followed by evaluation of its toxicity, absorption, metabolism, pharmacokinetics, etc in accordance with standard pharmaceutical practices.

As an alternative or preferred aspect of the present invention, there is provided a combination comprising gabapentin and an AChE inhibitor selected from donepezil (Aricept®), tacrine (cognex®), rivastigmine (Exelon®), physostigmine (Synapton®), galantamine (Reminyl), metrifonate (Promem), neostigmine (Prostigmin), icopezil, hupazine A, zanapezil (TAK 147), stacofylline, phenserine, (5*R*,9*R*)-5-(*r*-chloro-2-hydroxy-3-methoxybenzylidene-amino)-11-ethylidene-7-methyl-1,2,5,6,9,10-hexahydro-5,9-methanocycloocta[*b*]pyridin-2-one (ZT 1), the galantamine derivatives SPH 1371, SPH 1373 and SPH 1375, tolserine, 1-(3-fluorobenzyl)-4-[(2-fluoro-5,6-dimethoxy-1-indanone-2-yl)methyl]piperidine hydrochloride (ER 127528), thiatolserine, (-)-12-amino-3-chloro-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride



(huperine X), *N,N*-dimethylcarbamic acid 4- [1(*S*)-(methylamino)-3-(4-nitrophenoxy)propyl] phenyl ester hemifumarate (RS 1259), ipidacrine (Amiridin), velnacrine (Mentane®), eptastigmine (heptylphysostigmine), zifrosilone (2,2,2-trifluoro-1-[3-(trimethylsilyl)phenyl]ethanone), 2-[2-(1-benzylpiperidin-4-yl)ethyl]-2,3-dihydro-9-methoxy-1*H*-pyrrolo[3,4-*b*]quinolin-1-one hemifumerate (T 82), 1,3-dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-*b*]-quinazoline (CI 1002), *N*-heptylcarbamic acid 2,4a,9-trimethyl-2,3,4,4a,9,9a-hexahydro-1,2-oxazino[6,5-*b*]indol-6-yl ester-L-tartrate (CHF 2060), 3-(2-[1-(1,3-dioxolan-2-ylmethyl)piperidin-4-yl]ethyl)-3,4 -dihydro-2*H*-1,3-benzoxazine-2,4-dione hydrochloride (E 2030), *N*-[10-(diethylamino)decyl]carbamic acid (3*aS*,8*aR*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester (MF 247), 5-amino-6-chloro-4-hydroxy-3,4-dihydro-1*H*-thiopyrano-[3,4-*b*]quinoline (MF 8615), *N*-[8-(*cis*-2,6-dimethylmorpholin-4-yl)octyl]carbamic acid (3*aS*,8*aR*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester L-bitartrate hydrate (MF 268), (-)*N*-(3-piperidinopropyl)-*N*-demethylgalantamine (SPH 1286), *N*-propargyl- 3*R*-aminoindan-5-yl -ethyl methyl carbamate (TV 3326) and their pharmaceutically acceptable salts.

As an alternative or preferred aspect of the present invention, there is provided a combination comprising pregabalin and an AChE inhibitor selected from donepezil (Aricept®), tacrine (cognex®), rivastigmine (Exelon®), physostigmine (Synapton®), galantamine (Reminyl), metrifonate (Promem), neostigmine (Prostigmin), icopezil, hupazine A, zanapezil (TAK 147), stacofylline, phenserine, (5*R*,9*R*)-5-(*r*-chloro-2-hydroxy-3-methoxybenzylidene-amino)-11-ethlidene-7-methyl-1,2,5,6,9,10-hexahydro-5,9-methanocycloocta[*b*]pyridin-2-one (ZT 1), the galantamine derivatives SPH 1371, SPH 1373 and SPH 1375, tolserine, 1-(3-fluorobenzyl)-4-[(2-fluoro-5,6-dimethoxy-1-indanone-2-yl)methyl]piperidine hydrochloride (ER 127528), thiatolserine, (-)-12-amino-3-chloro-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride (huperine X), *N,N*-dimethylcarbamic acid 4- [1(*S*)-(methylamino)-3-(4-nitrophenoxy)propyl] phenyl ester hemifumarate (RS 1259), ipidacrine (Amiridin), velnacrine (Mentane®), eptastigmine (heptylphysostigmine), zifrosilone (2,2,2-trifluoro-1-[3-(trimethylsilyl)phenyl]ethanone), 2-[2-(1-benzylpiperidin-4-yl)ethyl]-2,3-dihydro-9-methoxy-1*H*-pyrrolo[3,4-*b*]quinolin-1-one hemifumerate (T 82), 1,3-dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-*b*]-quinazoline (CI 1002), *N*-heptylcarbamic acid

2,4a,9-trimethyl-2,3,4,4a,9,9a-hexahydro-1,2-oxazino[6,5-*b*]indol-6-yl ester-L-tartrate (CHF 2060), 3-(2-[1-(1,3-dioxolan-2-ylmethyl)piperidin-4-yl]ethyl)-3,4-dihydro-2H-1,3-benzoxazine-2,4-dione hydrochloride (E 2030), N-[10-(diethylamino)decyl]carbamic acid (3a*S*,8a*R*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester (MF 247), 5-amino-6-chloro-4-hydroxy-3,4-dihydro-1H-thiopyrano-[3,4-*b*]quinoline (MF 8615), N-[8-(*cis*-2,6-dimethylmorpholin-4-yl)octyl]carbamic acid (3a*S*,8a*R*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester L-bitartrate hydrate (MF 268), (-)N-(3-piperidinopropyl)-N-demethylgalantamine (SPH 1286), N-propargyl-3R-aminoindan-5-yl-ethyl methyl carbamate (TV 3326), and their pharmaceutically acceptable salts. A particularly preferred combination comprises gabapentin and donepezil.

Alternatively, there is provided a pharmaceutical composition for the curative, prophylactic or palliative treatment of pain, particularly neuropathic pain, comprising an alpha-2-delta ligand selected from pregabalin or gabapentin or a pharmaceutically acceptable salt thereof, and an AChE inhibitor selected from donepezil (Aricept®), tacrine (cognex®), rivastigmine (Exelon®), physostigmine (Synapton®), galantamine (Reminyl), metrifonate (Promem), neostigmine (Prostigmin), icopezil, hupazine A, zanapezil (TAK 147), stacofylline, phenserine, (5*R*,9*R*)-5-(*r*-chloro-2-hydroxy-3-methoxybenzylidene-amino)-11-ethylidene-7-methyl-1,2,5,6,9,10-hexahydro-5,9-methanocycloocta[*b*]pyridin-2-one (ZT 1), the galantamine derivatives SPH 1371, SPH 1373 and SPH 1375, tolserine, 1-(3-fluorobenzyl)-4-[(2-fluoro-5,6-dimethoxy-1-indanone-2-yl)methyl]piperidine hydrochloride (ER 127528), thiatolserine, (-)-12-amino-3-chloro-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride (huperine X), *N,N*-dimethylcarbamic acid 4-[1(*S*)-(methylamino)-3-(4-nitrophenoxy)propyl]phenyl ester hemifumarate (RS 1259), ipidacrine (Amiridin), velnacrine (Mentane®), eptastigmine (heptylphysostigmine), zifrosilone (2,2,2-trifluoro-1-[3-(trimethylsilyl)phenyl]ethanone), 2-[2-(1-benzylpiperidin-4-yl)ethyl]-2,3-dihydro-9-methoxy-1*H*-pyrrolo[3,4-*b*]quinolin-1-one hemifumerate (T 82), 1,3-dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-*b*]quinazoline (CI 1002), N-heptylcarbamic acid 2,4a,9-trimethyl-2,3,4,4a,9,9a-hexahydro-1,2-oxazino[6,5-*b*]indol-6-yl ester-L-tartrate (CHF 2060), 3-(2-[1-(1,3-dioxolan-2-ylmethyl)piperidin-4-yl]ethyl)-3,4-dihydro-2H-1,3-benzoxazine-2,4-dione hydrochloride (E 2030), N-[10-(diethylamino)decyl]carbamic acid

(3a*S*,8a*R*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester (MF 247), 5-amino-6-chloro-4-hydroxy-3,4-dihydro-1*H*-thiopyrano-[3,4-*b*]quinoline (MF 8615), N-[8-(*cis*-2,6-dimethylmorpholin-4-yl)octyl]carbamic acid (3a*S*,8a*R*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester L-bitartrate hydrate (MF 268), (-)N-(3-piperidinopropyl)-N-demethylgalantamine (SPH 1286), N-propargyl-3*R*-aminoindan-5-yl -ethyl methyl carbamate (TV 3326), and their pharmaceutically acceptable salts.

As a further alternative or preferred aspect of the present invention, there is provided a combination comprising [(1*R*,5*R*,6*S*)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid or a pharmaceutically acceptable salt thereof, and an AChE inhibitor. Suitably, there is provided a combination comprising [(1*R*,5*R*,6*S*)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid or a pharmaceutically acceptable salt thereof, and a AChE inhibitor selected from from donepezil (Aricept®), tacrine (cognex®), rivastigmine (Exelon®), physostigmine (Synapton®), galantamine (Reminyl), metrifonate (Promem), neostigmine (Prostigmin), icopezil, hupazine A, zanapezil (TAK 147), stacofylline, phenserine, (5*R*,9*R*)-5-(*r*-chloro-2-hydroxy-3-methoxybenzylidene-amino)-11-ethlidene-7-methyl-1,2,5,6,9,10-hexahydro-5,9-methanocycloocta[*b*]pyridin-2-one (ZT 1), the galantamine derivatives SPH 1371, SPH 1373 and SPH 1375, tolserine, 1-(3-fluorobenzyl)-4-[(2-fluoro-5,6-dimethoxy-1-indanone-2-yl)methyl]piperidine hydrochloride (ER 127528), thiatolserine, (-)-12-amino-3-chloro-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride (huperine X), *N,N*-dimethylcarbamic acid 4-[1(*S*)-(methylamino)-3-(4-nitrophenoxy)propyl] phenyl ester hemifumarate (RS 1259), ipidacrine (Amiridin), velnacrine (Mentane®), eptastigmine (heptylphysostigmine), zifrosilone (2,2,2-trifluoro-1-[3-(trimethylsilyl)phenyl]ethanone), 2-[2-(1-benzylpiperidin-4-yl)ethyl]-2,3-dihydro-9-methoxy-1*H*-pyrrolo[3,4-*b*]quinolin-1-one hemifumerate (T 82), 1,3-dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-*b*]quinazoline (CI 1002), N-heptylcarbamic acid 2,4a,9-trimethyl-2,3,4,4a,9,9a-hexahydro-1,2-oxazino[6,5-*b*]indol-6-yl ester-L-tartrate (CHF · 2060), 3-(2-[1-(1,3-dioxolan-2-yl)methyl]piperidin-4-yl)ethyl)-3,4 -dihydro-2*H*-1,3-benzoxazine-2,4-dione hydrochloride (E 2030), N-[10-(diethylamino)decyl]carbamic acid (3a*S*,8a*R*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester (MF 247), 5-amino-6-chloro-4-hydroxy-3,4-dihydro-1*H*-thiopyrano-[3,4-*b*]quinoline (MF 8615), N-[8-(*cis*-2,6-

dimethylmorpholin-4-yl)octyl]carbamic acid (3a*S*,8a*R*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester L-bitartrate hydrate (MF 268), (-)N-(3-piperidinopropyl)-N-demethylgalantamine (SPH 1286), N-propargyl-3R-aminoindan-5-yl-ethyl methyl carbamate (TV 3326) or a pharmaceutically acceptable salt thereof.

5 Preferably, the combination comprises [(1*R*,5*R*,6*S*)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid and donepezil or pharmaceutically acceptable salts thereof.

Alternatively, there is provided a pharmaceutical composition for the curative, prophylactic or palliative treatment of pain, particularly neuropathic pain, comprising a combination comprising [(1*R*,5*R*,6*S*)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid or a pharmaceutically acceptable salt thereof, and an AChE inhibitor.

10

As a yet further preferred aspect of the present invention, the combination is selected from:

- 15 gabapentin and donepezil;  
gabapentin and tacrine;  
gabapentin and rivastigmine;  
gabapentin and physostigmine;  
gabapentin and galantamine;
- 20 gabapentin and metrifonate;  
gabapentin and neostigmine;  
gabapentin and icopezil;  
pregabalin and donepezil;  
pregabalin and tacrine;
- 25 pregabalin and rivastigmine;  
pregabalin and physostigmine;  
pregabalin and galantamine;  
pregabalin and metrifonate;  
pregabalin and neostigmine;
- 30 pregabalin and icopezil;  
[(1*R*,5*R*,6*S*)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid and donepezil;  
[(1*R*,5*R*,6*S*)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid and tacrine;  
[(1*R*,5*R*,6*S*)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid and rivastigmine;

- [(1R,5R,6S)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid and physostigmine;  
[(1R,5R,6S)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid and galantamine;  
[(1R,5R,6S)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid and metrifonate;  
[(1R,5R,6S)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid and neostigmine;  
5 [(1R,5R,6S)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid and icopezil;  
(1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ )(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid and donepezil;  
(1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ )(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid and tacrine;  
(1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ )(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid and rivastigmine;  
(1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ )(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid and physostigmine;  
10 (1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ )(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid and galantamine;  
(1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ )(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid and metrifonate;  
(1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ )(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid and neostigmine; and  
(1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ )(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid and icopezil; (3S,4S)-(1-  
Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, and donepezil;  
15 (3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, and tacrine;  
(3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, and rivastigmine;  
(3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, and physostigmine;  
(3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, and galantamine;  
(3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, and metrifonate;  
20 (3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, and neostigmine; and  
(3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, and icopezil;  
or pharmaceutically acceptable salts or solvates of any thereof.

The combination of the present invention in a single dosage form is suitable for  
25 administration to any mammalian subject, preferably human. Administration may be  
once (o.d.), twice (b.i.d.) or three times (t.i.d.) daily, suitably b.i.d. or t.i.d., more suitably  
b.i.d, most suitably o.d.. Thus, as a further aspect of the present invention, there is  
provided a method of curative, prophylactic or palliative treatment of pain in a  
mammalian subject comprising once, twice or thrice, suitably twice or thrice, more  
30 suitably twice, most suitably once daily administration of an effective, particularly  
synergistic, combination of an alpha-2-delta ligand and an AChE inhibitor.

Determining a synergistic interaction between one or more components, the optimum range for the effect and absolute dose ranges of each component for the effect may be definitively measured by administration of the components over different w/w ratio ranges and doses to patients in need of treatment. For humans, the complexity and cost of carrying out clinical studies on patients renders impractical the use of this form of testing as a primary model for synergy. However, the observation of synergy in one species can be predictive of the effect in other species and animal models exist, as described herein, to measure a synergistic effect and the results of such studies can also be used to predict effective dose and plasma concentration ratio ranges and the absolute doses and plasma concentrations required in other species by the application of pharmacokinetic/pharmacodynamic methods. Established correlations between animal models and effects seen in man suggest that synergy in animals is best-demonstrated using static and dynamic allodynia measurements in rodents that have undergone surgical (e.g. chronic constriction injury) or chemical (e.g. streptozocin) procedures to induce the allodynia. Because of plateau effects in such models, their value is best assessed in terms of synergistic actions that in neuropathic pain patients would translate to dose-sparing advantages. Other models in which existing agents used for the treatment of neuropathic pain give only a partial response are more suited to predict the potential of combinations acting synergistically to produce increased maximal efficacy at maximally tolerated doses of the two components.

Thus, as a further aspect of the present invention, there is provided a synergistic combination for human administration comprising an alpha-2-delta ligand and an AChE inhibitor, or pharmaceutically acceptable salts or solvates thereof, in a w/w combination range which corresponds to the absolute ranges observed in a non-human animal model, preferably a rat model, primarily used to identify a synergistic interaction. Suitably, the ratio range in humans corresponds to a non-human range selected from between 1:50 to 50:1 parts by weight, 1:50 to 20:1, 1:50 to 10:1, 1:50 to 1:1, 1:20 to 50:1, 1:20 to 20:1, 1:20 to 10:1, 1:20 to 1:1, 1:10 to 50:1, 1:10 to 20:1, 1:10 to 10:1, 1:10 to 1:1, 1:1 to 50:1, 1.1 to 20:1 and 1:1 to 10:1. More suitably, the human range corresponds to a non-human range of 1:10 to 20:1 parts by weight.

For humans, several experimental pain models may be used in man to demonstrate that agents with proven synergy in animals also have effects in man compatible with that synergy. Examples of human models that may be fit for this purpose include the heat/capsaicin model (Petersen, K.L. & Rowbotham, M.C. (1999) *NeuroReport* 10, 1511-1516), the i.d capsaicin model (Andersen, O.L., Felsby, S., Nicolaisen, L., Bjerring, P., Jsen, T.S. & Arendt-Nielsen, L. (1996) *Pain* 66, 51-62), including the use of repeated capsaicin trauma (Witting, N., Svesson, P., Arendt-Nielsen, L. & Jensen, T.S. (2000) *Somatosensory Motor Res.* 17, 5-12), and summation or wind-up responses (Curatolo, M. et al. (2000) *Anesthesiology* 93, 1517 – 1530). With these models, subjective assessment of pain intensity or areas of hyperalgesia may be used as endpoints, or more objective endpoints, reliant on electrophysiological or imaging technologies (such as functional magnetic resonance imaging) may be employed (Bornhovd, K., Quante, M., Glauche, V., Bromm, B., Weiller, C. & Buchel, C. (2002) *Brain* 125, 1326-1336). All such models require evidence of objective validation before it can be concluded that they provide evidence in man of supporting the synergistic actions of a combination that have been observed in animal studies.

