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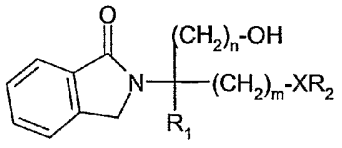
(54) Title: AMINOPROPANOL DERIVATIVES



(I)

(57) Abstract: Compounds of formula (I): wherein R_1, R_2, n and m are as defined in the specification, processes for their production, their uses and pharmaceutical compositions containing them.

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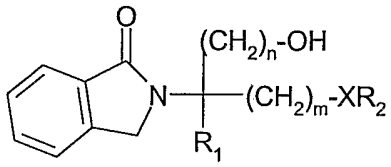
Aminopropanol derivatives

The present invention relates to organic compounds, a process for their production and pharmaceutical compositions containing them.

More particularly the present invention provides a compound of formula I:



I

wherein

each of m and n, independently, is 1, 2 or 3;

X is O or a direct bond;

R_1 is

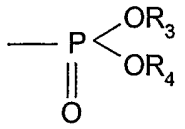
- a phenylalkyl wherein alkyl is a straight- or branched (C_{6-20})carbon chain; or
- a phenylalkyl wherein alkyl is a straight- or branched (C_{1-30})carbon chain wherein said phenylalkyl is substituted at the phenyl residue by
- a straight- or branched (C_{6-20})carbon chain optionally substituted by halogen,
- a straight- or branched (C_{6-20})alkoxy chain optionally substitued by halogen,
- a straight- or branched (C_{6-20})alkenyloxy,
- phenylalkoxy, halophenylalkoxy, phenylalkoxyalkyl, phenoxyalkoxy or phenoxyalkyl,
- cycloalkylalkyl substituted by C_{6-20}alkyl,
- heteroarylalkyl substituted by C_{6-20}alkyl,
- heterocyclic C_{6-20}alkyl or
- heterocyclic alkyl substituted by C_{2-20}alkyl,

and wherein

the alkyl moiety may have

- in the carbon chain, a bond or a heteroatom selected from a double bond, a triple bond, O, S, sulfinyl, sulfonyl, or NR_5, wherein R_5 is H, alkyl, aralkyl, acyl or alkoxycarbonyl, and
- as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxy or carboxy, and

R_2 is



wherein each of R_3 and R_4, independently, is H or C_{1-4}alkyl, wherein alkyl is optionally substituted by 1, 2 or 3 halogen atoms;

in free form or in salt form.

Halogen is F, Cl, Br or I. Alkyl or alkoxy may be straight or branched chain.

Cycloalkyl is preferably C_{3-10}cycloalkyl, more preferably C_{3-8}cycloalkyl and includes, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl.

Acyl may be a residue R_y-CO- wherein R_y is C_{1-6}alkyl, C_{3-6}cycloalkyl, phenyl or phenyl-C_{1-4}alkyl.

When in the compounds of formula I the carbon chain as R_1 is substituted, it is preferably substituted by halogen, nitro, amino, hydroxy or carboxy. When the carbon chain is interrupted by an optionally substituted phenylene, the carbon chain is preferably unsubstituted. When the phenylene moiety is substituted, it is preferably substituted by halogen, nitro, amino, methoxy, hydroxy or carboxy.

In the compounds of the invention, the following significances are preferred individually or in any sub-combination:

1. m and n are each 1 or 2, preferably 1.
2. X is O.
3. R_1 is C_{13-20}alkyl, optionally substituted by nitro, halogen, amino, hydroxy or carboxy, and, more preferably those wherein R_1 is phenylalkyl substituted by C_{6-14}-alkyl chain optionally substituted by halogen and the alkyl moiety is a C_{1-6}alkyl optionally substituted by hydroxy. More preferably, R_1 is phenyl-C_{1-6}alkyl, e.g. phenyl-C_{1-6}alkyl, e.g. phenyl-C_2alkyl , substituted on the phenyl by a straight or branched, preferably straight, C_{6-14}alkyl chain. The C_{6-14}alkyl chain may be in ortho, meta or para, preferably in para.
4. each of R_3 and R_4 is H.

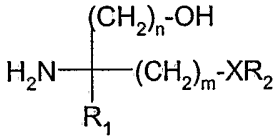
A particularly preferred compound is phosphoric acid mono-[2-hydroxymethyl-4-(4-octyl-phenyl)-2-(1-oxo-1,3-dihydro-isoindol-2-yl)-butyl] ester.

Compounds of formula I may exist in free form or in salt form, e.g. addition salts with e.g. inorganic acids, such as hydrochloride, hydrobromide or sulfate, salts with organic acids,

such as acetate, fumarate, maleate, benzoate, citrate, malate, methanesulfonate or benzenesulfonate salts; when R_3 or R_4 is H, R_2 may also be present in salt form, e.g. an ammonium salt or salts with metals such as sodium, potassium, calcium, zinc or magnesium, or a mixture thereof. Compounds of formula I and their salts in hydrate or solvate forms are also part of the invention.

The compounds of formula I have one or more asymmetric centers in the molecule, and thus various optical isomers may be obtained. The present invention also encompasses enantiomers, racemates, diastereoisomers and mixtures thereof. The central asymmetric carbon atom may have the R or S configuration. Moreover, when the compounds of formula I include geometric isomers, the present invention embraces cis-compounds, trans-compounds and mixtures thereof. Similar considerations apply in relation to starting materials exhibiting asymmetric carbon atoms or unsaturated bonds as mentioned above.

A compound of formula I may be prepared by reacting a compound of formula II:



II

wherein m, n, X, R_1 and R_2 are as defined in formula I;
with an aromatic 1,2-dicarbaldehyde, e.g. benzene-1,2-dicarbaldehyde, and recovering the resulting compound of formula I in free or salt form.

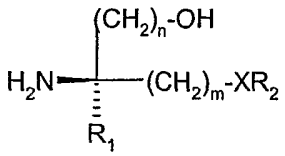
The process may be performed according to methods known in the art, e.g. as described in the examples.

A compound of formula II (e.g. a racemic mixture thereof) may be obtained as described in WO 02/18395 or WO 02/076995.

The present invention also provides a compound of formula I or formula II, wherein greater than 70% by weight of the compound is in the form of the S enantiomer, or greater than 70% by weight of the compound is in the form of the R enantiomer, e.g. greater than 90% is in the form of the R or S enantiomer. More preferably greater than 95% by weight, e.g. greater than 99% by weight of the compound is in the form of the R or S enantiomer. Thus the invention may relate to the substantially pure R or S enantiomer (e.g. the S enantiomer substantially free of the R enantiomer or vice versa), preferably the S enantiomer, of a compound of formula I or formula II. Particularly preferred are the substantially pure (e.g. greater than 99% by weight) R or S enantiomers, especially the S enantiomers, of phosphoric acid mono-[2-amino-2-hydroxymethyl-4-(4-octyl-phenyl)-butyl] ester (FTY720-

phosphate) and phosphoric acid mono-[2-hydroxymethyl-4-(4-octyl-phenyl)-2-(1-oxo-1,3-dihydro-isoindol-2-yl)-butyl] ester.

Compounds having the following 3-dimensional configuration are generally preferred:



II

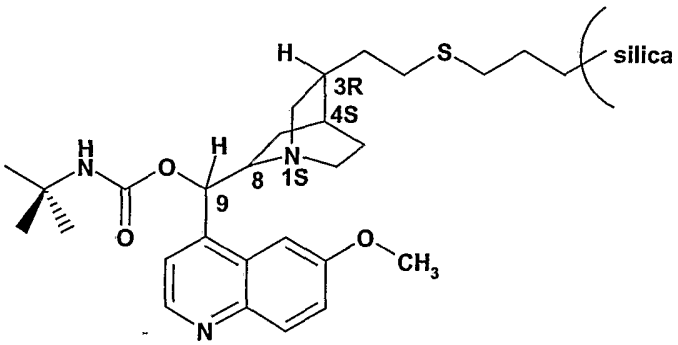
Enantiomers of the compounds of formula I and II cannot be satisfactorily separated by standard methods. According to the present invention, separation of the enantiomers is achieved by the use of novel separation techniques and synthesis strategies.

A compound of formula I, wherein greater than 70% by weight of the compound is in the form of the R or S enantiomer, e.g. the substantially pure R or S enantiomer, may be obtained by:

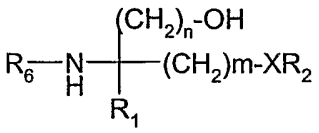
a) separation of the S enantiomer from the R enantiomer in a racemic mixture of a compound of formula I, using chromatography on a chiral stationary phase; or

b) reacting a compound of formula II, wherein greater than 70% by weight of the compound is in the form of the R or S enantiomer, e.g. the substantially pure R or S enantiomer of a compound of formula II, with an aromatic 1,2-dicarbaldehyde e.g. benzene-1,2-dicarbaldehyde.

According to method a), the chromatographic separation is preferably carried out using a chiral ion-exchange phase based on quinine carbamate or quinidine carbamate as chiral selector, e.g. a quinine carbamate phase (8S,9R) available commercially under the tradename ProntoSIL Chiral AX QN-1:



A compound of formula II, wherein greater than 70% by weight of the compound is in the form of the R or S enantiomer, may be obtained by deprotecting a compound of formula III, wherein greater than 70% by weight of the compound of formula III is in the form of the R or S enantiomer:

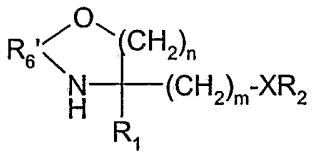


III

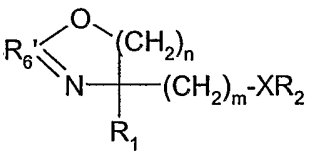
wherein m, n, X, R_1 and R_2 are as defined above and R_6 is an amino protecting group, and, where required, converting the compounds of formula I obtained in free form into the desired salt form, or vice versa.

Examples of suitable amino protecting groups as R_6 are e.g. as disclosed in "Protective Groups in Organic Synthesis" T.W. Greene, J.Wiley & Sons NY, 2nd ed., chapter 7, 1991, and references therein, e.g. acyl, e.g. tert.-butoxy-carbonyl, benzyloxycarbonyl, 9-fluorenyl methoxy carbonyl, trifluoroacetyl, trimethylsilylethanesulfonyl and the like.

