(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 August 2013 (22.08.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/120871 A1

(51) International Patent Classification:
C07D 209/52 (2006.01) *C07K 5/08* (2006.01)

(21) International Application Number:
PCT/EP2013/052819

(22) International Filing Date:
13 February 2013 (13.02.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
MI2012A000192 13 February 2012 (13.02.2012) IT

(71) Applicant: **DIPHARMA FRANCIS S.R.L.** [IT/IT]; Via Bissone, 5, I-20021 Baranzate (MI) (IT).

(72) Inventors: **TADDEI, Murizio**; c/o DIPHARMA FRANCIS S.R.L., Via Bissone, 5, I-20021 Baranzate (MI) (IT). **ATTOLINO, Emanuele**; c/o DIPHARMA FRANCIS S.R.L., Via Bissone, 5, I-20021 Baranzate (MI) (IT). **BALDUCCI, Evita**; c/o DIPHARMA FRANCIS S.R.L., Via Bissone, 5, I-20021 Baranzate (MI) (IT). **MICHIELETTI, Mario**; c/o DIPHARMA FRANCIS S.R.L., Via Bissone, 5, I-20021 Baranzate (MI) (IT).

(74) Agent: **BERTUCCIO, Silvia**; Bianchetti Bracco Minoja S.r.l., Via Plinio, 63, I-20129 Milano (IT).

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

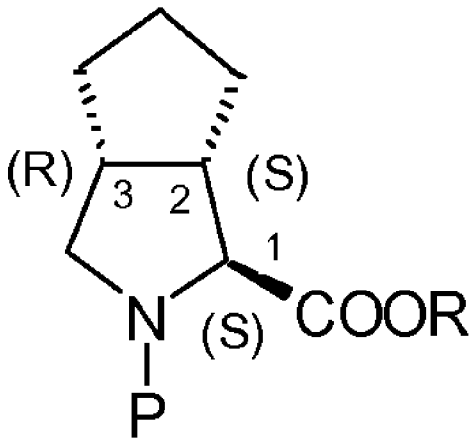
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2013/120871 A1

(54) Title: PROCESS FOR THE PREPARATION OF A VIRAL PROTEASE INHIBITOR AND ITS INTERMEDIATES



(II)

(57) Abstract: The application describes a process for the preparation of a compound of formula (II) useful for the preparation of a viral protease inhibitor. Intermediates of the process are also claimed.

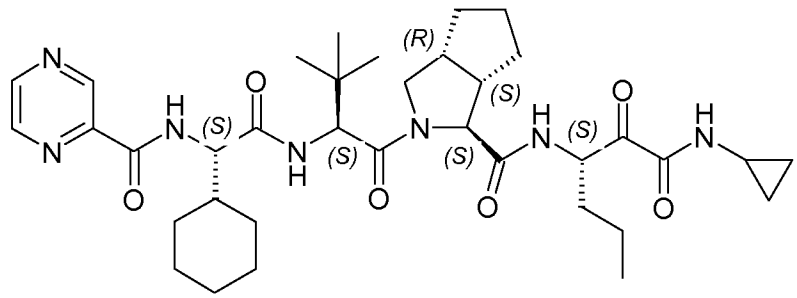
PROCESS FOR THE PREPARATION OF A VIRAL PROTEASE INHIBITOR AND ITS INTERMEDIATES

FIELD OF INVENTION

The present invention relates to a process for the preparation of a viral protease inhibitor and intermediates thereof.

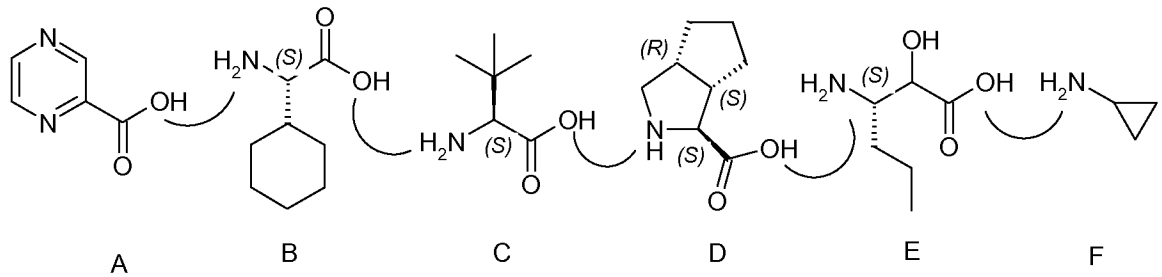
PRIOR ART

(1S, 3aR, 6aS)-2-[(2S)-2-[[(2S)-2-Cyclohexyl-2-[(2-pyrazinylcarbonyl) amino]acetyl]amino-3,3-dimethylbutanoyl]-N-[(1S)-1-[(cyclopropylamino) (oxo)acetylbutyl]-3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrole-3-carboxy amide of formula (**I**), also known as telaprevir, is a potent viral protease inhibitor used to treat hepatitis C infections.



(I)

The preparation of telaprevir, reported in US 7,820,671, involves the assembly of 6 different structural units to create 5 amide bonds (Scheme 1)



Scheme 1

and the subsequent oxidation of the alcohol hydroxyl group of compound of formula E. Compounds of formula A and F are pyrazine-carboxylic acid and cyclopropylamine respectively.

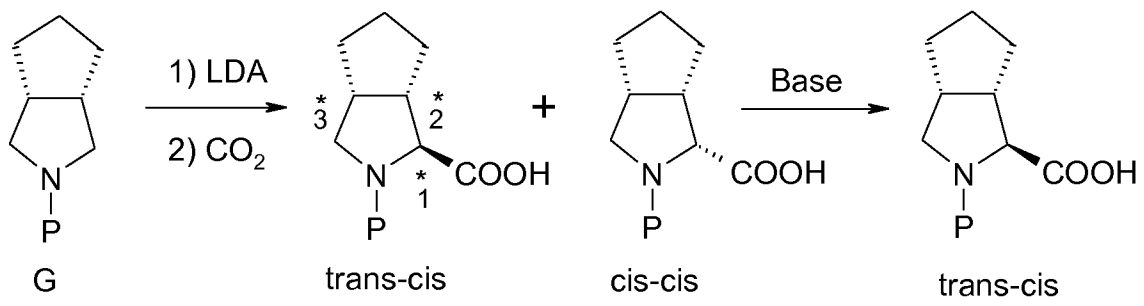
Compounds of formula B, C, D and E are four amino acids, all with configuration (S).

