



(51) International Patent Classification:

C07C 405/00 (2006.01) **A61K 31/557** (2006.01)
C07D 307/00 (2006.01) **A61P 27/00** (2006.01)

(21) International Application Number:

PCT/IN2011/000484

(22) International Filing Date:

21 July 2011 (21.07.2011)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

2109/CHE/2010 23 July 2010 (23.07.2010) IN

(71) Applicant (for all designated States except US): **APTU-IT LAURUS PRIVATE LIMITED** [IN/IN]; 2nd Floor, Serene Chambers, Road No: 7, Banjara Hills, Hyderabad 500034 (IN).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **DAMMALAPATI, Venkata Lakshmi Narasimha Rao** [IN/IN]; 2nd Floor, Serene Chambers, Road No: 7, Banjara Hills, Hyderabad 500034 (IN). **KOMMANA, Praveen** [IN/IN]; 2nd Floor, Serene Chambers, Road No: 7, Banjara Hills, Hyderabad 500034 (IN). **CHENNURU, Lakshmi Narayana** [IN/IN]; Daicel Chiral Technologies (India) Pvt. Ltd, Lab No. 4A, Phase III, ICICI Knowledge Park, Genome Valley, Turkapally, Shameerpet (Mandal), Ranga Reddy (District), Hyderabad 500078 (IN). **GUNNAM, Srinivasu** [IN/IN]; Daicel Chiral Technologies (India) Pvt. Ltd, Lab No. 4A, Phase III, ICICI Knowledge Park, Genome Valley, Turkapally, Shameerpet (Mandal), Ranga Reddy (District), Hyderabad 500078 (IN).

(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, 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, PE, PG, PH, PL, PT, RO, RS, RU, 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, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, 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).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- of inventorship (Rule 4.17(iv))

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: PREPARATION OF PROSTAGLANDIN DERIVATIVES

(57) Abstract: A process for preparing the prostaglandin derivatives is provided, wherein the benzoyl and p-nitrobenzoyl groups are used as the hydroxyl protective groups. A pharmaceutical composition comprising the prostaglandin derivatives is also provided.



PREPARATION OF PROSTAGLANDIN DERIVATIVES

PRIORITY

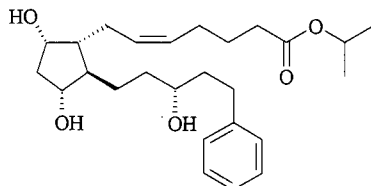
This application claims the benefit under Indian Provisional Application No. 2109/CHE/2010 filed July 23, 2010 and entitled "PROCESS FOR THE PREPARATION OF PROSTAGLANDIN DERIVATIVES ", the contents of which are incorporated by reference herein.

FIELD OF THE INVENTION

The present invention generally relates to a method for derivatization of intermediate of prostaglandins, a process for its conversion in to prostaglandins and prostaglandin analogs, namely bimatoprost, a purification process using preparative chiral chromatography and pharmaceutical compositions containing the same.

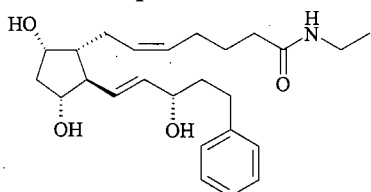
BACKGROUND OF THE INVENTION

Prostaglandins (PG) are a family of biologically active compounds that are found in virtually all tissues and organs. These naturally occurring prostaglandins have extremely complex biological functions (e.g. stimulation of smooth muscles, dilation of smaller arteries and bronchi, lowering blood pressure, etc.). Synthetic prostaglandins are for example clinically used to treatment of glaucoma and elevated ocular hypertension (IOP). The marketed PG analogs used to reduce IOP include Latanoprost, ((9S, 11R, 15R)-9, 11, 15-trihydroxy-17-phenyl-18, 19, 20-trinor-5Z-prostenoic acid, isopropyl ester), is available in the market under the brand name Xalatan® in the form of 0.005% ophthalmic solution.



Latanoprost

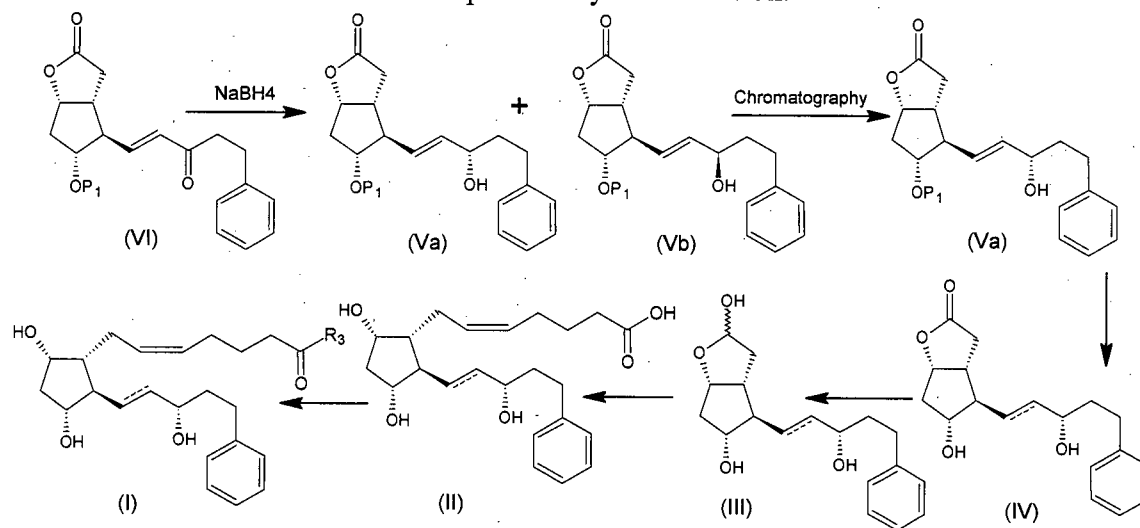
Bimatoprost, ((9S, 11R, 15S)-9, 11, 15-trihydroxy-17-phenyl-18, 19, 20-trinor-5Z, 13E-prostadienoic acid, ethyl amide), is available in the market under the brand name Lumigan® in the form of 0.03% ophthalmic solution.



Bimatoprost

The known methods for the synthesis of prostaglandin compounds (See US 3931279; US 5223537; US 5698733; US5688819; WO95/26729 and J. Med. Chem., 1993, 36, 243) are shown in scheme 1 and include the following stages i.e. reduction of the ketone of Formula VI forms the corresponding alcohol of Formula V as a mixture of diastereomers. Separating the unwanted isomer from the desired compound by column chromatography, followed by optional deprotection and reducing the keto group with

diisobutylaluminium hydride (DIBAL-H) to give lactol of compound of Formula III, reacting the lactol compound of Formula III with Wittig reagent forms acid of Formula II which is converted into the desired product by esterification.

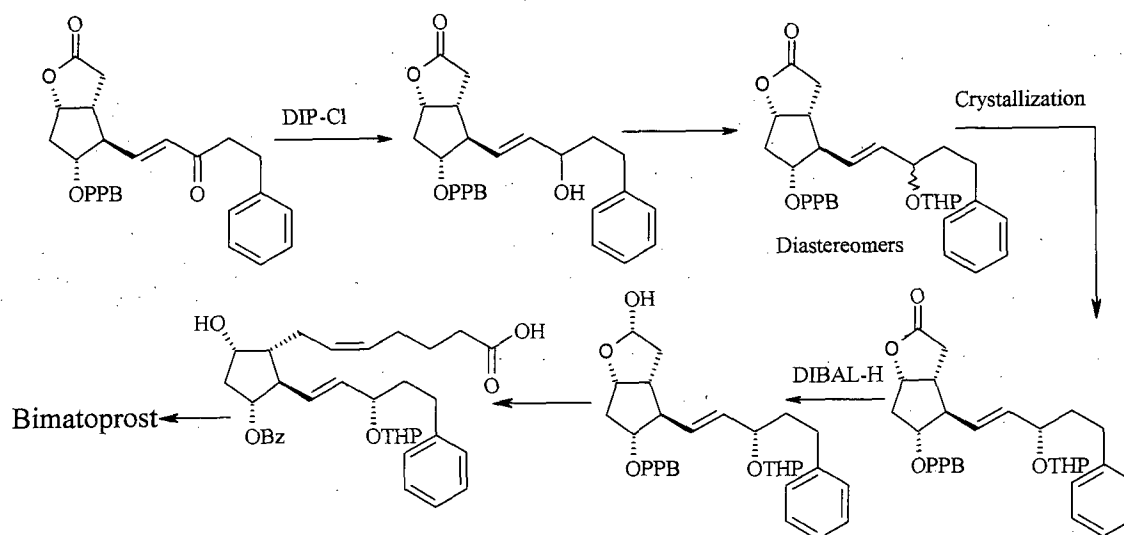


- 5 P₁ is p-phenyl benzoyl or benzoyl, “----” represents an optional double bond, R₃ is alkoxy or alkyl amino

Scheme 1

The scheme 1 method is difficult to operate in commercial scale since the required stereochemistry is obtained by column chromatography method. The chromatographic separation of diastereomers is an unfavorable process for scale up due to its multi step nature and cost. The difficulty in chromatographic separation stems from the fact that the two epimers do not differ greatly in their affinity for the adsorbent, and thus their retention times are too close to allow efficient separation in one chromatographic step.

U.S. Patent No. 7,157,590 (“the ‘590 patent”) disclosed a process for preparing prostaglandins by stereo selective reduction of carbonyl function of the PPB (p-phenyl benzoyl) protected compound with (-)-B-chlorodiisopinocampheyl borane (DIP-Cl) to yield a diastereomeric mixture of corresponding 3-hydroxyl compound. Separation of the desired isomer by derivatization of 3-hydroxyl group with silyl or tetrahydropyran groups and isolating the desired isomer by solvent crystallization, followed by reducing the lactone group to lactol with diisobutyl aluminum hydride at -20 to + 20°C and then Wittig reaction finally deprotection of the protecting groups to obtain Bimatoprost. The ‘590 patent further discloses a method of recycling of resulting mother liquor to the starting material by oxidizing the hydroxyl group. The process disclosed in the ‘590 patent is schematically represented by the scheme 2.