Alternatively and more preferably a compound of formula II, wherein greater than 70% by weight of the compound is in the form of the R or S enantiomer, may be obtained by deprotecting a compound of formula IIIa or IIIb, wherein greater than 70% by weight of the compound of formula IIIa or IIIb is in the form of the R or S enantiomer:



IIIa



IIIb

wherein n, m, X, R_1 and R_2 are as defined above and R_6' is a simultaneous OH and amino protecting group, e.g. such that R_6' together with the O and N atoms to which it is attached, the asymmetric carbon atom and 1 to 3 further carbon atoms forms a cyclic residue, e.g. a 5 to 7-membered heterocyclic ring, e.g oxazolidin-2-one (in IIIa R_6' is –C(O)-) or 2-methyl-4,5-dihydro-oxazole (in IIIb R_6' is $-C(CH_3)-$).

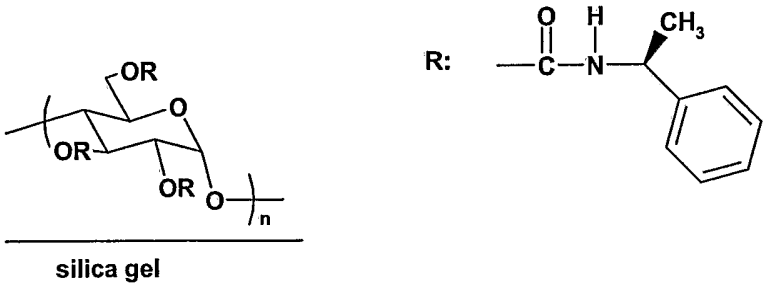
The removal of the R_6 or R_6' protecting group in a compound of formula III, IIIa or IIIb may conveniently be performed according to methods known in the art, e.g. by hydrolysis, e.g. in

a basic medium, for example using a hydroxide such as barium hydroxide. It may also be performed by hydrogenolysis, e.g. in the presence of Pearlman's catalyst, e.g. as disclosed in J.Org.Chem., 1998, 63, 2375-2377.

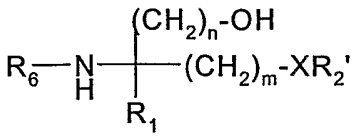
Thus in a further alternative aspect the present invention provides a compound of formula III, IIIa of IIIb as defined above, in free or salt form. The compounds of formula III, IIIa or IIIb have one or more asymmetric centers in the molecule, and thus various optical isomers may be obtained. The present invention also encompasses enantiomers, racemates, diastereoisomers and mixtures thereof.

The removal of the R_6 or R_6' protecting group in a compound of formula III, IIIa or IIIb may conveniently be performed according to methods known in the art, e.g. by hydrolysis, e.g. in a basic medium, for example using a hydroxide such as barium hydroxide. It may also be performed by hydrogenolysis, e.g. in the presence of Pearlman's catalyst, e.g. as disclosed in J.Org.Chem., 1998, 63, 2375-2377.

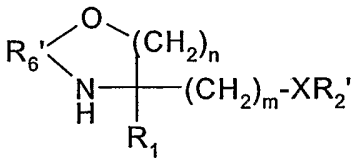
A compound of formula III, IIIa or IIIb comprising greater than 70% by weight of the R or S enantiomer may be obtained by separating the S enantiomer from the R enantiomer in a racemic mixture of a compound of formula III, IIIa or IIIb, using chromatography (MPLC, HPLC, SFC) or simulated moving bed (multi-column) chromatography with a polysaccharide-based chiral stationary phase, preferably an amylose-type phase, e.g. amylose tris[(S)-α-methylbenzyl carbamate coated on silica gel substrate, as available under the tradename CHIRALPAK AS and shown below, or in an immobilized form as prepared according to the processes described in WO 97/04011 and WO 97/49733:



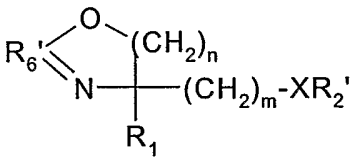
A compound of formula III, IIIa or IIIb comprising greater than 70% by weight of the R or S enantiomer may alternatively be obtained by removing the hydrolysable groups present in R_2' in a compound of formula IV, IVa or IVb comprising greater than 70% by weight of the R or S enantiomer:



IV

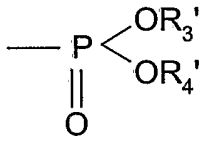


IVa



IVb

wherein m, n, X, R_1, R_6 and R_6' are as defined above and R_2' is

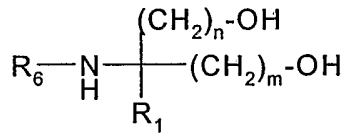


wherein each of R_3' and R_4' is a hydrolysable group.

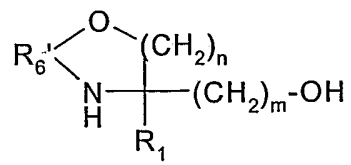
Preferably R_3' and R_4' are identical and have the significance of e.g. phenyl or benzyl or form together a cyclic system such as in 1,5-dihydro-2,4,3-benzodioxaphosphepin.

A compound of formula IV, IVa or IVb comprising greater than 70% by weight of the R or S enantiomer may be obtained by separating the S enantiomer from the R enantiomer in a racemic mixture of a compound of formula IV, IVa or IVb, e.g. as described above for the separation of enantiomers of compounds of formula III, IIIa or IIIb.

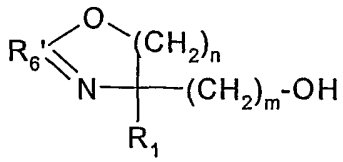
A compound of formula IV, IVa or IVb, e.g. a racemic mixture thereof, wherein X is O may be obtained by reacting a compound of formula V, Va or Vb:



V



Va

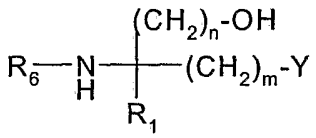


Vb

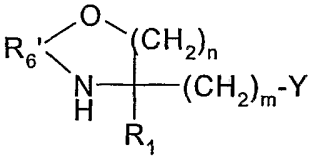
wherein m, n, R_1, R_6 and R_6' are as defined above,

with a phosphorylating agent, e.g. a phosphorochloridate, e.g.diphenylchlorophosphate or dibenzylchlorophosphate, cyanoethylphosphate, a phosphoramidate such as N-phenyl phosphoramidate, 3-(diethylamino)-1,5-dihydro-2,4,3-benzodioxaphosphepin and the like. The reaction may be carried out according to methods known in the art, e.g. as disclosed in J.Org.Chem. *supra*. In the compounds of formula IIIa the amino group is preferably in protected form, as R'_4 when R_4 is other than acyl.

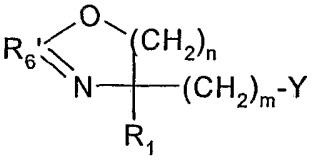
A compound of formula IV, IVa or IVb, e.g. a racemic mixture thereof, wherein X is a direct bond may be obtained by reacting a compound of formula V', Va' or Vb':



V'



Va'



Vb'

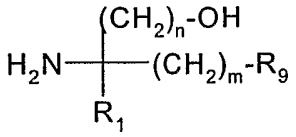
wherein m, n, R_1, R_6 and R_6' are as defined above, and
Y is a leaving group, e.g. Br,

with a phosphorylating agent, e.g. diethyl phosphite under reducing conditions, e.g. in the presence of NaH. The reaction may be performed in accordance with methods known in the art.

Alternatively the chiral separation may be performed at an earlier stage in the process. Thus a compound of formula IV, IVa or IVb comprising greater than 70% by weight of the R or S enantiomer may be obtained by reacting a compound of formula V, Va, Vb, V', Va' or Vb' comprising greater than 70% by weight of the R or S enantiomer with a phophorylating

agent. A compound of formula V, Va, Vb, V', Va' or Vb' comprising greater than 70% by weight of the R or S enantiomer may be obtained by separating the S enantiomer from the R enantiomer in a racemic mixture of a compound of formula V, Va, Vb, V', Va' or Vb', e.g. using HPLC or simulated moving bed (multi-column) chromatography with a polysaccharide-based chiral stationary phase as described above for separation of the enantiomers of a compound of formula IV, IVa or IVb.

A compound of formula V or V' may be prepared by reacting a compound of formula VI:



VI

wherein m, n and R_1 are as defined above and R_9 is OH or a leaving group, e.g. Br,

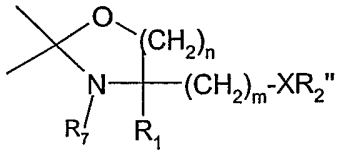
with an amino protecting group donor compound. A compound of formula Va, Va', Vb or Vb' may be prepared by reacting a compound of formula VI with an OH and amino protecting compound, e.g the OH and amino protection may be performed simultaneously by reacting the free aminoalcohol or aminodiol of formula VI in order to obtain a cyclic residue, e.g. a 5 to 7-membered heterocyclic ring, e.g oxazolidin-2-one or 2-methyl-4,5-dihydro-oxazole, e.g. by reaction with Cbo-Cl, Boc-anhydride, triethylortho acetate and acetonitrile, or phosgene under basic conditions.

At any stage in the process, R_1 in any of the formulae above may optionally be converted to an alternative R_1 group using known methods. For example, a compound of formula Vb wherein R_1 is (4-benzyloxy-phenyl)-ethyl may be converted to an alternative compound of formula Vb wherein R_1 is [4-(6-fluoro-hexyloxy)-phenyl]-ethyl by (a) removal of a benzyl group from R_1 by hydrogenation to leave a (4-hydroxy-phenyl)-ethyl residue, followed by (b) reaction with 1-bromo-6-fluorohexane. The alternative compound of formula Vb may then undergo chiral separation or be converted to a compound of formula IVb as described above.

The compounds of formulae III, IIIa, IIIb, IV, IVa and IVb comprising greater than 70% by weight of the R or S enantiomer used as starting materials, and salts thereof are also novel and form part of the present invention.

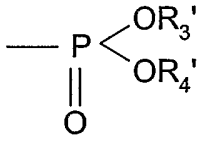
In a further alternative aspect, a compound of formula II wherein greater than 70% by weight of the compound is in the form of the R or S enantiomer, may be obtained by deprotecting

and hydrolyzing a compound of formula VII, wherein greater than 70% by weight of the compound is in the form of the R or S enantiomer:



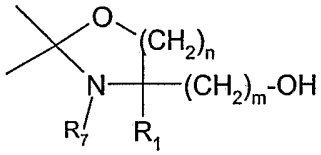
VII

wherein m, n, X and R_1 are as defined above, and R_2" is



wherein each of R_3' and R_4' is a hydrolysable group, e.g. tert-butyl, and R_7 is an amino protecting group, e.g. benzyloxycarbonyl.