For the present invention in humans, a suitable alpha-2-delta ligand:AChE inhibitor ratio range is selected from between 1:50 to 50:1 parts by weight, 1:50 to 20:1, 1:50 to 10:1, 1:50 to 1:1, 1:20 to 50:1, 1:20 to 20:1, 1:20 to 10:1, 1:20 to 1:1, 1:10 to 50:1, 1:10 to 20:1, 1:10 to 10:1, 1:10 to 1:1, 1:1 to 50:1, 1:1 to 20:1 and 1:1 to 10:1, more suitably 1:10 to 20:1, preferably, 1:1 to 10:1.

Optimal doses of each component for synergy can be determined according to published procedures in animal models. However, in man (even in experimental models of pain) the cost can be very high for studies to determine the entire exposure-response relationship at all therapeutically relevant doses of each component of a combination. It may be necessary, at least initially, to estimate whether effects can be observed that are consistent with synergy at doses that have been extrapolated from those that give optimal synergy in animals. In scaling the doses from animals to man, factors such as relative body weight/body surface area, relative absorption, distribution, metabolism and excretion of each component and relative plasma protein binding need to be considered and, for these reasons, the optimal dose ratio predicted for man (and also for patients) is

unlikely to be the same as the dose ratio shown to be optimal in animals. However, the relationship between the two can be understood and calculated by one skilled in the art of animal and human pharmacokinetics. Important in establishing the bridge between animal and human effects are the plasma concentrations obtained for each component used in the animal studies, as these are related to the plasma concentration of each component that would be expected to provide efficacy in man. Pharmacokinetic/ pharmacodynamic modeling (including methods such as isobolograms, interaction index and response surface modelling) and simulations may help to predict synergistic dose ratios in man, particularly where either or both of these components has already been studied in man.

It is important to ascertain whether any concluded synergy observed in animals or man is due solely to pharmacokinetic interactions. For example, inhibition of the metabolism of one compound by another might give a false impression of pharmacodynamic synergy

Thus, according to a further aspect of the present invention, there is provided a synergistic combination for administration to humans comprising an alpha-2-delta ligand and an AChE inhibitor or pharmaceutically acceptable salts or solvates thereof, where the dose range of each component corresponds to the absolute ranges observed in a non-human animal model, preferably the rat model, primarily used to identify a synergistic interaction. Suitably, the dose range of alpha-2-delta ligand in human corresponds to a dose range of 1-20mg/kg, more suitably 1-10mg/kg, in the rat.

Suitably, the dose of alpha-2-delta ligand for use in a human is in a range selected from 1-1200mg, 1-500mg, 1-100mg, 1-50mg, 1-25mg, 500-1200mg, 100-1200mg, 100-500mg, 50-1200mg, 50-500mg, or 50-100mg, suitably 50-100mg, b.i.d. or t.i.d., suitably t.i.d., and the dose of AChE inhibitor is in a range selected from 1-200mg,, 1-100mg, 1-50mg, 1-25mg, 10-100mg, 10-50mg or 10-25 mg, suitably 10-100mg, b.i.d or t.i.d, suitably t.i.d..

It will be apparent to the skilled reader that the plasma concentration ranges of the alpha-2-delta ligand and AChE inhibitor combinations of the present invention required to



provide a therapeutic effect depend on the species to be treated, and components used. For example, for gabapentin in the rat the C<sub>max</sub> values range from 0.520 $\mu$ g/ml to 10.5 $\mu$ g/ml.

5 It is possible, using standard PK/PD and allometric methods, to extrapolate the plasma concentration values observed in an animal model to predict the values in a different species, particularly human. Thus, as a further aspect of the present invention, there is provided a synergistic combination for administration to humans comprising an alpha-2-delta ligand and an AChE inhibitor, where the plasma concentration range of  
10 each component corresponds to the absolute ranges observed in a non-human animal model, preferably the rat model, primarily used to identify a synergistic interaction.

Particularly preferred combinations of the invention include those in which each variable of the combination is selected from the suitable parameters for each variable.  
15 Even more preferable combinations of the invention include those where each variable of the combination is selected from the more suitable, most suitable, preferred or more preferred parameters for each variable.

## 20 DETAILED DESCRIPTION OF THE INVENTION

The compounds of the present combination invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms, including hydrated forms, which may contain isotopic substitutions (e.g. D<sub>2</sub>O, d<sub>6</sub>-  
25 acetone, d<sub>6</sub>-DMSO), are equivalent to unsolvated forms and are encompassed within the scope of the present invention.

Certain of the compounds of the present invention possess one or more chiral centers and each center may exist in the R(D) or S(L) configuration. The present  
30 invention includes all enantiomeric and epimeric forms as well as the appropriate mixtures thereof. Separation of diastereoisomers or cis and trans isomers may be achieved by conventional techniques, e.g. by fractional crystallisation, chromatography or

H.P.L.C. of a stereoisomeric mixture of a compound of the invention or a suitable salt or derivative thereof.

A number of the alpha-2-delta ligands of the present invention are amino acids. Since amino acids are amphoteric, pharmacologically compatible salts can be salts of appropriate non-toxic inorganic or organic acids or bases. Suitable acid addition salts are the acetate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate, camsylate, citrate, edisylate, esylate, fumarate, gluceptate, gluconate, glucuronate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, hydrogen phosphate, isethionate, D- and L-lactate, malate, maleate, malonate, mesylate, methylsulphate, 2-napsylate, nicotinate, nitrate, orotate, palmoate, phosphate, saccharate, stearate, succinate sulphate, D- and L-tartrate, and tosylate salts. Suitable base salts are formed from bases which form non-toxic salts and examples are the sodium, potassium, aluminium, calcium, magnesium, zinc, choline, diolamine, olamine, arginine, glycine, tromethamine, benzathine, lysine, meglumine and diethylamine salts. Salts with quaternary ammonium ions can also be prepared with, for example, the tetramethyl-ammonium ion. The compounds of the invention may also be formed as a zwitterion. Furthermore, since a number of the AChE inhibitors of the present invention are amines and a number of the alpha-2-delta ligands have an acid functionality, a further aspect of the present invention comprises a salt form containing the 2 components, particularly in a 1:1 combination. A suitable combination salt form is the salt formed by a 1:1 combination of gabapentin and donepezil.

A suitable salt for amino acid compounds of the present invention is the hydrochloride salt. For a review on suitable salts see Stahl and Wermuth, Handbook of Pharmaceutical Salts: Properties, Selection, and Use, Wiley-VCH, Weinheim, Germany (2002).

Also within the scope of the invention are clathrates, drug-host inclusion complexes wherein, in contrast to the aforementioned solvates, the drug and host are present in non-stoichiometric amounts. For a review of such complexes, see J Pharm Sci, 64 (8), 1269-1288 by Haleblian (August 1975).

Hereinafter all references to compounds of the invention include references to salts thereof and to solvates and clathrates of compounds of the invention and salts thereof.

5 Also included within the present scope of the compounds of the invention are polymorphs thereof.

Prodrugs of the above compounds of the invention are included in the scope of the instant invention. The chemically modified drug, or prodrug, should have a different  
10 pharmacokinetic profile to the parent, enabling easier absorption across the mucosal epithelium, better salt formulation and/or solubility, improved systemic stability (for an increase in plasma half-life, for example). These chemical modifications may be

- 15 (1) Ester or amide derivatives which may be cleaved by, for example, esterases or lipases. For ester derivatives, the ester is derived from the carboxylic acid moiety of the drug molecule by known means. For amide derivatives, the amide may be derived from the carboxylic acid moiety or the amine moiety of the drug molecule by known means.
- 20 (2) Peptides which may be recognized by specific or nonspecific proteinases. A peptide may be coupled to the drug molecule via amide bond formation with the amine or carboxylic acid moiety of the drug molecule by known means.
- (3) Derivatives that accumulate at a site of action through membrane selection of a prodrug form or modified prodrug form.
- (4) Any combination of 1 to 3.

25 Aminoacyl-glycolic and -lactic esters are known as prodrugs of amino acids (Wermuth C.G., *Chemistry and Industry*, 1980:433-435). The carbonyl group of the amino acids can be esterified by known means. Prodrugs and soft drugs are known in the art (Palomino E., *Drugs of the Future*, 1990;15(4):361-368). The last two citations are hereby incorporated by reference.

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The combination of the present invention is useful for the general treatment of pain, particularly neuropathic pain. Physiological pain is an important protective mechanism designed to warn of danger from potentially injurious stimuli from the

external environment. The system operates through a specific set of primary sensory neurones and is exclusively activated by noxious stimuli via peripheral transducing mechanisms (Millan 1999 *Prog. Neurobio.* 57: 1-164 for an integrative Review). These sensory fibres are known as nociceptors and are characterised by small diameter axons with slow conduction velocities. Nociceptors encode the intensity, duration and quality of noxious stimulus and by virtue of their topographically organised projection to the spinal cord, the location of the stimulus. The nociceptors are found on nociceptive nerve fibres of which there are two main types, A-delta fibres (myelinated) and C fibres (non-myelinated). The activity generated by nociceptor input is transferred after complex processing in the dorsal horn, either directly or via brain stem relay nuclei to the ventrobasal thalamus and then on to the cortex, where the sensation of pain is generated.

Intense acute pain and chronic pain may involve the same pathways driven by pathophysiological processes and as such cease to provide a protective mechanism and instead contribute to debilitating symptoms associated with a wide range of disease states. Pain is a feature of many trauma and disease states. When a substantial injury, via disease or trauma, to body tissue occurs the characteristics of nociceptor activation are altered. There is sensitisation in the periphery, locally around the injury and centrally where the nociceptors terminate. This leads to hypersensitivity at the site of damage and in nearby normal tissue. In acute pain these mechanisms can be useful and allow for the repair processes to take place and the hypersensitivity returns to normal once the injury has healed. However, in many chronic pain states, the hypersensitivity far outlasts the healing process and is normally due to nervous system injury. This injury often leads to maladaptation of the afferent fibres (Woolf & Salter 2000 *Science* 288: 1765-1768). Clinical pain is present when discomfort and abnormal sensitivity feature among the patient's symptoms. Patients tend to be quite heterogeneous and may present with various pain symptoms. There are a number of typical pain subtypes: 1) spontaneous pain which may be dull, burning, or stabbing; 2) pain responses to noxious stimuli are exaggerated (hyperalgesia); 3) pain is produced by normally innocuous stimuli (allodynia) (Meyer et al., 1994 *Textbook of Pain* 13-44). Although patients with back pain, arthritis pain, CNS trauma, or neuropathic pain may have similar symptoms, the underlying mechanisms are different and, therefore, may require different treatment strategies. Therefore pain can be divided into a number of different areas because of

differing pathophysiology, these include nociceptive, inflammatory, neuropathic pain etc. It should be noted that some types of pain have multiple aetiologies and thus can be classified in more than one area, e.g. Back pain, Cancer pain have both nociceptive and neuropathic components.

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Nociceptive pain is induced by tissue injury or by intense stimuli with the potential to cause injury. Pain afferents are activated by transduction of stimuli by nociceptors at the site of injury and sensitise the spinal cord at the level of their termination. This is then relayed up the spinal tracts to the brain where pain is perceived (Meyer et al., 1994 Textbook of Pain 13-44). The activation of nociceptors activates two types of afferent nerve fibres. Myelinated A-delta fibres transmitted rapidly and are responsible for the sharp and stabbing pain sensations, whilst unmyelinated C fibres transmit at a slower rate and convey the dull or aching pain. Moderate to severe acute nociceptive pain is a prominent feature of, but is not limited to pain from strains/sprains, post-operative pain (pain following any type of surgical procedure), posttraumatic pain, burns, myocardial infarction, acute pancreatitis, and renal colic. Also cancer related acute pain syndromes commonly due to therapeutic interactions such as chemotherapy toxicity, immunotherapy, hormonal therapy and radiotherapy. Moderate to severe acute nociceptive pain is a prominent feature of, but is not limited to, cancer pain which may be tumour related pain, (e.g. bone pain, headache and facial pain, viscera pain) or associated with cancer therapy (e.g. postchemotherapy syndromes, chronic postsurgical pain syndromes, post radiation syndromes), back pain which may be due to herniated or ruptured intervertebral discs or abnormalities of the lumbar facet joints, sacroiliac joints, paraspinal muscles or the posterior longitudinal ligament

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Neuropathic pain is defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system (IASP definition). Nerve damage can be caused by trauma and disease and thus the term 'neuropathic pain' encompasses many disorders with diverse aetiologies. These include but are not limited to, Diabetic neuropathy, Post herpetic neuralgia, Back pain, Cancer neuropathy, HIV neuropathy, Phantom limb pain, Carpal Tunnel Syndrome, chronic alcoholism, hypothyroidism, trigeminal neuralgia, uremia, or vitamin deficiencies. Neuropathic pain is pathological as it has no protective role. It is often present well after the original cause has dissipated, commonly lasting for

years, significantly decreasing a patients quality of life (Woolf and Mannion 1999 Lancet 353: 1959-1964). The symptoms of neuropathic pain are difficult to treat, as they are often heterogeneous even between patients with the same disease (Woolf & Decosterd 1999 Pain Supp. 6: S141-S147; Woolf and Mannion 1999 Lancet 353: 1959-1964). They  
5 include spontaneous pain, which can be continuous, or paroxysmal and abnormal evoked pain, such as hyperalgesia (increased sensitivity to a noxious stimulus) and allodynia (sensitivity to a normally innocuous stimulus).

The inflammatory process is a complex series of biochemical and cellular events  
10 activated in response to tissue injury or the presence of foreign substances, which result in swelling and pain (Levine and Taiwo 1994: Textbook of Pain 45-56). Arthritic pain makes up the majority of the inflammatory pain population. Rheumatoid disease is one of the commonest chronic inflammatory conditions in developed countries and rheumatoid arthritis is a common cause of disability. The exact aetiology of RA is unknown, but  
15 current hypotheses suggest that both genetic and microbiological factors may be important (Grennan & Jayson 1994 Textbook of Pain 397-407). It has been estimated that almost 16 million Americans have symptomatic osteoarthritis (OA) or degenerative joint disease, most of whom are over 60 years of age, and this is expected to increase to 40 million as the age of the population increases, making this a public health problem of  
20 enormous magnitude (Houge & Mersfelder 2002 Ann Pharmacother. 36: 679-686; McCarthy et al., 1994 Textbook of Pain 387-395). Most patients with OA seek medical attention because of pain. Arthritis has a significant impact on psychosocial and physical function and is known to be the leading cause of disability in later life. Other types of inflammatory pain include but are not limited to inflammatory bowel diseases (IBD),

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Other types of pain include but are not limited to;

Musculo-skeletal disorders including but not limited to myalgia, fibromyalgia, spondylitis, sero-negative (non-rheumatoid) arthropathies, non-articular rheumatism,  
30 dystrophinopathy, Glycogenolysis, polymyositis, pyomyositis.

Central pain or 'thalamic pain' as defined by pain caused by lesion or dysfunction of the nervous system including but not limited to central post-stroke pain, multiple sclerosis, spinal cord injury, Parkinson's disease and epilepsy.

5 Heart and vascular pain including but not limited to angina, myocardical infarction, mitral stenosis, pericarditis, Raynaud's phenomenon, scleredoma, scleredoma, skeletal muscle ischemia.

10 Visceral pain, and gastrointestinal disorders. The viscera encompasses the organs of the abdominal cavity. These organs include the sex organs, spleen and part of the digestive system. Pain associated with the viscera can be divided into digestive visceral pain and non-digestive visceral pain. Commonly encountered gastrointestinal (GI) disorders include the functional bowel disorders (FBD) and the inflammatory bowel diseases (IBD). These GI disorders include a wide range of disease states that are  
15 currently only moderately controlled, including – for FBD, gastro-esophageal reflux, dyspepsia, the irritable bowel syndrome (IBS) and functional abdominal pain syndrome (FAPS), and – for IBD, Crohn's disease, ileitis, and ulcerative colitis, which all regularly produce visceral pain. Other types of visceral pain include the pain associated with  
20 dysmenorrhea, pelvic pain, cystitis and pancreatitis.

Head pain including but not limited to migraine, migraine with aura, migraine without aura cluster headache, tension-type headache.

25 Orofacial pain including but not limited to dental pain, temporomandibular myofascial pain.

As an alternative aspect, there is provided the simultaneous, sequential or separate use of a synergistic combination of an alpha-2-delta ligand and an AChE inhibitor in the manufacture of a medicament for the curative, prophylactic or palliative treatment of pain,  
30 particularly neuropathic pain. As a preferred feature, the use suitably comprises any one of the combinations mentioned herein above.

As a further alternative aspect, there is provided a method for the curative, prophylactic or palliative treatment of pain, particularly neuropathic pain, comprising simultaneous, sequential or separate administration of a therapeutically synergistic amount of an alpha-2-delta ligand and an AChE inhibitor to a mammal in need of said treatment. As a preferred feature, the method suitably comprises any one of the combinations mentioned herein above.

The biological activity of the alpha-2-delta ligands of the invention may be measured in a radioligand binding assay using [<sup>3</sup>H]gabapentin and the  $\alpha_2\delta$  subunit derived from porcine brain tissue (Gee N.S., Brown J.P., Dissanayake V.U.K., Offord J., Thurlow R., Woodruff G.N., *J. Biol. Chem.*, 1996;271:5879-5776). Results may be expressed in terms of  $\mu$ M or nM  $\alpha_2\delta$  binding affinity.