Compounds of formula B and C are (S)-cyclohexylglycine and (S)-tert-leucine respectively, namely simple amino acids commercially available, while compounds of formula D and E are two synthetic amino acids with a more complex structure.

The preparation of the compounds of formula D and E is reported in US 7,820,671.

While the preparation of the amino acid derived from norvaline of formula E has long been reported in the literature and can be effected by known methods, the preparation of the key amino acid of formula D is rather complex, and uses expensive reagents which are often not commercially available, especially if the expensive chiral phase-transfer catalysts (i.e. chiral PTC) are used.

The synthesis of the dicyclic amino acid of formula D, reported more recently in US 7,776,887, involves the formation of the organolithium intermediate of the protected amine compound of formula G, and its subsequent carboxylation, according to Scheme 2 reported below.



Scheme 2

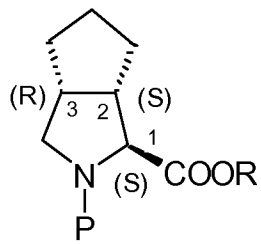
The mixture of diastereoisomers of the carboxyl intermediate thus formed, due to the formation of three *trans-cis*, *cis-cis* stereocentres at the 1-, 2- and 3- positions, is then subjected to partial epimerisation of stereocentre 1 to give only the racemic *trans-cis* isomer, which is then subjected to resolution via diastereomeric salts to give the desired resolved amino acid compound of formula D.

In this case too, the process is rather long, laborious and inefficient in terms of chemical and stereochemical yields. Moreover, the specific reaction conditions prevent its use on an industrial scale. In addition, the starting amine compound of formula G, used in the synthesis, is not commercially available.

There is consequently a need for a more advantageous alternative method to prepare Telaprevir, and its synthetic intermediates, on an industrial scale. Said novel method should in particular be more industrially scalable, involve the use of cheaper, safer, easier to handle reagents and mild reaction conditions, and at the same time provide high yields of the desired compounds.

SUMMARY OF THE INVENTION

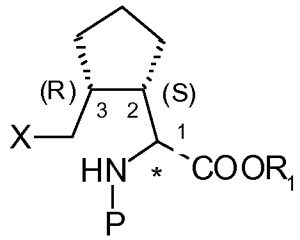
It has now surprisingly been found that a compound of formula (**II**) or a salt thereof



(**II**)

where R is H or an optionally substituted C_1-C_{12} alkyl group, an optionally substituted C_3-C_{10} cycloalkyl group or an optionally substituted aryl group, and P is an amino protecting group, can be advantageously prepared with a process comprising intramolecular cyclisation of a compound of

formula (**III**)



(**III**)

where P is as defined above; R_1 is an optionally substituted C_1-C_{12} alkyl group, an optionally substituted C_3-C_{10} cycloalkyl group or an optionally substituted aryl group, X is an OH group or a leaving group, and the asterisk * indicates the presence of a stereocentre with configuration (R) or (S) at the 1- position.

Said compound of formula (**III**), and the novel intermediates for its preparation, are further object of the invention.

The intramolecular cyclisation reaction of a compound of formula (**III**) in accordance with the process according to the invention is particularly advantageous because it allows operation at low temperatures under particularly mild reaction conditions, thus obtaining high yields of the desired compound of formula (**II**) with high chemical and stereochemical purity.

BRIEF DESCRIPTION OF ANALYSIS METHODS

The 1H-NMR and 13C-NMR spectra were recorded with a Bruker AC 200 spectrometer with operational frequency of 200 MHz, and with a Bruker Avance spectrometer with operational frequency of 400 MHz. The chemical shift values (δ) are expressed in parts per million (ppm) from the tetramethylsilane (TMS) used as internal standard. The coupling constants (J) are expressed in Hz. When reporting the spectra, the following terminology has been used:

s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet.

The mass spectra were recorded by the LC/MSD chromatography system with an Agilent series 1100 inline UV detector. Analysis conditions: 95% MeOH + 5% H2O, flow rate 0.4 mL/min, direct injection, ESI ionisation, desiccant gas (N2) flow rate 9 L/min, temperature 350°C, nebulisation pressure 40 psi, frag 70.

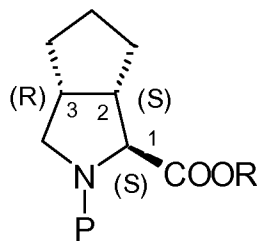
The flash chromatography separations were performed with the methodologies and instrumentation described by W.C. Still et al., using silica gel 60 with a particle size of 230-400 mesh (Fluka) and appropriate elements as stationary phase.

Thin-layer chromatography (TLC) was performed on sheets of glass covered with silica gel 60 F254 (Merck) and viewed by exposure under a UV lamp (254 nm) and development with a 1% alkaline solution of KMnO4 (yellow spots on a purple background).

An HPLC Varian Pro Star Model 210, with UV lamp detector at the wavelength of 220 nm, was used to monitor the enzymatic hydrolysis reactions.

DETAILED DESCRIPTION OF THE INVENTION

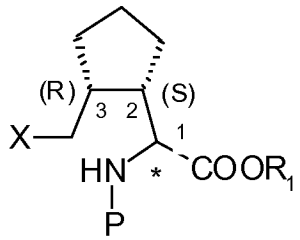
The invention relates to a process for the preparation of a compound of formula (**II**) or a salt thereof,



(**II**)

where R is H or an optionally substituted C_1-C_{12} alkyl group, an optionally substituted C_3-C_{10} cycloalkyl group, or an optionally substituted aryl group; P is an amino protecting group; comprising the intramolecular

cyclisation reaction of a compound of formula (**III**)



(**III**)

where P is as defined above, R_1 is an optionally substituted C_1-C_{12} alkyl group, an optionally substituted C_3-C_{10} cycloalkyl group, or an optionally substituted aryl group; X is an OH group or a leaving group, and the asterisk * indicates the presence of a stereocentre with configuration (R) or (S) at the 1- position; and, if the case, the conversion of a compound of formula (**II**) into another compound of formula (**II**), and/or, the conversion of a compound of formula (**II**) to a salt thereof, and/or the conversion of a salt of a compound of formula (**II**) to the free acid.

A salt of a compound of formula (**II**) is typically a pharmaceutically acceptable salt thereof.