A compound of formula VII, wherein greater than 70% by weight of the compound is in the form of the R or S enantiomer, may be obtained by reacting a compound of formula VIII, wherein greater than 70% by weight of the compound is in the form of the R or S enantiomer,



VIII

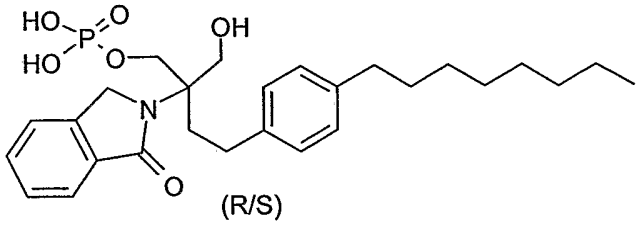
with a phosphorylating agent, e.g. as described above.

Insofar as the production of the starting materials is not particularly described, the compounds are known or may be prepared analogously to methods known in the art or as disclosed in the Examples hereinafter.

The following Examples are illustrative of the invention.

RT	=	room temperature
CBO	=	benzyloxycarbonyl
FTY720	=	2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol
EDTA	=	ethylenediaminetetraacetic acid
OPA	=	ortho-phthalaldehyde (benzene-1,2-dicarbaldehyde)

Example 1: Phosphoric acid mono-[(R/S)-2-hydroxymethyl-4-(4-octyl-phenyl)-2-(1-oxo-1,3-dihydro-isoindol-2-yl)-butyl] ester



(R/S)

a) (R/S)-4-Hydroxymethyl-4-[2-(4-octyl-phenyl)-ethyl]-oxazolidin-2-one:

Benzyl chloroformate (0.45 ml; 3.2 mmol) is added to a suspension of FTY720.HCl (1.03 g, 3 mmol) in 2N NaOH (20 ml). The reaction is kept at RT over night and in order to complete the reaction further benzyl chloroformate (0.9 ml; 6.4 mmol) is added. After 2 days at RT the reaction is acidified with 1N HCl, extracted with methylenchloride and purified on a silica gel column using methylenchloride/methanol/acetic $acid_{50\%}$ (9/1/0.125) as mobile phase.

$[M+H]^+$: 334 (ESI-MS)

b) (R/S)-4-[2-(4-Octyl-phenyl)-ethyl]-4-(3-oxo-1,5-dihydro-3lambda*5*-benzo[e][1,3,2]dioxaphosphepin-3-yloxymethyl)-oxazolidin-2-one:

To a solution of the endproduct of a) (2.4 g; 7.2 mmol) in methylenchloride/THF 1/1 (100 ml) at 0 °C is added tetrazole (recrystallized; 2.52 g; 36 mmol) and 3-(diethylamino)-1,5-dihydro-2,4,3-benzodioxaphosphepintriphenyl-phosphite (5.17 g; 21.6 mmol). After 18 hours at RT, H_2O_2 (8.2 ml [30% in water]; 72 mmol) is added (cooling) to the solution and kept at RT for additional 90 minutes. After quenching with saturated $Na_2S_2O_3$ solution (100 ml) the reaction is extracted with ethylacetate (three times). The organic layer is dried over Na_2SO_4 and the compound is purified on silica gel using cyclohexane/ethylacetate 1/1 as mobile phase.

c) Phosphoric acid mono-{(R/S)-4-[2-(4-octyl-phenyl)-ethyl]-2-oxo-oxazolidin-4-ylmethyl} ester

The endproduct of step b) (1.03 g; 2 mmol) is hydrogenated at normal pressure ($Pd/C_{10\%}$; 50 mg) over a period of 90 minutes. After filtration the reaction is concentrated and used in step d) without further purification.

d) Phosphoric acid mono-[(R/S)-2-amino-2-hydroxymethyl-4-(4-octyl-phenyl)-butyl] ester ((R/S)-FTY720-phosphate)

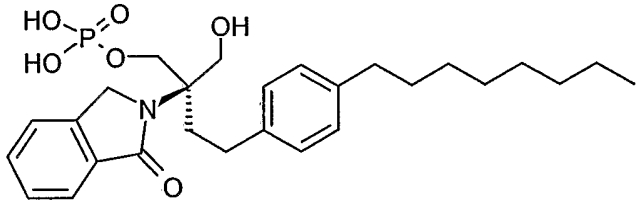
To a solution of the endproduct of step c) in ethanol (20 ml) LiOH (20 ml; 10% solution in water) is added. After 24 hours at reflux the reaction is neutralized with HCl (1N in water) and concentrated. The residue is treated with glacial acetic acid (5 ml) and precipitation of the endproduct occurs after addition water (50 ml). After filtering, washing (water) and drying pure endproduct is obtained without any further purification.

e) Phosphoric acid mono-[(R/S)-2-hydroxymethyl-4-(4-octyl-phenyl)-2-(1-oxo-1,3-dihydro-isoindol-2-yl)-butyl] ester (OPA-derivatization)

The endproduct of step d) ((R/S)-FTY720-phosphate) (50 mg; 0.125 mmol) is suspended in a solution of EDTA (0.5 ml; 10 mM in water) and aqueous boric acid (0.5 ml; 3% in water; adjusted to pH 10.5 with aqueous $KOH_{10\%}$). After addition of OPA (33 mg, 0.25 mmol), dissolved in ethanol (0.5 ml), the reaction is kept at RT for 1 hour (ultrasound). After that the pH is adjusted to 3.5 (aqueous HCl; 1N) and extracted with ethylacetate (three times). The organic layer is dried over Na_2SO_4 and the compound is purified on silica gel using methylenechloride/methanol (95/5 → 0/100) as mobile phase.

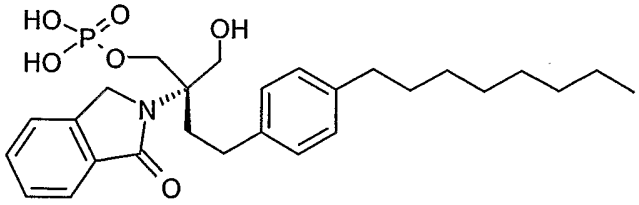
$[M-H]^-$: 502.5 (ESI-MS)

Example 2: Phosphoric acid mono-[(R)-2-hydroxymethyl-4-(4-octyl-phenyl)-2-(1-oxo-1,3-dihydro-isoindol-2-yl)-butyl] ester



Example 2 is obtained after separation of the final product of step b) by HPLC on CHIRALPAK AS column at a preparative scale (ethanol/n-hexane 40/60 as mobile phase), or by simulated moving bed chromatography on HPLC columns packed with immobilized amylose tris[(S)-α-methylbenzyl carbamate coated on silica gel (n-hexane/ethanol /chloroform 60/20/20 as the mobile phase; feed concentration, 1 %) and applying steps c), d) and e) as described for example 1.

Example 3: Phosphoric acid mono-[(S)-2-hydroxymethyl-4-(4-octyl-phenyl)-2-(1-oxo-1,3-dihydro-isoindol-2-yl)-butyl] ester



Example 3 is obtained after separation of the final product of step b) by HPLC on CHIRALPAK AS column at a preparative scale (ethanol/n-hexane 40/60 as mobile phase), or by simulated moving bed chromatography on HPLC columns packed with immobilized amylose tris[(S)-α-methylbenzyl carbamate coated on silica gel (n-hexane/ethanol /chloroform 60/20/20 as the mobile phase; feed concentration, 1 %) and applying steps c), d) and e) as described for example 1.

Example 4: ((R/S)-4-{2-[4-(6-Fluoro-hexyloxy)-phenyl]-ethyl}-2-methyl-4,5-dihydro-oxazol-4-yl)-methanol

a) To a solution of 4-(2-hydroxy-ethyl)-phenol (50g, 0.36mol) in ethanol (400ml) is added potassium carbonate (75g, 0.54mol, 1.5eq) and benzyl bromide (47.2ml, 0.39mol, 1.1eq), the reaction mixture is stirred at RT overnight. The reaction mixture is then filtered off through celite and concentrated under vacuum. 2-(4-Benzyloxy-phenyl)-ethanol is isolated after crystallization with diethyl ether.

b) To a solution of 2-(4-benzyloxy-phenyl)-ethanol (78.72g, 0.34mol) in methylene chloride (400ml) is added triethylamine (67.3ml, 0.44mol, 1.4eq), then at 0°C is added mesylchloride (34.8ml, 0.44mol, 1.3eq). The reaction mixture is stirred at 0°C for 30 minutes and allowed to rise to RT. The reaction mixture is extracted with methylene chloride (2 x 300ml), the combined organic layers are then washed with brine (2 x 300ml) and concentrated under vacuum.

c) To the crude product in solution in ethyl acetate (600ml) is added sodium iodide (67.2g, 0.44mol, 1.3eq) and the reaction mixture is stirred under reflux for 6 hours. After filtration, the organic layer is washed with brine (3 x 400ml), dried with Na_2SO_4, filtered and concentrated under vacuum. 1-Benzyloxy-4-(2-iodo-ethyl)-benzene is isolated after crystallization with diethyl ether.

d) To a solution of acetamidomalonate (59.4g, 0.27mol, 2eq) in dry dimethylformamide (400ml) is added at 0°C under inert atmosphere sodium hydride (60% in oil) (9.94g, 0.49mol, 1.8eq), the reaction mixture is stirred for 3 hours at 0°C. 1-Benzyloxy-4-(2-iodo-ethyl)-benzene (46.8g, 0.13mol, 1eq) in solution in dry dimethylformamide (250ml) is then slowly added at 0°C and the reaction mixture is stirred at RT overnight. The reaction mixture is quenched with few drops of methanol and concentrated almost to dryness under vacuum, then extracted with ethyl acetate and washed subsequently with 1N HCl (2 x 500ml), saturated solution of $NaHCO_3$ (2 x 500ml) and brine (2 x 500ml), dried with Na_2SO_4, filtered and concentrated under vacuum. 2-Acetylamino-2-[2-(4-benzyloxy-phenyl)-ethyl]-malonic acid diethyl ester is isolated after multiple crystallization using diethyl ether.

e) To a solution of 2-acetylamino-2-[2-(4-benzyloxy-phenyl)-ethyl]-malonic acid diethyl ester (44.1g, 0.1mol) in ethanol water (2/1) (285ml / 285ml) is added $CaCl_2$ (28.5g, 0.26mol, 2.5eq) and $NaBH_4$ by portion (19.4g, 0.52mol, 5.0eq), the reaction mixture is stirred overnight at RT. At 0°C the reaction mixture is carefully quenched with drop wise methanol (10ml) and concentrated to almost dryness under vacuum. The crude mixture is extracted with ethyl acetate (4 x 500ml) and washed subsequently with 1N HCl (2 x 300ml), saturated solution of $NaHCO_3$ (2 x 300ml) and brine (2 x 300ml). The combined organic layers are then dried with Na_2SO_4, filtered and concentrated under vacuum. N-[3-(4-Benzyloxy-phenyl)-1,1-bis-hydroxymethyl-propyl]-acetamide is carried on without further purification.

f) To a solution of crude N-[3-(4-benzyloxy-phenyl)-1,1-bis-hydroxymethyl-propyl]-acetamide in a mixture of tetrahydrofuran, methanol, water (1/2/2) (450ml / 900ml / 900ml) is added at RT lithium hydroxide (32.7g, 1.36mol, 8.0eq). The reaction mixture is stirred at 55°C for 5 hours, then extracted with ethyl acetate (500ml) and washed with brine (2 x 300ml), the combined organic layers are then dried with Na_2SO_4, filtered and concentrated under vacuum. 2-Amino-2-[2-(4-benzyloxy-phenyl)-ethyl]-propane-1,3-diol is isolated after crystallization using ethyl acetate.