Scheme 2

The disadvantages associated with the '590 patent involves use of expensive protecting agents such as silyl or tetrahydropyran groups at the 3-hydroxy position.

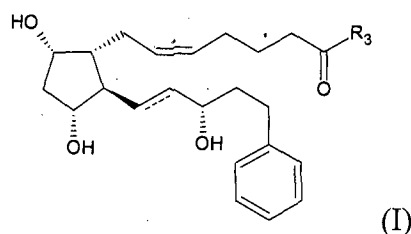
U.S. patent No. 7,611,886 ("the '886 patent") disclosed a method of selectively enzymatically esterifying and selectively enzymatically solvolizing epimers at 3-hydroxy analogs using enzymes. The enzymatic esterification synthesis is not commercially favorable since it requires costly enzymes and depending on the selectivity of enzyme requires additional reaction steps involved.

Hence, there remains a need for a simple, industrially feasible, inexpensive, and scalable process for the synthesis of prostaglandins and prostaglandin analogs, namely bimatoprost that would avoid the aforementioned difficulties.

The present invention provides a process for the preparation of prostaglandins and prostaglandin analogs by derivatization of 3-hydroxy analogs with simple protecting groups such as p-nitrobenzoyl and benzoyl groups, avoids expensive column chromatography to increase the desired isomer ratio. The process of the present invention can be practiced on an industrial scale, and also can be carried out without sacrifice of overall yield.

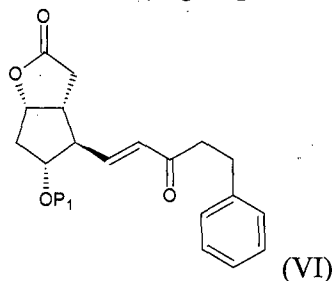
SUMMARY OF THE INVENTION

In accordance with one embodiment, the present invention provides a process for the preparation of compound of Formula I

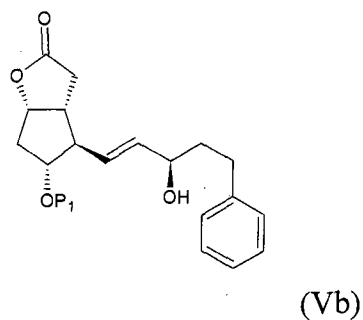
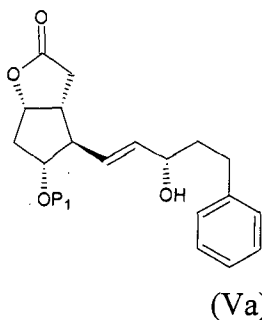


wherein R_3 is selected from alkoxy or alkyl amino and “----” represents an optional double bond; the process comprising:

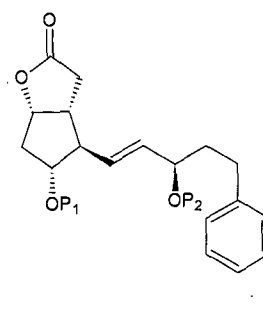
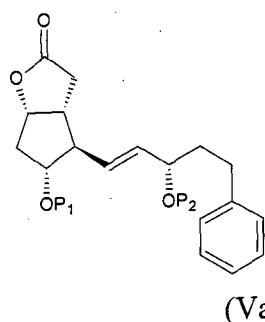
- a) stereo selective reduction of the carbonyl group of the compound of Formula VI



wherein ‘ P_1 ’ represents a benzoyl (Bz) or a p-nitrobenzoyl group (PNB); to yield a mixture of compounds of Formula Va and Vb;



- b) derivatizing the mixture of compounds of Formula Va and Vb into a mixture of compounds of Formula Vaa and Vbb:



wherein one of P_1 and P_2 represents a benzoyl group and the other one represents a p-nitrobenzoyl group;

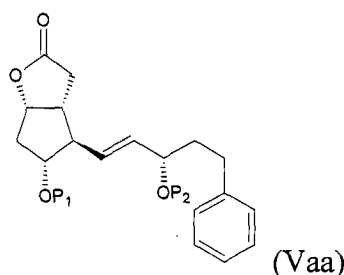
- c) isolating the compound of Formula Vaa from the mixture of compounds of Formula Vaa and Vbb;
d) converting the compound of Formula Vaa into a compound of Formula I.

In accordance with a second embodiment, the present invention provides a process for isolating the compound of Formula Vaa by solvent crystallization; comprising:

- i) dissolving or slurring the mixture of compounds of Formula Vaa and Vbb in an organic solvent S1,
- ii) optional heating the resultant reaction mixture at ambient temperature,
- iii) cooling to precipitate and filter the precipitated product.

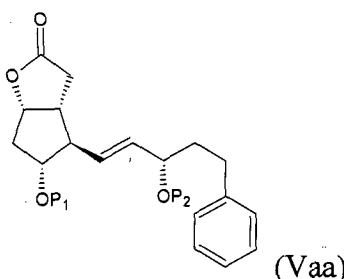
wherein the organic solvent S1 is selected from the group consisting of C₁₋₄ alcohols, esters, ethers, hydrocarbons, nitriles, ketones, water and their mixtures.

In accordance with a third embodiment, the present invention provides a compound of the Formula Vaa.



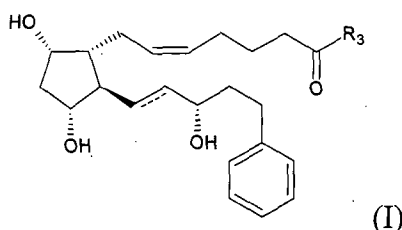
wherein one of P₁ and P₂ is benzoyl group and the other one is p-nitrobenzoyl group.

In accordance with a fourth embodiment, the present invention provides a compound of Formula Vaa



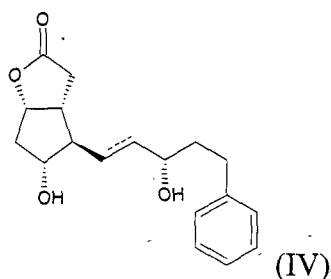
wherein P₁ is benzoyl group and P₂ is p-nitrobenzoyl group.

In accordance with a fifth embodiment, the present invention provides a process for the preparation of compound of Formula I, comprising:



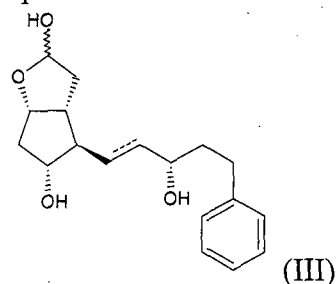
wherein R₃ is selected from alkoxy or alkyl amino and “----” represents an optional double bond; the process comprising:

- a) deprotecting the compound of Formula Vaa, obtained by a process of the present invention described above to obtain a diol compound of Formula IV



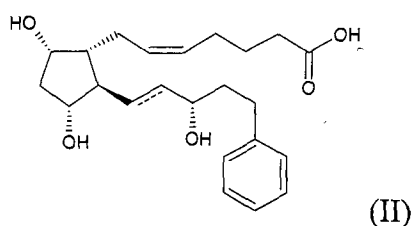
“----” represents an optional double bond;

- b) reducing the carbonyl group of the resulting compound of Formula IV with DIBAL-H to obtain a triol compound of Formula III



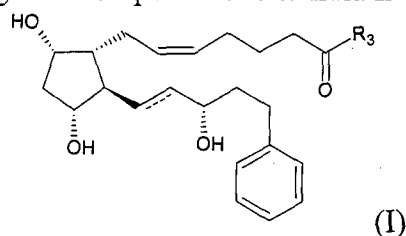
“----” represents an optional double bond;

- c) reacting the resulting triol compound of Formula III with 5-(triphenylphosphoranylidene) pentanoic acid bromide to obtain acid compound of Formula II



“----” represents an optional double bond;

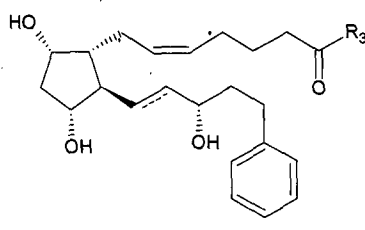
- d) converting the resulting acid compound of Formula II to compound of Formula I



wherein R_3 is selected from alkoxy or alkyl amino and “----” represents an optional double bond.

- e) purifying the compound of Formula I with preparative chiral high performance liquid chromatography (Preparative chiral HPLC).

In accordance with a sixth embodiment, the present invention provides a process for purification of compound of Formula I

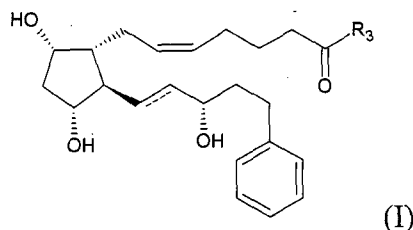


wherein R_3 is selected from alkoxy or alkyl amino and “----” represents an optional double bond, comprising; subjecting the compound of Formula I to a preparative chiral HPLC with a suitable eluent selected from C_{1-4} alcohol, water or a mixture thereof, to obtain the pure compound of Formula I.

In accordance with a seventh embodiment, the present invention provides a method for preparing the pharmaceutical composition for treating ocular hypertension by combining therapeutically effective amount of compound of Formula I prepared according to the present invention with ophthalmically acceptable vehicle.