A C_1-C_{12} alkyl group, which may be straight or branched, can be for example a C_1-C_4 alkyl group optionally substituted by one to three substituents such as a halogen atom, typically fluorine, and tert-butyl, preferably tert-butyl.

A C_3-C_{10} cycloalkyl group, which can be optionally substituted for example by one to three halogen atoms, is preferably a C_3-C_7 cycloalkyl group, in particular cyclopropyl and cyclohexyl.

An aryl group is, for example, phenyl, naphthyl or a heteromonocyclic or heterobicyclic group containing one to three heteroatoms independently selected from oxygen, nitrogen and sulphur, optionally substituted, for example, by one to three halogen atoms; preferably it is phenyl.

A amino protecting group can be, for example, a protecting group known from peptide chemistry, preferably tert-butyloxycarbonyl (Boc) or

benzyloxycarbonyl (Cbz).

A leaving group according to the present invention can be, for example, a halogen atom, preferably bromine and iodine, or a C_1-C_6 alkyl- or aryl-sulphonyloxy group, possibly substituted by one or more fluorine atoms, preferably methanesulphonate (mesylate), p-toluenesulphonate (tosylate) or trifluoromethanesulphonate (triflate).

In a compound of formula (**III**) X is preferably an OH group.

The stereocentres at the 2- and 3- positions in a compound of formula (**III**) present on the cycle must have the (S) and (R) configuration respectively, so that the two bonds present on the cyclopentane residue are *cis* to one another, namely on the same side as the cycle, whereas the absolute configuration of the stereocentre at the 1- position marked with an asterisk * can be (R) or (S), preferably (R).

If in a compound of formula (**III**) X is a leaving group as defined above, the cyclisation reaction can be effected in a solvent in the presence of a base.

A base can be organic or inorganic. An organic base can be, for example, a cyclic or acyclic tertiary amine, DBU (diazabicycloundecene), imidazole or a C_1-C_6 alkaline metal alkoxide, for example of sodium or potassium, preferably potassium tert-butoxide.

An inorganic base can be, for example, a carbonate, hydroxide or hydride of an alkali-metal or alkaline-earth metal, for example of sodium, potassium or calcium, preferably sodium or potassium.

The reaction can be effected in a polar aprotic solvent; an apolar aprotic solvent, such as toluene; or a polar protic solvent; or in a mixture of two or more, preferably two or three, of said solvents.

Examples of a polar aprotic solvent include amides, for instance dimethylformamide and dimethylacetamide; N-methylpyrrolidone; acetonitrile

or dimethyl sulphoxide; cyclic and acyclic ethers, for example methyl tert-butyl ether, tetrahydrofuran, dioxane; chlorinated solvents, for examole dichloromethane, dichloroethane, chloroform, chlorobenzene; and esters, for example ethyl or methyl acetate. Examples of polar protic solvents include straight-chain or branched C_1-C_8 alkanols, such as C_1-C_5 alkanol or water.

The reaction can be effected in a mixture of two or more, preferably two or three, of said solvents.

The cyclisation reaction can be conducted at a temperature of between about 0°C and the reflux temperature of the solvent, preferably between about 10°C and about 40°C, and more preferably at room temperature.

Alternatively, if in a compound of formula (III) X is OH, the cyclisation reaction can be effected, for example, by treating a compound of formula (**III**) with iodine and triphenylphosphine in the presence of a base and a solvent as defined above; or with the use of a dialkylazadicarboxylate, such as diethylazadicarboxylate, and triphenylphosphine, under Mitsunobu cyclisation conditions. The cyclisation reaction can be typically carried out in a single step.

When in a compound of formula (**III**) X is OH, the cyclisation reaction is preferably carried out by treatment with iodine and triphenylphosphine, under the conditions reported above.

A compound of formula (**II**) can be converted to a salt thereof, and similarly, the conversion of a salt thereof to the free compound can be effected according to known methods.

A compound of formula (**II**) can be converted into another compound of formula (**II**) according to known methods.

For example the conversion of a compound of formula (**II**), where R, being as defined above, is other than hydrogen, can be converted to a compound of formula (**II**), where R is H, by treatment in a strongly basic,

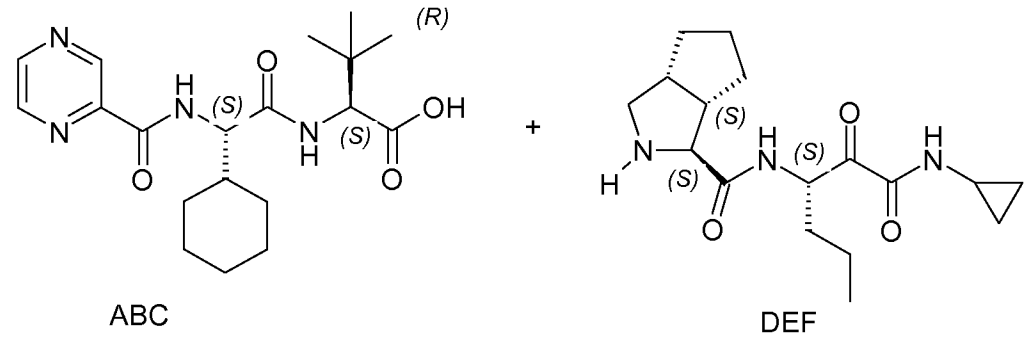
aqueous or anhydrous, environment, such as treatment with an aqueous solution of sodium hydroxide or with a solution of potassium tert-butoxide in an organic solvent.

Regardless of the stereochemistry of the stereocentre at the 1- position in a compound of formula (**III**), the basic conditions of the cyclisation reaction are such that, even if the reaction begins with a compound of formula (**III**) wherein the absolute stereochemistry of the stereocentre at the 1- position is (R), a compound of formula (**II**) is obtained wherein the stereochemistry of the stereocentre at the 1- position is only (S). This is because total epimerisation of the stereocentre at the 1- position takes place according to the process to which the present invention relates.

A compound of formula **(II)** can be used in a process for the preparation of telaprevir of formula (**I**), as defined above, according to known methods, such as those reported in US 7,820,671.

The invention therefore also discloses a process for the preparation of a compound of formula (**I**), as defined above, comprising utilizing, as starting material, a compound of formula (**II**), obtained by the process according to the present invention.