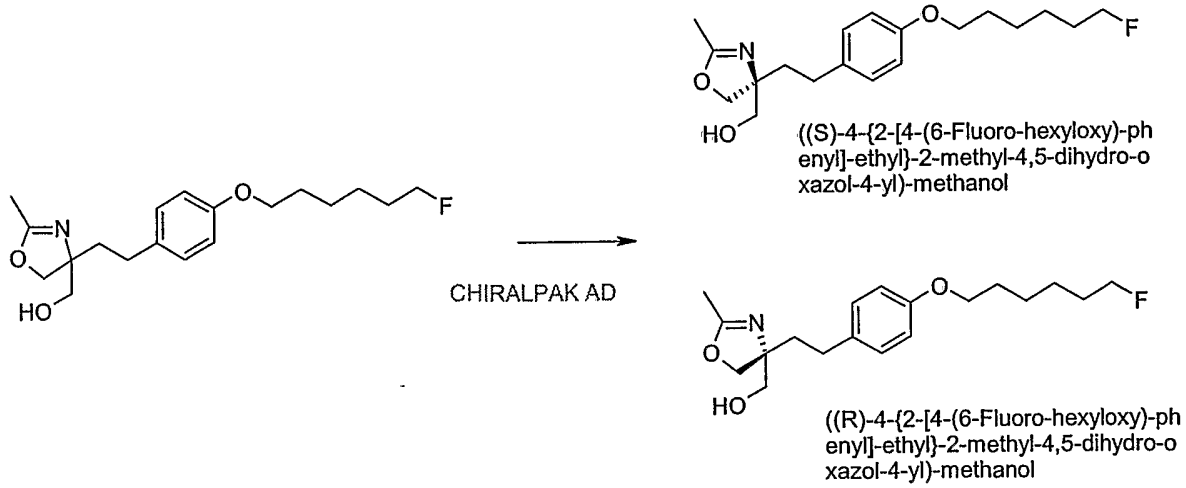
g) To a solution of 2-amino-2-[2-(4-benzyloxy-phenyl)-ethyl]-propane-1,3-diol (31.1g, 0.10mol) in acetonitrile (2.38l) is added triethylortho acetate (17.1ml, 0.12mol, 1.2eq) and acetic acid (5.48ml, 0.11mol, 1.1eq), the reaction mixture is then stirred at 80°C for 5 hours.

The reaction mixture is then concentrated under vacuum, {4-[2-(4-benzyloxy-phenyl)-ethyl]-2-methyl-4,5-dihydro-oxazol-4-yl}-methanol is isolated after crystallization with ethyl acetate.

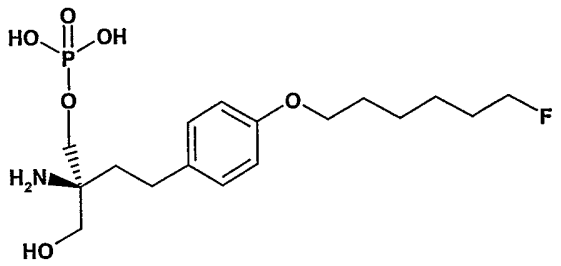
h) To a solution of {4-[2-(4-benzyloxy-phenyl)-ethyl]-2-methyl-4,5-dihydro-oxazol-4-yl}-methanol (26.1g, 0.08mol) in methanol (800ml) is added palladium on charcoal (2.6g, 10% wt), and the reaction mixture is stirred under hydrogen atmosphere at RT for 5 hours. The reaction mixture is then filtered through celite and concentrated under vacuum. (R/S)-4-[2-(4-Hydroxymethyl-2-methyl-4,5-dihydro-oxazol-4-yl)-ethyl]-phenol is isolated in quantitative yield after crystallization with ethyl acetate and hexanes.

i) To a solution of (R/S)-4-[2-(4-hydroxymethyl-2-methyl-4,5-dihydro-oxazol-4-yl)-ethyl]-phenol (500mg, 2.12mmol) in dry DMF (8ml) is added under inert atmosphere Cs_2CO_3 (901mg, 2.76mmol, 1.3eq.) and 1-bromo-6-fluoro-hexane (464.1mg, 2.55mmol, 1.2eq.). The reaction mixture is stirred under inert atmosphere at 85°C overnight. A saturated solution of $NaHCO_3$ (20ml) and ethyl acetate (40ml) are then added. The organic layer is separated and the aqueous phase is extracted with ethyl acetate (3 x 40ml). The combined organic extracts are washed with brine and 1M HCl, dried over $MgSO_4$, and evaporated to dryness. Purification by flash chromatography (cy Hexane / ethyl acetate (9/1) to (1/1) and (0/1)) affords (R/S)-(4-{2-[4-(6-fluoro-hexyloxy)-phenyl]-ethyl}-2-methyl-4,5-dihydro-oxazol-4-yl)-methanol as colorless oil.

Separation of enantiomers of ((R/S)-4-{2-[4-(6-Fluoro-hexyloxy)-phenyl]-ethyl}-2-methyl-4,5-dihydro-oxazol-4-yl)-methanol is performed by HPLC on CHIRALPAK AD column at a preparative scale (n-hexane/2-propanol 96/6 as the mobile phase).



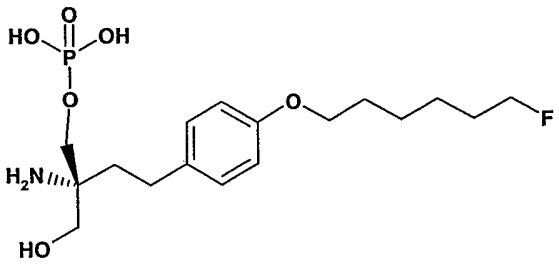
Example 5: Phosphoric acid mono-{(S)-2-amino-4-[4-(6-fluoro-hexyloxy)-phenyl]-2-hydroxymethyl-butyl} ester



a) To a solution of chiral ((S)-4-{2-[4-(6-fluoro-hexyloxy)-phenyl]-ethyl}-2-methyl-4,5-dihydro-oxazol-4-yl)-methanol (300mg, 0.80mmol) and tetrazole (337.4mg, 4.82mmol, 6eq., recrystallized from toluene) in dry THF (6ml) at -25°C is added 3-diethylamino-1,5-dihydro-benzo[e][1,3,2]dioxaphosphepine (433.5µL, 1.56mmol, 1.95eq.). The reaction mixture is stirred under argon at -25°C for 3h, then allowed to come back to RT. Then, H_2O_2 (30%, 75µL, 4.0mmol, 5eq.) is injected at 0°C with vigorous stirring. The reaction mixture is stirred for further 30 min, followed by addition of saturated sodium thiosulfate solution (1ml). The organic layer is separated and the aqueous phase is extracted with ether (3 x 20ml). The combined organic extracts are washed with brine, dried over $MgSO_4$, and evaporated to dryness. Purification by flash chromatography (ethyl acetate) affords phosphoric acid di-tert-butyl ester (S)-4-{2-[4-(6-fluoro-hexyloxy)-phenyl]-ethyl}-2-methyl-4,5-dihydro-oxazol-4-ylmethyl ester as colorless oil.

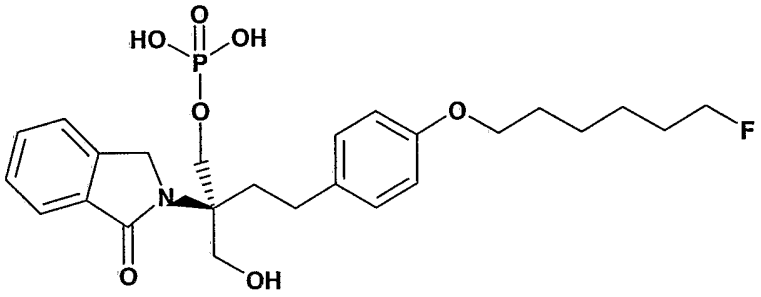
b) To a solution of phosphoric acid di-tert-butyl ester (S)-4-{2-[4-(6-fluoro-hexyloxy)-phenyl]-ethyl}-2-methyl-4,5-dihydro-oxazol-4-ylmethyl ester (33mg, 0.050mmol) in ethanol (2ml) is added conc. HCl (2ml). The reaction mixture is stirred at 85°C for 2 hours, then concentrated to dryness. The residue is re-dissolved in ethyl acetate and precipitated with hexanes. The solid is filtered off, washed with dry ether and dried under vacuum to afford phosphoric acid mono-{(S)-2-amino-4-[4-(6-fluoro-hexyloxy)-phenyl]-2-hydroxymethyl-butyl} ester as a colorless powder.

Example 6: Phosphoric acid mono-{(R)-2-amino-4-[4-(6-fluoro-hexyloxy)-phenyl]-2-hydroxymethyl-butyl} ester



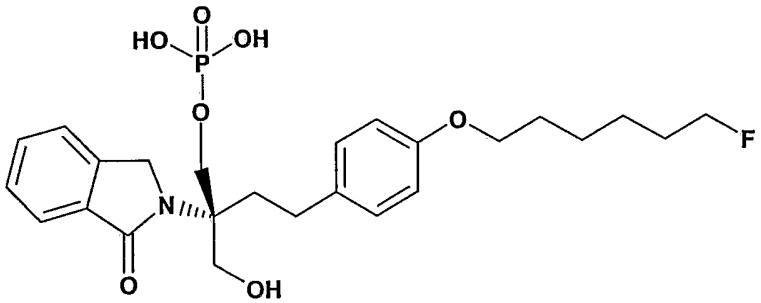
The synthesis is performed applying the chemistry described for example 5.