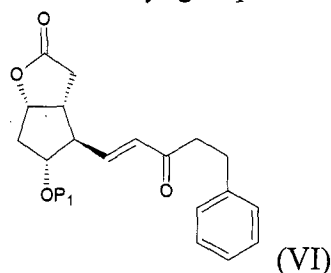
DETAILED DESCRIPTION OF THE INVENTION

The present invention provides, a process for the preparation of compound of Formula I

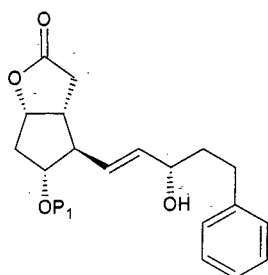


wherein R_3 is selected from alkoxy or alkyl amino and “----” represents an optional double bond; the process comprising:

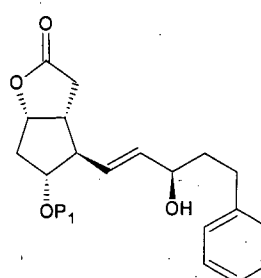
- a) stereo selective reduction of the carbonyl group of the compound of Formula VI



wherein ‘ P_1 ’ represents a benzoyl or a p-nitrobenzoyl group; to yield a mixture of compounds of Formula Va and Vb;

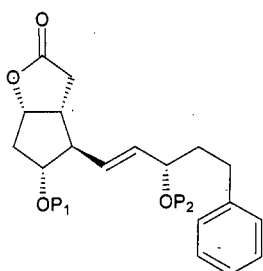


(Va)

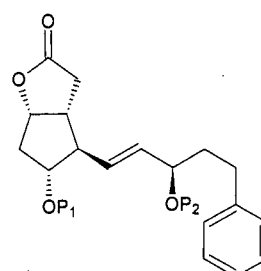


(Vb)

- b) derivatizing the mixture of compounds of Formula Va and Vb into a mixture of compounds of Formula Vaa and Vbb;



(Vaa)



(Vbb)

wherein one of P₁ and P₂ represents a benzoyl group and the other one represents a p-nitrobenzoyl group;

- c) isolating the compound of Formula Vaa from the mixture of compounds of Formula Vaa and Vbb;
- d) converting the compound of Formula Vaa into a compound of Formula I.

The starting material compound of Formula VI is known in the art and can be prepared by any known method, for example, a compound of Formula VI can be synthesized by the process mentioned in US 5698733, the content of which is incorporated herein by reference.

The step of stereo selective reduction of the carbonyl group of the compound of Formula VI, wherein 'P₁' represents a benzoyl or a p-nitrobenzoyl group, preferably benzoyl group, is carried out with (-)-B-chlorodiisopinocampheylborane or with borane in the presence of 2-alkyl-CBS-oxazaborolydines at temperature suitable for keto reduction. More preferably the reduction is carried out with (-)-B-chlorodiisopinocampheylborane in an organic solvent at a temperature of about -50°C to about +10°C, preferably at about -35°C to about -25°C. Preferably the organic solvent is selected from tetrahydrofuran (THF), ether, 1,2-dimethoxyethane, toluene, hexane, dichloromethane or mixtures of these solvents; more preferably the organic solvent is THF.

Optionally, unwanted process impurities such as pinines formed during the stereo selective reduction of the carbonyl group of the compound of Formula VI, can be removed by a column chromatography method known in the art and unwanted R-isomer

can be separated by a derivatization process of the present invention that involves use of benzoyl or p-nitrobenzoyl groups.

The conversion of the mixture of compounds of Formula Va and Vb into a mixture of compounds of Formula Vaa and Vbb, wherein P₁ represents a benzoyl group, it may be carry forward from the previous process steps, P₂ represents a p-nitrobenzoyl group; may be carried out with a suitable p-nitrobenzoyl protection forming agents known in the art, for example p-nitrobenzoyl halides such as p-nitrobenzoyl chloride, p-nitrobenzoyl bromide and the like preferably p-nitrobenzoyl chloride; optionally in the presence of a catalyst and a base in an organic solvent.

The organic solvent includes, but is not limited to C₁₋₄ alcohols such as methanol, ethanol, isopropanol and the like; esters such as ethyl acetate, isopropyl acetate and the like; ketones such as acetone, methyl isobutyl ketone, methyl ethyl ketone and the like; ethers such as diethyl ether, THF, methyl tertiary butyl ether, diisopropyl ether and the like, hydrocarbons such as hexane, heptane, cyclohexane, toluene and the like; water and their mixtures. Preferably the organic solvent is selected from the group consisting of methanol, ethyl acetate, acetone, THF, methyl tertiary butyl ether, hexane, toluene and mixtures thereof; more preferably ethyl acetate. The catalyst used, such as dimethyl amino pyridine (DMAP) and the like and the base include organic bases such as triethyl amine, diisopropylamine, diisopropyl ethylamine and the like and mixtures thereof; inorganic bases such as sodium hydroxide, potassium hydroxide, sodium carbonate, potassium carbonate and the like and mixtures thereof; preferably the base is triethyl amine.

The reaction temperature should be sufficient to effect the reaction. Typically the reaction temperature may be from about ambient temperature to about reflux temperature. Preferably the reaction temperature is at about 20°C to about 65°C, more preferably at about 25°C to about 35°C.

The present invention provides a process for isolating the compound of Formula Vaa, wherein P₁ represents a benzoyl group, P₂ represents a p-nitrobenzoyl group, obtained by a process comprising crystallizing the mixture of compounds of Formula Vaa and Vbb, where the resultant compound of Formula Vaa may have a chiral purity of 99.9: 0.1 % of S: R ratio as determined by chiral HPLC.

The present invention further provides a process for isolating the compound of Formula Vaa, wherein P₁ represents a benzoyl group, P₂ represents a p-nitrobenzoyl group, comprising:

- i) dissolving or slurring the mixture of compounds of Formula Vaa and Vbb obtained by the process described above in an organic solvent S1,
- ii) optional heating the resultant reaction mixture at ambient temperature,
- iii) cooling to precipitate and filter the precipitated product.

Preferably, the organic solvent S1 include, but are not limited to C₁₋₄ alcohols such as methanol, ethanol, isopropanol, n-propanol, isobutanol, n-butanol, tertiary butanol and the like; esters such as methyl acetate, ethyl acetate, isopropyl acetate, n-propyl acetate, isobutyl acetate, n-butyl acetate, and the like; ethers such as THF, 1,4-dioxane, diethyl ether, diisopropyl ether, methyl isobutyl ether, methyl tertiary butyl ether (MTBE) and the like; hydrocarbons selected from the group consisting of aromatic hydrocarbons such as benzene, toluene, xylene and the like; cyclic hydrocarbons such as n-hexane, n-heptane, cyclohexane and the like; halogenated hydrocarbons such as methylene chloride, ethylene chloride, chloroform, carbon tetrachloride and the like; nitriles such as acetonitrile, propionitrile and the like, ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone, and the like; water and their mixtures. Preferably the organic solvent S1 is selected from the group consisting of methanol, isopropanol, ethyl acetate, methyl tertiary butyl ether, hexane, heptane, acetonitrile, acetone, water and their mixtures; more preferably a mixture of ethyl acetate and n-hexane.

Optionally, the resultant reaction mass may be heated. Typically, the solution is heated at a temperature of at least about 30°C to about reflux temperature. Preferably, the solution is heated at about 40°C to about 80°C; more preferably at temperature about 50°C to about 60°C.

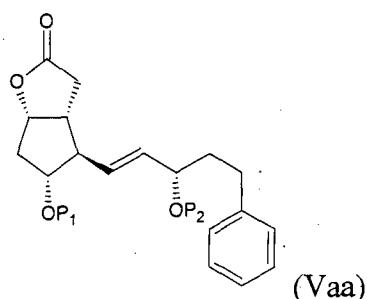
Then, cooling the resultant reaction mass at a temperature from about 30°C or less; such that the resulting compound of formula Vaa can be isolated by conventional techniques, for example filtration. In the event that stirring is involved, the temperature during stirring can range from about -10°C to about + 40°C, preferably at about 25°C to about 35°C.

The resultant product may optionally be further dried. Drying can be suitably carried out in a tray dryer, vacuum oven, air oven, fluidized bed drier, spin flash dryer, flash dryer and the like. The drying can be carried out at a temperature ranging from about 30°C to about 70°C, preferably at about 25°C to about 35°C.

Optionally the above crystallization process may be repeated one or more times to get a chiral purity typically equal to or greater than about 99.9% of S-isomer by weight as determined using chiral HPLC.

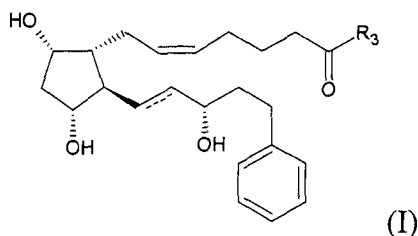
The compound of formula Vaa recovered using the process of the present invention, having chiral purity equal to or greater than about 99.9% of S-isomer by weight as determined using chiral HPLC.

The present invention provides a compound of formula Vaa



wherein P_1 is benzoyl group, P_2 is p-nitrobenzoyl group; obtained by the process of the present invention.

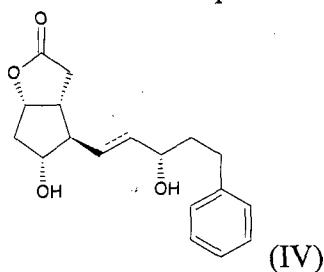
- 5 The present invention provides a process for the preparation of a compound of Formula I



- 10 wherein R_3 is selected from alkoxy or alkyl amino and “----” represents an optional double bond, obtained by a process comprising providing a compound of Formula Vaa, as a starting material, where the resultant compound of Formula I may have a purity of greater than about 99.5% as determined by chiral HPLC.