AChE inhibitor activity may be determined by the methods described by Ellman, GL et al, *Biochem. Pharmacol.* 1961,7 88-95. The assay solution consists of a 0.1 M sodium phosphate buffer, pH 8.0, with the addition of 100  $\mu$ M tetraisopropylpyrophosphoramidate (*iso*-OMPA), 100  $\mu$ M 5,5<sup>I</sup>-dithiobis (2-nitrobenzoic acid) (DTNB), 0.02 units/mL AChE (Sigma Chemical Co., from human erythrocytes) and 200  $\mu$ M acetylthiocholine iodide. The final assay volume was 0.25 mL. Test compounds were added to the assay solution prior to enzyme addition, whereupon a 20-min preincubation period with enzyme was followed by addition of substrate. Changes in absorbance at 412 nM were recorded for 5 min. The reaction rates were compared, and the percent inhibition due to the presence of test compounds was calculated.

Inhibition of butyrylcholinesterase was measured as described above for AChE by omitting addition of *iso*-OM-PA and substitution 0.02 units/mL of BuChE (Sigma Chemical Co., from horse serum) and 200  $\mu$ M butyrylthiocholine for enzyme and substrate, respectively.

*In vivo* Microdialysis. Male Sprague-Dawley rats were implanted in the corpus striatum with guide cannulae and dialysis probes (Bioanalytical Systems, West Lafayette, IN) and superfused at a rate 3 mL/minute. The dialysis fluid was a Ringer's buffer (pH



7.2) containing 500 nM physostigmine to reduce degradation of Ach by AChE. Fractions (60 µl) were collected every 20 minutes for 2 hours before drug administration and for 3 hours following oral administration of drug. Samples (50 µl) were used directly for HPLC analysis of Ach content as described above. Basal Ach release was defined as the average Ach content in the three fractions just prior to drug administration. Ach content in all fractions was converted to a percentage of these basal control values.

The elements of the combination of the instant invention may be administered separately, simultaneously or sequentially. As a further aspect of the present invention, there is provided a package comprising a synergistic combination of an alpha-2-delta ligand and an AChE inhibitor and a suitable container.

The combination may also optionally be administered with one or more other pharmacologically active agents. Suitable optional agents include:

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(i) opioid analgesics, e.g. morphine, heroin, hydromorphone, oxymorphone, levorphanol, levallorphan, methadone, meperidine, fentanyl, cocaine, codeine, dihydrocodeine, oxycodone, hydrocodone, propoxyphene, nalmeferne, nalorphine, buprenorphine, butorphanol, nalbuphine and pentazocine;

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(ii) Opioid antagonists, e.g. naloxone, naltrexone

(iii) nonsteroidal antiinflammatory drugs (NSAIDs), e.g. aspirin, diclofenac, difluinsal, etodolac, fenbufen, fenoprofen, flufenisal, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, meclofenamic acid, mefenamic acid, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, sulindac, tolmetin, zomepirac, and their pharmaceutically acceptable salts or solvates;

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(iv) barbiturate sedatives, e.g. amobarbital, aprobarbital, butabarbital, butabital, mephobarbital, metharbital, methohexital, pentobarbital, phenobarbital, secobarbital, talbutal, theamylal, thiopental and their pharmaceutically acceptable salts or solvates;

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(v) benzodiazepines having a sedative action, e.g. chlordiazepoxide, clorazepate, diazepam, flurazepam, lorazepam, oxazepam, temazepam, triazolam and their pharmaceutically acceptable salts or solvates,

(vi) H<sub>1</sub> antagonists having a sedative action, e.g. diphenhydramine, pyrilamine, promethazine, chlorpheniramine, chlorcyclizine and their pharmaceutically acceptable salts or solvates;

(vii) miscellaneous sedatives such as glutethimide, meprobamate,  
5 methaqualone, dichloralphenazone and their pharmaceutically acceptable salts or solvates;

(viii) skeletal muscle relaxants, e.g. baclofen, tolperisone, carisoprodol, chlorzoxazone, cyclobenzaprine, methocarbamol, orphenadine and their pharmaceutically acceptable salts or solvates,

(ix) NMDA receptor antagonists, e.g. dextromethorphan ((+)-3-hydroxy-N-methylmorphinan) and its metabolite dextrorphan ((+)-3-hydroxy-N-methylmorphinan), ketamine, memantine, pyrroloquinoline quinone and cis-4-(phosphonomethyl)-2-piperidinecarboxylic acid and their pharmaceutically acceptable salts or solvates; alpha-adrenergic active compounds, e.g. doxazosin, tamsulosin,  
10 clonidine and 4-amino-6,7-dimethoxy-2-(5-methanesulfonamido-1,2,3,4-tetrahydroisoquinol-2-yl)-5-(2-pyridyl) quinazoline;

(x) tricyclic antidepressants, e.g. desipramine, imipramine, amitriptyline and nortriptyline;

(xi) anticonvulsants, e.g. carbamazepine, valproate, lamotrigine;

(xii) serotonin reuptake inhibitors, e.g. fluoxetine, paroxetine,  
20 citalopram and sertraline;

(xiii) mixed serotonin-noradrenaline reuptake inhibitors, e.g. milnacipran, venlafaxine and duloxetine;

(xiv) noradrenaline reuptake inhibitors, e.g. reboxetine;

(xv) Tachykinin (NK) antagonists, particularly Nk-3, NK-2 and NK-1 antagonists e.g., ( $\alpha$ R,9R)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7H-[1,4]diazocino[2,1-g][1,7]naphthridine-6-13-dione (TAK-637), 5-[[[(2R,3S)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro-3H-1,2,4-triazol-3-one (MK-869), lanepitant, dapitant  
25 and 3-[[2-methoxy-5-(trifluoromethoxy)phenyl]methylamino]-2-phenyl-piperidine  
30 (2S,3S)

(xvi) Muscarinic antagonists, e.g. oxybutin, tolterodine, propiverine, tropsium chloride and darifenacin;

(xvii) COX-2 inhibitors, e.g. celecoxib, rofecoxib and valdecoxib;

(xviii) Non-selective COX inhibitors (preferably with GI protection), e.g. nitroflurbiprofen (HCT-1026);

(xix) coal-tar analgesics, in particular, paracetamol;

(xx) neuroleptics, such as droperidol;

(xxi) Vanilloid receptor agonists, e.g. resiniferatoxin;

(xxii) Beta-adrenergic compounds such as propranolol;

(xxiii) Local anaesthetics, such as mexiletine, lidocaine;

(xxiv) Corticosteroids, such as dexamethasone

(xxv) serotonin receptor agonists and antagonists;

(xxvi) cholinergic (nicotinic) analgesics; and

(xxvii) miscellaneous agents such as Tramadol®.

15

Thus, the present invention extends to a product comprising an alpha-2-delta ligand, an AChE inhibitor, and one or more other therapeutic agents, such as those listed above, for simultaneous, separate or sequential use in the curative, prophylactic treatment of pain, particularly neuropathic pain.

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The combination of the invention can be administered alone but one or both elements will generally be administered in an admixture with suitable pharmaceutical excipient(s), diluent(s) or carrier(s) selected with regard to the intended route of administration and standard pharmaceutical practice. If appropriate, auxiliaries can be added. Auxiliaries are preservatives, anti-oxidants, flavours or colourants. The compounds of the invention may be of immediate-, delayed-, modified-, sustained-, pulsed- or controlled-release type.

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The elements of the combination of the present invention can be administered, for example but not limited to, the following route: orally, buccally or sublingually in the form of tablets, capsules, multi- and nano-particulates, gels, films (incl. muco-adhesive), powder, ovules, elixirs, lozenges (incl. liquid-filled), chews, solutions, suspensions and

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sprays. The compounds of the invention may also be administered as osmotic dosage form, or in the form of a high energy dispersion or as coated particles or fast-dissolving, fast -disintegrating dosage form as described in Ashley Publications, 2001 by Liang and Chen. The compounds of the invention may be administered as crystalline or amorphous products, freeze dried or spray dried. Suitable formulations of the compounds of the invention may be in hydrophilic or hydrophobic matrix, ion-exchange resin complex, coated or uncoated form and other types as described in US 6,106,864 as desired.

Such pharmaceutical compositions, for example, tablets, may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate, glycine and starch (preferably corn, potato or tapioca starch), mannitol, disintegrants such as sodium starch glycolate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), triglycerides, hydroxypropylcellulose (HPC), bentonite sucrose, sorbitol, gelatin and acacia. Additionally, lubricating agents may be added to solid compositions such as magnesium stearate, stearic acid, glyceryl behenate, PEG and talc or wetting agents, such as sodium lauryl sulphate. Additionally, polymers such as carbohydrates, phospholipids and proteins may be included.

Fast dispersing or dissolving dosage formulations (FDDFs) may contain the following ingredients: aspartame, acesulfame potassium, citric acid, croscarmellose sodium, crospovidone, diascorbic acid, ethyl acrylate, ethyl cellulose, gelatin, hydroxypropylmethyl cellulose, magnesium stearate, mannitol, methyl methacrylate, mint flavouring, polyethylene glycol, fumed silica, silicon dioxide, sodium starch glycolate, sodium stearyl fumarate, sorbitol or xylitol. The terms dispersing or dissolving as used herein to describe FDDFs are dependent upon the solubility of the drug substance used, i.e. where the drug substance is insoluble a fast dispersing dosage form can be prepared and where the drug substance is soluble a fast dissolving dosage form can be prepared.

The solid dosage form, such as tablets are manufactured by a standard process, for example, direct compression or a wet, dry or melt granulation, melt congealing and extrusion process. The tablet cores which may be mono or multi-layer may be coated with appropriate overcoats known in the art.

Solid compositions of a similar type may also be employed as fillers in capsules such as gelatin, starch or HPMC capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. Liquid compositions may be employed as fillers in soft or hard capsules such as gelatin capsule. For aqueous and oily suspensions, solutions, syrups and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol, methylcellulose, alginic acid or sodium alginate, glycerin, oils, hydrocolloid agents and combinations thereof. Moreover, formulations containing these compounds and excipients may be presented as a dry product for constitution with water or other suitable vehicles before use.

Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water propylene glycol solutions. For parenteral injection, liquid preparations can be formulated in solution in aqueous polyethylene glycol solution. Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizing and thickening agents as desired. Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents.

The elements of the combination of the present invention can also be administered by injection, that is, intravenously, intramuscularly, intracutaneously, intraduodenally, or intraperitoneally, intraarterially, intrathecally, intraventricularly, intraurethrally, intrasternally, intracranially, intraspinally or subcutaneously, or they may be administered by infusion, needle-free injectors or implant injection techniques. For such parenteral administration they are best used in the form of a sterile aqueous solution, suspension or emulsion (or system so that can include micelles) which may contain other substances known in the art, for example, enough salts or carbohydrates such as glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. For some forms of parenteral administration they may be used in the form of a sterile non-aqueous system such as fixed

oils, including mono- or diglycerides, and fatty acids, including oleic acid. The preparation of suitable parenteral formulations under sterile conditions for example lyophilisation is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (e.g. sterile, pyrogen-free water) before use.

Also, the elements of the combination of the present invention can be administered intranasally or by inhalation. They are conveniently delivered in the form of a dry powder (either alone, as a mixture, for example a dry blend with lactose, or a mixed component particle, for example with phospholipids) from a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray, atomiser (preferably an atomiser using electrohydrodynamics to produce a fine mist) or nebuliser, with or without the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A [trade mark]) or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA [trade mark]), carbon dioxide, a further perfluorinated hydrocarbon such as Perflubron (trade mark) or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray, atomiser or nebuliser may contain a solution or suspension of the active compound, e.g. using a mixture of ethanol (optionally, aqueous ethanol) or a suitable agent for dispersing, solubilising or extending release and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules, blisters and cartridges (made, for example, from gelatin or HPMC) for use in an inhaler or insufflator may be formulated to contain a powder mix of the compound of the invention, a suitable powder base such as lactose or starch and a performance modifier such as l-leucine, mannitol or magnesium stearate.

Prior to use in a dry powder formulation or suspension formulation for inhalation the elements of the combination of the invention will be micronised to a size suitable for delivery by inhalation (typically considered as less than 5 microns). Micronisation could be achieved by a range of methods, for example spiral jet milling, fluid bed jet milling, use of supercritical fluid crystallisation or by spray drying.

A suitable solution formulation for use in an atomiser using electrohydrodynamics to produce a fine mist may contain from 1µg to 10mg of the compound of the invention per actuation and the actuation volume may vary from 1 to 100µl. A typical formulation may comprise the elements of the combination of the invention, propylene glycol, sterile  
5 water, ethanol and sodium chloride. Alternative solvents may be used in place of propylene glycol, for example glycerol or polyethylene glycol.

Alternatively, the elements of the combination of the invention may be administered topically to the skin, mucosa, dermally or transdermally, for example, in the  
10 form of a gel, hydrogel, lotion, solution, cream, ointment, dusting powder, dressing, foam, film, skin patch, wafers, implant, sponges, fibres, bandage, microemulsions and combinations thereof. For such applications, the compounds of the invention can be suspended or dissolved in, for example, a mixture with one or more of the following:  
15 mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax, fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid, water, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol, alcohols such as ethanol. Alternatively, penetration enhancers may be used. The following may also be used  
20 polymers, carbohydrates, proteins, phospholipids in the form of nanoparticles (such as niosomes or liposomes) or suspended or dissolved. In addition, they may be delivered using iontophoresis, electroporation, phonophoresis and sonophoresis.

Alternatively, the elements of the combination of the invention can be  
25 administered rectally, for example in the form of a suppository or pessary. They may also be administered by vaginal route. For example, these compositions may be prepared by mixing the drug with a suitable non-irritant excipients, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquefy and/or dissolve in the cavity to release the drug.

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The elements of the combination of the invention may also be administered by the ocular route. For ophthalmic use, the compounds can be formulated as micronised

suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline. A polymer may be added such as crossed-linked polyacrylic acid, polyvinylalcohol, hyaluronic acid, a cellulosic polymer (e.g. hydroxypropylmethylcellulose, hydroxyethylcellulose, methyl cellulose), or a  
5 heteropolysaccharide polymer (e.g. gelan gum). Alternatively, they may be formulated in an ointment such as petrolatum or mineral oil, incorporated into bio-degradable (e.g. absorbable gel sponges, collagen) or non-biodegradable (e.g. silicone) implants, wafers, drops, lenses or delivered via particulate or vesicular systems such as niosomes or liposomes. Formulations may be optionally combined with a preservative, such as  
10 benzalkonium chloride. In addition, they may be delivered using iontophoresis. They may also be administered in the ear, using for example but not limited to the drops.

The elements of the combination of the invention may also be used in combination with a cyclodextrin. Cyclodextrins are known to form inclusion and non-inclusion  
15 complexes with drug molecules. Formation of a drug-cyclodextrin complex may modify the solubility, dissolution rate, taste-masking, bioavailability and/or stability property of a drug molecule. Drug-cyclodextrin complexes are generally useful for most dosage forms and administration routes. As an alternative to direct complexation with the drug the cyclodextrin may be used as an auxiliary additive, e.g. as a carrier, diluent or solubiliser.  
20 Alpha-, beta- and gamma-cyclodextrins are most commonly used and suitable examples are described in WO-A-91/11172, WO-A-94/02518 and WO-A-98/55148.

The term 'administered' includes delivery by viral or non-viral techniques. Viral delivery mechanisms include but are not limited to adenoviral vectors, adeno- associated  
25 viral (AAV) vectors, herpes viral vectors, retroviral vectors, lentiviral vectors, and baculoviral vectors. Non-viral delivery mechanisms include lipid mediated transfection, liposomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical or sublingual routes.

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Thus, as a further aspect of the present invention, there is provided a pharmaceutical composition comprising a combination comprising an alpha-2-delta



ligand, an AChE inhibitor and a suitable excipient, diluent or carrier. Suitably, the composition is suitable for use in the treatment of pain, particularly neuropathic pain.

As an alternative aspect of the present invention, there is provided a  
5 pharmaceutical composition comprising a synergistic combination comprising an alpha-2-  
delta ligand, an AChE inhibitor and a suitable excipient, diluent or carrier. Suitably, the  
composition is suitable for use in the treatment of pain, particularly neuropathic pain.

For non-human animal administration, the term 'pharmaceutical' as used herein  
10 may be replaced by 'veterinary.'

The element of the pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation,  
15 the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsules, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. The quantity of active component in a unit dose preparation may be varied or adjusted from 0.1 mg to 1 g according to the particular application and the  
20 potency of the active components. In medical use the drug may be administered three times daily as, for example, capsules of 100 or 300 mg. In therapeutic use, the compounds utilized in the pharmaceutical method of this invention are administered at the initial dosage of about 0.01 mg to about 100 mg/kg daily. A daily dose range of about 0.01 mg to about 100 mg/kg is preferred. The dosages, however, may be varied depending upon  
25 the requirements of the patient, the severity of the condition being treated, and the compounds being employed. Determination of the proper dosage for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compounds. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. For  
30 convenience, the total daily dosage may be divided and administered in portions during the day, if desired.

For veterinary use, a combination according to the present invention or veterinarily acceptable salts or solvates thereof, is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

## BIOLOGY EXAMPLES

### **METHODS**

10

The following biological methods may be used to determine the activity of the combinations of the present invention.

#### **Animals**

15 Male Sprague Dawley rats (200-250g), obtained from Charles River, (Margate, Kent, U.K.) are housed in groups of 6. All animals are kept under a 12h light/dark cycle (lights on at 07h 00min) with food and water *ad libitum*. All experiments are carried out by an observer unaware of drug treatments.