Example 7: Phosphoric acid mono-[(S)-4-[4-(6-fluoro-hexyloxy)-phenyl]-2-hydroxymethyl-2-(1-oxo-1,3-dihydro-isoindol-2-yl)-butyl] ester



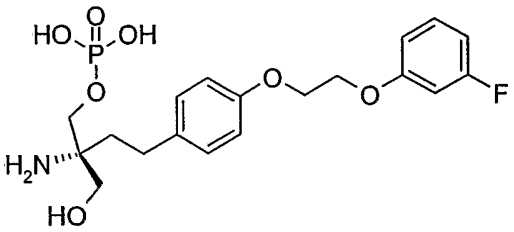
OPA-derivatization is performed according to the procedure given in example 1, step e.

Example 8: Phosphoric acid mono-[(R)-4-[4-(6-fluoro-hexyloxy)-phenyl]-2-hydroxymethyl-2-(1-oxo-1,3-dihydro-isoindol-2-yl)-butyl] ester



OPA-derivatization is performed according to the procedure given in example 1, step e.

Example 9: Phosphoric acid mono-((R)-2-amino-4-{4-[2-(3-fluoro-phenoxy)-ethoxy]-phenyl}-2-hydroxymethyl-butyl) ester.



To a solution of 4-(2-hydroxy-ethyl)-phenol (50g, 0.36mol) in ethanol (400ml) is added potassium carbonate (75g, 0.54mol, 1.5eq) and benzyl bromide (47.2ml, 0.39mol, 1.1eq), the reaction mixture is stirred at room temperature overnight. The reaction mixture is then filtered off trough celite and concentrated under vacuum. 2-(4-Benzyloxy-phenyl)-ethanol is isolated after crystallization with diethyl ether (82.6g, 95%).

To a solution of 2-(4-benzyloxy-phenyl)-ethanol (78.72g, 0.34mol) in methylene chloride (400ml) is added triethylamine (67.3ml, 0.44mol, 1.4eq), then at 0°C is added mesylchloride (34.8ml, 0.44mol, 1.3eq). The reaction mixture is stirred at 0°C for 30 minutes and allowed to rise to room temperature. The reaction mixture is extracted with methylene chloride (2 x 300ml), the combined organic layers are then washed with brine (2 x 300ml) and concentrated under vacuum. To the crude product in solution in ethyl acetate (600ml) is added sodium iodide (67.2g, 0.44mol, 1.3eq) and the reaction mixture is stirred under reflux for 6 hours. After filtration, the organic layer is washed with brine (3 x 400ml), dried with Na_2SO_4, filtered and concentrated under vacuum. 1-Benzyloxy-4-(2-iodo-ethyl)-benzene is isolated after crystallization with diethyl ether (116.5g, 86%).

To a solution of acetamidomalonate (59.4g, 0.27mol, 2eq) in dry dimethylformamide (400ml) is added at 0°C under inert atmosphere sodium hydride (60% in oil) (9.94g, 0.49mol, 1.8eq), the reaction mixture is stirred for 3 hours at 0°C. 1-Benzyloxy-4-(2-iodo-ethyl)-benzene (46.8g, 0.13mol, 1eq) in solution in dry dimethylformamide (250ml) is then slowly added at 0°C and the reaction mixture is stirred at room temperature overnight. The reaction mixture is quenched with few drops of methanol and concentrated almost to dryness under vacuum, then extracted with ethyl acetate and washed subsequently with 1N HCl (2 x 500ml), saturated solution of $NaHCO_3$ (2 x 500ml) and brine (2 x 500ml), dried with Na_2SO_4, filtered and concentrated under vacuum. 2-Acetylamino-2-[2-(4-benzyloxy-phenyl)-ethyl]-malonic acid diethyl ester is isolated after multiple crystallization using diethyl ether (47.3g, 80%).

To a solution of 2-acetylamino-2-[2-(4-benzyloxy-phenyl)-ethyl]-malonic acid diethyl ester (44.1g, 0.1mol) in ethanol water (2/1) (285ml / 285ml) is added $CaCl_2$ (28.5g, 0.26mol, 2.5eq) and $NaBH_4$ by portion (19.4g, 0.52mol, 5.0eq), the reaction mixture is stirred overnight at room temperature. At 0°C the reaction mixture is carefully quenched with drop wise methanol (10ml) and concentrated to almost dryness under vacuum. The crude mixture is extracted with ethyl acetate (4 x 500ml) and washed subsequently with 1N HCl (2 x 300ml), saturated solution of $NaHCO_3$ (2 x 300ml) and brine (2 x 300ml). The combined organic layers are then dried with Na_2SO_4, filtered and concentrated under vacuum. N-[3-(4-Benzyloxy-phenyl)-1,1-bis-hydroxymethyl-propyl]-acetamide is carried on without further purification.

To a solution of crude N-[3-(4-benzyloxy-phenyl)-1,1-bis-hydroxymethyl-propyl]-acetamide in a mixture of tetrahydrofuran, methanol, water (1/2/2) (450ml / 900ml / 900ml) is added at room temperature lithium hydroxide (32.7g, 1.36mol, 8.0eq). The reaction mixture is stirred at 55°C for 5 hours, then extracted with ethyl acetate (500ml) and washed with brine (2 x 300ml), the combined organic layers are then dried with Na_2SO_4, filtered and concentrated under vacuum. 2-Amino-2-[2-(4-benzyloxy-phenyl)-ethyl]-propane-1,3-diol is isolated after crystallization using ethyl acetate (28.8g, 97%).

To a solution of 2-amino-2-[2-(4-benzyloxy-phenyl)-ethyl]-propane-1,3-diol (200mg, 0.66mmol) in dioxane (10ml) is added a solution of 1M NaOH (0.73ml, 0.73mmol, 1.1eq) and Boc anhydride (217mg, 0.99mmol, 1.5eq). The reaction mixture is then stirred overnight at room temperature. Extraction with ethyl acetate (80ml) and washed with brine (2 x 50ml), the combined organic layers are then dried with Na_2SO_4, filtered and concentrated under vacuum. 2-Amino-2-[2-(4-benzyloxy-phenyl)-ethyl]-propane-1,3-diol is isolated as a white solid after flash chromatography (Ethyl acetate/Hexane (3/1)) and crystalization with diethyl ether (204mg, 87%).

To a solution of 2-amino-2-[2-(4-benzyloxy-phenyl)-ethyl]-propane-1,3-diol (6.31g, 0.16mol) in dichloromethane (120ml) and pyridine (1.26ml, 0.16mol) is added at room temperature o-nitrobenzoylchloride (2.27ml. 0.017mol). The reaction mixture is then stirred overnight at room temperature. Extraction with dichloromethane (300ml) and washed with brine (2 x 200ml), the combined organic layers are then dried with Na_2SO_4, filtered and concentrated

under vacuum. 2-Nitro-benzoic acid 4-(4-benzyloxy-phenyl)-2-tert-butoxycarbonylamino-2-hydroxymethyl-butyl ester is isolated as a white solid after flash chromatography (Ethyl acetate/Hexane (1/1)) and crystalization with diethyl ether (6.55mg, 76%). 1.40g of starting material could be isolated.

To a solution of 2-nitro-benzoic acid 4-(4-benzyloxy-phenyl)-2-tert-butoxycarbonylamino-2-hydroxymethyl-butyl ester (105mg, 0.19mmol) in toluene (2ml) is added 2,2 dimethoxypropane (0.06ml, 0.57mmol, 3eq) and a catalytic amount of *p*-toluene sulfonic acid. The reaction mixture is stirred at 95°C for 6 hours, then concentrated to dryness under negative pressure. 4-[2-(4-Benzyloxy-phenyl)-ethyl]-2,2-dimethyl-4-(2-nitro-benzoyloxy-methyl)-oxazolidine-3-carboxylic acid tert-butyl ester is isolated as an oil (88mg, 78%) after purification by flash chromatography (Ethyl acetate/Hexane (1/3)).

To a solution of 4-[2-(4-benzyloxy-phenyl)-ethyl]-2,2-dimethyl-4-(2-nitro-benzoyloxymethyl)-oxazolidine-3-carboxylic acid tert-butyl ester (80mg, 0.13mmol) in methanol (1ml) and THF (1ml) is added K_2CO_3 (1.5mg, 0.076eq), the reaction mixture is stirred overnight at room temperature. The reaction mixture is concentrated to dryness and 4-[2-(4-benzyloxy-phenyl)-ethyl]-4-hydroxymethyl-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester (51mg, 85%) is isolated as a white solid after flash chromatography (Ethyl acetate/Hexane (1/4)).

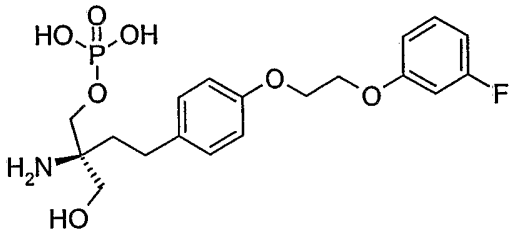
To a solution of 4-[2-(4-benzyloxy-phenyl)-ethyl]-4-hydroxymethyl-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester (25mg, 0.05mmol) in methanol (10ml) is added a catalytic amount of paladium on charcoal (10%wt). The reaction mixture is stirred under H_2 atmosphere at room temperature for 2 hours. 4-Hydroxymethyl-4-[2-(4-hydroxy-phenyl)-ethyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester is isolated as a white solid after filtration of the suspension through celite and concentration to dryness.

To a solution of (S)-4-hydroxymethyl-4-[2-(4-hydroxy-phenyl)-ethyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester (100mg, 0.28mmol) in DMF (5ml) is added $CsCO_3$ (120.5mg, 0.37mmol, 1.3eq) and 1-(2-bromo-ethoxy)-3-fluoro-benzene (80.7mg, 0.37 mmol, 1.3eq). The reaction mixture is stirred at 85°C for 4 hours. Ethyl acetate and water are then added, the organic layer is separated and the aqueous phase is extracted with ethylacetate (3 x 50 ml). The combined organic extracts are washed with brine, dried over $MgSO_4$, and evaporated to dryness. Purification by flash chromatography (AcOEt / Hx 9:1) affords (S)-4-(2-{4-[2-(3-fluoro-phenoxy)-ethoxy]-phenyl}-ethyl)-4-hydroxymethyl-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester as colorless oil.

To a solution of (S)-4-(2-{4-[2-(3-fluoro-phenoxy)-ethoxy]-phenyl}-ethyl)-4-hydroxymethyl-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester (70mg, 0.14mmol) and tetrazole (49mg, 0.7mmol, 5eq., recrystallized from toluene) in dry THF (5 ml) is added di-tBu-N,N-diisopropylphosphoramide (155mg, 0.56mmol, 4 eq.). After stirring under argon at RT for 3h, H_2O_2 (30%, 10 eq.) is slowly added at 0°C with vigorous stirring. The reaction mixture is stirred for further 30 min, followed by addition of saturated sodium thiosulfate solution (5 ml). The organic layer is separated and the aqueous phase is extracted with ether (3 x 20 ml). The combined organic extracts are washed with brine, dried over $MgSO_4$, and evaporated to dryness. Purification by flash chromatography (AcOEt / Hx 1:1) affords (R)-4-(di-tert-butoxy-phosphoryloxymethyl)-4-(2-{4-[2-(3-fluoro-phenoxy)-ethoxy]-phenyl}-ethyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester as colorless crystals.