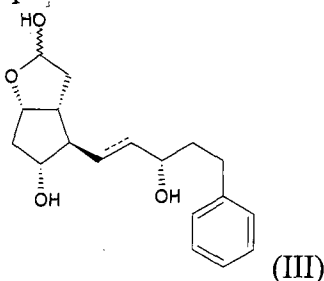
The present invention further provides a process for a preparation of a compound of Formula I, comprising:

- 15 a) deprotecting the compound of Formula Vaa, obtained by a process of the present invention described above to obtain a diol compound of Formula IV



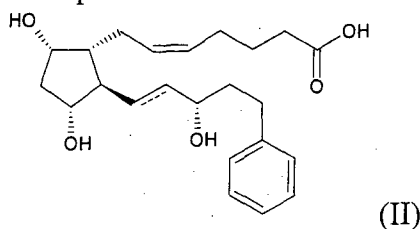
“----” represents an optional double bond

- 20 b) reducing the carbonyl group of the resulting compound of Formula IV with DIBAL-H to obtain a triol compound of Formula III



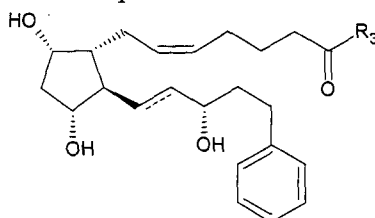
“----” represents an optional double bond

- c) reacting the resulting compound with 5-(triphenylphosphoranylidene) pentanoic acid bromide to obtain acid compound of Formula II



“----” represents an optional double bond

- d) converting the resulting acid compound of Formula II to compound of Formula I



wherein R_3 is selected from alkoxy or alkyl amino and “----” represents an optional double bond.

- e) purifying the compound of Formula I with preparative chiral high performance liquid chromatography (Preparative chiral HPLC).

The step a) of the foregoing process of deprotection of the compound of formula Vaa may include with a base in an organic solvent. The base include, but are not limited to organic bases such as triethyl amine, diisopropyl amine, diethyl amine and the like; inorganic bases such as sodium hydroxide, potassium hydroxide, sodium carbonate, potassium carbonate and the like. Preferably the base is potassium carbonate. The organic solvent include, but are not limited to C_{1-4} alcohols such as methanol, ethanol, isopropanol and the like; esters such as ethyl acetate, isopropyl acetate and the like; chlorinated solvents such as methylene chloride, ethylene chloride, chloroform and the like; hydrocarbons such as n-hexane, n-heptane, cyclohexane, toluene, xylene and the like; and mixtures thereof. Preferably the organic solvent is selected from the group consisting of methanol, ethyl acetate, methylene chloride, n-hexane; more preferably methanol, methylene chloride and mixtures thereof.

The step b) of the foregoing process may include reduction in presence of DIBAL-H in an organic solvent at a temperature of about -80°C to about -10°C . The organic solvent include, but are not limited to toluene, THF, diethyl ether, methyl tertiary butyl ether, methylene chloride, ethyl acetate, isobutyl acetate and the like and mixtures thereof. Preferably the organic solvent is methylene chloride, THF and mixtures thereof; and the temperature is about -80°C to -75°C .

Then, the resultant triol compound may be crystallized from a suitable organic solvent at a temperature of about 20°C to about 45°C . The suitable organic solvent

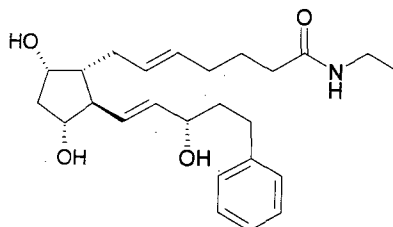
include, but is not limited to ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone and the like; C₁₋₄ alcohols such as methanol, ethanol, isopropanol, n-propanol, isobutanol, n-butanol, tertiary butanol and the like; esters such as methyl acetate, ethyl acetate, isopropyl acetate, n-propyl acetate, isobutyl acetate, n-butyl acetate and the like; hydrocarbons such as n-hexane, n-heptane, cyclohexane, and the like; and mixtures thereof. Preferably the suitable organic solvent is selected from acetone, ethyl acetate, n-hexane and mixtures thereof, more preferably mixture of acetone and ethyl acetate.

The step c) of the foregoing process may include reacting the resultant triol compound with 5-(triphenylphosphoranylidene) pentanoic acid bromide in presence of alkali metal tertiary butoxide in an organic solvent at a temperature of about -20°C to about 0°C, to obtain acid compound of Formula II. The alkali metal tertiary butoxide include, but are not limited to potassium tertiary butoxide, magnesium tertiary butoxide, lithium tertiary butoxide and the like, preferably the alkali metal tertiary butoxide is potassium tertiary butoxide. The organic solvent include, but is not limited to toluene, THF, diethyl ether, methyl tertiary butyl ether, methylene chloride, and the like and mixtures thereof. Preferably the organic solvent is THF and the temperature is about 5°C to about 10°C.

The present invention further provides a process for the preparation of a compound of Formula I (wherein R₃ is alkoxy, preferably methoxy or isopropoxy and "----" represents an optional double bond) includes esterifying an acid compound of Formula II. The esterification of the acid compound of Formula II can be carried out in the presence of an alkyl iodide and a base in an organic solvent. A useful alkyl iodide includes, but is not limited to methyl iodide or isopropyl iodide. The base includes, but is not limited to sodium carbonate, potassium carbonate, sodium hydroxide, potassium hydroxide, diazabicyclo [5.4.0] undec-7-ene; preferably the base is sodium carbonate. The organic solvent includes, but is not limited to methanol, ethanol, acetone, ethyl acetate, toluene, THF, diethyl ether, methyl tertiary butyl ether, methylene chloride, dimethyl formamide, and the like and mixtures thereof; preferably the organic solvent is dimethyl formamide.

The present invention further provides a process for the preparation of bimatoprost (a compound of Formula I, wherein R₃ is alkyl amino, preferably ethylamino and "----" represents a double bond) includes amidation of a compound of Formula I (wherein R₃ is alkoxy, preferably methoxy and "----" represents a double bond). The amidation of the compound of Formula I (wherein R₃ is alkoxy, preferably methoxy and "----" represents a double bond) can be carried out with ethyl amine at temperature about 20°C to about 50°C, preferably at about 25°C to about 35°C to obtain crude bimatoprost, where the ethyl amine may be in the form of an aqueous or anhydrous form, for example aqueous ethyl amine can be used, preferably 70% aqueous ethyl amine can be used.

The crude bimatoprost as obtained by a process of the present invention may contain about 3% of 15 (S)-5,6-trans bimatoprost of Formula A. The quality of the crude bimatoprost can be improved by purifying the bimatoprost using preparative chiral column chromatography method or solvent purification method to selectively reducing the content of undesired 15 (S)-5,6-trans bimatoprost of Formula A.



Formula A

The present invention provides, crude bimatoprost thus obtained may be purified by preparative chiral column chromatography method such as normal phase or reverse phase chromatography; or solvent purification method. The normal phase preparative chiral column chromatography method can be performed using preparative chiral column and an eluent comprising an alcohol. The eluent alcohol is selected from methanol, ethanol, or isopropanol and the like. Preferably the eluent is methanol. The normal phase preparative chiral chromatography column may be selected by any chiral columns known in the art, for example from 250 × 30 mm to 500 × 50 mm, preferably 500 × 50 mm of CHIRALPAK AD-H with about 5 μm particles, manufactured by Daicel, Japan. Flow rate of the mobile phase may be selected from about 10 ml to 80 ml per minute, preferably about 30 ml to 60 ml per minute, more preferably about 50 ml per minute. Conditions for the normal phase preparative chiral column chromatography are known to the person skilled in the art.

After collecting fractions containing bimatoprost from the chromatography, evaporating the fractions completely under vacuum and resultant residue may be crystallized from an organic solvent as known in the person skilled in the art. The organic solvent includes, but is not limited to ethers such as diethyl ether, isopropyl ether, methyl isobutyl ether, methyl tertiary butyl ether, THF, 1,4-dioxane and the like; hydrocarbons such as n-hexane, n-heptane, cyclohexane, and the like, and mixture thereof; preferably the organic solvent is methyl tertiary butyl ether.

The reverse phase preparative chiral column chromatography method can be performed using reverse phase column chromatography and an eluent comprising an alcohol and water. The eluent used comprises an alcohol, such as methanol, ethanol, or isopropanol and the like. Preferably the alcohol and water are used in a ratio of about 1:30 to about 30:1, preferably about 5:20 to about 20:5 or in a gradient mode. The reverse phase preparative chiral chromatography column may be selected by any chiral columns known in the art, for example, preferably 250 × 50 mm Inertsil Prep ODS with 10 μm particles. The mobile phase additive is added at a level of 0.01%, 0.05%, 0.10%, 0.25%

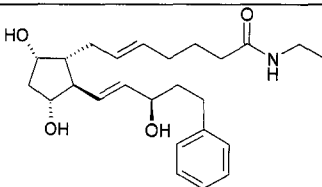
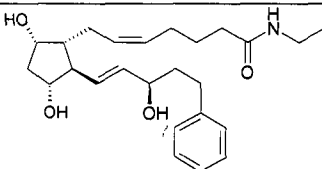
or 0.5%. Conditions for the reverse phase preparative chromatography are known to the person skilled in the art.

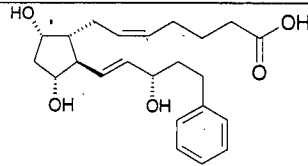
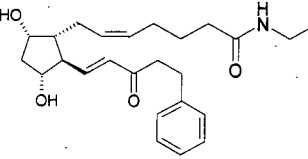
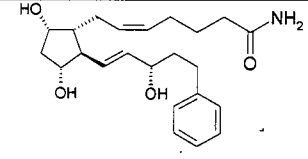
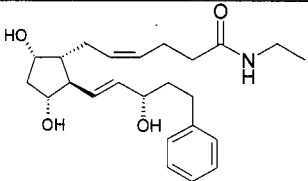
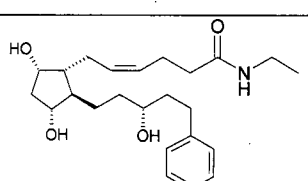
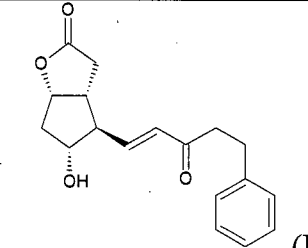
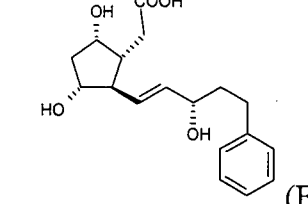
The solvent purification method can be performed by dissolving the crude bimatoprost obtained by the process of the present invention in an organic solvent, wherein the organic solvent include, but are not limited to C₁₋₄ alcohols such as methanol, ethanol, isopropanol, butanol and the like and mixtures thereof; esters such as methyl acetate, ethyl acetate, isopropyl acetate, n-propyl acetate, isobutyl acetate, n-butyl acetate, and the like and mixtures thereof; ethers such as THF, 1,4-dioxane, diethyl ether, diisopropyl ether, methyl isobutyl ether, MTBE and the like and mixtures thereof. Preferably the solvent selected from the group consisting of methanol, isopropanol, ethyl acetate, and MTBE; more preferably ethyl acetate.