#### **20 CCI surgery in the rat**

Animals are anaesthetised with isoflurane. The sciatic nerve is ligated as previously described by Bennett and Xie, 1988. Animals are placed on a homeothermic blanket for the duration of the procedure. After surgical preparation the common sciatic nerve is exposed at the middle of the thigh by blunt dissection through biceps femoris. Proximal to the sciatic trifurcation, about 7mm of nerve is freed of adhering tissue and 4 ligatures (4-0 silk) are tied loosely around it with about 1mm spacing. The incision is closed in layers and the wound treated with topical antibiotics.

#### **30 Effect of combinations on the maintenance of CCI-induced static and dynamic allodynia**

Dose-responses to the alpha-2-delta ligand and the AChE inhibitor may be first performed alone in the CCI model. Combinations are examined following a fixed ratio design. A dose-response to each fixed dose ratio of the combination is performed. On each test day,

baseline paw withdrawal thresholds (PWT) to von Frey hairs and paw withdrawal latencies (PWL) to a cotton bud stimulus are determined prior to drug treatment. The alpha-2-delta ligand is administered p.o. directly followed by s.c. administration of the AChE inhibitor and PWT and PWL re-examined for up to 5h. The data are expressed at the 2h time point  
5 for both the static and dynamic data as this timepoint represent the peak antiallodynic effects.

### **Evaluation of allodynia**

Static allodynia may be measured using Semmes-Weinstein von Frey hairs (Stoelting,  
10 Illinois, U.S.A.). Animals are placed into wire mesh bottom cages allowing access to the underside of their paws. Animals are habituated to this environment prior to the start of the experiment. Static allodynia is tested by touching the plantar surface of the animals right hind paw with von Frey hairs in ascending order of force for up to 6 sec. Once a withdrawal response is established, the paw is re-tested, starting with the next descending  
15 von Frey hair until no response occurs. The highest force, which lifts the paw as well as eliciting a response, thus represents the cut off point. The lowest amount of force required to elicit a response is recorded as the PWT in grams.

Dynamic allodynia is assessed by lightly stroking the plantar surface of the hind paw with  
20 a cotton bud. Care is taken to perform this procedure in fully habituated rats that are not active to avoid recording general motor activity. At least three measurements are taken at each time point the mean of which represents the paw withdrawal latency (PWL). If no reaction is exhibited within 15s the procedure is terminated and animals are assigned this withdrawal time. Thus 15s effectively represents no withdrawal. A withdrawal response is  
25 often accompanied with repeated flinching or licking of the paw. Dynamic allodynia is considered to be present if animals respond to the cotton stimulus before 8s of stroking.

### **Combination studies**

Dose responses are first performed to both the alpha-2-delta ligand and the AChE  
30 inhibitor alone. A number of fixed dose ratios of alpha-2-delta ligand:AChE inhibitor are then examined. Dose responses to each fixed dose ratio are performed with the time-course for each experiment determined by the duration of antiallodynic-action of each separate ratio.

Suitable AChE inhibitors of the present invention may be prepared as described in the references or are obvious to those skilled in the art on the basis of these documents.

5            Suitable alpha-2-delta ligand compounds of the present invention may be prepared as described herein below or in the aforementioned patent literature references, particularly in PCT/IB02/01146, which are illustrated by the following non-limiting examples and intermediates.

10    Chemistry Examples

**Example 1. (3S,5R)-3-Amino-5-methyl-octanoic acid hydrochloride(R)-2,6-Dimethyl-non-2-ene.** To (*S*)-citronellyl bromide (50 g, 0.228 mol) in THF (800 mL) at 0°C was added LiCl (4.3 g) followed by CuCl<sub>2</sub> (6.8 g). After 30 minutes  
15 methylmagnesium chloride (152 mL of a 3 M solution in THF, Aldrich) was added and the solution warmed to room temperature. After 10 hours the solution was cooled to 0°C and a saturated aqueous solution of ammonium chloride carefully added. The resultant two layers were separated and the aqueous phase extracted with ether. The combined organic phases were dried (MgSO<sub>4</sub>) and concentrated to give (R)-2,6-dimethyl-non-2-  
20 ene. 32.6 g; 93%. Used without further purification. <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 5.1 (m, 1H), 1.95 (m, 2H), 1.62 (s, 3H), 1.6 (s, 3H), 1.3 (m, 4H), 1.2 (m, 2H), 0.8 (s, 6H); <sup>13</sup>C NMR (100 MHz; CDCl<sub>3</sub>) δ 131.13, 125.28, 39.50, 37.35, 32.35, 25.92, 25.77, 20.31, 19.74, 17.81, 14.60.

25            **(R)-4-Methyl-heptanoic acid.** To (R)-2,6-dimethyl-non-2-ene (20 g, 0.13 mol) in acetone (433 mL) was added a solution of CrO<sub>3</sub> (39 g, 0.39 mol) in H<sub>2</sub>SO<sub>4</sub> (33 mL)/H<sub>2</sub>O (146 mL) over 50 minutes. After 6 hours a further amount of CrO<sub>3</sub> (26 g, 0.26 mol) in H<sub>2</sub>SO<sub>4</sub> (22 mL)/H<sub>2</sub>O (100 mL) was added. After 12 hours the solution was diluted with brine and the solution extracted with ether. The combined organic phases  
30 were dried (MgSO<sub>4</sub>) and concentrated. Flash chromatography (gradient of 6:1 to 2:1

hexane/EtOAc) gave (R)-4-methyl-heptanoic acid as an oil. 12.1 g; 65%. MS, *m/z* (relative intensity): 143 [M-H, 100%].

**(4R,5S)-4-Methyl-3-((R)-4-methyl-heptanoyl)-5-phenyl-oxazolidin-2-one.** To  
5 (R)-4-methyl-heptanoic acid (19 g, 0.132 mol) and triethylamine (49.9 g, 0.494 mol) in THF (500 mL) at 0°C was added trimethylacetylchloride (20 g, 0.17 mol). After 1 hour LiCl (7.1 g, 0.17 mol) was added followed by (4R,5S)-(+)-4-methyl-5-phenyl-2-oxazolidinone) **3** (30 g, 0.17 mol). The mixture was warmed to room temperature and after 16 hours the filtrate was removed by filtration and the solution concentrated under  
10 reduced pressure. Flash chromatography (7:1 hexane/EtOAc) gave (4R,5S)-4-methyl-3-((R)-4-methyl-heptanoyl)-5-phenyl-oxazolidin-2-one as an oil. 31.5 g; 79%.  $[\alpha]_D = +5.5$  (c 1 in CHCl<sub>3</sub>). MS, *m/z* (relative intensity): 304 [M+H, 100%].

**(3S,5R)-5-Methyl-3-((4R,5S)-4-methyl-2-oxo-5-phenyl-oxazolidine-3-carbonyl)-octanoic acid tert-butyl ester.** To (4R,5S)-4-methyl-3-((R)-4-methyl-heptanoyl)-5-phenyl-oxazolidin-2-one (12.1 g, 0.04 mol) in THF (200 ml) at -50°C was added sodium bis(trimethylsilyl)amide (48 mL of a 1 M solution in THF). After 30 min t-butylbromoacetate (15.6 g, 0.08 mol) was added. The solution was stirred for 4 hours at -50°C and then warmed to room temperature. After 16 hours a saturated aqueous solution  
20 of ammonium chloride was added and the two layers separated. The aqueous phase was extracted with ether and the combined organic phases dried (MgSO<sub>4</sub>) and concentrated. Flash chromatography (9:1 hexane/EtOAc) gave (3S,5R)-5-methyl-3-((4R,5S)-4-methyl-2-oxo-5-phenyl-oxazolidine-3-carbonyl)-octanoic acid tert-butyl ester as a white solid  
25 12 g; 72%.  $[\alpha]_D = +30.2$  (c 1 in CHCl<sub>3</sub>). <sup>13</sup>C NMR (100 MHz; CDCl<sub>3</sub>)  $\delta$  176.47, 171.24, 152.72, 133.63, 128.87, 125.86, 80.85, 78.88, 55.34, 39.98, 38.77, 38.15, 37.58, 30.60, 28.23, 20.38, 20.13, 14.50, 14.28.

**(S)-2-((R)-2-Methyl-pentyl)-succinic acid 4-tert-butyl ester.** To (3S,5R)-5-methyl-3-((4R,5S)-4-methyl-2-oxo-5-phenyl-oxazolidine-3-carbonyl)-octanoic acid tert-butyl ester (10.8 g, 0.025 mol) in H<sub>2</sub>O (73 mL) and THF (244 mL) at 0°C was added a  
30 premixed solution of LiOH (51.2 mL of a 0.8 M solution) and H<sub>2</sub>O<sub>2</sub> (14.6 mL of a 30%

solution). After 4 hours a further 12.8 mL LiOH (0.8 M solution) and 3.65 mL of H<sub>2</sub>O<sub>2</sub> (30% solution) was added. After 30 minutes sodium bisulfite (7 g), sodium sulfite (13 g), and water (60 mL) was added followed by hexane (100 mL) and ether (100 mL). The two layers were separated and the aqueous layer extracted with ether. The combined organic phases were concentrated to an oil that was dissolved in heptane (300 mL). The resultant solid was filtered off and the filtrate dried (MgSO<sub>4</sub>) and concentrated to afford (S)-2-  
5 ((R)-2-methyl-pentyl)-succinic acid 4-tert-butyl ester (6 g, 93%) which was used immediately without further purification. MS, *m/z* (relative intensity): 257 [M+H, 100%].

10 **(3S, 5R)-3-Benzoyloxycarbonylamino-5-methyl-octanoic acid, tert-butyl ester.**

A solution of (S)-2-((R)-2-methyl-pentyl)-succinic acid 4-tert-butyl ester (6.0 g, 23.22 mmol) and triethylamine (3.64 mL, 26.19 mmol) in toluene (200 mL) was treated with diphenylphosphoryl azide (5.0 mL, 23.22 mL) and stirred at room temperature for 0.5 hours. After the reaction mixture was then heated at reflux for 3h and cooled briefly,  
15 benzyl alcohol was added (7.2 mL, 69.7 mmol) and the solution heated for another 3 h. After the reaction mixture was allowed to cool, it was diluted with ethyl ether (200 mL) and the combined organic layer was washed successively with saturated NaHCO<sub>3</sub> and brine and dried (Na<sub>2</sub>SO<sub>4</sub>). The concentrated organic component was purified by chromatography (MPLC) eluting with 8:1 hexanes: ethyl acetate to provide (3S, 5R)-3-  
20 benzyloxycarbonylamino-5-methyl-octanoic acid, *tert*-butyl ester (6.4 g, 75.8%). MS: M+1: 364.2, 308.2.

**(3S, 5R)-3-Amino-5-methyl-octanoic acid, tert-butyl ester.** A solution of (3S, 5R)-3-benzyloxycarbonylamino-5-methyl-octanoic acid, *tert*-butyl ester (2.14g, 5.88  
25 mmol) in THF (50 mL) was treated with Pd/C (0.2 g) and H<sub>2</sub> at 50 psi for 2 hours. The reaction mixture was then filtered and concentrated to an oil in vacuo to give (3S, 5R)-3-amino-5-methyl-octanoic acid, *tert*-butyl ester in quantitative yield. MS: M+1: 230.2, 174.1.

30 **(3S, 5R)-3-Amino-5-methyl-octanoic acid hydrochloride.** A slurry of (3S, 5R)-amino-5-methyl-octanoic acid, *tert*-butyl ester (2.59g, 11.3 mmol) in 6N HCl (100 mL) was heated under reflux 18 hours, cooled, and filtered over Celite. The filtrate was

concentrated in vacuo to 25 mL and the resulting crystals were collected and dried to provide (3S, 5R)-3-amino-5-methyl-octanoic acid hydrochloride, mp 142.5-142.7°C (1.2g, 50.56%). A second crop (0.91g) was obtained from the filtrate. Anal. Calc'd for C<sub>9</sub>H<sub>19</sub>NO<sub>2</sub>·HCl: C: 51.55, H: 9.61, N: 6.68, Cl: 16.91. Found: C: 51.69, H: 9.72, N: 6.56, Cl: 16.63.

**(3S, 5R)-3-Amino-5-methyl-octanoic acid hydrochloride acid salt.** 5.3 g of 2S-(2R-methyl-pentyl)-succinic acid-4-tert-butyl ester contained in 30 mL methyltertbutyl ether is reacted at room temperature with 3.5 mL triethylamine followed by 6.4 g of diphenylphosphoryl azide. After allowing the reaction to exotherm to 45°C and stirring for at least 4 hours, the reaction mixture is allowed to cool to room temperature and stand while the phases separated. The lower layer is discarded and the upper layer is washed with water, followed by dilute aqueous HCl. The upper layer is then combined with 10 mL of 6 N aqueous HCl, and stirred at 45-65°C. The reaction mixture is concentrated by vacuum distillation to about 10 -14 mL and allowed to crystallize while cooling to about 5°C. After collecting the product by filtration, the product is washed with toluene and reslurried in toluene. The product is dried by heating under vacuum resulting in 2.9 g (67%) of white crystalline product. The product may be recrystallized from aqueous HCl. mp 137°C, HNMR (400 MHz, D6 DMSO) δ 0.84 - 0.88 (overlapping d and t, 6H), 1.03 - 1.13 (m, 1H), 1.16 - 1.37 (m, 4H), 1.57 - 1.68 (m, 2H), 2.55 (dd, 1H, J = 7, 17 Hz), 2.67 (dd, 1H, J = 6, 17 Hz), 3.40 (m, 1H), 8.1 (br s, 3H), 12.8 (br s, 1H).

#### **Example 2. (3S, 5R)-Amino-5-methyl-heptanoic acid**

**Methanesulfonic acid (S)-3,7-dimethyl-oct-6-enyl ester.** To S-(-)-citronellol (42.8 g, 0.274 mol) and triethylamine (91 mL, 0.657 mol) in CH<sub>2</sub>Cl<sub>2</sub> (800 mL) at 0°C was added methanesulphonyl chloride (26 mL, 0.329 mol) in CH<sub>2</sub>Cl<sub>2</sub> (200 mL). After 2 hours at 0°C the solution was washed with 1N HCl then brine. The organic phase was dried (MgSO<sub>4</sub>) and concentrated to afford the titled compound an oil (60.5 g, 94%) which was used without further purification. MS, m/z (relative intensity): 139 [100%], 143 [100%].

**(R)-2,6-Dimethyl-oct-2-ene.** To methanesulfonic acid (S)-3,7-dimethyl-oct-6-enyl ester (60 g, 0.256 mol) in THF (1 L) at 0°C was added lithium aluminum hydride (3.8 g, 0.128 mol). After 7 hours, a further 3.8 g of lithium aluminum hydride was added and the solution warmed to room temperature. After 18 hours, a further 3.8 g of lithium aluminum hydride was added. After a further 21 hours, the reaction was carefully quenched with 1N citric acid and the solution diluted further with brine. The resultant two phases were separated and the organic phase was dried (MgSO<sub>4</sub>) and concentrated to afford the titled compound as an oil which was used without further purification. MS, *m/z* (relative intensity): 139 [M+H, 100%].

10

**(R)-4-Methyl-hexanoic acid.** A procedure similar to the synthesis of (R)-4-methyl-heptanoic acid was utilized giving the acid as an oil (9.3 g, 56%). IR (film) 2963, 2931, 2877, 2675, 1107, 1461, 1414 cm<sup>-1</sup>; MS, *m/z* (relative intensity): 129 [M-H, 100%].

15

**(4R,5S)-4-Methyl-3-((R)-4-methyl-hexanoyl)-5-phenyl-oxazolidin-2-one.** A procedure similar to the synthesis of (4R,5S)-4-methyl-3-((R)-4-methyl-heptanoyl)-5-phenyl-oxazolidin-2-one was utilized giving the titled compound as an oil (35.7 g, 95%). MS, *m/z* (relative intensity): 290 [M+H, 100].

20

**(3S,5R)-5-Methyl-3-[1-((4R,5S)-4-methyl-2-oxo-5-phenyl-oxazolidin-3-yl)-methanoyl]-heptanoic acid tert-butyl ester.** A procedure similar to the preparation of (3S,5R)-5-methyl-3-((4R,5S)-4-methyl-2-oxo-5-phenyl-oxazolidine-3-carbonyl)-octanoic acid tert-butyl ester was followed giving the titled compound as an oil (7.48 g; 31%). MS, *m/z* (relative intensity): 178 [100%], 169 [100%]; [α]<sub>D</sub> = + 21.6 (c 1 in CHCl<sub>3</sub>).

25

**(S)-2-((R)-2-Methyl-butyl)-succinic acid 4-tert-butyl ester.** (3S,5R)-5-Methyl-3-[1-((4R,5S)-4-methyl-2-oxo-5-phenyl-oxazolidin-3-yl)-methanoyl]-heptanoic acid tert-butyl ester (7.26 g, 0.018 mol) in H<sub>2</sub>O (53 mL) and THF (176 mL) at 0°C was added a premixed solution of LiOH (37 mL of a 0.8 M solution) and H<sub>2</sub>O<sub>2</sub> (10.57 mL of a 30% solution) and the solution warmed to room temperature. After 2 hours sodium bisulfite (7 g), sodium sulfite (13 g), and water (60 mL) was added and the two layers were separated and the aqueous layer extracted with ether. The combined organic phases were

30



concentrated to an oil that was dissolved in heptane (200 mL). The resultant solid was filtered off and the filtrate dried ( $\text{MgSO}_4$ ) and concentrated to afford the titled compound as an oil (4.4 g) that was used without further purification. MS,  $m/z$  (relative intensity): 243 [100%].