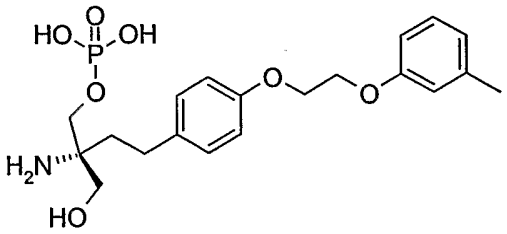
Finally, a solution of (R)-4-(di-tert-butoxy-phosphoryloxymethyl)-4-(2-{4-[2-(3-fluoro-phenoxy)-ethoxy]-phenyl}-ethyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester (70 mg, 0.10mmol) in conc. HCl (2 ml). is stirred at room temperature for one hour and is then heated to 95°C for 2 hours, then concentrated to dryness. The residue is re-dissolved in ethyl acetate and precipitated with hexanes. The solid is filtered off, washed with dry ether and dried in vacuo to afford phosphoric acid mono-((R)-2-amino-4-{4-[2-(3-fluoro-phenoxy)-ethoxy]-phenyl}-2-hydroxymethyl-butyl) ester as a colorless powder.

Example 10: Phosphoric acid mono-((S)-2-amino-4-{4-[2-(3-fluoro-phenoxy)-ethoxy]-phenyl}-2-hydroxymethyl-butyl) ester.



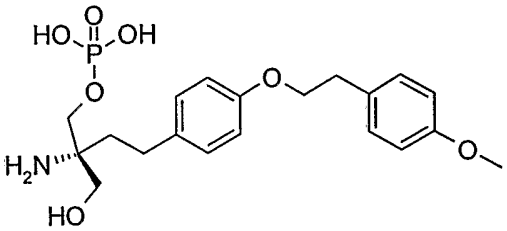
Phosphoric acid mono-((S)-2-amino-4-{4-[2-(3-fluoro-phenoxy)-ethoxy]-phenyl}-2-hydroxymethyl-butyl) ester is prepared as described in example 9 using (R)-4-hydroxymethyl-4-[2-(4-hydroxy-phenyl)-ethyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester instead of (S)-4-hydroxymethyl-4-[2-(4-hydroxy-phenyl)-ethyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester.

Example 11: Phosphoric acid mono-{(R)-2-amino-2-hydroxymethyl-4-[4-(2-m-tolyloxy-ethoxy)-phenyl]-butyl} ester



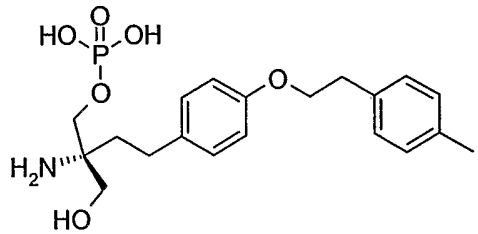
Phosphoric acid mono-{(R)-2-amino-2-hydroxymethyl-4-[4-(2-m-tolyloxy-ethoxy)-phenyl]-butyl} ester is prepared using an analogous method to that described in example 9.

Example 12: Phosphoric acid mono-((R)-2-amino-2-hydroxymethyl-4-{4-[2-(4-methoxy-phenyl)-ethoxy]-phenyl}-butyl) ester



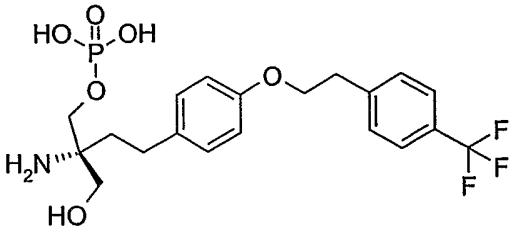
Phosphoric acid mono-((R)-2-amino-2-hydroxymethyl-4-{4-[2-(4-methoxy-phenyl)-ethoxy]-phenyl}-butyl) ester is prepared using an analogous method to that described in example 9.

Example 13: Phosphoric acid mono-{(R)-2-amino-2-hydroxymethyl-4-[4-(2-p-tolyl-ethoxy)-phenyl]-butyl} ester



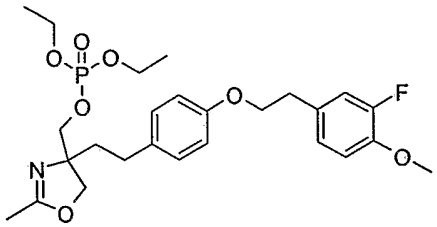
Phosphoric acid mono-{(R)-2-amino-2-hydroxymethyl-4-[4-(2-p-tolyl-ethoxy)-phenyl]-butyl} ester is prepared using an analogous method to that described in example 9.

Example 14: Phosphoric acid mono-((R)-2-amino-2-hydroxymethyl-4-{4-[2-(4-trifluoromethyl-phenyl)-ethoxy]-phenyl}-butyl) ester



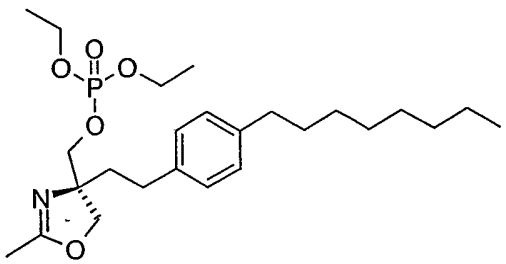
Phosphoric acid mono-((R)-2-amino-2-hydroxymethyl-4-{4-[2-(4-trifluoromethyl-phenyl)-ethoxy]-phenyl}-butyl) ester is prepared using an analogous method to that described in example 9.

Example 15: Phosphoric acid diethyl ester 4-(2-{4-[2-(3-fluoro-4-methoxy-phenyl)-ethoxy]-phenyl}-ethyl)-2-methyl-4,5-dihydro-oxazol-4-ylmethyl ester



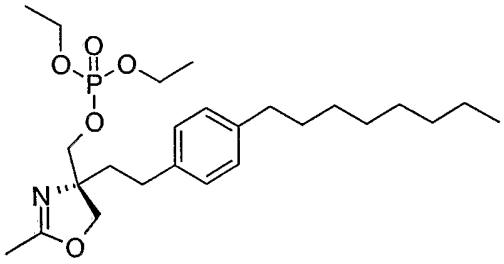
To a solution of phosphoric acid mono-(2-amino-4-{4-[2-(3-fluoro-4-methoxy-phenyl)-ethoxy]-phenyl}-2-hydroxymethyl-butyl) ester (100mg, 0.22mmol) in triethyl orthoacetate (5ml) is added acetic acid (13ul, 0.22mmol). The reaction mixture is stirred at 80°C for 2 hours and concentrated to dryness. Purification by flash chromatography (AcOEt / Hx 1:4) affords phosphoric acid diethyl ester 4-(2-{4-[2-(3-fluoro-4-methoxy-phenyl)-ethoxy]-phenyl}-ethyl)-2-methyl-4,5-dihydro-oxazol-4-ylmethyl ester as colorless oil.

Example 16: Phosphoric acid diethyl ester (S)-2-methyl-4-[2-(4-octyl-phenyl)-ethyl]-4,5-dihydro-oxazol-4-ylmethyl ester



Phosphoric acid diethyl ester (S)-2-methyl-4-[2-(4-octyl-phenyl)-ethyl]-4,5-dihydro-oxazol-4-ylmethyl ester is prepared as described in example 15 using of phosphoric acid mono-(2-amino-4-{4-[2-(3-fluoro-4-methoxy-phenyl)-ethoxy]-phenyl}-2-hydroxymethyl-butyl) ester.

Example 17: Phosphoric acid diethyl ester (R)-2-methyl-4-[2-(4-octyl-phenyl)-ethyl]-4,5-dihydro-oxazol-4-ylmethyl ester



Phosphoric acid diethyl ester (R)-2-methyl-4-[2-(4-octyl-phenyl)-ethyl]-4,5-dihydro-oxazol-4-ylmethyl ester is prepared as described in example 15 using phosphoric acid mono-(2-amino-4-{4-[2-(3-fluoro-4-methoxy-phenyl)-ethoxy]-phenyl}-2-hydroxymethyl-butyl) ester.

Example 18: Chromatographic resolution of racemic 4-hydroxymethyl-4-[2-(4-hydroxy-phenyl)-ethyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester.

10 micro-litre of a 0.1 % ethanol solution of racemic 4-hydroxymethyl-4-[2-(4-hydroxy-phenyl)-ethyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid tert-butyl ester are injected on a Chiralcel OD-H column (0.46 x 25 cm; commercially available from Chiral Technologies). The chromatographic separation is achieved at room temperature and at a flow rate of 1 ml/min using a mixture of n-hexane/ethanol 90/10 (volume) containing 0.1% trifluoroacetic acid (TFA) as the mobile phase. Detection is performed by UV at 210 nm. The enantiomers elute respectively after 7.23 min and 9.39 min (Separation factor α: 1.52)

Example 19: Chromatographic resolution of racemic Phosphoric acid diethyl ester 4-(2-{4-[2-(3-fluoro-4-methoxy-phenyl)-ethoxy]-phenyl}-ethyl)-2-methyl-4,5-dihydro-oxazol-4-ylmethyl ester.

10 micro-litre of a 0.1 % ethanol solution of racemic phosphoric acid diethyl ester 4-(2-{4-[2-(3-fluoro-4-methoxy-phenyl)-ethoxy]-phenyl}-ethyl)-2-methyl-4,5-dihydro-oxazol-4-ylmethyl ester are injected on a Chiralcel OD-H column (0.46 x 25 cm; commercially available from Chiral Technologies). The chromatographic separation is achieved at room temperature and at a flow rate of 1 ml/min using a mixture of n-hexane/ethanol 95/5 (volume) as the mobile

phase. Detection is performed by UV at 210 nm. The enantiomers elute respectively after 31.17 min and 35.44 min (Separation factor α: 1.15)

Example 20: Chromatographic separation of the enantiomers phosphoric acid diethyl ester 4-(2-{4-[2-(3-fluoro-4-methoxy-phenyl)-ethoxy]-phenyl}-ethyl)-2-methyl-4,5-dihydro-oxazol-4-ylmethyl ester

10 micro-litre of a 0.1 % ethanol solution of a mixture of the enantiomers phosphoric acid diethyl ester 4-(2-{4-[2-(3-fluoro-4-methoxy-phenyl)-ethoxy]-phenyl}-ethyl)-2-methyl-4,5-dihydro-oxazol-4-ylmethyl ester are injected on a Chiralcel OJ-H column (0.46 x 25 cm; commercially available from Chiral Technologies). The chromatographic separation is achieved at room temperature and at a flow rate of 1 ml/min using a mixture of n-hexane/2-propanol 75/25 (volume) as the mobile phase. Detection is performed by UV at 210 nm. The enantiomers elute respectively after 13.89 min and 16.77 min (Separation factor α: 1.27)

Example 21: Chromatographic separation of the enantiomers phosphoric acid diethyl ester (S)-2-methyl-4-[2-(4-octyl-phenyl)-ethyl]-4,5-dihydro-oxazol-4-ylmethyl ester.