The solvent may be heated to obtain a solution at a temperature of from about ambient temperature to about reflux temperature, preferably at about 25° to about 80°C, more preferably at about 45°C to 65°C. Then, optionally, adding an antisolvent to the resultant solution, wherein the antisolvent include, but are not limited to hydrocarbons such as hexane, heptane, toluene and the like and mixture thereof, preferably hexane. The reaction solution may be cooled at a temperature from about 30°C or less such that the bimatoprost can be isolated by conventional techniques.

The present invention provides a bimatoprost, obtained by the process described herein, having a chiral purity of at least about 98% as measured by chiral HPLC, preferably at least about 99% as measured by chiral HPLC; more preferably at least about 99.5% as measured by chiral HPLC; a 15 (S) – 5,6-trans bimatoprost of Formula A of less than about 0.4% as measured by HPLC, preferably less than about 0.2 % as measured by HPLC, more preferably less than about 0.15% as measured by HPLC; and substantially free of one or more of impurities as described in Table 1.

Table 1:

15 (R)- 5,6 -trans bimatoprost	 (Formula B)
15-(R)-5,6-cis bimatoprost	 (Formula C)

(Z)- 7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(1E,3S)-3-hydroxy-5-phenyl-1-pentenyl] cyclopentyl]-5-heptenoic acid	 (Formula D)
(Z)- 7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(1E)-3-oxo-5-phenyl-1-pentenyl] cyclopentyl] - 5-N-ethylheptenamide	 (Formula E)
(Z)- 7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(1E,3S)-3-hydroxy-5-phenyl-1-pentenyl] cyclopentyl]-5-heptenamide	 (Formula F)
(Z)- 7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(1E,3S)-3-hydroxy-5-phenyl-1-pentenyl] cyclopentyl]-5-N-ethyl hexenamide	 (Formula G)
(Z)- 7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(3R)-3-hydroxy-5-phenyl-1-pentenyl] cyclopentyl]-5-N-ethyl heptenamide	 (Formula H)
(3aR,4R,5R,6aS)-4-(3-oxo-5-phenyl-1E-pentenyl)-5-(hydroxy)-hexahydro-2H-cyclopenta[b]furan-2-one	 (Formula I)
2-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(3R)-3-hydroxy-5-phenylpentyl] cyclopentyl]acetic acid	 (Formula J)

wherein the word "substantially free" refers to bimatoprost having individual or total of less than about 0.1% of Formula B, Formula C, Formula D, Formula E, Formula F, Formula G, Formula H, Formula I, or Formula J, as measured by HPLC, more preferably

individual or total of less than about 0.05% Formula B, Formula C, Formula D, Formula E, Formula F, Formula G, Formula H, Formula I, or Formula J, as measured by HPLC.

As used herein, the term "alkoxy" refers to a methoxy, ethoxy, propoxy and isopropoxy group.

5

As used herein, the term "alkylamino" refers to a methyl amino, ethyl amino, propylamino, isopropylamino group.

As used herein, the term "not detected" refers to bimatoprost having less than 0.01% by HPLC of one or more of impurities.

10

The present invention provides a bimatoprost, obtained by the above process, as analyzed using chiral high performance liquid chromatography ("chiral HPLC") with the conditions described below:

Column	Chiralcel OD-RH (150 X 4.6) mm, 5 μ m
Column temperature	25 °C
Mobile phase	Prepare a mixture of degassed Water and Acetonitrile in the ratio of 80:20(% v/v)
Diluent	Water and Acetonitrile in the ratio of 1:1(v/v)
Flow rate	1.0 mL/min
Wavelength	UV at 210 nm
Injection Volume	25 μ L
Run time	60 min

15

The present invention provides a bimatoprost, obtained by the above process, as analyzed using high performance liquid chromatography ("HPLC") with the conditions described below:

Column	Waters X-Bridge C18 (250 X 4.6) mm, 5 μ m
Column temperature	40 °C
Mobile phase	Mixture of degassed Water, Methanol and acetic acid in the ratio of 50:50:0.1 (% v/v/v)
Diluent	Water and Acetonitrile in the ratio of 1:1(v/v)
Flow rate	1.0 mL/min
Wavelength	UV at 210 nm
Injection Volume	50 μ L
Run time	100 min

20

The present invention also encompasses a pharmaceutical composition comprising bimatoprost, obtained by a process of the present invention in the manufacture of a medicament for topical ocular use. The composition is prepared by combining a therapeutically effective amount of bimatoprost of the present invention, as an active ingredient, with conventional pharmaceutically-acceptable excipients, e.g. an ophthalmically-acceptable vehicle. The therapeutically efficient amount typically is

25

between about 0.0001 and about 5% (w/v), preferably about 0.001 to about 1.0% (w/v) in liquid formulations.

In addition to the above-described principal active ingredients, the compositions of the present invention may further comprise various formulatory ingredients, such as ophthalmically acceptable diluents, buffers, hydrochloric acid, sodium hydroxide, antimicrobial preservatives, stabilizers, tonicity adjustors, viscosity-enhancing agents, chelating agents, antioxidants, surfactants and/or solubilizers and combinations thereof. Preferably, the diluent is purified water.

Preservatives are used in multi-use ophthalmic formulations to prevent microbial contamination of the composition after the packaging has been opened. A number of preservatives have been used including quaternary ammonium salts, mercury compounds, alcohols and stabilized chlorine dioxide. Preferred preservatives that may be used in the pharmaceutical compositions of the present invention include benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric acetate, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, phenylmercuric nitrate, Polyquad(TM).

Viscosity-enhancing agents may be added as needed or convenient. They include, but are not limited to, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, povidone, polyvinyl alcohol, polyethylene glycol, or combinations thereof.

Tonicity adjustors may be added as needed or convenient. They include, but are not limited to, salts, particularly sodium chloride, potassium chloride, mannitol, dextrose, propylene glycol and glycerin, or any other suitable ophthalmically acceptable tonicity adjustor.

Various buffers and means for adjusting pH may be used so long as the resulting preparation is ophthalmically acceptable. Accordingly, buffers include acetate buffers, citrate buffers, phosphate buffers and borate buffers. Hydrochloric acid or sodium hydroxide may be used to adjust the pH of these formulations as needed.

Preferred surfactants are, for example, polyethoxylated castor oil, Tween 80, polyvinyl alcohol, povidone, hydroxypropyl methyl cellulose, poloxamers, carboxymethyl cellulose and hydroxyethyl cellulose cyclodextrin.

Other excipient components, which may be included in the ophthalmic preparations, are chelating agents. The preferred chelating agent is edentate disodium, although other chelating agents may also be used in place of or in conjunction with it.

EXAMPLES

The following examples are provided to enable one skilled in the art to practice the invention and are merely illustrative of the invention. The examples should not be read as limiting the scope of the invention as defined in the claims.

- 5 **Example 1:** Preparation of (3aR, 4R, 5R, 6aS)-4-[3S-hydroxy-5-phenyl-1E-pentenyl]-5-(benzoyloxy)-hexahydro-2H-cyclopenta[b]furan-2-one.

10 Into a 3 liter 4 necked round bottom flask fitted with a mechanical stirrer and a reflux condenser was charged THF (1000 ml) and (3aR,4R,5R,6aS)-4-[3-oxo-5-phenyl-1E-pentenyl]-5-(benzoyloxy)-hexahydro-2H-cyclopenta[b]furan-2-one (100 gm). Cooled the
15 reaction mass to -28° to -32°C and added (-)-DIP-Cl (814 ml of 70% (-)-DIP-Cl in toluene). Maintained for 2 hours at same temperature and after completion of the reaction, quenched the reaction mass with methanol (300 ml). Heated the solution at temperature 25°C to 30°C and solvent was removed partially under vacuum and charged DM water (1000 ml). Extracted the product with ethyl acetate (2×500 ml) and washed the
20 organic layer with 20% ammonium chloride (2×500 ml). Combined the entire organic layer and removed the solvent by distillation under vacuum at temperature about 40°C to about 45°C to provide the title compound as residue. The residue was performed chromatographic purification to remove the undesired pinine impurities with using ethyl acetate and n-hexane as elutes.

20 Ratio of elutes:

30% ethyl acetate in hexanes, 35% ethyl acetate in hexanes, 40% ethyl acetate in hexanes, followed by 100% ethyl acetate.

Yield: 70 gms

HPLC purity (chiral): 94.03%

25 R-isomer: 5.97%

Example 2 : Preparation of (3aR, 4R, 5R, 6aS)-4-[3-S-(p-nitrobenzoyloxy)-5-phenyl-1E-pentenyl]-5-(benzoyloxy)-hexahydro-2H-cyclopenta[b]furan-2-one.