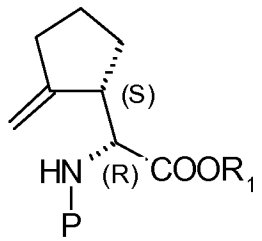
According to a further object of the invention, a compound of formula (**I**), as defined above, can be obtained by a process comprising coupling of tripeptide ABC and tripeptide DEF in the presence of DMTMM (i.e. 4-(4,6-dimethoxy (1,3,5) triazin-2-yl)-4-methylmorpholinium chloride).



ABC DEF

A compound of formula (**III**) is novel, and represents a further subject of the invention.

A compound of formula (**III**), where X is OH and the stereocentre at the 1- position is, for example, (R), can be prepared by a process comprising the anti-Markovnikov hydration reaction of the olefin compound of formula (**IV**),



(**IV**)

where R_1 and P are as defined above.

Said conversion can be performed, for example, by a hydroboration/oxidation process.

The hydroboration step can be performed by treating the olefin compound of formula (**IV**) with a commercially available hydroborating agent, for example using a complex of borane BH_3 with THF, Me_2S, or an amine, or using an alkylborane of formula R_2BH, where R is a C_1-C_6 alkyl, or 9-BBN.

The oxidation step can be conducted, for example, by treatment with hydrogen peroxide in a basic aqueous environment.

A compound of formula (**III**) where X is OH can be converted to a compound of formula (**III**) where X is a leaving group by known methods, such as by activating the alcoholic function to the leaving group, as defined

above.

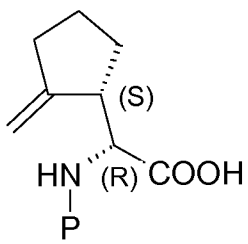
A compound of formula (**IV**) as defined above is novel, and represents a further subject of the invention.

A compound of formula (**IV**) can be prepared by resolution of its corresponding racemic mixture, comprising enantioselective enzymatic hydrolysis of the ester function of an enantiomer present in said racemic mixture in the presence of a enzyme, in a solvent mixture.

The enzyme can be a hydrolase, such as a lipase, a protease or an esterase.

Enantioselective enzymatic hydrolysis of the ester function in an enantiomer, present in the above-mentioned racemic mixture, can preferably be effected with a protease obtainable from various sources, such as bacteria, fungi, animals or plants, preferably a protease active at a pH of between about 5 and about 9.

In this way, one of the two enantiomers which is not a substrate for the enzyme remains unchanged, while the other, which is the substrate for the enzyme, is hydrolysed to obtain a compound of formula (**V**),



(V)

where P is as defined above.

The enantioselective enzymatic hydrolysis can preferably be effected using a protease, in particular a protease obtained from bacteria of the genus *Bacillus,* preferably *Bacillus licheniformis*, such as the protease called PROTIN® supplied by Amano®, or PROTEX® supplied by Genencor® and supplied by Clea®, or the alkalases® supplied by Clea® or Novozyme®, such as FE201®.

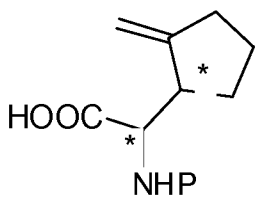
A solvent mixture is formed, for example, by a solution comprising an aqueous buffer at a pH of between approximately 5.0 and approximately 9.0, more preferably around a pH of approximately 7.5; and possibly an organic co-solvent, miscible or immiscible with the buffer.

A solution of an aqueous buffer may, for example, be a phosphate buffer, ammonium bicarbonate, ethanolamine/HCl or borate; the reaction is preferably conducted in phosphate buffer.

An organic co-solvent may, for example, be an aprotic polar solvent such as dimethylformamide, dimethylacetamide, acetonitrile or dimethyl sulphoxide; a ketone, such as acetone or methyl isobutyl ketone; an ether, such as tetrahydrofuran or methyl tert-butyl ether; or an aprotic apolar solvent such as toluene, preferably an ether.

The reaction can be conducted at a temperature of between about 15 and about 60°C, preferably between about 20 and about 40°C, and more preferably at about 25°C. The reaction times depend on the reaction temperature and the type of enzyme used. Typically, the enzyme is left to react until about 50% conversion of the starting racemate is detected by HPLC. If the reaction is conducted in the presence of an automatic titrator (pH-stat), the endpoint of the reaction can be set, for example, at pH 7.5, and the reaction mixture left under stirring until the titrator no longer corrects the pH of the mixture. According to the preferred operating conditions, indicated above, enzymatic hydrolysis is normally complete in about 1-2 days.

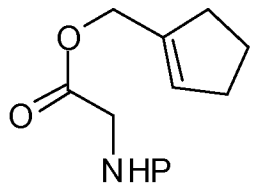
A compound of formula (**IV**) in racemic mixture can be prepared from a racemic mixture of formula (**VI**) with relative *syn*-configuration



(**VI**)

where P is as defined above and each of the asterisks * indicates a stereocentre, by esterification of the carboxyl function with an alcohol of formula R_1OH, where R_1 is as defined above, according to known techniques.

The racemic mixture of formula (**VI**) with relative *syn*-configuration can be prepared by chelate Claisen rearrangement of a compound of formula (**VII**)



(**VII**)

where P is as defined above.

Although up to 4 stereoisomers of formula (**VI**) can be obtained by rearranging a compound of formula (**VII**), in practice the chelate Claisen rearrangement reaction is highly diastereoselective (see Kazmaier, *Angew. Chem. Int. And.* **1994**, *33*, 998) and only leads to the formation of the racemic mixture of formula (**VI**) with relative *syn* stereochemistry, the two enantiomers having an absolute configuration (R,S) and (S,R).

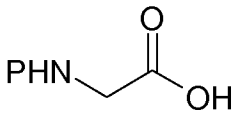
The chelate Claisen rearrangement reaction can be conducted in a solvent in the presence of a strong organic base and a zinc salt, such as zinc chloride.

In this rearrangement reaction a solvent can be, for example, a polar or apolar aprotic solvent as specified above, either alone or as a mixture of two or more, preferably two or three, of said solvents. The reaction is preferably conducted in tetrahydrofuran.

A strong base can be, for example, lithium diisopropylamide (LDA) or lithium bis(trimethylsilyl)amide (LiHMDS).

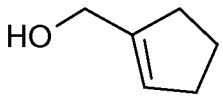
The reaction can be conducted at a temperature of between about -78°C and the reflux temperature of the solvent, preferably between -78°C and about 0°C.