5

**(3S, 5R)-3-Benzoyloxycarbonylamino-5-methyl-heptanoic acid, *tert*-butyl ester-**

This compound was prepared as described above starting with (S)-2-((R)-2-methyl-butyl) succinic acid, 4-*tert*-butyl ester to give (3S, 5R)-3-benzoyloxycarbonylamino-5-methyl-heptanoic acid, *tert*-butyl ester as an oil (73.3% yield).  $^1\text{H}$  NMR (400 MHz;  $\text{CDCl}_3$ )  $\delta$  0.84(t, 3H,  $J = 7.33$  Hz), 0.89(d, 3H,  $J = 6.60$  Hz), 1.12-1.38 (m, 4H), 1.41 (s, 9H), 1.43-1.59 (m, 2H), 2.42 (m, 2H), 4.05 (m, 1H), 5.07 (t, 2H  $J = 12.95$  Hz), and 7.28-7.34 (m, 5H).

**(3S, 5R)-Amino-5-methyl-heptanoic acid, *tert*-butyl ester-** This compound was prepared as described above starting with (3S, 5R)-3-benzoyloxycarbonylamino-5-methyl-heptanoic acid, *tert*-butyl ester instead of (3S, 5R)-3-benzoyloxycarbonylamino-5-methyl-octanoic acid, *tert*-butyl ester to give the titled compound.  $^1\text{H}$  NMR (400 MHz;  $\text{CDCl}_3$ )  $\delta$  0.84 (overlapping t and d, 6H), 1.08-1.16(m, 2H), 1.27-1.30(m, 2H), 1.42(s, 9H), 1.62 (br s, 2H), 2.15 (dd, 1H,  $J = 8.54$  and  $15.62$  Hz), 2.29(dd, 1H,  $J = 4.15$  and  $15.37$  Hz), and 3.20(br s, 2H).

**(3S, 5R)-Amino-5-methyl-heptanoic acid hydrochloride-**A slurry of (3S, 5R)-amino-5-methyl-heptanoic acid, *tert*-butyl ester (1.44g, 6.69 mmol) in 3N HCl was heated at reflux for 3 hours, filtered hot over Celite, and concentrated to dryness. Trituration of the resulting solid in ethyl ether provided (3S, 5R)-3-amino-5-methyl-heptanoic acid hydrochloride, (0.95g, 85%) mp 126.3-128.3°C. Anal. Calc'd for  $\text{C}_8\text{H}_{17}\text{NO}_2 \cdot \text{HCl} \cdot 0.1\text{H}_2\text{O}$ : C: 48.65, H: 9.29, N: 7.09, Cl: 17.95. Found: C: 48.61, H: 9.10, N: 7.27, Cl: 17.87MS: M+1: 160.2

30 **Example 3. (3S, 5R)-3-Amino-5-methyl-nonanoic acid**

**(R)-4-Methyl-octanoic acid.** Lithium chloride (0.39 g, 9.12 mmol) and copper (I) chloride (0.61 g, 4.56 mmol) were combined in 45 ml THF at ambient temperature and

stirred 15 minutes, then cooled to 0°C at which time ethylmagnesium bromide (1 M solution in THF, 45 mL, 45 mmol) was added. (*S*)-citronellyl bromide (5.0 g, 22.8 mmol) was added dropwise and the solution was allowed to warm slowly to ambient temperature with stirring overnight. The reaction was quenched by cautious addition of sat. NH<sub>4</sub>Cl (aq), and stirred with Et<sub>2</sub>O and sat. NH<sub>4</sub>Cl (aq) for 30 minutes. The phases were separated and the organic phase dried (MgSO<sub>4</sub>) and concentrated. The crude (*R*)-2,6-dimethyl-dec-2-ene was used without purification. To a solution of (*R*)-2,6-dimethyl-dec-2-ene (3.8 g, 22.8 mmol) in 50 mL acetone at 0°C was added Jones' reagent (2.7 M in H<sub>2</sub>SO<sub>4</sub> (aq), 40 mL, 108 mmol) and the solution was allowed to warm slowly to ambient temperature with stirring overnight. The mixture was partitioned between Et<sub>2</sub>O and H<sub>2</sub>O, the phases were separated, and the organic phase washed with brine, dried (MgSO<sub>4</sub>), and concentrated. The residue was purified by flash chromatography (8:1 hexanes:EtOAc) to afford 2.14 g (59%) of the titled compound as a colorless oil: LRMS: *m/z* 156.9 (M<sup>+</sup>). Jones' reagent was prepared as a 2.7M solution by combining 26.7g CrO<sub>3</sub>, 23 mL H<sub>2</sub>SO<sub>4</sub>, and diluting to 100 mL with H<sub>2</sub>O.

**(4*R*, 5*S*)-4-Methyl-3-((*R*)-4-methyl-octanoyl)-5-phenyl-oxazolidin-2-one.** To (*R*)-4-methyl-octanoic acid (2.14 g, 13.5 mmol) in 25 mL CH<sub>2</sub>Cl<sub>2</sub> at 0°C was added 3 drops DMF, followed by oxalyl chloride (1.42 mL, 16.2 mmol) resulting in vigorous gas evolution. The solution was warmed directly to ambient temperature, stirred 30 minutes, and concentrated. Meanwhile, to a solution of the oxazolidinone (2.64 g, 14.9 mmol) in 40 mL THF at -78°C was added *n*-butyllithium (1.6 M soln in hexanes, 9.3 mL, 14.9 mmol) dropwise. The mixture was stirred for 10 minutes at which time the acid chloride in 10 mL THF was added dropwise. The reaction was stirred 30 minutes at -78°C, then warmed directly to ambient temperature and quenched with sat. NH<sub>4</sub>Cl. The mixture was partitioned between Et<sub>2</sub>O and sat. NH<sub>4</sub>Cl (aq), the phases were separated, and the organic phase dried (MgSO<sub>4</sub>), and concentrated to furnish 3.2 g of the titled compound as a colorless oil. LRMS: *m/z* 318.2 (M<sup>+</sup>).

**(3S,5R)-5-Methyl-3-((4R,5S)-4-methyl-2-oxo-5-phenyl-oxazolidine-3-carbonyl)-nonanoic acid tert-butyl ester.** To a solution of diisopropylamine (1.8 mL, 12.6 mmol) in 30 mL THF at -78°C was added *n*-butyllithium (1.6 M soln in hexanes, 7.6 mL, 12.1 mmol), and the mixture stirred 10 minutes at which time (4R, 5S)-4-Methyl-3-((R)-4-methyl-octanoyl)-5-phenyl-oxazolidin-2-one (3.2 g, 10.1 mmol) in 10 mL THF was added dropwise. The solution was stirred for 30 minutes, *t*-butyl bromoacetate (1.8 mL, 12.1 mmol) was added quickly dropwise at -50°C, and the mixture was allowed to warm slowly to 10°C over 3 hours. The mixture was partitioned between Et<sub>2</sub>O and sat. NH<sub>4</sub>Cl (aq), the phases were separated, and the organic phase dried (MgSO<sub>4</sub>), and concentrated. The residue was purified by flash chromatography (16:1 to 8:1 hexanes:EtOAc) to provide 2.65 g (61%) of the titled compound as a colorless crystalline solid, mp = 84-86°C.  $[\delta]_D^{23} +17.1$  (c = 1.00, CHCl<sub>3</sub>).

**(S)-2-((R)-2-Methyl-hexyl)-succinic acid 4-tert-butyl ester.** To a solution of (3S,5R)-5-Methyl-3-((4R,5S)-4-methyl-2-oxo-5-phenyl-oxazolidine-3-carbonyl)-nonanoic acid tert-butyl ester (2.65 g, 6.14 mmol) in 20 mL THF at 0°C was added a precooled (0°C) solution of LiOH monohydrate (1.0 g, 23.8 mmol) and hydrogen peroxide (30 wt% aqueous soln, 5.0 mL) in 10 mL H<sub>2</sub>O. The mixture was stirred vigorously for 90 minutes, then warmed to ambient temperature and stirred 90 minutes. The reaction was quenched at 0°C by addition of 100 mL 10% NaHSO<sub>3</sub> (aq), then extracted with Et<sub>2</sub>O. The phases were separated, and the organic phase washed with brine, dried (MgSO<sub>4</sub>), and concentrated. The titled compound was used without purification.

**(3S, 5R)-3-Benzoyloxycarbonylamino-5-methylnonanoic acid, tert-butyl ester-** This compound was prepared similarly as described above starting with (S)-2-((R)-2-methylhexyl) succinic acid, 4-*tert*-butyl ester instead of (S)-2-((R)-2-methylpentyl) succinic acid, 4-*tert*-butyl ester to provide the titled compound as an oil (71.6% yield). <sup>1</sup>HNMR (400 MHz; CDCl<sub>3</sub>) δ 0.81(t, 3H, *J* = 4.40 Hz), 0.85(d, 3H, *J* = 6.55 Hz), 1.06-1.20(m, 7H), 1.36(s, 9H), 1.38-1.50(m, 2H), 2.36(m, 2H), 3.99(m, 1H), 5.02(m+s, 3H), and 7.28-7.28(m, 5H).

**(3S, 5R)-3-Amino-5-methyl-nonanoic acid, tert-butyl ester-** This compound was prepared as described above starting with (3S, 5R)-benzyloxycarbonylamino-5-methyl-nonanoic acid, *tert*-butyl ester instead of (3S, 5R)-3-benzyloxycarbonylamino-5-methyl-octanoic acid, *tert*-butyl ester. Yield = 97%. <sup>1</sup>HNMR (400 MHz; CDCl<sub>3</sub>) δ 0.82(overlapping d and t, 6H), 1.02-1.08(m, 1H), 1.09-1.36(m, 6H), 1.39(s, 9H), 1.47(br s, 1H), 1.80(s, 2H), 2.13(dd, 1H, *J* = 8.54 and 15.61 Hz), and 2.27(dd, 1H, *J* = 4.15 and 15.38 Hz).

10 **(3S, 5R)-3-Amino-5-methyl-nonanoic acid hydrochloride-**A mixture of (3S, 5R)-3-amino-5-methyl-nonanoic acid, *tert*-butyl ester (1.50g, 6.16 mmol) in 3N HCl (100 mL) was heated at reflux for 3hours, filtered hot over Celite, and concentrated to 30mL in vacuo. The resulting crystals were collected, washed with additional 3N HCl, and dried to provide the title compound, mp 142.5-143.3°C. Additional crops were obtained from the  
15 filtrate to provide 1.03g (70.4%). Anal. Calc'd for C<sub>10</sub>H<sub>21</sub>NO<sub>2</sub>·HCl: C: 53.68, H: 9.91, N: 6.26, Cl: 15.85. Found: C: 53.89, H: 10.11, N: 6.13. MS: M+1: 188.1.

**Example 4. (2R, 4R)-2-Aminomethyl-4-methyl-heptanoic acid**

**5R-Methyl-3R-(4S-methyl-2-oxo-5R-phenyloxazolidine-3-carbonyl)octanoic acid.** A solution of (3R,5R)-5-Methyl-3-((4S,5R)-4-methyl-2-oxo-5-phenyl-oxazolidine-3-carbonyl)-octanoic acid *tert*-butyl ester(3.9 g, 9.34 mmol) in dichloromethane (150 mL) was treated with trifluoroacetic acid (7.21 mL, 93.4 mL) and stirred 18 hours at ambient temperature. After the solvents and reagent were removed in vacuo, the resulting residue was triturated in 100 mL hexanes to provide 3.38g of the title compound (100%) mp  
20 142-143°C.  
25

**[4R-Methyl-2R-(4S-methyl-2-oxo-5R-phenyloxazolidine-3-carbonyl)heptyl]carbamic acid benzyl ester.** A solution of 5R-methyl-3R-(4S-methyl-2-oxo-5R-phenyloxazolidine-3-carbonyl)octanoic acid (1.98 g; 5.48 mmol) and triethylamine (0.92mL, 6.57 mmol) was treated with diphenylphosphorylazide (1.2 mL, 5.48 mmol),  
30 stirred 30 min at ambient temperature and then heated at reflux for 3 hours. After cooling briefly, the reaction mixture was treated with benzyl alcohol (2.8 mL, 27.4 mmol) and

heated for an additional 3 h at reflux. The reaction mixture was cooled, diluted with ethyl ether (150 mL), washed successively with sat'd NaHCO<sub>3</sub> and brine, dried (MgSO<sub>4</sub>) and concentrated in vacuo to an oil. Chromatography (MPLC, elution with 4:1 hexanes:ethyl acetate) provided the title compound (2.0 g, 78.3%) as an oil. MS M+1 = 467.1.

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**2R-(Benzyloxycarbonylaminomethyl)-4R-methylheptanoic acid.** A solution of 4R-methyl-2R-(4S-methyl-2-oxo-5R-phenyloxazolidine-3-carbonyl)heptyl]carbamic acid benzyl ester (4.12 g, 8.83 mmol) in 3:1 THF:water (100 mL) was cooled to 0°C and treated with a mixture of 0.8 N LiOH (17.5 mL, 14 mmol) and 30% H<sub>2</sub>O<sub>2</sub> (4.94 mL, 44 mmol). After the reaction mixture was stirred in the cold 3 hours, it was quenched with a slurry of NaHSO<sub>3</sub> (2.37g) and Na<sub>2</sub>SO<sub>3</sub> (4.53g) in water (30 mL) and stirred 1 hour. The reaction mixture was diluted with ethyl ether (200 mL), partitioned, and the organic layer washed with brine and dried (MgSO<sub>4</sub>). The concentrated organic extract was chromatographed (MPLC) eluting with ethyl acetate to give 1.25g of 2R-  
10 (benzyloxycarbonylaminomethyl)-4R-methylheptanoic acid (46%). MS M+1 = 308.1.  
15

**(2R,4R)-2-Amino-4-methyl-heptanoic acid hydrochloride.** A mixture of 2R-(benzyloxycarbonylaminomethyl)-4R-methyl-heptanoic acid (1.25g, 4.07 mmol) and Pd/C (20%, 0.11 g) in methanol (50 mL) was hydrogenated at 50 psi for 18 hours. After the catalyst was removed by filtration, the solvent was removed in vacuo and the resulting solid triturated in ether to provide (2S, 4R)-2-amino-4-methyl-heptanoic acid hydrochloride (0.28g, 40%) mp 226.3-228.0°C. MS M+1 = 174.0. Anal. Calc'd for C<sub>9</sub>H<sub>19</sub>NO<sub>2</sub>·0.1 H<sub>2</sub>O C: 61.75 H: 11.06 N: 8.00. Found C: 61.85 H: 10.83 N: 8.01.  
20

25 **Example 5. 2-Aminomethyl-4,4-dimethyl-heptanoic acid hydrochloride.**

**2-Cyano-4,4-dimethyl-hepta-2,6-dienoic acid ethyl ester.** A solution of 2,2-dimethyl-pent-4-enal (5.0g, 44 mmol), cyano-acetic acid ethyl ester (5.12 mL, 48 mmol), piperidine (1.3 mL, 14 mmol) and acetic acid (4.52 mL, 80 mmol) in 170 mL of toluene was heated under reflux for 18 hours in a flask equipped with a Dean-Stark separator. Several mL of water was collected in the trap. The reaction was cooled and washed with 1N HCl, NaHCO<sub>3</sub> and brine, successively. The organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to an oil. This oil was chromatographed eluting with 20% of EtOAc in  
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hexane to give a combination of two lots total 8.3g (91%). <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) 1.28 (s, 6H), 1.32 (t, 3H, *J* = 7 Hz), 2.26 (d, 2H, *J* = 7.6 Hz), 4.27 (q, 2H, *J* = 7.2 Hz), 5.08 (d, 1H, *J* = 12 Hz), 5.10 (d, 1H, *J* = 4 Hz), 5.72 (m, 1H).

5           **2-Aminomethyl-4,4-dimethyl-heptanoic acid hydrochloride.** 2-Cyano-4,4-dimethyl-hepta-2,6-dienoic acid ethyl ester (5.88g, 28 mmol) was dissolved in the mixture of 91 mL of ethanol and 6 mL of HCl and treated with 0.4g of PtO<sub>2</sub>. The reaction was carried out under 100 psi of hydrogen pressure at room temperature for 15 hours. The catalyst was filtered and filtrate was concentrated to give 3.8g of the desired product 2-aminomethyl-4,4-dimethyl-heptanoic acid ethyl ester as an oil. MS (APCI): 216.2 (M+1)<sup>+</sup>. This oil was refluxed in 75 mL of 6N HCl for 18 hours. While the reaction was cooled, a precipitate formed. The solid was filtered, washed with additional HCl solution and triturated with ether to give the clean title compound. MS (APCI): 188.1 (M+1)<sup>+</sup>. 186.1 (M-1)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz; CD<sub>3</sub>OD): 0.91 (9H, m), 1.30 (5H, m), 1.81 (dd, 1H, *J* = 7.2 Hz, 14.4 Hz), 2.72 (1H, m), 3.04 (2H, m); Anal. Calc'd for C<sub>10</sub>H<sub>21</sub>NO<sub>2</sub>·HCl: C: 53.68, H: 9.91, N: 6.26, Cl: 15.85; Found: C: 53.83, H: 10.15, N: 6.22, Cl: 15.40. MP: 229.5-231.0°C.