30 Into a 3 liter 4 necked round bottom flask fitted with a mechanical stirrer and a reflux condenser was charged ethyl acetate (1000 ml), Example 1 product (100 gm), Triethylamine (103 ml), DMAP (0.6 gm) and p-nitrobenzoyl chloride (68 gm) at 25°C to 30°C. Stirred for 3 hours at same temperature and after completion of the reaction, charged DM water (1000 ml) and extracted the product with ethyl acetate (3×1000 ml). Combined the entire organic layer and washed with 1N HCl (500 ml). Solvent was
35 removed from the organic layer under vacuum at temperature 35°C to 40°C to yield a title compound as residue Yield: 160 gms

HPLC purity: 93.75%

R-isomer: 6.25%

40 **Example 3:** Purification of (3aR, 4R, 5R, 6aS)-4-[3-S-(p-nitrobenzoyloxy)-5-phenyl-1E-pentenyl]-5-(benzoyloxy)-hexahydro-2H-cyclopenta[b]furan-2-one by solvent crystallization with using ethyl acetate and n-hexane.

Into a 3 liter 4 necked round bottom flask fitted with a mechanical stirrer and a reflux condenser was charged Example 2 residue (150 gms), ethyl acetate (600 ml) and n-hexane (900 ml) and heated to 60°C and maintained for 15 minutes. Cooled to 25°C to 30°C and maintained for 3 hours at same temperature and filtered the product and washed with chilled n-hexane (200 ml). The wet product was dried at 45°C to 50°C under reduced pressure to provide the title compound. Yield: 100 gms.

HPLC purity (chiral): 99.91%

R-isomer: 0.09%

Example 4: Preparation of (3aR, 4R, 5R, 6aS)-4-[3-S-hydroxy-5-phenylpentyl]-5-(hydroxy)-hexahydro-2H-cyclopenta[b]furan-2-one.

Into a 3 liter 4 necked round bottom flask fitted with a mechanical stirrer and a reflux condenser was charged methanol (800 ml), MDC (800 ml) and Example 3 product (100 gm). Charged potassium carbonate (37 gm) and maintained the reaction mass at temperature 25°C to 30°C. After completion of the reaction adjusted pH of the solution to 4 by addition of 1N HCl solution (16 ml) and concentrated the reaction mass to 300 ml under reduced pressure at temperature 40°C to 45°C. Extracted the product with MDC and separated the organic layer. Extracted aqueous layer with MDC (2×500 ml) and combined the entire organic layer and washed with 10 % sodium bicarbonate (2×500 ml). The organic layer was removed by distillation from the reaction mass under vacuum at temperature 35°C to 45°C to yield title compound as residue. The residue was performed chromatographic purification with using ethyl acetate and n-hexane as elutes.

Ratio of elutes: 40% ethyl acetate in hexanes, 60% ethyl acetate in hexanes, followed by 100% ethyl acetate.

Yield: 40 gms

HPLC purity (chiral): 99.90%

R-isomer: 0.10%

Example 5: Preparation of (3aR, 4R, 5R, 6aS)-4-[3S-hydroxy-5-phenyl-1E-pentenyl]-5-(hydroxy)-hexahydro-2H-cyclopenta[b]furan-2-ol.

Into a 3 liter 4 necked round bottom flask fitted with a mechanical stirrer and a reflux condenser was charged THF (1000 ml), MDC (650 ml) and Example 4 product (50 gm). Cooled to -76°C to -80°C and added DIBAL-H solution (825 ml) over a period of 4 hours. Maintained the reaction for 2 hours at same temperature and after completion of the reaction added methanol (20 ml) at same temperature -76°C to -80°C. Charged a solution of 20% sodium potassium tartrate (500 ml) and extracted the product with ethyl acetate (3×500 ml). Separated the layers and washed the organic layer with 10% brine solution (1000 ml). The organic layer was removed by distillation from the reaction mass under vacuum at temperature 35°C to 45°C to obtain a residue. The residue was performed chromatographic purification with using ethyl acetate and n-hexane as elutes.

Ratio of elutes: ethyl acetate in hexane, 70% ethyl acetate in hexane, 80% ethyl acetate in hexane, followed by 100% ethyl acetate.

Yield: 30 gms

HPLC purity (chiral): 99.90%

R-isomer: 0.10%

5 **Example 6: Preparation of Bimatoprost (crude).**

10 Into a 3 liter 4 necked round bottom flask fitted with a mechanical stirrer and a reflux condenser was charged THF (275 ml), 4-carboxybutyl triphenylphosphonium bromide (90gm) at temperature 25° to 30°C. Cooled the reaction mass to -8°C to -12°C and added potassium tert-butoxide solution (400 ml) (1 molar solution in THF). Charged Example 5 product (15 gm in 75 ml THF) at 5°C to 10°C and maintained the reaction mass for 2 hours at same temperature. After completion of the reaction charged DM water (300 ml) at 5°C to 10°C and washed the reaction mass with ether (3×300 ml). Separated the aqueous layer and adjusted pH to 3 with 20% citric acid solution (70 g of citric acid, water to make 350 ml). Extracted the product with ethyl acetate (3×300 ml) and removed the solvent by distillation under vacuum at temperature 35°C to 45°C. Charged acetone (600 ml) to the obtained residue and filtered the precipitated product and washed with acetone (150 ml). Combined the acetone layer and evaporated under vacuum at temperature below 45°C to obtain a residue. To the resultant residue charged dimethyl formamide (150 ml) and sodium carbonate (30 gms). Heated the solution to 25°C to 30°C and charged methyl iodide (40 ml) and stirred for 12 hours. After completion of the reaction the solvent was removed from the solution under vacuum at 40 °C to 45°C and charged DM water (300 ml) and extracted the product with ethyl acetate (2×300 ml). Separated the organic layer and washed with 10% citric acid solution (450 ml) (45 g of citric acid, water to make 450 ml). Separated the organic layer and removed the solvent under vacuum at 40 °C to 45°C to yield the bimatoprost methyl ester as residue. To the residue added 70 % aqueous ethylamine solution (500 ml) at temperature 25°C to 30°C and stirred for 48 hours at same temperature. After completion of the reaction, concentrated the reaction solution to half of the volume by distillation under vacuum at 40 °C to 45°C. Charged DM water and adjusted the pH of the reaction mass to 3 to 5 with 30% sodium bisulphate solution (125 ml) (37.5 g of sodium bisulphate, water to make 125 ml) and extracted the product with ethyl acetate (2×125 ml). Separated the organic layer and washed with 10% sodium chloride solution (125 ml). The organic layer was removed by distillation from the reaction mass under vacuum at temperature 35°C to 45°C to yield title compound as residue. Charged methyl tertiary butyl ether (125 ml) at temperature 25°C to 30°C and stirred for 60 minutes. Filtered the precipitated product and washed with chilled methyl tertiary butyl ether (25 ml). The wet product was dried at 35°C to 40°C under reduced pressure to provide the title compound.

Yield: 11.5 gms.

HPLC purity (chiral): 97.00%

40 Formula A: 2.80%

Formula B: 0.20%

Formula C: Not detected

Example 7: Purification of Bimatoprost using normal phase preparative chiral chromatography.

- 5 Input: 5 gms from example -6
- Preparative HPLC System : YMC K-prep 100
- Preparative Column : 500 x 50 mm CHIRALPAK AD-H 5 μ
- Mobile phase : methanol
- Flow rate : 50 ml/min
- 10 Wavelength : UV at 210 nm
- Sample preparation : 400-500 mg/ml
- Injection Volume : 2-10 ml
- Run time : 30 min
- Column temperature : ambient temperature
- 15 5 ml of sample solution was injected to the preparative HPLC and recorded up to 30 minutes. The bimatoprost and its isomers peak collection was done from 10 to 25 minutes of retention time. CHIRALPAK AD-H (250 x 4.6) 5 μ m was used to monitor the purity of collected fractions. Collected fractions of bimatoprost and its isomers were concentrated to obtain a residue. To the residue charged methyl tertiary butyl ether (10
- 20 volumes of residue) and stirred for 30 minutes at 25°C to 30°C. Cooled the solution to 0°C to 5°C and stirred for 60 minutes. Filtered the precipitated product and washed with chilled methyl tertiary butyl ether (10 ml). The wet product was dried at 25°C to 30°C under reduced pressure to provide the title compound.
- Yield: 1.8 gms
- 25 Formula A: 0.16%,
Formula C: 0.17%
Formula D: 0.02%
Formula F: 0.01%
Formula B, Formula E, Formula G, Formula H, Formula I, and Formula J: Not detected

30 **Example 8:** Purification of Bimatoprost using reverse phase preparative chiral chromatography.

Input: 3 gms from example -6

- Preparative Column : Inertsil Prep ODS (250x50) mm, 10 μ
- 35 Mobile phase : A) Degassed Milli Q Water & B) Methanol
- Elution : Gradient
- Gradient (T/%B) : 0/20, 5/20, 35/80, 40/80, 42/20, 45/20

Diluent : Methanol.
 Flow rate : 80.0 ml/min
 Wavelength : UV at 210 nm
 Sample Conc. : 300 mg in 1.7 ml methanol
 5 Injection Volume : 1.7 ml
 Run time : 45 min

Six fractions of Bimatoprost were collected and the pure fractions were concentrated to remove methanol and the product was then extracted with MDC (2×100 ml). The organic layer was concentrated under vacuum and crystallized from methyl tertiary butyl ether to
 10 obtain a solid material (1.6 gms).

HPLC purity (chiral): 99.66%

Formula A: 0.19%,

Formula C: 0.16%

Formula D: 0.03%

15 Formula F: 0.01%

Formula B, Formula E, Formula G, Formula H, Formula I, and Formula J: Not detected

Example 9: Purification of Bimatoprost using solvent-antisolvent method.

Into a 1 liter 4 necked round bottom flask fitted with a mechanical stirrer and a reflux
 20 condenser was charged crude bimatoprost (2 gms, obtained from Example 6 above) and ethyl acetate (30 ml). Heated the solution to about 65°C to about 70°C and stirred for 15 minutes at the same temperature. Added hexane (30 ml) at temperature about 65°C to about 70°C and cooled the solution to about 25°C to about 30°C over a period of 2 hours. Filtered the resultant product and washed with chilled ethyl acetate (10 ml) and hexane
 25 (10 ml).). The wet product was dried at 35°C to 40°C under reduced pressure to provide the title compound Yield: 1.2 gms.