10 micro-litre of a 0.1 % ethanol solution of a mixture of the enantiomers phosphoric acid diethyl ester (S)-2-methyl-4-[2-(4-octyl-phenyl)-ethyl]-4,5-dihydro-oxazol-4-ylmethyl ester are injected on a Chiralpak AD-H column (0.46 x 25 cm; commercially available from Chiral Technologies). The chromatographic separation is achieved at room temperature and at a flow rate of 1 ml/min using a mixture of n-hexane/ethanol 95/5 (volume) as the mobile phase. Detection is performed by UV at 210 nm. The enantiomers elute respectively after 10.35 min and 12.32 min (Separation factor α: 1.27).

The compounds of formula II comprising greater than 70% of the R or S enantiomer, especially the S enantiomer, in free form or in pharmaceutically acceptable salt form, (hereinafter referred to as the compounds of the invention) exhibit valuable pharmacological properties, e.g. lymphocyte recirculation modulating properties, e.g. as indicated in in vitro and in vivo tests and are therefore indicated for therapy.

A. In vitro

The compounds of the invention have binding affinity to individual human S1P receptors as determined in following assays:

Transient transfection of human S1P receptors into HEK293 cells

S1P receptors and G_i proteins are cloned, and equal amounts of 4 cDNAs for the S1P receptor, G_i-α, G_i-β and G_i-γ are mixed and used to transfect monolayers of HEK293 cells using the calcium phosphate precipitate method (M. Wigler et al., Cell. 1977,;11;223 and DS. Im et al., Mol. Pharmacol. 2000;57;753). Briefly, a DNA mixture containing 25 µg of DNA and 0.25 M CaCl is added to HEPES-buffered 2 mM Na_2HPO_4. Subconfluent monolayers of HEK293 cells are poisoned with 25 mM chloroquine, and the DNA precipitate is then applied to the cells. After 4 h, the monolayers are washed with phosphate-buffered saline and refed media (90% 1:1 Dulbecco's modified essential media (DMEM):F-12 + 10% fetal bovine serum). The cells are harvested 48-72 h after addition of the DNA by scraping in HME buffer (in mM: 20 HEPES, 5 $MgCl_2$, 1 EDTA, pH 7.4) containing 10% sucrose on ice, and disrupted using a Dounce homogenizer. After centrifugation at 800×g, the supernatant is diluted with HME without sucrose and centrifuged at 100,000×g for 1 h. The resulting pellet is rehomogenized and centrifuged a second hour at 100,000×g. This crude membrane pellet is resuspended in HME with sucrose, aliquoted, and snap-frozen by immersion in liquid nitrogen. The membranes are stored at 70 °C. Protein concentration is determined spectroscopically by Bradford protein assay.

GTPγS binding assay using S1P receptor/HEK293 membrane preparations

GTPγS binding experiments are performed as described by DS. Im et al., Mol. Pharmacol. 2000; 57:753. Ligand-mediated GTPγS binding to G-proteins is measured in GTP binding buffer (in mM: 50 HEPES, 100 NaCl, 10 $MgCl_2$, pH 7.5) using 25 µg of a membrane preparation from transiently transfected HEK293 cells. Ligand is added to membranes in the presence of 10 µM GDP and 0.1 nM [^{35}S]GTPγS (1200 Ci/mmol) and incubated at 30 °C for 30 min. Bound GTPγS is separated from unbound using the Brandel harvester (Gaithersburg, MD) and counted with a liquid scintillation counter.

In these assays, the compounds of the invention have binding affinities to S1P receptors in the sub-microM range.

In particular, the EC_{50} values in nM for the following compounds at various S1P receptors are shown in the table below:

	S1P-1	S1P-3	S1P-2	S1P-4	S1P-5
	EC_{50} nM	EC_{50} nM	EC_{50} nM	EC_{50} nM	EC_{50} nM
(R)-FTY720-P	191.15	28.91	>10000	80.13	>10000
(S)-FTY720-P	0.33	3.21	>10000	0.75	0.33
Ex. 14	0.4	140	>10000	6.6	7.7

wherein (R)-FTY720-P is (R)-FTY720-phosphate, (S)-FTY720-P is (S)-FTY720-phosphate, In contrast to (R)-FTY720-phosphate, which shows agonistic effects on S1P receptors only at very high concentrations, (S)-FTY720-phosphate is a full agonist on S1P1 and S1P3 and a partial agonist on S1P4 and S1P5 in the low nanomolar range.

B. In vivo: Blood Lymphocyte Depletion

A compound of the invention or the vehicle is administered orally by gavage to rats. Tail blood for hematological monitoring is obtained on day –1 to give the baseline individual values, and at 2, 6, 24, 48 and 72 hours after drug application. In this assay, the compounds of the invention deplete peripheral blood lymphocytes when administered at a dose of 0.03 to 3 mg/kg.

The compounds of the invention are, therefore, useful in the treatment and/or prevention of diseases or disorders mediated by lymphocytes interactions, e.g. in transplantation, such as acute or chronic rejection of cell, tissue or organ allo- or xenografts or delayed graft function, graft versus host disease, autoimmune diseases, e.g. rheumatoid arthritis, systemic lupus erythematosus, hashimoto's thyroidis, multiple sclerosis, myasthenia gravis, diabetes type I or II and the disorders associated therewith, vasculitis, pernicious anemia, Sjoegren syndrome, uveitis, psoriasis, Graves ophthalmopathy, alopecia areata and others, allergic diseases, e.g. allergic asthma, atopic dermatitis, allergic rhinitis/conjunctivitis, allergic contact dermatitis, inflammatory diseases optionally with underlying aberrant reactions, e.g. inflammatory bowel disease, Crohn's disease or ulcerative colitis, intrinsic asthma, inflammatory lung injury, inflammatory liver injury, inflammatory glomerular injury, atherosclerosis, osteoarthritis, irritant contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, cutaneous manifestations of immunologically-mediated disorders, inflammatory eye disease, keratoconjunctivitis, myocarditis or hepatitis, ischemia/reperfusion injury, e.g. myocardial infarction, stroke, gut ischemia, renal failure or hemorrhage shock, traumatic shock, others, cancer, e.g. T cell lymphomas or T cell

leukemias, infectious diseases, e.g. toxic shock (e.g. superantigen induced), septic shock, adult respiratory distress syndrome or viral infections, e.g. AIDS, viral hepatitis or chronic bacterial infection. Examples of cell, tissue or solid organ transplants include e.g. pancreatic islets, stem cells, bone marrow, corneal tissue, neuronal tissue, heart, lung, combined heart-lung, kidney, liver, bowel, pancreas, trachea or oesophagus.

For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired. In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.03 to 2.5 mg/kg per body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5 mg to about 100 mg, conveniently administered, for example, in divided doses up to four times a day or in retard form. Suitable unit dosage forms for oral administration comprise from ca. 1 to 50 mg active ingredient.

The compounds of the invention may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, or parenterally, e.g. in the form of injectable solutions or suspensions, topically, e.g. in the form of lotions, gels, ointments or creams, or in a nasal or a suppository form. Pharmaceutical compositions comprising a compound of the invention in free form or in pharmaceutically acceptable salt form in association with at least one pharmaceutical acceptable carrier or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent.

The compounds of the invention may be administered in free form or in pharmaceutically acceptable salt form e.g. as indicated above. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds.

In accordance with the foregoing the present invention further provides:

1.1 A method for preventing or treating disorders or diseases mediated by lymphocytes, e.g. such as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula II comprising greater than 70% by weight of the R or S enantiomer, or a pharmaceutically acceptable salt thereof;

1.2 A method for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases, e.g. as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an

effective amount of a compound of formula II comprising greater than 70% by weight of the R or S enantiomer, or a pharmaceutically acceptable salt thereof;

2. A compound of formula II comprising greater than 70% by weight of the R or S enantiomer, in free form or in a pharmaceutically acceptable salt form for use as a pharmaceutical, e.g. in any of the methods as indicated under 1.1 or 1.2 above.

3. A pharmaceutical composition, e.g. for use in any of the methods as in 1.1 or 1.2 above comprising a compound of formula II comprising greater than 70% by weight of the R or S enantiomer in free form or pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent or carrier therefor.

4. A compound of formula II comprising greater than 70% by weight of the R or S enantiomer or a pharmaceutically acceptable salt thereof for use in the preparation of a pharmaceutical composition for use in any of the method as in 1.1 or 1.2 above.

The compounds of the invention may be administered as the sole active ingredient or in conjunction with, e.g. as an adjuvant to, other drugs e.g. immunosuppressive or immunomodulating agents or other anti-inflammatory agents, e.g. for the treatment or prevention of allo- or xenograft acute or chronic rejection or inflammatory or autoimmune disorders, or a chemotherapeutic agent, e.g a malignant cell anti-proliferative agent. For example, the compounds of the invention may be used in combination with a calcineurin inhibitor, e.g. cyclosporin A or FK 506; a mTOR inhibitor, e.g. rapamycin, 40-O-(2-hydroxyethyl)-rapamycin, CCI779 or ABT578; an ascomycin having immunosuppressive properties, e.g. ABT-281, ASM981, etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; leflunomide; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine or an immunosuppressive homologue, analogue or derivative thereof; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to leukocyte receptors, e.g., MHC, CD2, CD3, CD4, CD7, CD8, CD25, CD28, CD40. CD45, CD58, CD80, CD86 or their ligands; other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g. CTLA4Ig (for ex. designated ATCC 68629) or a mutant thereof, e.g. LEA29Y ; adhesion molecule inhibitors, e.g. LFA-1 antagonists, ICAM-1 or -3 antagonists, VCAM-4 antagonists or VLA-4 antagonists; or a chemotherapeutic agent, e.g. paclitaxel, gemcitabine, cisplatinum, doxorubicin or 5-fluorouracil.