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**Example 6. (S)-3-Amino-5,5-dimethyl-octanoic acid.**

20           **3-(4,4-Dimethyl-heptanoyl)-(R)-4-methyl-(S)-5-phenyl-oxazolidin-2-one:** A solution of 4,4-dimethyl-heptanoic acid (1.58g, 10mmol) and triethylamine (4.6 mL) in 50 mL THF was cooled to 0°C and treated with 2,2-dimethyl-propionyl chloride (1.36 mL). After one hour, 4R-methyl-5S-phenyl-oxazolidin-2-one (1.95g, 11mmol) and lithium chloride (0.47g, 11 mmol) was added and the mixture was stirred for 18 hours. 25 The precipitate was filtered and washed thoroughly with additional THF. The filtrate was concentrated *in vacuo* to give an oily solid. This solid was dissolved in 200 mL Et<sub>2</sub>O, washed successively with saturated NaHCO<sub>3</sub>, 0.5N HCl and saturated NaCl, dried (MgSO<sub>4</sub>) and concentrated *in vacuo* to give the title compound as an oil (3.0g, 95%). <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>): 0.73-0.84 (m, 12H), 1.10-1.22 (m, 4H), 1.46-1.54 (m, 2H), 2.75-2.87 (m, 2H), 4.70 (m, 1H, *J* = 7 Hz), 5.59 (d, 1H, *J* = 7 Hz), 7.22-7.37 (m, 5H).

30

**5,5-Dimethyl-(S)-3-((R)-4-methyl-2-oxo-(S)-5-phenyl-oxazolidine-3-carbonyl)-octanoic acid tert-butyl ester:** According to example 1, 5.07g (16 mmol) of 3-(4,4-

dimethyl-heptanoyl)-4-methyl-5-phenyl-oxazolidin-2-one, 18 mL (1N, 18 mmol) of NaHMDS solution and 4.72 mL (32 mmol) of bromo-acetic acid tert-butyl ester gave 3.40g (49.3%) of the title compound as a crystalline solid. m.p.: 83-85°C.

5        **(S)-2-(2,2-Dimethyl-pentyl)-succinic acid 4-tert-butyl ester:** According to example 1, 3.4g (7.9 mmol) of 5,5-dimethyl-3-(4-methyl-2-oxo-5-phenyl-oxazolidine-3-carbonyl)-octanoic acid tert-butyl ester, 16 mL (12.8 mmol) of 0.8N LiOH and 4.5 mL of 30% H<sub>2</sub>O<sub>2</sub> gave 2.42g (>100%) of the title compound as an oil. <sup>1</sup>HNMR (400 MHz; CDCl<sub>3</sub>): 0.77-0.82 (m, 9H), 1.14-1.29 (m, 5H), 1.42 (s, 9H), 1.77 (dd, 1H, *J* = 8 Hz, 16 Hz), 2.36 (dd, 1H, *J* = 6 Hz, 16 Hz), 2.59 (dd, 1H, *J* = 8 Hz, 16 Hz), 2.75-2.85 (m, 1H).

15        **(S)-3-Benzyloxycarbonylamino-5,5-dimethyl-octanoic acid tert-butyl ester:** According to example 1, 2.14g (7.9 mmol) of 2-(2,2-dimethyl-pentyl)-succinic acid 4-tert-butyl ester, 1.7 mL of DPPA, 1.1 mL of Et<sub>3</sub>N and 2.44 mL of BnOH provided 1.63g (54.8% in two steps) of the title compound as an oil. <sup>1</sup>HNMR (400 MHz; CDCl<sub>3</sub>): 0.78-0.89 (m, 9H), 1.10-1.30 (m, 5H), 1.36 (s, 9H), 2.39 (t, 2H, *J* = 5 Hz), 4.95-4.05 (m, 1H), 5.00 (s, 2H), 5.09 (d, 1H, *J* = 9.6 Hz), 7.22-7.30 (m, 5H).

20        **(S)-3-Amino-5,5-dimethyl-octanoic acid tert-butyl ester:** According to example 1, 1.63g of 3-benzyloxycarbonylamino-5,5-dimethyl-octanoic acid tert-butyl ester and 0.2g of 20% Pd/C furnished the title compound. MS, *m/z*, 244.2 (M+1)<sup>+</sup>.

25        **(S)-3-Amino-5,5-dimethyl-octanoic acid hydrochloride:** According to example 1, 3-amino-5,5-dimethyl-octanoic acid tert-butyl ester was treated with 3N HCl to provide 286mg of the title compound as a solid. MS (APCI), *m/z*: 188.1 (M+1)<sup>+</sup>. 186.1 (M-1)<sup>+</sup>. Anal. Calc'd for C<sub>10</sub>H<sub>21</sub>NO<sub>2</sub>·HCl·0.12H<sub>2</sub>O: C: 53.17, H: 9.92, N: 6.20, Cl: 15.69; Found: C: 53.19, H: 10.00, N: 6.08, Cl: 15.25. α = +20° (MeOH). MP: 194.2-195.2°C.

**Example 7. 2-Aminomethyl-3-(1-methyl-cyclopropyl)-propionic acid.**

30        **2-Cyano-3-(1-methyl-cyclopropyl)-acrylic acid ethyl ester.** To 1-methylcyclopropane-methanol (Aldrich, 1.13mL, 11.6mmol) in 50mL CH<sub>2</sub>Cl<sub>2</sub> was added neutral alumina (2.5g) and then PCC (2.5g, 11.6 mmol), and the mixture stirred 3h at

ambient temperature. The mixture was filtered through a 1cm plug of silica gel under vacuum, and rinsed with Et<sub>2</sub>O. The filtrate was concentrated to ca. 5mL total volume. To the residue was added THF (10mL), ethyl cyanoacetate (1.2mL, 11.3 mmol), piperidine (5 drops), and finally acetic acid (5 drops). The whole was stirred at ambient temperature overnight, then partitioned between Et<sub>2</sub>O and sat. aq. NaHCO<sub>3</sub>. The phases were separated and the organic phase washed with brine, dried (MgSO<sub>4</sub>), and concentrated. Flash chromatography of the residue (10→15% EtOAc/hexanes) provided 0.53g (25%) of the ester as a colorless oil that crystallized on standing. Anal. Calcd for C<sub>10</sub>H<sub>13</sub>NO<sub>2</sub>: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.86; H, 7.47; N, 7.70.

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**2-Aminomethyl-3-(1-methyl-cyclopropyl)-propionic acid ethyl ester.** To 2-cyano-3-(1-methyl-cyclopropyl)-acrylic acid ethyl ester (0.45g, 2.51mmol) in 16 mL EtOH:THF (1:1) was added RaNi (0.4g), and the mixture was hydrogenated in a Parr shaker at 48 psi for 15.5 h. Pearlman's catalyst (0.5g) was then added and hydrogenation was continued for an additional 15h. The mixture was filtered and concentrated. Flash chromatography of the residue 2→3→4→5→6→8% MeOH/CH<sub>2</sub>Cl<sub>2</sub> provided 0.25g (54%) of the aminoester as a colorless oil. LRMS: m/z 186.1 (M+1).

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**2-Aminomethyl-3-(1-methyl-cyclopropyl)-propionic acid.** To a solution of 2-aminomethyl-3-(1-methyl-cyclopropyl)-propionic acid ethyl ester (0.25g, 1.35mmol) in 10mL methanol at 0°C was added 10% aq. NaOH (10mL). The mixture was stirred at ambient temperature overnight, then concentrated to remove the methanol. The residue was cooled to 0°C and acidified to pH 2 with conc. HCl. After allowing to warm to ambient temperature the mixture was loaded onto DOWEX-50WX8-100 ion exchange resin and eluted with H<sub>2</sub>O until neutral to litmus. Elution was continued with 5% aq. NH<sub>4</sub>OH (100mL) and the alkaline fractions concentrated to provide 0.15g (71%) of the amino acid as a colorless solid. LRMS: m/z 158.0 (M+1).

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**Example 8. (3S,5R)-3-Amino-5-methyl-octanoic acid.**

**(5S)-5-Methyl-octa-2,6-dienoic acid tert-butyl ester.** To a solution of (S)-3-methyl-hex-4-enoic acid ethyl ester\* (1.0g, 6.4mmol) in 30mL toluene at -78 °C was added DIBAH (1.0M in THF, 6.4mL) dropwise over 5 min. The mixture was stirred at -

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78 °C 45 min at which time 5 drops of methanol were added, resulting in vigorous H<sub>2</sub> evolution. Methanol was added until no more gas evolution was observed (ca. 5mL). At this time the cold bath was removed and ca. 5mL of sat. aq. Na<sup>+</sup>K<sup>+</sup> tartrate was added. When the mixture reached room temperature, additional sat. aq. Na<sup>+</sup>K<sup>+</sup> tartrate and Et<sub>2</sub>O were added and stirring was continued until the phases were mostly clear (ca. 1h). The phases were separated, and the organic phase washed with brine, dried (MgSO<sub>4</sub>), and concentrated to ca. 10mL total volume owing to volatility concerns. The crude mixture was combined with an additional batch of aldehyde prepared from 10mmol of the ester by the method described above and the whole used without purification. To a suspension of sodium hydride (60% dispersion in mineral oil) in 25mL THF was added *t*-butyl-*P,P*-dimethylphosphonoacetate (3.0mL, 15mmol) dropwise over 1h such that the evolution of H<sub>2</sub> was under control. After the addition was complete, the crude aldehyde in toluene (ca. 20mL total volume) was added quickly dropwise and the mixture stirred at ambient temperature overnight. The mixture was partitioned between Et<sub>2</sub>O and sat. aq. NH<sub>4</sub>Cl, the phases separated, the organic phase washed with brine, dried (MgSO<sub>4</sub>), and concentrated. Flash chromatography of the residue (0→3→5% EtOAc/hexanes) afforded 1.0g (29%, two steps) of the unsaturated ester as a pale yellow oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.75 (m, 1H), 5.66 (m, 1H), 5.30 (m, 2H), 2.03-2.29 (m, 3H), 1.58 (d, *J* = 6.1 Hz, 3H), 1.41 (s, 9H), 0.91 (d, *J* = 6.6 Hz, 3H).

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\*(*S*)-3-methyl-hex-4-enoic acid ethyl ester was prepared from (*S*)-*trans*-3-Penten-2-ol [Liang, J.; Hoard, D. W.; Van Khau, V.; Martinelli, M. J.; Moher, E. D.; Moore, R. E.; Tius, M. A. *J. Org. Chem.*, **1999**, *64*, 1459] via Johnson-Claisen rearrangement with triethylorthoacetate according to the literature protocol [Hill, R. K.; Soman, R.; Sawada, S., *J. Org. Chem.*, **1972**, *37*, 3737].

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**(3*R*,5*S*)-3-[Benzyl-(1-phenyl-ethyl)-amino]-5-methyl-oct-6-enoic acid tert-butyl ester.** To a solution of (*S*)-(-)-*N*-benzyl- $\alpha$ -methylbenzylamine (0.60mL, 2.85mmol) in 9.0mL THF at -78 °C was added *n*-butyllithium (1.6M in hexanes, 1.6 mL) quickly dropwise resulting in a deep pink color. The mixture was stirred at -78 °C for 30 min at which time (*S*)-5-Methyl-octa-2,6-dienoic acid tert-butyl ester (0.5g, 2.38mmol) in 1.0mL THF was added slowly dropwise, resulting in a pale tan color which darkened

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over 3h. The mixture was stirred 3h at  $-78\text{ }^{\circ}\text{C}$ , then quenched with sat. aq.  $\text{NH}_4\text{Cl}$ . The mixture was allowed to warm to rt and stirred overnight, then partitioned between EtOAc and sat. aq.  $\text{NH}_4\text{Cl}$ . The phases were concentrated, and the organic phase dried ( $\text{MgSO}_4$ ), and concentrated. Flash chromatography of the residue (3 $\rightarrow$ 5% EtOAc/hexanes) provided 0.52g (52%) of the aminoester as a yellow oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.34 (m, 2H), 7.20 (m, 8H), 5.27 (m, 2H), 3.74 (m, 1H), 3.72 (d,  $J = 15.9$  Hz, 1H), 3.41 (d,  $J = 14.9$  Hz, 1H), 3.27 (m, 1H), 2.38 (m, 1H), 1.98 (dd,  $J = 3.7, 14.2$  Hz, 1H), 1.81 (dd,  $J = 9.3, 14.4$  Hz, 1H), 1.54 (d,  $J = 4.9$  Hz, 3H), 1.32 (s, 9H), 1.24 (d,  $J = 7.1$  Hz, 3H), 0.99 (m, 2H), 0.74 (d,  $J = 6.6$  Hz, 3H).

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**(3S,5R)-3-Amino-5-methyl-octanoic acid.** To a solution of (3R,5S)-3-[Benzyl-(1-phenyl-ethyl)-amino]-5-methyl-oct-6-enoic acid tert-butyl ester (0.92g, 2.18mmol) in 50mL MeOH was added 20% Pd/C (0.20g), and the mixture was hydrogenated in a Parr shaker at 48 psi for 23 h. The mixture was filtered and concentrated. To the crude aminoester in 10mL  $\text{CH}_2\text{Cl}_2$  was added 1.0mL trifluoroacetic acid, and the solution stirred at ambient temperature overnight. The mixture was concentrated, and the residue dissolved in the minimum amount of  $\text{H}_2\text{O}$ , and loaded onto DOWEX-50WX8-100 ion exchange resin. The column was eluted with  $\text{H}_2\text{O}$  until neutral to litmus, then continued with 5% aq.  $\text{NH}_4\text{OH}$  (100mL). The alkaline fractions were concentrated to provide 0.25g (66%, two steps) of the amino acid as an off-white solid.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  3.41 (m, 1H), 2.36 (dd,  $J = 5.1, 16.6$  Hz, 1H), 2.25 (dd,  $J = 8.1, 16.6$  Hz, 1H), 1.42 (m, 2H), 1.24 (m, 1H), 1.12 (m, 2H), 1.00 (m, 1H), 0.73 (d,  $J = 6.4$  Hz, 3H), 0.68 (t,  $J = 6.8$  Hz, 3H). LRMS:  $m/z$  172.1 (M-1).

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**Example 9. 2-Aminomethyl-8-methyl-nonanoic acid.**

A procedure similar to that of 2-Aminomethyl-4,4,8-trimethyl-nonanoic acid was utilized to prepare 2-Aminomethyl-8-methyl-nonanoic acid from 6-methyl-1-heptanol  $m/z$  202.1 (M+).

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**2-Aminomethyl-4,8-dimethyl-nonanoic acid**

**(R)-2,6-dimethyl heptan-1-ol:** Magnesium turnings (2.04 g, 84 mmol) and a crystal of iodine were suspended in 5 mL THF for the addition of 1-bromo-3-methyl

butane (0.3 mL, neat). The mixture was heated to start the Grignard formation. The remaining 1-bromo-3-methyl butane (8.63 mL, 72 mmol) was diluted in THF (60 mL) and added dropwise. The mixture was stirred at ambient temperature for 2 hours and cooled to  $-5\text{ }^{\circ}\text{C}$ . A solution of copper chloride (1.21 g, 9 mmol) and LiCl (0.76 g, 18 mmol) in THF (50 mL) was added dropwise keeping the temperature below  $0\text{ }^{\circ}\text{C}$ . The resulting mixture was stirred for 20 min, and (R)-3-bromo-2-methylpropanol in THF (20 mL) was added dropwise while keeping the temperature below  $0\text{ }^{\circ}\text{C}$ . The mixture was allowed to slowly reach ambient temperature overnight. The reaction mixture was quenched with ammonium hydroxide and water. The mixture was diluted with EtOAc and extracted with 3x20 mL EtOAc. The organics were washed with brine, dried ( $\text{MgSO}_4$ ), filtered and concentrated. The residual oil was purified via silica gel chromatography (90/10 Hexane/EtOAc) to give 2.67 g (R)-2,6-dimethyl heptan-1-ol.

**(R)-1-iodo-2,6-dimethyl heptane:** To a mixture of supported triphenyl phosphine (6.55 g, 19.67 mmol) in  $\text{CH}_2\text{Cl}_2$  at  $0\text{ }^{\circ}\text{C}$  was added iodine (4.99 g, 19.67 mmol) and imidazole (1.33 g, 19.67 mmol). The mixture was warmed to ambient temperature, stirred for 1 h and cooled to  $0\text{ }^{\circ}\text{C}$  for the dropwise addition of (R)-2,6-dimethyl heptan-1-ol in  $\text{CH}_2\text{Cl}_2$  (5 mL). The mixture was allowed to reach ambient temperature and stirred for 1 h, at which time it was filtered through a pad of celite and the solids were washed with  $\text{CH}_2\text{Cl}_2$ . The filtrate was concentrated, and the crude product was purified via silica gel chromatography to give (R)-1-iodo-2,6-dimethyl heptane (2.44 g).

**(4R)-4,8-dimethyl nonanoic acid t-butyl ester:** To diisopropyl amine (0.827 mL, 5.9 mmol) in THF (8 mL) at  $-78\text{ }^{\circ}\text{C}$  was added nBuLi (2.65 mL of a 2.6 M solution in pentane). The solution was stirred for 30 min at  $-78\text{ }^{\circ}\text{C}$ , followed by the addition of t-butyl acetate (0.8 mL, 5.9 mmol). The mixture was stirred at  $-78\text{ }^{\circ}\text{C}$  for 2 h, and then (R)-1-iodo-2,6-dimethyl heptane (0.3 g, 1.18 mmol) and HMPA (1.5 mL) in THF (1 mL) was added. The reaction was stirred at  $-78\text{ }^{\circ}\text{C}$  and allowed to slowly reach ambient temperature overnight, then heated at  $35\text{ }^{\circ}\text{C}$  to drive the reaction to completion. The reaction was quenched by the addition of ammonium chloride (saturated aqueous solution), and the mixture was extracted with EtOAc (2x10 mL). The organics were combined, washed with water, dried ( $\text{MgSO}_4$ ), filtered and concentrated. Silica gel

chromatography (98/2 hexane/EtOAc) provided 0.25 g of (4R)-4,8-dimethyl nonanoic acid t-butyl ester.