HPLC purity (chiral): 99.61%

Formula A: 0.35%

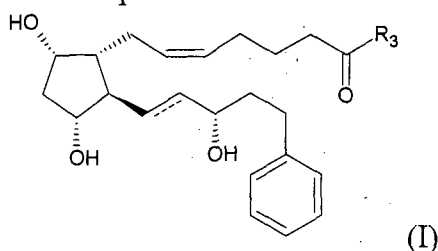
Formula D: 0.03%

30 Formula G: 0.01%.

It will be understood that various modifications may be made to the embodiments disclosed herein. Therefore the above description should not be construed as limiting, but merely as exemplifications of preferred embodiments. For example, the
 35 functions described above and implemented as the best mode for operating the present invention are for illustration purposes only. Other arrangements and methods may be implemented by those skilled in the art without departing from the scope and spirit of this invention. Moreover, those skilled in the art will envision other modifications within the scope and spirit of the specification appended hereto.

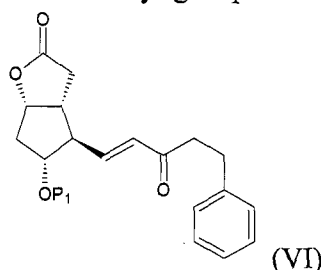
WE CLAIM:

1. A process for the preparation of compound of Formula I

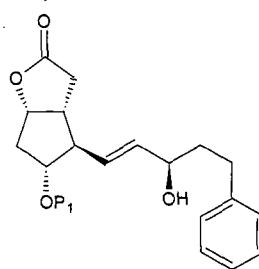
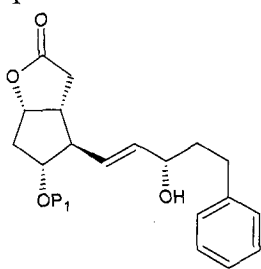


wherein R_3 is selected from alkoxy or alkyl amino and “----” represents an optional double bond, the process comprising:

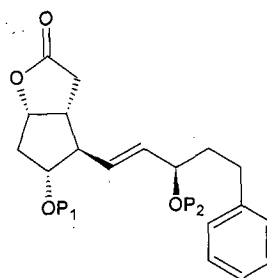
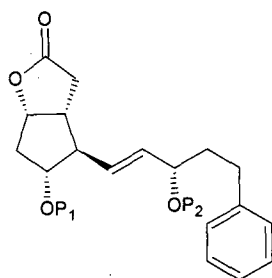
- a) stereo selective reduction of the carbonyl group of the compound of Formula VI



wherein ‘ P_1 ’ represents a benzoyl group or a p-nitrobenzoyl group; to yield a mixture of compounds of Formula Va and Vb;



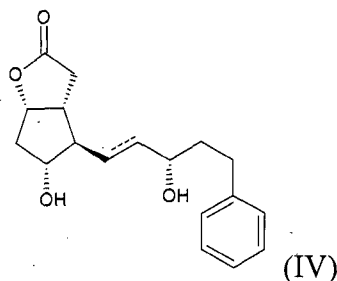
- b) derivatizing the mixture of compounds of Formula Va and Vb into a mixture of compounds of Formula Vaa and Vbb;



wherein one of P_1 and P_2 represents a benzoyl group and the other one represents a p-nitrobenzoyl group;

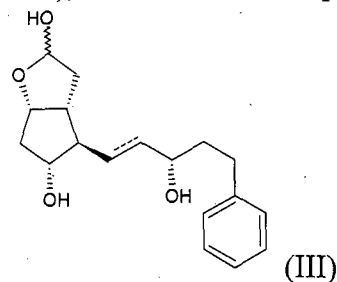
- c) isolating the compound of Formula Vaa from the mixture of compounds of Formula Vaa and Vbb;
d) converting the compound of Formula Vaa into a compound of Formula I.

2. The process of claim 1, wherein the P_1 is benzoyl group and the P_2 is p-nitrobenzoyl group.
3. The process of claim 1, wherein the step of derivatizing the mixture of compounds of Formula Va and Vb into a mixture of compounds of Formula Vaa and Vbb, using p-nitrobenzoyl chloride.
4. The process of claim 1, wherein the stereo selective reduction of the carbonyl group of the compound of Formula VI is carried out in presence of (-)-B-chlorodiisopinocampheylborane.
5. The process of claim 1, wherein isolating the compound of Formula Vaa is carried out by subjecting the mixture of compounds of Formula Vaa and Vbb to a solvent crystallization.
6. The process of claim 5, wherein the solvent crystallization comprising the steps of:
 - i) dissolving or slurring the mixture of compounds of Formula Vaa and Vbb in an organic solvent S1,
 - ii) optional heating the resultant reaction mixture at ambient temperature,
 - iii) cooling to precipitate and filter the precipitated product.
 wherein the organic solvent S1 is selected from the group consisting of C_{1-4} alcohols, esters, ethers, hydrocarbons, nitriles, ketones, water and mixtures thereof.
7. The process of claim 1, wherein the step of converting comprises:
 - a) subjecting the compound of Formula Vaa to deprotection with a base to obtain a compound of Formula IV



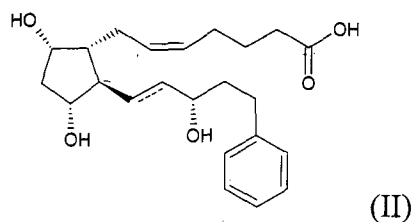
“----“ represents an optional double bond;

- b) reducing the carbonyl group of the compound of Formula IV with a diisobutyl aluminum hydride (DIBAL-H), to obtain triol compound of Formula III



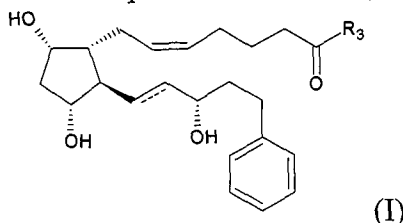
“----“ represents an optional double bond;

- c) reacting the triol compound of Formula III with a metal salt of 5-(triphenylphosphoranylidene) pentanoic acid, to obtain acid compound of Formula II;



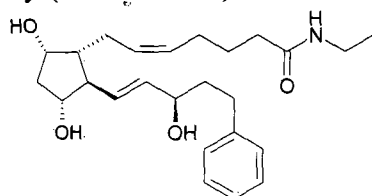
5 "----" represents an optional double bond

- d) converting the resulting acid compound of Formula II to a compound of Formula I;
- e) subjecting the compound of Formula I to a preparative chiral high performance liquid chromatography, to give the compound of Formula I.
- 10 8. The process of claim 7, wherein the base is selected from organic base such as triethylamine, diisopropyl amine, diethyl amine and mixtures thereof; inorganic bases such as sodium or potassium hydroxide, sodium or potassium carbonate and mixtures thereof.
9. The process of claim 7, wherein the step of converting comprises:
- 15 a) esterifying the compound of Formula II with alkyl iodide and a base to obtain a compound of Formula I, wherein R_3 represents alkoxy;
- b) amidation of the compound of Formula I, wherein R_3 represents alkoxy; with alkylamine to obtain compound of Formula I, wherein R_3 represents alkylamino.
- 20 10. The process of claim 9, wherein the alkyl iodide is methyl iodide and the alkylamine is ethylamine.
11. A process for purification of a compound of Formula I



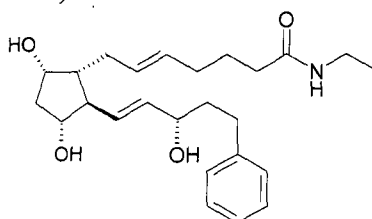
- 25 wherein R_3 is selected from alkoxy or alkyl amino and "----" represents an optional double bond, comprising; subjecting the compound of Formula I to a preparative chiral high performance liquid chromatography (preparative chiral HPLC) with using suitable eluent selected from C_{1-4} alcohol, water or mixtures thereof, to obtain the pure compound of Formula I.
12. The process of claim 11, wherein the preparative chiral HPLC is selected from normal phase or reverse phase preparative chiral HPLC.
- 30 13. The process of claim 11, wherein the preparative chiral HPLC is normal phase preparative chiral HPLC.
14. The process of claim 11, wherein the preparative chiral HPLC is normal phase preparative chiral HPLC and the eluent is methanol.

15. The process of claims 1 - 14, wherein the compound of Formula I having less than 0.1% of 15-(R)-5,6-cis bimatoprost of Formula C by chiral high performance liquid chromatography (chiral HPLC).



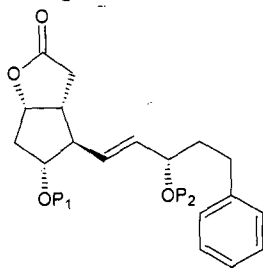
(Formula C)

- 5 16. The process of claim 1 - 14, wherein the compound of Formula I having less than 0.2% of 15-(S)-5,6-trans bimatoprost of Formula A by high performance liquid chromatography (HPLC).



(Formula A)

17. A process for isolation of a compound of Formula Vaa



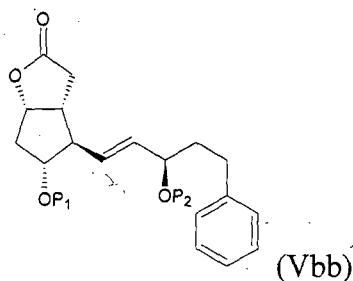
(Vaa)

wherein one of P₁ and P₂ is benzoyl group and the other one is p-nitrobenzoyl group; which process comprises:

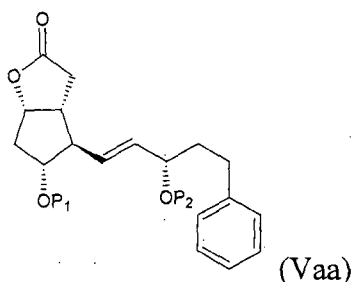
- i) dissolving or slurring the mixture of compounds of Formula Vaa and Vbb in an organic solvent S₁,
- ii) optional heating the resultant reaction mixture at ambient temperature,
- iii) cooling to precipitate and filter the precipitated product.

wherein the organic solvent S₁ is selected from the group consisting of C₁₋₄ alcohols, esters, ethers, hydrocarbons, nitriles, ketones, water and mixtures thereof

18. The process of claim 17, wherein the organic solvent is selected from methanol, ethyl acetate, n-hexane, acetonitrile, acetone, water and their mixtures.
19. The process of claim 17, wherein the organic solvent is a mixture of ethyl acetate and n-hexane.
20. The process of claim 17, wherein the compound of Formula Vaa having less than 0.1 % of R-isomer of Formula Vbb by chiral HPLC.