A compound of formula (**VII**) can be prepared by condensing a compound of formula (**VIII**) where P is as defined above



(**VIII**)

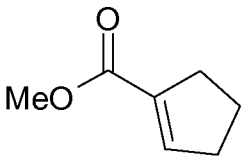
with a compound of formula (**IX**)



(**IX**)

by known procedures.

The compound of formula (**IX**) is commercially available or can be prepared by reducing the compound of formula (**X**)



(**X**)

which is commercially available, or can be prepared in turn from methyl adipate by known methods.

The following examples illustrate the invention.

Example 1: Synthesis of compound of formula (IX)

A solution of methyl-1-cyclopentene-1-carboxylate (**X**) (10 mL, 81.88 mmol) in CH_2Cl_2 (200 mL) under N_2 atmosphere is brought to 0°C, and a solution of 1M DIBALH (diisobutylaluminium hydride) in hexane (204.7 mL, 204.7 mmol) is added drop by drop. The mixture is kept under stirring a 0°C for 1 hour. H_2O is added to the reaction, insolubles are filtered through celite, and the phases are separated. The organic phase is washed with brine, dried over Na_2SO_4, filtered and evaporated under low pressure. The pure compound of formula (**IX**) is obtained.

Yield: 95% (7.62 g of a colourless liquid).

R_f: 0.28 (EtPet/EtOAc 9:1).

^{1}H-NMR 400 MHz ($CDCl_3$) δ (ppm): 5.56 (s, 1H); 4.13 (s, 2H); 2.76 (bs, 1H); 2.30-2.26 (m, 4H); 1.90-1.82 (m, 2H).

^{13}C-NMR 100 MHz ($CDCl_3$) δ (ppm): 143.67; 125.06; 61.60; 32.08; 31.86; 22.97.

MS(ES^+): m/z 121 $[M+Na]^+$.

Example 2: Preparation of cyclopentenylmethyl 2-(*tert*-butoxy carbonylamino) acetate of formula (VII)

Dimethylaminopyridine (828 mg, 6.78 mmol) is added to a solution of alcohol (**IX**) (6.65 g, 67.85 mmol) in CH_2Cl_2 (100 mL) under N_2 atmosphere, followed by a solution of dicyclohexylcarbodiimide (15.4 g, 74.64 mmol) in CH_2Cl_2 (80 mL) and a solution of Boc-glycine (**VIII**) (13 g, 74.64 mmol) in CH_2Cl_2 (100 mL). The mixture is kept under magnetic stirring at room temperature for 3 h, then filtered through celite to remove insolubles. The solution is evaporated under low pressure. The crude product is purified by flash chromatography on silica gel (eluent: EtPet/EtOAc 85:15) to obtain a chemically pure product of formula (**VII**).

Yield: 95% (16.43 g of a colourless oil).

R_f: 0.43 (EtPet/EtOAc 85:15).

^{1}H-NMR 400 MHz ($CDCl_3$) δ (ppm): 5.53 (s, 1H); 5.26 (bs, 1H); 4.54 (s, 2H); 3.75 (d, J = 4, 2H); 2.20-2.14 (m, 4H); 1.79-1.74 (m, 2H); 1.29 (s, 9H).

^{13}C-NMR 100 MHz ($CDCl_3$) δ (ppm): 169.76; 155.32; 138.00; 128.56; 79.14; 63.26; 41.83; 32.25; 31.82; 27.72; 22.69.

MS(ES^+): m/z 278 $[M+Na]^+$.

Example 3: Synthesis of (R,S)-(S,R) (2-(*tert*-butoxycarbonylamino)-2-(2-methylenecyclopentyl) acetic acid racemic mixture of formula (VI)

Preparation of lithium diisopropylamide (LDA): a solution of

diisopropylamine (24.6 mL, 174.12 mmol) in THF (150 mL) under N_2 atmosphere is brought to 0°C, and a 2.5 M solution of BuLi in hexane (69.65 mL, 174.12 mmol) is added drop by drop. The mixture is kept under stirring at 0°C for 20 minutes.

$ZnCl_2$ (8.7 g, 63.84 mmol), previously oven-dried at 140°C overnight, is added to a solution of compound (**VII**) (14.8 g, 58.04 mmol) in THF (200 mL) under N_2 atmosphere, and the mixture is brought to -78°C. LDA is added drop by drop, and the temperature is allowed to rise gradually to room temperature. The reaction is left under magnetic stirring for 12 h. 1N $KHSO_4$ is then added, and the mixture is diluted with Et_2O. The organic phase is washed with 1N $KHSO_4$ (3x), and then extracted with 1N NaOH (4x). The combined basic aqueous phases are adjusted to pH = 2 with 1N $KHSO_4$ and extracted with Et_2O (4x). The combined organic phases are washed with brine (1x), dried over Na_2SO_4, filtered and evaporated under low pressure. The chemically pure product (**VI**) is obtained.

Yield: 90% (13.3 g of a white solid).

Pf: 119-121 °C.

R_f: 0.69 (EtOAc).

^{1}H-NMR 400 MHz (MeOD) δ (ppm): 4.94 (s, 1H); 4.86-4.85 (m, 2H); 2.89-2.87 (m, 2H); 2.32-2.29 (m, 2H); 1.77-1.70 (m, 2H); 1.61-1.48 (m, 2H); 1.40 (s, 9H).

^{13}C-NMR 100 MHz (MeOD) δ (ppm): 173.83; 155.75; 150.70; 105.39; 78.65; 54.78; 45.24; 32.46; 27.32; 26.88; 23.28.

MS(ES^+): m/z 278 $[M+Na]^+$.

Example 4: Synthesis of (R,S)-(S,R) (2-(*tert*-butoxycarbonylamino)-2-(2-methylene cyclopentyl)acetic acid methyl ester racemic mixture of formula (IV)

2M trimethylsilyl diazomethane ($TMSCH_2N_2$) in Et_2O is added drop by

drop to a solution of a compound of formula **(VI)** (380 mg, 1.49 mmol) obtained according to example 3 in toluene (9 mL) and MeOH (4.5 mL), until the colour remains yellow. The reaction is left under stirring at room temperature for 30 minutes. The mixture is evaporated under low pressure, and the chemically pure racemic mixture of formula (**IV**) is obtained.

Yield: 98% (393 mg of a colourless oil).