Where the compounds of the invention are administered in conjunction with other immunosuppressive / immunomodulatory, anti-inflammatory or chemotherapeutic therapy, dosages of the co-administered immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic compound will of course vary depending on the type of co-drug employed, e.g. whether it is a steroid or a calcineurin inhibitor, on the specific drug employed, on the condition being treated and so forth. In accordance with the foregoing the present invention provides in a yet further aspect:

5. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective non-toxic amount of a compound of the invention and at least a second drug substance, e.g. an immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic drug, e.g. as indicated above.

6. A pharmaceutical combination, e.g. a kit, comprising a) a first agent which is a compound of the invention as disclosed herein, in free form or in pharmaceutically acceptable salt form, and b) at least one co-agent, e.g. an immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic drug. The kit may comprise instructions for its administration.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a compound of the invention and a co-agent, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a compound of the invention and a co-agent, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the 2 compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of 3 or more active ingredients.

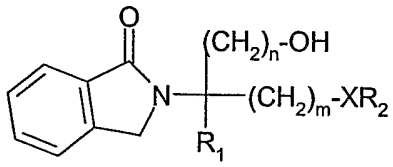
In a further aspect, the present invention provides a method of separating the R and S enantiomers of a compound of formula I, comprising separating the enantiomers by high performance liquid chromatography (HPLC) using a chiral ion-exchange phase e.g. based on quinine carbamate or quinidine carbamate as described above.

In a further aspect, the present invention provides a method of determining the amount of the R and/or S isomers of a compound of formula II present in a sample, comprising (a) reacting the compound of formula II present in the sample with benzene-1,2-dicarbaldehyde to form a compound of formula I, and separating the R and S isomers of the compound of formula I by HPLC. The HPLC is preferably performed as described above, using a chiral ion-exchange phase e.g. based on quinine carbamate or quinidine carbamate. The compounds of formula I are useful as intermediates in such a method. The sample may be e.g. a sample derived from a bodily fluid e.g. blood, plasma, saliva, or urine, and may be first subjected to one or more separation steps, (e.g. extraction with methanol and/or non-chiral HPLC) in order to separate the compound of formula II from the fluid. By such a method the amount of the active R or S enantiomer in the sample may be determined. Thus the method may be useful e.g. for monitoring the blood concentration of the active S enantiomer of a compound of formula II in a subject, following administration of a compound of formula II (e.g. a racemic mixture thereof) or following administration of a precursor of a compound of formula II (forming the compound of formula II as a metabolite in the body) to the subject.

For example, achiral 14C-labeled FTY720 is administered to rats either orally (7.5 mg/kg) or by intravenous infusion (4 mg/kg). Chiral 14C-labeled FTY720-phosphate is formed in the body of the rat as a metabolite of 14C-labeled FTY720. Blood samples are taken at different times (e.g. 3 or 72 hours) after dosing. Each blood sample is extracted with methanol and [14C]FTY720-phosphate is isolated by non-chiral HPLC. The isolated [14C]FTY720-phosphate is derivatized with OPA. The derivative is spiked with unlabelled OPA-derivatized R and S FTY720-phosphate as retention time markers (monitored by UV at 215 nm) and subjected to chiral HPLC separation using a ProntoSIL Chiral AX QN-1 column. The [14C]FTY720-phosphate in all blood samples represents exclusively the pharmacologically active S-enantiomer. The inactive R-enantiomer is not detectable.

CLAIMS

1. A compound of formula I:



I

wherein

each of m and n, independently, is 1, 2 or 3;

X is O or a direct bond;

R_1 is

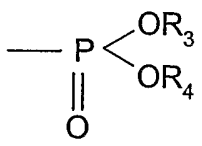
- a phenylalkyl wherein alkyl is a straight- or branched (C_{6-20})carbon chain; or
- a phenylalkyl wherein alkyl is a straight- or branched (C_{1-30})carbon chain wherein said phenylalkyl is substituted at the phenyl residue by
- a straight- or branched (C_{6-20})carbon chain optionally substituted by halogen,
- a straight- or branched (C_{6-20})alkoxy chain optionally substitued by halogen,
- a straight- or branched (C_{6-20})alkenyloxy,
- phenylalkoxy, halophenylalkoxy, phenylalkoxyalkyl, phenoxyalkoxy or phenoxyalkyl,
- cycloalkylalkyl substituted by C_{6-20}alkyl,
- heteroarylalkyl substituted by C_{6-20}alkyl,
- heterocyclic C_{6-20}alkyl or
- heterocyclic alkyl substituted by C_{2-20}alkyl,

and wherein

the alkyl moiety may have

- in the carbon chain, a bond or a heteroatom selected from a double bond, a triple bond, O, S, sulfinyl, sulfonyl, or NR_5, wherein R_5 is H, alkyl, aralkyl, acyl or alkoxycarbonyl, and
- as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxy or carboxy, and

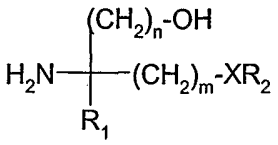
R_2 is



wherein each of R_3 and R_4, independently, is H or C_{1-4}alkyl, wherein alkyl is optionally substituted by 1, 2 or 3 halogen atoms;

in free form or in salt form.

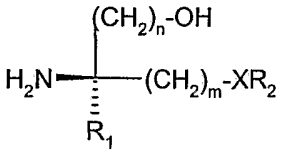
2. A compound of formula II:



II

wherein m, n, X, R_1 and R_2 are as defined in formula I, and greater than 70% of the compound by weight is in the form of the R or S enantiomer.

3. A compound of formula I according to claim 1 or of formula II according to claim 2 in the form of the essentially pure enantiomer having the following 3-dimensional configuration



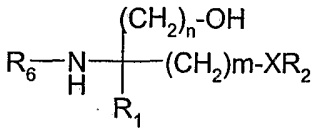
II

,

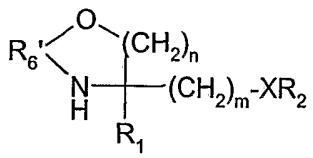
preferably the S enantiomers of phosphoric acid mono-[2-amino-2-hydroxymethyl-4-(4-octyl-phenyl)-butyl] ester and phosphoric acid mono-[2-hydroxymethyl-4-(4-octyl-phenyl)-2-(1-oxo-1,3-dihydro-isoindol-2-yl)-butyl] ester.

4. A process for producing a compound of formula I, comprising reacting a compound of formula II as defined in claim 2 with an aromatic 1,2-dicarbaldehyde, and recovering the resulting compound of formula I in free or salt form.

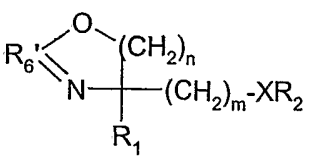
5. A process for producing a compound as defined in claim 2, comprising deprotecting a compound of formula III, IIIa or IIIb, wherein greater than 70% by weight of the compound of formula III, IIIa or IIIb is in the form of the R or S enantiomer:



III



IIIa



IIIb

wherein n, m, X, R_1 and R_2 are as defined above, R_6 is an amino protecting group, and R_6' is a simultaneous OH and amino protecting group;
and, where required, converting the compounds of formula II obtained in free form into the desired salt form, or vice versa.

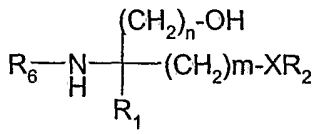
6. A method for separating the R and S enantiomers of a compound of formula I, comprising separating the enantiomers by high performance liquid chromatography (HPLC) using a chiral ion-exchange phase based on quinine carbamate or quinidine carbamate.

7. A method for determining the amount of the R and/or S isomers of a compound of formula II present in a sample, comprising (a) reacting the compound of formula II present in the sample with an aromatic 1,2-dicarbaldehyde to form a compound of formula I, and separating the R and S isomers of the compound of formula I by HPLC.

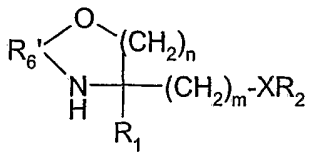
8. A compound as defined in claim 2, in free form or in a pharmaceutically acceptable salt form for use as a pharmaceutical.

9. A method for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound as defined in claim 2, or a pharmaceutically acceptable salt thereof.

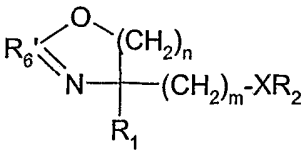
10. A compound of formula III, IIIa or IIIb:



III



IIIa



IIIb

wherein n, m, X, R_1 and R_2 are as defined in claim 1 and R_6' is a simultaneous OH and amino protecting group;
in free or salt form.

INTERNATIONAL SEARCH REPORT

Int ›nal Application No
PCT/EP2004/009589

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D209/46 C07F9/09 A61K31/137 A61K31/661 A61P37/02

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C C07F A61K A61P

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)

EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2004/024673 A (NOVARTIS PHARMA GMBH ; NOVARTIS AG (CH); BUEHLMAYER PETER (CH); SPANKA) 25 March 2004 (2004-03-25) page 30 - page 33; examples 79-86,89,93,94	2,5,8-10
X	WO 02/076995 A (PRIESCHL-STRASSMAYR EVA ERIKA ; BAUMRUKER THOMAS (AT); NOVARTIS ERFIND) 3 October 2002 (2002-10-03) page 23- - page 26; examples 1-16	2,5,8-10
X	WO 02/18395 A (PARSONS WILLIAM H ; HAJDU RICHARD (US); BERGSTROM JAMES (US); CARD DEB) 7 March 2002 (2002-03-07) claims 31-35; example 20; table 1; compounds 1-4	2,3,8-10

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

☒ Patent family members are listed in annex.

° Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 December 2004	31/01/2005

Name and mailing address of the ISA	Authorized officer
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	Richter, H

INTERNATIONAL SEARCH REPORT

Int ınal Application No
PCT/EP2004/009589

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004024673 A	25-03-2004	WO 2004024673 A1	25-03-2004
WO 02076995 A	03-10-2002	BR 0208365 A	09-03-2004
		CA 2442178 A1	03-10-2002
		CZ 20032560 A3	17-12-2003
		WO 02076995 A2	03-10-2002
		EP 1377593 A2	07-01-2004
		HU 0303601 A2	01-03-2004
		JP 2004531501 T	14-10-2004
		NO 20034291 A	24-11-2003
		SK 11942003 A3	02-03-2004
		US 2004147490 A1	29-07-2004
		ZA 200306914 A	05-05-2004
WO 0218395 A	07-03-2002	AU 8533101 A	13-03-2002
		CA 2421893 A1	07-03-2002
		EP 1315735 A1	04-06-2003
		JP 2004507552 T	11-03-2004
		WO 0218395 A1	07-03-2002
		US 2002091105 A1	11-07-2002