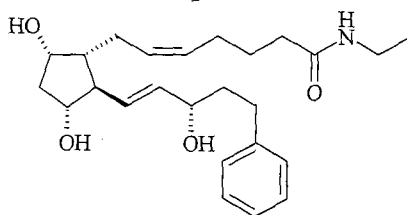


21. A compound of Formula Vaa.



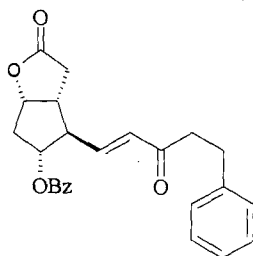
wherein one of P_1 and P_2 is benzoyl group and the other one is p-nitrobenzoyl group.

22. The compound of claim 21, wherein the P_1 is benzoyl group, P_2 is p-nitrobenzoyl group.
23. A process for the preparation of bimatoprost of the Formula,

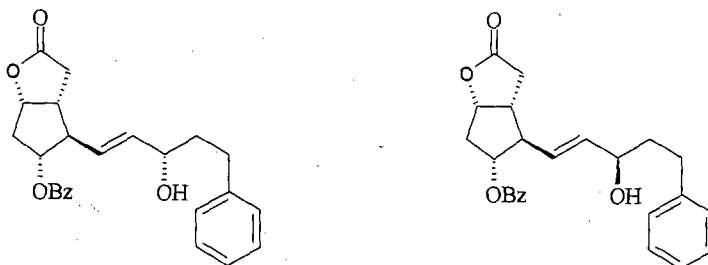


the process comprising:

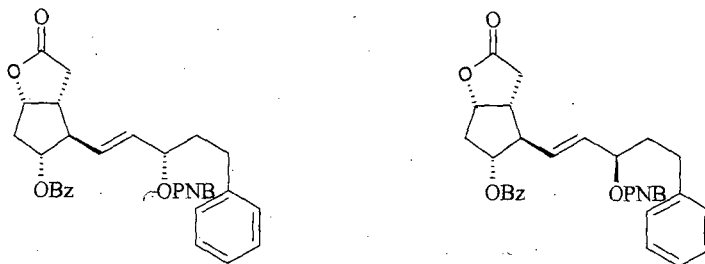
- a) stereo selective reduction of the carbonyl group of the compound of Formula



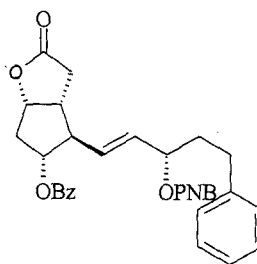
with (-)-B-chlorodiisopinocampheylborane, to yield a mixture of compounds of Formula:



- b) derivatizing the resulting mixture of compounds with p-nitrobenzoyl chloride to obtain a mixture of compounds of Formula:



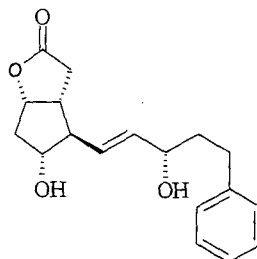
- c) isolating the compound of Formula



5

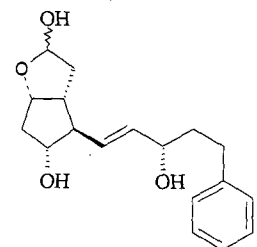
by solvent crystallization.

- d) deprotection of the resulting compound with potassium carbonate to obtain a diol compound of Formula:

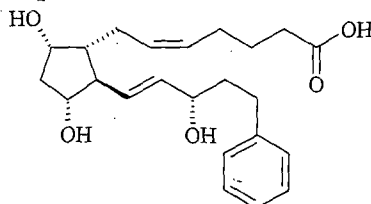


10

- e) reducing the carbonyl group of the resulting compound with diisobutylaluminium hydride (DIBAL-H) to obtain a triol compound of Formula:

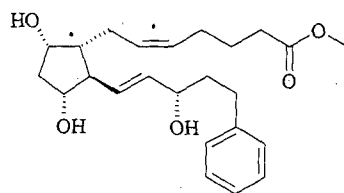


- f) reacting the resulting compound with 5-(triphenylphosphoranylidene) pentanoic acid bromide to obtain bimatoprost acid of Formula:

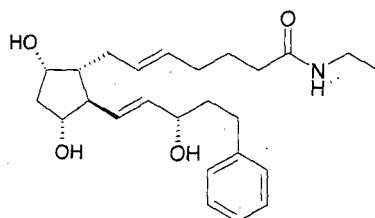


15

- g) reacting the resultant bimatoprost acid with methyl iodide to obtain bimatoprost methyl ester of Formula:



- h) amidation of the resulting bimatoprost methyl ester with ethyl amine to obtain bimatoprost,
- i) purifying the bimatoprost with preparative chiral high performance liquid chromatography using methanol as eluent.
24. Bimatoprost having less than about 0.2% by HPLC of 15 (S)-5,6-trans bimatoprost of Formula A.



(Formula A)

25. Bimatoprost substantially free of one or more of each of the impurities by HPLC:
- i) 15-(R)-5,6-trans bimatoprost;
 - ii) 15-(R)-5,6-cis bimatoprost;
 - iii) (Z)-7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(1E,3S)-3-hydroxy-5-phenyl-1-pentenyl]cyclopentyl]-5-heptenoic acid;
 - iv) (Z)-7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(1E)-3-oxo-5-phenyl-1-pentenyl]cyclopentyl]-5-N-ethylheptenamide;
 - v) (Z)-7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(1E,3S)-3-hydroxy-5-phenyl-1-pentenyl]cyclopentyl]-5-heptenamide;
 - vi) (Z)-7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(1E,3S)-3-hydroxy-5-phenyl-1-pentenyl]cyclopentyl]-5-N-ethyl hexenamide;
 - vii) (Z)-7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(3R)-3-hydroxy-5-phenyl-1-pentenyl]cyclopentyl]-5-N-ethyl heptenamide;
 - viii) (3aR,4R,5R,6aS)-4-(3-oxo-5-phenyl-1E-pentenyl)-5-(hydroxy)-hexahydro-2H-cyclopenta[b]furan-2-one;
 - ix) 2-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(3R)-3-hydroxy-5-phenylpentyl]cyclopentyl] acetic acid.
26. A method for preparing the pharmaceutical composition for treating ocular hypertension by combining therapeutically effective amount of bimatoprost according to any of claims 1 to 25 with ophthalmically acceptable vehicle.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IN2011/000484

A. CLASSIFICATION OF SUBJECT MATTER

See Extra Sheet

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: C07C 405/00; C07D 307/00; A61K31/557; A61P 27/00

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)

WPI, EPODOC, CNKI, CNPAT, CA; prostaglandin, bimatoprost, latanoprost, benzoyl, nitro, hydroxyl, protect, FINETECH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO02096868 A2 (FINETECH LABORATORIES LTD.) 05 Dec. 2002 (05.12.2002), claims 1-12, see page 9, Para. 1-page 14, Para. 3, Examples	1-26
X	WO2009153206 A2 (SANDOZ AG) 23 Dec. 2009 (23.12.2009), claims 1-10, Example 5	24-25
A	WO0155101 A2 (FINETECH LTD.) 02 Aug. 2001 (02.08.2001), claims 1-15, page 6, Para. 1 - page 9, Para. 3, Scheme 2	1-26

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:	“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
“A” document defining the general state of the art which is not considered to be of particular relevance	“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
“E” earlier application or patent but published on or after the international filing date	“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
“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)	“&” document member of the same patent family
“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	

Date of the actual completion of the international search
17 Nov. 2011 (17. 11. 2011)Date of mailing of the international search report
29 Dec. 2011 (29.12.2011)Name and mailing address of the ISA/CN
The State Intellectual Property Office, the P.R.China
6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China
100088
Facsimile No. 86-10-62019451

Authorized officer

ZHANG, Bingnan

Telephone No. (86-10)82245569

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IN2011/000484

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
WO02096868 A2	05.12.2002	EP1397347 A2	17.03.2004
		IL143477 A	20.07.2009
		JP2004529973 T	30.09.2004
		US7157590 B2	02.01.2007
		HU0400447 A2	28.04.2005
		US7166730 B2	23.01.2007
		US2005209337 A1	22.09.2005
		CN1774417 A	17.05.2006
		AU2002309221 A1	09.12.2002
		US2004171873 A1	02.09.2004
		IN200302034 P1	16.12.2005
WO2009153206 A2	23.12.2009	EP2135860 A1	23.12.2009
		TW201006792 A	16.02.2010
		WO2009153206 A3	01.04.2010
		US20110178340 A1	21.07.2011
		EP2321260 A2	18.05.2011
WO0155101 A2	02.08.2001	US2003149294 A1	07.08.2003
		HU0204395 A2	28.04.2003
		AU778887 B2	23.12.2004
		EP1252138 A2	30.10.2002
		US6927300 B2	09.08.2005
		IL134241 A	11.06.2006
		JP2003520848 T	08.07.2003

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IN2011/000484

Continuation of : **CLASSIFICATION OF SUBJECT MATTER**

C07C 405/00 (2006.01) i

C07D 307/00 (2006.01) i

A61K31/557 (2006.01) i

A61P 27/00 (2006.01) i