R_f: 0.62 (EtPet/EtOAc 4:1).

^{1}H-NMR 400 MHz ($CDCl_3$) δ (ppm): 4.93 (s, 2H); 4.82 (s, 1H); 4.40 (m, 1H); 3.65 (s, 3H); 2.79-2.77 (m, 1H); 2.27-2.25 (m, 2H); 1.70-1.66 (m, 2H); 1.53-1.50 (m, 2H), 1.37 (s, 9H).

^{13}C-NMR 100 MHz ($CDCl_3$) δ (ppm): 172.44; 155.13; 150.15; 106.87; 79.35; 54.78; 51.53; 46.07; 46.00; 32.67; 27.92; 23.54.

MS(ES^+): m/z 292 $[M+Na]^+$.

Example 5: Enzymatic resolution of the (R,S)-(S,R) of (2-(*tert*-butoxycarbonylamino)-2-(2-methylene cyclopentyl)acetic acid methyl ester racemic mixture of formula (IV)

The phosphate buffer (60 mM, pH 7.66, 285 mL) and the enzymatic solution PROTEX 6L (15 mL) are added to a solution of the racemic mixture of formula **(IV)** (2.31 g, 8.58 mmol) obtained according to example 4 in methyl tert-butyl ether (28.5 mL). The mixture is left under magnetic stirring at room temperature for 24 hours until 50% of the starting product has been converted (determined by HPLC). Et_2O is then added to the reaction, and the phases are separated. The organic phase is dried over Na_2SO_4, filtered and evaporated, to obtain enantiomerically pure methyl ester of formula (**IV**) (1.1 g, yield 50%). The buffered aqueous phase is adjusted to pH = 2 by adding 1N $KHSO_4$, and ethyl ether is added. A large emulsion forms which is removed by adding celite to the mixture, leaving it under stirring for 30 minutes and filtering out the celite through a porous septum. The phases of the

clear biphasic solution thus obtained are separated and the organic phase is dried over Na_2SO_4, filtered and evaporated, to obtain the enantiomerically pure compound of formula (**V**) (1.1 g, yield 50%).

HPLC: T_r (methyl ester 1): 23.28 min, T_r(methyl ester 2): 26.70 min (hexane/isopropanol 99:1, flow rate 1 mL/min, Chiralpak IC chiral column).

Example 6: Synthesis of (*R*)-*tert*-butyl 2-(*tert*-butoxycarbonyl amino)-2-((*S*)-2-methylenecyclopentyl) acetate of formula (IV)

Boc_2O (5.7 g, 26.12 mmol) is added to a solution of a compound of formula **(V)** (3.33 mg, 13.06 mmol) in *t*BuOH (30 mL) under N_2 atmosphere, followed by dimethylaminopyridine (478 mg, 3.92 mmol). The reaction is left under stirring for 3 h. The mixture is evaporated under low pressure and the crude reaction product is purified by flash chromatography on silica gel (eluent: EtPet) to obtain the compound of formula (**IV**).

Yield: 40% (1.62 g of a yellow oil).

R_f: 0.40 (EtPet/EtOAc 95:5).

^{1}H-NMR 400 MHz ($CDCl_3$) δ (ppm): 4.95 (s, 1H); 4.89 (s, 2H); 4.36-4.34 (m, 1H); 4.82-4.80 (m, 1H); 2.28-2.23 (m, 2H); 1.73-1.70 (m, 2H); 1.49-1.47 (m, 2H); 1.42 (s, 9H); 1.39 (s, 9H).

^{13}C-NMR 100 MHz ($CDCl_3$) δ (ppm): 170.97; 155.18; 150.28; 106.71; 81.20; 79.02; 55.01; 45.97; 32.99; 27.84; 27.57; 27.22; 23.70.

MS(ES$^+$): m/z 334 $[M+Na]^+$.

Example 7: Synthesis of (*R*)-*tert*-butyl 2-(*tert*-butoxy carbonylamino)-2-((1*S*,2*R*)-2-hydroxymethyl) cyclopentyl)acetate of formula (III)

BH_3·THF (1M solution in THF, 17.1 mL, 17.10 mmol) is added drop by drop to a solution of a compound **(IV)** (2.66 g, 8.55 mmol), such as the one prepared in example 6, in THF (50 mL) at -25°C under N_2 atmosphere. The mixture is brought to 0°C and left under magnetic stirring for 2 h. H_2O

(7.5 mL), 20% NaOH (7.5 mL) and 35% H_2O_2 (17.5 mL) are then added, and the mixture is left under stirring at 0°C for 1 h. The mixture is diluted with EtOAc and washed with brine. The organic phase is dried over Na_2SO_4, filtered and evaporated under low pressure. The crude product is purified by flash chromatography on silica gel (eluent: EtPet/EtOAc 7:3) to obtain an optically pure compound of formula (**III**).

Yield: 65% (1.8 g of a white solid).

Pf: 101-104 °C.

R_f: 0.29 (EtPet/EtOAc 7:3).

^{1}H-NMR 400 MHz ($CDCl_3$) δ (ppm): 5.07 (bd, J = 8, 1H); 4.22-4.20 (m, 1H); 3.71 (dd, J_1 = 6, J_2 = 10, 1H); 3.41 (dd, J_1 = 7, J_2 = 10, 1H); 2.78-2.76 (m, 1H); 2.16-2.08 (m, 2H); 2.63-2.61 (m, 4H); 1.53-1.48 (m, 1H); 1.37 (s, 9H); 1.33 (s, 9H).

^{13}C-NMR 100 MHz ($CDCl_3$) δ (ppm): 171.98; 155.21; 81.37; 79.32; 61.66; 54.23; 44.19; 43.06; 28.28; 27.81; 27.44; 26.62; 21.99.

MS(ES^+): m/z 352 $[M+Na]^+$.

Example 8: Synthesis of (1*R*,3a*R*,6a*S*)-di-*tert*-butylhexahydrocyclopenta [*c*]pyrrol-1,2(1*H*)-dicarboxylate of formula (II)

A solution of triphenylphosphine (598 mg, 2.28 mmol), iodine (579 mg, 2.28 mmol) and imidazole (310 mg, 4.56 mmol) in CH_2Cl_2 (8 mL) under N_2 atmosphere is left under magnetic stirring at 0°C for 15 minutes, and a solution of a compound of formula (**III**), obtained according to example 7 (500 mg, 1.52 mmol) in CH_2Cl_2 (5 mL), is added. The reaction is brought to room temperature and left under magnetic stirring for 12 h. The mixture is washed with $Na_2S_2O_3$ (2x). The organic phase is dried over Na_2SO_4, filtered and evaporated under low pressure. The crude product is purified by flash chromatography on silica gel (eluent: EtPet/EtOAc 85:15) to obtain an enantiomerically pure compound of formula (**II**).