**(4R)-4,8-dimethyl nonanoic acid:** (4R)-4,8-dimethyl nonanoic acid t-butyl ester  
5 in 25 mL CH<sub>2</sub>Cl<sub>2</sub> at 0 °C was treated with TFA (6 mL). The mixture was allowed to reach ambient temperature and stir overnight. The solvent was removed by rotary evaporation, and the mixture was purified by silica gel chromatography (95/5 hexane/EtOAc) to give 0.962 g (4R)-4,8-dimethyl nonanoic acid. *m/z* 185 (M<sup>-</sup>).

10 **3-(4R,8-Dimethyl-nonanoyl)-4(S)-methyl-5(R)-phenyl-oxazolidin-2-one:** A procedure similar to (4R,5S)-4-Methyl-3-(R)-4-methyl-heptanoyl)-5-oxazolidin-2-one was utilized to give 3-(4R,8-Dimethyl-nonanoyl)-4(S)-methyl-5(R)-phenyl-oxazolidin-2-one (1.35 g) *m/z* 346.5 (M<sup>+</sup>).

15 **[4R,8-Dimethyl-2R-(4R-methyl-2-oxo-5R-phenyl-oxazolidine-3-carbonyl)-nonyl]-carbamic acid benzyl ester:** To a solution of 3-(4(R),8-Dimethyl-nonanoyl)-4(S)-methyl-5(R)-phenyl-oxazolidin-2-one (1.05 g, 3.04 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (12 mL) and TiCl<sub>4</sub> (3.04 mL of a 1 M solution in CH<sub>2</sub>Cl<sub>2</sub>) was added diisopropyl ethyl amine (0.55 mL, 3.19 mmol) at -20 °C. The resulting dark red solution was stirred at -20 °C for 30  
20 min prior to the addition of a solution of N-methoxymethyl benzyl carbamate (0.652 g, 3.34 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3.5 mL) and TiCl<sub>4</sub> (3.34 mL). The mixture was stirred at 0 °C for 4 h. The reaction was quenched by the addition of saturated aqueous ammonium chloride solution. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x15 mL). The organics were combined and washed with 1 N HCl and neutralized with NaOH, followed by washing  
25 with brine. The organics were dried (MgSO<sub>4</sub>), filtered, concentrated and purified by silica gel chromatography (95/5 hexane /EtOAc) to give 0.555 g [4R,8-Dimethyl-2R-(4R-methyl-2-oxo-5R-phenyl-oxazolidine-3-carbonyl)-nonyl]-carbamic acid benzyl ester.

**2(R)-(Benzyloxycarbonylamino-methyl)-4(R),8-dimethyl-nonanoic acid:** A  
30 procedure similar to that of (S)-2-((R)-2-Methyl=pentyl)succinic acid t-butyl ester was utilized to provide 0.198 g 2(R)-(Benzyloxycarbonylamino-methyl)-4(R),8-dimethyl-nonanoic acid.

**2-aminomethyl-4,8-dimethyl nonanoic acid:** 2(R)-(Benzyloxycarbonylamino-methyl)-4(R),8-dimethyl-nonanoic acid (0.148 g, 0.566 mmol) was treated with hydrogen in the presence of 20% pd/C to give 0.082 g of 2-aminomethyl-4,8-dimethyl nonanoic acid after filtration and purification via silica gel chromatography (85/15 CH<sub>2</sub>Cl<sub>2</sub>/MeOH).  
5 *m/z* 216.3 (M+).

**Example 10. 2-Aminomethyl-4,4,8-trimethyl-nonanoic acid.**

**2,2,6-Trimethyl-heptanoic acid methyl ester:** To diisopropyl amine (1.54 mL, 11.03 mmol) in THF (22 mL) at -78 °C was added nBuLi (6.89 mL of a 1.6 M solution in hexane). The solution was stirred for 30 min at -78 °C, followed by the addition of methyl isobutyrate (0.97 mL, 8.48 mmol). The mixture was stirred at -78 °C for 2 h, and then 1-iodo-4-methyl pentane (1.8 g, 8.48 mmol) and DMPU (0.55 mL, 4.24 mmol) in THF (6 mL) was added. The reaction was stirred at -78 °C and allowed to slowly reach  
15 ambient temperature over 16 h. The reaction was quenched by the addition of ammonium chloride (saturated aqueous solution), and the mixture was extracted with EtOAc (2x10 mL). The organics were combined, washed with water, dried (MgSO<sub>4</sub>), filtered and concentrated. Silica gel chromatography (99/1 hexane/EtOAc) provided 1.57 g of 2,2,6-Trimethyl-heptanoic acid methyl ester.

20

**2,2,6-Trimethyl-heptan-1-ol:** 2,2,6-Trimethyl-heptanoic acid methyl ester (1.97 g, 10.6 mmol) was taken up in toluene (65 mL) and cooled to -78 °C. DiBALH (12.7 mL of a 1 N solution in toluene) was added dropwise. After 45 min, 1.5 mL DiBALH was added. After 2 h, the reaction was quenched by the addition of 15 mL MeOH at -78 °C.  
25 The mixture was warmed to ambient temperature, and then cooled again to -78 °C for the addition of 10 mL 1 N HCl. The mixture was extracted with EtOAc (3x15 mL). The combined organics were washed with brine, dried (MgSO<sub>4</sub>), filtered and concentrated. The residual oil was purified via silica gel chromatography (95/5 Hexane/EtOAc) to give 2,2,6-Trimethyl-heptan-1-ol (0.88 g). *m/z* 159 (M+).

30

**2,2,6-Trimethyl-heptanal:** Pyridinium chlorochromate (PCC, 4.17 g, 19.4 mmol) was combined with neutral alumina (14.6 g) in CH<sub>2</sub>Cl<sub>2</sub> and stirred at ambient temperature

for 15 min. The alcohol was diluted in  $\text{CH}_2\text{Cl}_2$ , and the mixture was stirred at ambient temperature for 2h. The solution was filtered through a pad of silica, and the solids were washed with  $\text{CH}_2\text{Cl}_2$ . The filtrate was evaporated to give 1.05 g  $m/z$  157 ( $\text{M}^+$ ). 2,2,6-Trimethyl-heptanal which was carried on without further purification.

5

**2-Cyano-4,4,8-trimethyl-non-2-enoic acid benzyl ester:** To a mixture of 2,2,6-Trimethyl-heptanal (1.05 g, 6.73 mmol), piperidine (0.19 mL, 2.01 mmol) and benzyl cyanoacetate (1.29 g, 7.4 mmol) in toluene (50 mL) was added glacial acetic acid (0.72 g, 12.1 mmol). The flask was fitted with a Dean-Stark trap, and the mixture was heated at reflux for 18. The mixture was cooled, treated with dilute HCl, and the layers were separated. The organics were washed with a saturated sodium bicarbonate solution followed by brine, and dried ( $\text{MgSO}_4$ ), filtered and concentrated. The residual oil was purified by silica gel chromatography (98/2 hexane/EtOAc) to give 1.3 g of 2-Cyano-4,4,8-trimethyl-non-2-enoic acid benzyl ester  $m/z$  314 ( $\text{M}^+$ ).

15

**2-aminomethyl-4,4,8-trimethyl-nonanoic acid:** 2-Cyano-4,4,8-trimethyl-non-2-enoic acid benzyl ester (1.3 g, 4.14 mmol) in THF (50 mL) was treated with hydrogen in the presence of 20% Pd/C to give a mixture of the cyano acid and the cyano methyl ester. The mixture was purified by silica gel chromatography to give 278 mg of 80105x41-1-2. The acid was then treated with hydrogen in the presence of Raney Ni in MeOH/ $\text{NH}_4\text{OH}$  to give 0.16 g of 2-aminomethyl-4,4,8-trimethyl-nonanoic acid.  $m/z$  230.3 ( $\text{M}^+$ ).

20

**Example 11. 2-Aminomethyl-4-ethyl-octanoic acid.**

A procedure similar to that of 2-Aminomethyl-4,4,8-trimethyl-nonanoic acid was utilized to prepare 2-Aminomethyl-4-ethyl-octanoic acid from 2-ethylhexanal.  $m/z$  202.1 ( $\text{M}^+$ ).

25

**Example 12. 2-Aminomethyl-4-ethyl-8-methyl-nonanoic acid.**

A procedure similar to that of 2-Aminomethyl-4,4,8-trimethyl-nonanoic acid was utilized to prepare 2-Aminomethyl-8-methyl-nonanoic acid from 2,6-di-*t*-butyl-4-methylphenyl cyclopropylcarboxylate.  $m/z$  230.2 ( $\text{M}^+$ ).

30

**Example 13. 3-Amino-2-[1-(4-methyl-pentyl)-cyclopropylmethyl]-propionic acid.**

A procedure similar to that of 2-Aminomethyl-4,4,8-trimethyl-nonanoic acid was utilized to prepare 2-Aminomethyl-8-methyl-nonanoic acid from 2,6-di-*t*-butyl-4-methylphenyl cyclopropylcarboxylate. *m/z* 228.2 (M+).

**Example 14. 2-Aminomethyl-4-ethyl-hexanoic acid.**

A procedure similar to 2-aminomethyl-4,8-dimethyl-nonanoic acid was used to prepare 2-aminomethyl-4-ethyl-hexanoic acid from 4-ethyl hexanoic acid. *m/z* 174.1.

**Example 15. 3(S)-Amino-3,5-dimethyl-heptanoic acid.**

**2-Methyl-propane-2(S)-sulfinic acid (1,3-dimethyl-pentylidene)-amide:** A solution of (S)-(-)-2-methyl-2-propanesulfonamide (500 mg, 4.1 mmol), 4-methyl-2-hexanone (470 mg, 4.1 mmol), and Titanium(IV) ethoxide (1.7 mL, 8.3 mmol) was heated at reflux for 18 h. The reaction mixture was poured into 20 mL brine with rapid stirring. The resulting solution was filtered through celite, and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (2x20 mL). The combined organics were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The resultant oil was purified by silica gel chromatography (25% EtOAc in hexane) to give 575 mg of 2-Methyl-propane-2(S)-sulfinic acid (1,3-dimethyl-pentylidene)-amide as a yellow oil.

**3,5-Dimethyl-3-(2-methyl-propane-2(S)-sulfinylamino)-heptanoic acid methyl ester:** To a -78 °C solution of lithium bis(trimethylsilyl)amide (5.1 ml of a 1 M solution in THF) in THF (6 mL) was added methyl acetate ((0.41 mL, 5.1 mmol) dropwise. After stirring for 20 min, a solution of chlorotitanium triisopropoxide (2.5 ml, 10 mmol) in THF (3 mL) was added dropwise. After 1 hour, 2-Methyl-propane-2(S)-sulfinic acid (1,3-dimethyl-pentylidene)-amide (560 mg, 2.6 mmol) in THF (3 mL) was added dropwise at -78 °C. The reaction was stirred at -78 °C for 5 h, and then quenched by the addition of 10 mL ammonium chloride solution and warmed to room temperature. The mixture was diluted with 10 mL water, and filtered. The aqueous layer was extracted with ethyl acetate (2x20 mL). The combined organics were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The resultant oil was purified by silica gel chromatography (30% EtOAc in

hexane) to give 360 mg of 3,5-Dimethyl-3-(2-methyl-propane-2(S)-sulfinylamino)-heptanoic acid methyl ester.

**3(S)-Amino-3,5-dimethyl-heptanoic acid:** 3,5-Dimethyl-3-(2-methyl-propane-2(S)-sulfinylamino)-heptanoic acid methyl ester (360 mg, 1.2 mmol) was dissolved in 6 N HCl (2 mL) and dioxane (2 mL) and heated at 100 C for 6 h. The mixture was cooled to room temperature, diluted with water, and extracted with EtOAc (15 mL). The organics were purified by ion exchange chromatography to give 3(S)-Amino-3,5-dimethyl-heptanoic acid (270 mg) and then repurification by silica gel chromatography (70:25:5 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH) to give 203 mg of 3(S)-Amino-3,5-dimethyl-heptanoic acid as a white solid. *m/z* 174 (C<sub>9</sub>H<sub>19</sub>NO<sub>2</sub>+H).

**Example 16. 3(S)-Amino-3,5-dimethyl-nonanoic acid.**

A procedure similar to that of 3(S)-Amino-3,5-dimethyl-heptanoic acid was used to prepare 3(S)-Amino-3,5-dimethyl-nonanoic acid. *m/z* 202.1 (C<sub>11</sub>H<sub>23</sub>NO<sub>2</sub>+H).

Pharmaceutical Composition Examples

In the following Examples, the term 'active compound' or 'active ingredient' refers to a suitable combination or individual element of an alpha-2-delta ligand and an AChE inhibitor and/or a pharmaceutically acceptable salt or solvate, according to the present invention.

(i) Tablet compositions

The following compositions A and B can be prepared by wet granulation of ingredients (a) to (c) and (a) to (d) with a solution of povidone, followed by addition of the magnesium stearate and compression.

30

Composition A

	<u>mg/tablet</u>	<u>mg/tablet</u>
(a) Active ingredient	250	250



56

	(b) Lactose B.P.	210	26
	(c) Sodium Starch Glycollate	20	12
	(d) Povidone B.P.	15	9
	(e) Magnesium Stearate	<u>5</u>	<u>3</u>
5		500	300

Composition B

		<u>mg/tablet</u>	<u>mg/tablet</u>
	(a) Active ingredient	250	250
10	(b) Lactose 150	150	-
	(c) Avicel PH 101	60	26
	(d) Sodium Starch Glycollate	20	12
	(e) Povidone B.P.	15	9
	(f) Magnesium Stearate	<u>5</u>	<u>3</u>
15		500	300

Composition C

		<u>mg/tablet</u>
	Active ingredient	100
20	Lactose	200
	Starch	50
	Povidone	5
	Magnesium Stearate	<u>4</u>
25		359

The following compositions D and E can be prepared by direct compression of the admixed ingredients. The lactose used in formulation E is of the direct compression type.

Composition D

		<u>mg/tablet</u>
30	Active ingredient	250
	Magnesium Stearate	4

Pregelatinised Starch NF15	<u>146</u>
	400

Composition E

	<u>mg/tablet</u>
5 Active ingredient	250
Magnesium Stearate	5
Lactose	145
Avicel	<u>100</u>
	500

10

Composition F (Controlled release composition)

	<u>mg/tablet</u>
(a) Active ingredient	500
15 (b) Hydroxypropylmethylcellulose (Methocel K4M Premium)	112
(c) Lactose B.P.	53
(d) Povidone B.P.C.	28
(e) Magnesium Stearate	<u>7</u>
20	700

The composition can be prepared by wet granulation of ingredients (a) to (c) with a solution of povidone, followed by addition of the magnesium stearate and compression.

25 Composition G (Enteric-coated tablet)

Enteric-coated tablets of Composition C can be prepared by coating the tablets with 25mg/tablet of an enteric polymer such as cellulose acetate phthalate, polyvinylacetate phthalate, hydroxypropylmethyl-cellulose phthalate, or anionic polymers of methacrylic acid and methacrylic acid methyl ester (Eudragit L). Except for Eudragit L, these  
30 polymers should also include 10% (by weight of the quantity of polymer used) of a plasticizer to prevent membrane cracking during application or on storage. Suitable plasticizers include diethyl phthalate, tributyl citrate and triacetin.

Composition H (Enteric-coated controlled release tablet)

5 Enteric-coated tablets of Composition F can be prepared by coating the tablets with  
50mg/tablet of an enteric polymer such as cellulose acetate phthalate, polyvinylacetate  
phthalate, hydroxypropylmethyl- cellulose phthalate, or anionic polymers of methacrylic  
acid and methacrylic acid methyl ester (Eudragit L). Except for Eudragit L, these  
10 polymers should also include 10% (by weight of the quantity of polymer used) of a  
plasticizer to prevent membrane cracking during application or on storage. Suitable  
plasticizers include diethyl phthalate, tributyl citrate and triacetin.

(ii) Capsule compositions

15

Composition A

Capsules can be prepared by admixing the ingredients of Composition D above and filling  
two-part hard gelatin capsules with the resulting mixture. Composition B (infra) may be  
20 prepared in a similar manner.

Composition B

		<u>mg/capsule</u>
25	(a) Active ingredient	250
	(b) Lactose B.P.	143
	(c) Sodium Starch Glycollate	25
	(d) Magnesium Stearate	<u>2</u>
		420

30

Composition Cmg/capsule

59

(a)	Active ingredient	250
(b)	Macrogol 4000 BP	<u>350</u>
		600

- 5 Capsules can be prepared by melting the Macrogol 4000 BP, dispersing the active ingredient in the melt and filling two-part hard gelatin capsules therewith.

#### Composition D

	<u>mg/capsule</u>	
10		
	Active ingredient	250
	Lecithin	100
	Arachis Oil	<u>100</u>
		450

- 15 Capsules can be prepared by dispersing the active ingredient in the lecithin and arachis oil and filling soft, elastic gelatin capsules with the dispersion.