Yield: 75% (354 mg of a yellow oil).

R_f: 0.42 (EtPet/EtOAc 85:15).

^{1}H-NMR 400 MHz ($CDCl_3$) δ (ppm): 4.25 (d, *J* = 8, 1H); 3.74-3.72 (m, 1H); 2.97 (t, *J* = 9, 1H); 2.91-2.83 (m, 1H); 2.64-2.62 (m, 1H); 1.74-1.66 (m, 2H); 1.60-1.54 (m, 4H); 1.42 (s, 9H); 1.38 (s, 9H).

^{13}C-NMR 100 MHz ($CDCl_3$) δ (ppm): 170.39; 153.30; 80.44; 78.94; 62.55; 51.56; 46.77; 42.10; 29.15; 27.90; 27.67; 27.26; 25.81.

MS(ES$^+$): m/z 334 $[M+Na]^+$.

Example 9: (1*S*,3a*R*,6a*S*)-2-(*tert*-butoxycarbonyl) octahydrocyclopenta[*c*]pyrrole-1-carboxylic acid of formula (II)

Potassium tert-butoxide (159 mg, 1.42 mmol) is added to a solution of an ester compound of formula (**II**) prepared according to example 8 (221 mg, 0.71 mmol) in THF (4 mL) in N_2 atmosphere, and left under magnetic stirring at room temperature for 12 hours. Ammonium chloride is added to the reaction diluted with EtOAc, and the phases are separated. The aqueous phase is extracted with EtOAc (2x) and the combined organic phases are washed with brine (1x), dried over Na_2SO_4, filtered and evaporated. The crude product is purified by flash chromatography on silica gel (eluent: EtPet/EtOAc 1:4) to obtain an optically pure acid compound of formula (**II**).

Yield: 60% (109 mg of a pale yellow oil).

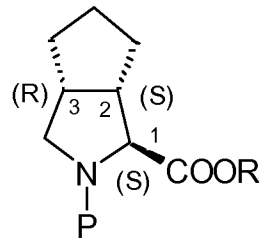
R_f: 0.23 (EtPet/EtOAc 3:7).

^{1}H-NMR 400 MHz ($CDCl_3$) δ (ppm): 8.34 (bs, 1H); 4.09-3.97 (m, 1H); 3.64-3.61 (m, 1H); 3.29-3.19 (m, 1H); 2.70-2.65 (m, 2H); 1.94-1.92 (m, 1H); 1.80-1.74 (m, 2H); 1.58-1.56 (m, 2H); 1.39 (d, *J* = 14, 10H).

MS(ES$^+$): m/z 278 $[M+Na]^+$.

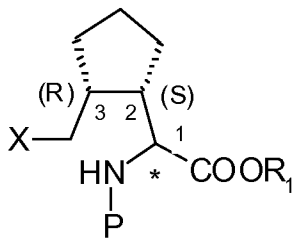
CLAIMS

1. A process for the preparation of a compound of formula (**II**) or a salt thereof,



(**II**)

wherein R is H or an optionally substituted C_1-C_{12} alkyl group, an optionally substituted C_3-C_{10} cycloalkyl group, or an optionally substituted aryl group; P is a amino protecting group; comprising the intramolecular cyclisation reaction of a compound of formula (**III**)



(**III**)

wherein P is as defined above, R_1 is an optionally substituted C_1-C_{12} alkyl group, an optionally substituted C_3-C_{10} cycloalkyl group, or an optionally substituted aryl group; X is an OH group or a leaving group; and the asterisk * indicates the presence of the stereocentre in (R) or (S) configuration at the 1- position; and, if appropriate, the conversion of a compound of formula (**II**) into another compound of formula (**II**), and/or, the conversion of a compound of formula (**II**) to a salt thereof, and/or the conversion of a salt of a compound of formula (**II**) to the free acid.

2. A process according to claim 1, wherein when in a compound of formula (**III**) X is a leaving group, the cyclisation reaction is carried out in a

solvent in the presence of a base.

3. A process according to claim 1, wherein when in a compound of formula (III) X is OH the cyclisation reaction is carried out by using iodine and triphenylphosphine in the presence of a base and a solvent or by using a dialkylazadicarboxylate and triphenylphosphine.

4. A process according to claim 3, wherein the cyclisation reaction is carried out in one step.

5. A process according to claims 2 or 3, wherein the base is an organic base selected from a cyclic or acyclic tertiary amine, DBU (diazabicycloundecene), imidazole and a C_1-C_6 alkoxide of an alkali metal or an inorganic base selected from an alkali or alkaline-earth metal carbonate, hydroxide and hydride.

6. A process according to claim 5, wherein the base is imidazole or potassium terbutoxide.

7. A process according to claims 2 or 3, wherein the solvent is selected from a polar aprotic solvent, an apolar aprotic solvent, a polar protic solvent, and a mixture of two or more of said solvents.

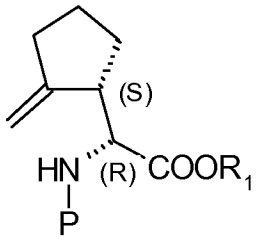
8. A process according to claim 7, wherein the apolar aprotic solvent is toluene; the polar aprotic solvent is selected from an amide, acetonitrile, dimethylsulphoxide, a cyclic or acyclic ether, a chlorinated solvent and an ester; and the polar protic solvent is selected from a straight or branched C_1-C_8 alkanol and water.

9. A process according to claim 8, wherein the solvent is selected from dichloromethane and tetrahydrofuran.

10. A process according to claims 1-9, wherein the cyclisation reaction is carried out at a temperature between about 0 °C and the reflux temperature of the solvent, preferably between about 10 °C and about 40°C.

11. A process according to claims 1-10, further comprising the preparation

of a compound of formula (**III**), as defined in claim 1, wherein X is OH and the stereocentre at the 1- position is (R), by a process comprising the anti-Markovnikov hydration reaction of an olefin compound of formula (IV),

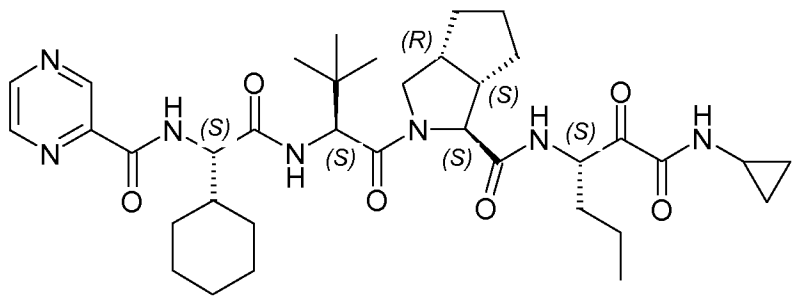


(**IV**)

wherein R_1 and P are as defined in claim 1.

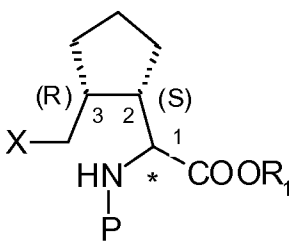
12. A process according to claim 11, wherein the anti-Markovnikov hydration reaction is performed by hydroboration/oxidation process, preferably by hydroboration of a compound of formula (IV) with a hydroborating agent selected from a complex of borane BH_3 with THF, Me_2S, or an amine, and an alkylborane of formula R_2BH, where R is a C_1-C_6 alkyl, or 9-BBN.

13. A process according to claims 1-12, further comprising the conversion of a so obtained compound of formula (**II**) to a compound of formula (**I**)

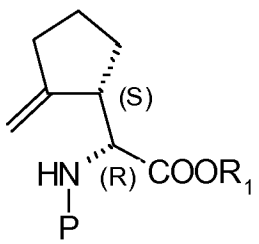


(**I**)

14. A compound of formula (**III**) or (**IV**)



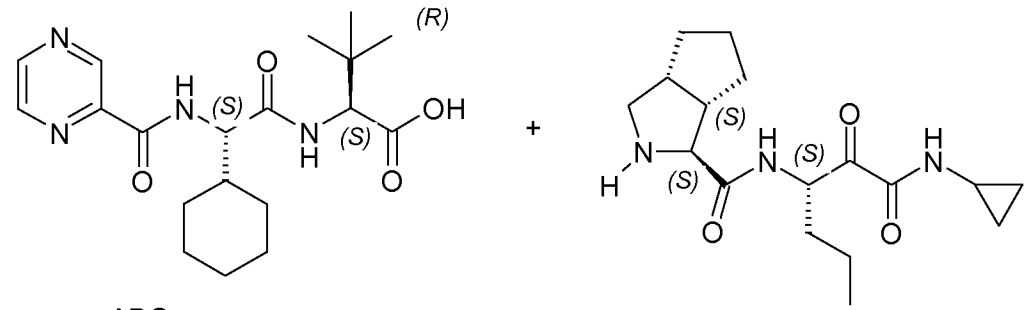
(**III**)



(**IV**)

wherein R_1, P and X are as defined in claim 1.

15. A process for the preparation of a compound of formula (**I**), as defined in claim 13, comprising coupling between a tripeptide ABC and the tripeptide DEF



ABC + DEF

in the presence of 4-(4,6-dimethoxy (1,3,5) triazine-2-yl)-4-methylmorpholinium chloride (DMTMM).

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/052819

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D209/52 C07K5/08
ADD.

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)
C07D C07K

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 practicable, search terms used)
EPO-Internal, WPI Data, 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.
A	WO 2007/022459 A2 (VERTEX PHARMA [US]; TANOURY GERALD J [US]; CHEN MINZHANG [US]; COCHRAN) 22 February 2007 (2007-02-22) cited in the application claims 1-64	1-14
A	DAVID R. WILLIAMS ET AL: "Strategies for the Synthesis of Fusicoccanes by Nazarov Reactions of Dolabelladienones: Total Synthesis of (+)-Fusicoauritone", ANGEWANDTE CHEMIE INTERNATIONAL EDITION, vol. 46, no. 6, 29 January 2007 (2007-01-29), pages 915-918, XP055032805, ISSN: 1433-7851, DOI: 10.1002/anie.200603853 Scheme 2, page 916	1-14

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent 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
5 April 2013	03/06/2013

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, Fax: (+31-70) 340-3016	Marzi, Elena

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/052819

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2010/075064 A1 (SEPRACOR INC [US]; SHAO LIMING [US]; WANG FENGJIANG [US]; MALCOLM SCOT) 1 July 2010 (2010-07-01) page 36; example 6.1.5	1-14
A	PRASAD: "Synthesis of Hydroxyethylene Dipeptide Isosters That Mimic a Cyclic Amino Acid at the PI' Subsite", TETRAHEDRON LETTERS, vol. 32, no. 42, 1 January 1991 (1991-01-01), pages 5857-5860, XP055032916, Scheme 1, page 5859	1-14

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/052819

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14

A process for the synthesis of the compounds of formula (II) and intermediates thereof.

---

2. claim: 15

A process for the synthesis of compound of formula (I).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/052819

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007022459 A2	22-02-2007	AR 058025 A1	23-01-2008
		AT 463480 T	15-04-2010
		AU 2006279357 A1	22-02-2007
		BR PI0615029 A2	16-06-2009
		CA 2619659 A1	22-02-2007
		CN 102382170 A	21-03-2012
		DK 1934179 T3	09-08-2010
		EP 2194043 A2	09-06-2010
		EP 2357170 A1	17-08-2011
		EP 2364970 A1	14-09-2011
		ES 2344156 T3	19-08-2010
		JP 2009504780 A	05-02-2009
		KR 20080047396 A	28-05-2008
		NZ 566049 A	29-07-2011
		PT 1934179 E	12-07-2010
		TW 200800889 A	01-01-2008
		TW 201245149 A	16-11-2012
		US 2007087973 A1	19-04-2007
		WO 2007022459 A2	22-02-2007
WO 2010075064 A1	01-07-2010	AU 2009330355 A1	03-11-2011
		CA 2746055 A1	01-07-2010
		CN 102317261 A	11-01-2012
		EP 2370405 A1	05-10-2011
		JP 2012512251 A	31-05-2012
		US 2011313013 A1	22-12-2011
		WO 2010075064 A1	01-07-2010