#### Composition E (Controlled release capsule)

	<u>mg/capsule</u>	
20		
(a)	Active ingredient	250
(b)	Microcrystalline Cellulose	125
(c)	Lactose BP	125
(d)	Ethyl Cellulose	<u>13</u>
25		513

- The controlled release capsule formulation can be prepared by extruding mixed ingredients (a) to (c) using an extruder, then spheronising and drying the extrudate. The dried pellets are coated with a release controlling membrane (d) and filled into two-part, hard gelatin capsules.

#### Composition F (Enteric capsule)

mg/capsule

	(a)	Active ingredient	250
	(b)	Microcrystalline Cellulose	125
	(c)	Lactose BP	125
	(d)	Cellulose Acetate Phthalate	50
5	(e)	Diethyl Phthalat	<u>5</u>
			555

The enteric capsule composition can be prepared by extruding mixed ingredients (a) to (c) using an extruder, then spheronising and drying the extrudate. The dried pellets are coated with an enteric membrane (d) containing a plasticizer (e) and filled into two-part, hard gelatin capsules.

#### Composition G (Enteric-coated controlled release capsule)

Enteric capsules of Composition E can be prepared by coating the controlled-release pellets with 50mg/capsule of an enteric polymer such as cellulose acetate phthalate, polyvinylacetate phthalate, hydroxypropylmethylcellulose phthalate, or anionic polymers of methacrylic acid and methacrylic acid methyl ester (Eudragit L). Except for Eudragit L, these polymers should also include 10% (by weight of the quantity of polymer used) or a plasticizer to prevent membrane cracking during application or on storage. Suitable plasticizers include diethyl phthalate, tributyl citrate and triacetin.

#### (iii) Intravenous injection composition

25	Active ingredient	0.200g
	Sterile, pyrogen-free phosphate buffer (pH 9.0) to	10 ml

The active ingredient is dissolved in most of the phosphate buffer at 35-40<sup>o</sup> C, then made up to volume and filtered through a sterile micropore filter into sterile 10 ml glass vials (Type 1) which are sealed with sterile closures and overseals.

(iv) Intramuscular injection composition

	Active ingredient	0.20 g
5	Benzyl Alcohol	0.10 g
	Glycofurol 75	1.45 g
	Water for Injection q.s. to	3.00 ml

The active ingredient is dissolved in the glycofurol. The benzyl alcohol is then added and  
 10 dissolved, and water added to 3 ml. The mixture is then filtered through a sterile  
 micropore filter and sealed in sterile 3 ml glass vials (Type 1).

(v) Syrup composition

15	Active ingredient	0.25g
	Sorbitol Solution	1.50g
	Glycerol	1.00g
	Sodium Benzoate	0.005g
	Flavour	0.0125ml
20	Purified Water q.s. to	5.0ml

The sodium benzoate is dissolved in a portion of the purified water and the sorbitol  
 solution added. The active ingredient is added and dissolved. The resulting solution is  
 mixed with the glycerol and then made up to the required volume with the purified water.  
 25

(vi) Suppository composition

		<u>mg/suppository</u>
	Active ingredient	250
	Hard Fat, BP (Witepsol H15 - Dynamit NoBel)	<u>1770</u>
30		2020

One-fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45<sup>o</sup> C maximum. The active ingredient is sifted through a 200lm sieve and added to the molten base with mixing, using a Silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at 45<sup>o</sup> C, the remaining Witepsol H15 is added to the suspension which is stirred to ensure a homogenous mix. The entire suspension is then passed through a 250lm stainless steel screen and, with continuous stirring, allowed to cool to 40<sup>o</sup> C. At a temperature of 38-40<sup>o</sup> C, 2.02g aliquots of the mixture are filled into suitable plastic moulds and the suppositories allowed to cool to room temperature.

10 (vii) Pessary composition

	<u>mg/pessary</u>
Active ingredient (63lm)	250
Anhydrous Dextrose	380
Potato Starch	363
15 Magnesium Stearate	<u>7</u>
	1000

The above ingredients are mixed directly and pessaries prepared by compression of the resulting mixture.

20

(viii) Transdermal composition

Active ingredient	200mg
Alcohol USP	0.1ml
Hydroxyethyl cellulose	

25

The active ingredient and alcohol USP are gelled with hydroxyethyl cellulose and packed in a transdermal device with a surface area of 10cm<sup>2</sup>.

CLAIMS

1. A combination comprising an alpha-2-delta ligand and an AChE inhibitor or a pharmaceutically acceptable salt or solvate of either thereof.
- 5
2. A combination according to claim 1, wherein the components are in a synergistic ratio.
3. A combination according to claim 1 or claim 2, wherein the alpha-2-delta ligand is selected from gabapentin, pregabalin, [(1R,5R,6S)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid, 3-(1-Aminomethyl-cyclohexylmethyl)-4H-[1,2,4]oxadiazol-5-one, C-[1-(1H-Tetrazol-5-ylmethyl)-cycloheptyl]-methylamine, (3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, (1 $\alpha$ ,3 $\alpha$ ,5 $\alpha$ )(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid, 10 (3S,5R)-3-Aminomethyl-5-methyl-octanoic acid, (3S,5R)-3-amino-5-methyl-heptanoic acid, (3S,5R)-3-amino-5-methyl-nonanoic acid and (3S,5R)-3-Amino-5-methyl-octanoic acid, or a pharmaceutically acceptable salt thereof.
4. A combination according to any one of claims 1-3 wherein the AChE inhibitor is selected from donepezil (Aricept®), tacrine (cognex®), 20 rivastigmine (Exelon®), physostigmine (Synapton®), galantamine (Reminyl), metrifonate (Promem), neostigmine (Prostigmin), icopezil, hupazine A, zanapezil (TAK 147), stacofylline, phenserine, (5R,9R)-5-(*r*-chloro-2-hydroxy-3-methoxybenzylidene-amino)-11-ethylidene-7-methyl- 1,2,5,6,9,10- 25 hexahydro-5,9-methanocycloocta[*b*]pyridin-2-one (ZT 1), the galantamine derivatives SPH 1371, SPH 1373 and SPH 1375, tolserine, 1- (3-fluorobenzyl)-4-[(2-fluoro-5,6-dimethoxy-1-indanone-2-yl)methyl]piperidine hydrochloride (ER 127528), thiatolserine, (-)-12-amino-3-chloro-9-ethyl- 6,7,10,11-tetrahydro-7,11- 30 methanocycloocta[*b*]quinoline hydrochloride (huperine X), *N*, *N*-dimethylcarbamic acid 4- [1(*S*)-(methylamino)-3-(4 nitrophenoxy)propyl]



phenyl ester hemifumarate (RS 1259), ipidacrine (Amiridin), velnacrine (Mentane®), eptastigmine (heptylphysostigmine), zifrosilone (2,2,2-trifluoro-1-[3-(trimethylsilyl)phenyl]ethanone), 2-[2-(1-benzylpiperidin-4-yl)ethyl]-2,3-dihydro-9-methoxy-1*H*-pyrrolo[3,4-*b*]quinolin-1-one hemifumerate (T 82), 1,3-dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-*b*]-quinazoline (CI 1002), N-heptylcarbamic acid 2,4a,9-trimethyl-2,3,4,4a,9,9a-hexahydro-1,2-oxazino[6,5-*b*]indol-6-yl ester-L-tartrate (CHF 2060), 3-(2-[1-(1,3-dioxolan-2-ylmethyl)piperidin-4-yl]ethyl)-3,4-dihydro-2*H*-1,3-benzoxazine-2,4-dione hydrochloride (E 2030), N-[10-(diethylamino)decyl]carbamic acid (3*aS*,8*aR*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester (MF 247), 5-amino-6-chloro-4-hydroxy-3,4-dihydro-1*H*-thiopyrano[3,4-*b*]quinoline (MF 8615), N-[8-(*cis*-2,6-dimethylmorpholin-4-yl)octyl]carbamic acid (3*aS*,8*aR*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester L-bitartrate hydrate (MF 268), (-)N-(3-piperidinopropyl)-N-demethylgalantamine (SPH 1286) and N-propargyl-3*R*-aminoindan-5-yl-ethyl methyl carbamate (TV 3326), or a pharmaceutically acceptable salt thereof.

5. A combination according to any one of claims 1-4 wherein the AChE inhibitor is selected from:
- Donepezil  
Tacrine;  
Rivastigmine;  
Physostigmine;  
Galantamine;  
Metrifonate;  
Neostigmine; and  
Icopezil; or a pharmaceutically acceptable salt thereof..
6. A combination according to any one of claims 1-5 wherein the AChE inhibitor is donepezil or a pharmaceutically acceptable salt thereof.

7. A pharmaceutical composition comprising a therapeutically effective amount of a combination according to any one of claims 1-6.
8. Use of a combination as claimed in any of claims 1-6 in the manufacture of a medicament for the curative, prophylactic or palliative treatment of pain.
9. Use according to claim 8 where the pain is neuropathic pain.
10. A combination comprising gabapentin, or a pharmaceutically acceptable salt thereof, and a AChE inhibitor selected from donepezil (Aricept®), tacrine (cognex®), rivastigmine (Exelon®), physostigmine (Synapton®), galantamine (Reminyl), metrifonate (Promem), neostigmine (Prostigmin), icopezil, hupazine A, zanapezil (TAK 147), stacofylline, phenserine, (5*R*,9*R*)-5-(*r*-chloro-2-hydroxy-3-methoxybenzylidene-amino)-11-ethlidene-7-methyl-1,2,5,6,9,10-hexahydro-5,6,7,8,9,10-hexahydroazepino[2,1-*b*]quinazoline (CI 1002), N-heptylcarbamic acid 2,4a,9-trimethyl-2,3,4,4a,9,9a-hexahydro-1,2-oxazino[6,5-*b*]indol-6-yl ester-L-tartrate (CHF 2060), 3-(2-[1-(1,3-dioxolan-2-ylmethyl)piperidin-4-yl]ethyl)-3,4-dihydro-2*H*-1,3-benzoxazine-2,4-dione hydrochloride (E 2030), N-[10-(diethylamino)decyl]carbamic acid (3*aS*,8*aR*)-1,3*a*,8-trimethyl-1,2,3,3*a*,8,8*a*-hexahydropyrrolo[2,3-*b*]indol-5-yl ester (MF 247), 5-amino-6-chloro-4-hydroxy-3,4-dihydro-1*H*-thiopyrano-[3,4-*b*]quinoline (MF 8615), N-[8-(*cis*-2,6-dimethylmorpholin-4-yl)octyl]carbamic acid (3*aS*,8*aR*)-1,3*a*,8-trimethyl-1,2,3,3*a*,8,8*a*-hexahydropyrrolo[2,3-*b*]indol-5-yl ester L-bitartrate hydrate (MF

268), (-)N-(3-piperidinopropyl)-N-demethylgalantamine (SPH 1286) and N-propargyl-3R-aminoindan-5-yl-ethyl methyl carbamate (TV 3326), or a pharmaceutically acceptable salt thereof.

- 5 11. A combination according to claim 10 comprising gabapentin and donepezil or pharmaceutically acceptable salts thereof.
12. A combination comprising pregabalin and a AChE inhibitor selected from donepezil (Aricept®), tacrine (cognex®), rivastigmine (Exelon®), physostigmine (Synapton®), galantamine (Reminyl), metrifonate (Promem), neostigmine (Prostigmin), icopezil, hupazine A, zanapezil (TAK 147), stacofylline, phenserine, (5*R*,9*R*)-5-(*r*-chloro-2-hydroxy-3-methoxybenzylidene-amino)-11-ethlidene-7-methyl-1,2,5,6,9,10-hexahydro-5,9-methanocycloocta[*b*]pyridin-2-one (ZT 1), the galantamine derivatives SPH 1371, SPH 1373 and SPH 1375, tolserine, 1-(3-fluorobenzyl)-4-[(2-fluoro-5,6-dimethoxy-1-indanone-2-yl)methyl]piperidine hydrochloride (ER 127528), thiatolserine, (-)-12-amino-3-chloro-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride (huperine X), *N,N*-dimethylcarbamic acid 4-[1(*S*)-(methylamino)-3-(4-nitrophenoxy)propyl]phenyl ester hemifumarate (RS 1259), ipidacrine (Amiridin), velnacrine (Mentane®), eptastigmine (heptylphysostigmine), zifrosilone (2,2,2-trifluoro-1-[3-(trimethylsilyl)phenyl]ethanone), 2-[2-(1-benzylpiperidin-4-yl)ethyl]-2,3-dihydro-9-methoxy-1*H*-pyrrolo[3,4-*b*]quinolin-1-one hemifumerate (T 82), 1,3-dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-*b*]quinazoline (CI 1002), *N*-heptylcarbamic acid 2,4a,9-trimethyl-2,3,4,4a,9,9a-hexahydro-1,2-oxazino[6,5-*b*]indol-6-yl ester-L-tartrate (CHF 2060), 3-(2-[1-(1,3-dioxolan-2-ylmethyl)piperidin-4-yl]ethyl)-3,4-dihydro-2*H*-1,3-benzoxazine-2,4-dione hydrochloride (E 2030), *N*-[10-(diethylamino)decyl]carbamic acid (3*aS*,8*aR*)-1,3*a*,8-trimethyl-1,2,3,3*a*,8,8*a*-hexahydropyrrolo[2,3-*b*]indol-5-yl ester (MF 247), 5-amino-6-chloro-4-hydroxy-3,4-dihydro-1*H*-thiopyrano-[3,4-*b*]quinoline (MF 8615), *N*-[8-(*cis*-2,6-dimethylmorpholin-4-yl)octyl]carbamic acid (3*aS*,8*aR*)-1,3*a*,8-trimethyl-1,2,3,3*a*,8,8*a*-hexahydropyrrolo[2,3-*b*]indol-5-yl ester L-bitartrate hydrate (MF 268), (-)N-(3-piperidinopropyl)-N-demethylgalantamine (SPH 1286) and N-
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propargyl- 3R-aminoindan-5-yl -ethyl methyl carbamate (TV 3326), or a pharmaceutically acceptable salt thereof.

13. A combination according to claim 12 comprising pregabalin and donepezil or pharmaceutically acceptable salts thereof.
14. A pharmaceutical composition for the curative, prophylactic or palliative treatment of pain, comprising an alpha-2-delta ligand selected from pregabalin or gabapentin and a AChE inhibitor selected from donepezil (Aricept®), tacrine (cognex®), rivastigmine (Exelon®), physostigmine (Synapton®), galantamine (Reminyl), metrifonate (Promem), neostigmine (Prostigmin), icopezil, hupazine A, zanapezil (TAK 147), stacofylline, phenserine, (5*R*,9*R*)-5-(*r*-chloro-2-hydroxy-3-methoxybenzylidene-amino)-11-ethlidene-7-methyl-1,2,5,6,9,10-hexahydro-5,9-methanocycloocta[*b*]pyridin-2-one (ZT 1), the galantamine derivatives SPH 1371, SPH 1373 and SPH 1375, tolserine, 1-(3-fluorobenzyl)-4-[(2-fluoro-5,6-dimethoxy-1-indanone-2-yl)methyl]piperidine hydrochloride (ER 127528), thiatolserine, (-)-12-amino-3-chloro-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride (huperine X), *N,N*-dimethylcarbamic acid 4- [1(*S*)-(methylamino)-3-(4-nitrophenoxy)propyl] phenyl ester hemifumarate (RS 1259), ipidacrine (Amiridin), velnacrine (Mentane®), eptastigmine (heptylphysostigmine), zifrosilone (2,2,2-trifluoro-1-[3-(trimethylsilyl)phenyl]ethanone), 2-[2-(1 benzylpiperidin-4-yl)ethyl]-2,3-dihydro-9-methoxy-1*H*-pyrrolo[3,4-*b*]quinolin-1-one hemifumerate (T 82), 1,3-dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-*b*]-quinazoline (CI 1002), *N*-heptylcarbamic acid 2,4*a*,9-trimethyl-2,3,4,4*a*,9,9*a*-hexahydro-1,2-oxazino[6,5-*b*]indol-6-yl ester-L-tartrate (CHF 2060), 3-(2-[1-(1,3-dioxolan-2-ylmethyl)piperidin-4-yl]ethyl)-3,4-dihydro-2*H*-1,3-benzoxazine-2,4-dione hydrochloride (E 2030), *N*-[10-(diethylamino)decyl]carbamic acid (3*aS*,8*aR*)-1,3*a*,8-trimethyl-1,2,3,3*a*,8,8*a*-hexahydropyrrolo[2,3-*b*]indol-5-yl ester (MF 247), 5-amino-6-chloro-4-hydroxy-3,4-dihydro-1*H*-thiopyrano-[3,4-*b*]quinoline (MF 8615), *N*-[8-(*cis*-2,6-dimethylmorpholin-4-yl)octyl]carbamic acid (3*aS*,8*aR*)-1,3*a*,8-trimethyl-1,2,3,3*a*,8,8*a*-hexahydropyrrolo[2,3-*b*]indol-5-yl ester L-bitartrate hydrate (MF 268), (-)-*N*-(3-piperidinopropyl)-*N*-demethylgalantamine (SPH 1286) and *N*-

propargyl- 3R-aminoindan-5-yl -ethyl methyl carbamate (TV 3326), or a pharmaceutically acceptable salt thereof.

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<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
IPC 7	A61K45/06 A61K31/27	A61K31/196 A61K31/407
A61K31/197	A61K31/55	A61K31/445 A61K31/662
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According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols)		
IPC 7 A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
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<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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° Special categories of cited documents :		
*A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed		*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family
Date of the actual completion of the international search		Date of mailing of the international search report
13 December 2004		21/12/2004
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  Kanbier, D

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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