



(51) International Patent Classification:

C07C 59/54 (2006.01) A61K 31/192 (2006.01)
C07C 215/10 (2006.01) A61P 27/06 (2006.01)

(21) International Application Number:

PCT/US2014/044658

(22) International Filing Date:

27 June 2014 (27.06.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(71) Applicant: **ALLERGAN, INC.** [US/US]; 2525 Dupont Drive, Irvine, California 92612 (US).

(72) Inventors: **KARAMI, Thomas, K.**; 117 Mayfair, Aliso Viejo, California 92656 (US). **SMITH, Scott, W.**; 45 Windswept Way, Mission Viejo, California 92692 (US). **DUBAS-FISHER, Fiona**; 35 Monkswood, Littleport, Ely Cambridgeshire CB6 1JD (GB). **ST CLAIR BROWN, Adrian**; 1 Crauden Gardens, Ely Cambridgeshire CB6 2XJ (GB).

(74) Agents: **POTTS, Barbara, C.** et al.; Allergan, Inc., 2525 Dupont Drive, Irvine, California 92612 (US).

(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, IR, IS, JP, KE, KG, 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, SA, 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, KM, 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))

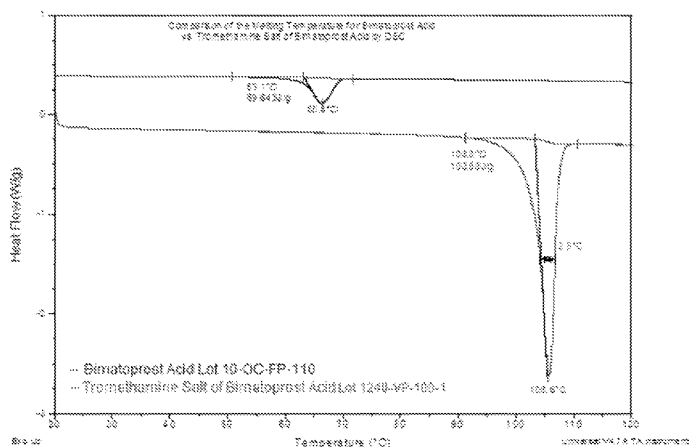
— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

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

(54) Title: TROMETHAMINE SALT OF BIMATOPROST ACID IN CRYSTALLINE FORM 1, METHODS FOR PREPARATION, AND METHODS FOR USE THEREOF

Figure 1: DSC data for Bimatoprost Acid (crystalline anhydrous) (top trace) compared to the Tromethamine Salt of Bimatoprost Acid (crystalline Form 1) (bottom trace), showing a melting temperature for the salt at about 40°C higher than the melting temperature of the free acid.



(57) Abstract: The present invention provides tromethamine salt of (Z)-7-[3,5-Dihydroxy-2-((E)-3-hydroxy-5-phenyl-pent-1-enyl)-cyclopentyl]-hept-5-enoic acid in crystalline Form 1 and amorphous form. This compound is may also be referred to as "tromethamine salt of bimatoprost acid." The invention crystalline form is useful for solid ocular implant or topical formulations, utilized in the treatment of various ocular conditions, such as, for example, ocular hypertension.

**TROMETHAMINE SALT OF BIMATOPROST ACID IN CRYSTALLINE
FORM 1, METHODS FOR PREPARATION, AND METHODS FOR USE
THEREOF**

5

FIELD OF THE INVENTION

The present invention relates generally to tromethamine salt of bimatoprost acid and its crystalline form (Form 1) and its amorphous form. The present invention further relates to methods for its preparation and to methods for treating disorders associated with ocular hypertension, hair growth, and fat reduction.

10

BACKGROUND OF THE INVENTION

Ocular hypotensive agents are useful in the treatment of a number of various ocular hypertensive conditions, such as post-surgical and post-laser trabeculectomy ocular hypertensive episodes, glaucoma, and as presurgical adjuncts.

15

Glaucoma is a disease of the eye characterized by increased intraocular pressure. On the basis of its etiology, glaucoma has been classified as primary or secondary. For example, primary glaucoma in adults (congenital glaucoma) may be either open-angle or acute or chronic angle-closure. Secondary glaucoma results from pre-existing ocular diseases such as uveitis, intraocular tumor or an enlarged cataract.

20

The underlying causes of primary glaucoma are not yet known. The increased intraocular tension is due to the obstruction of aqueous humor outflow. In chronic open-angle glaucoma, the anterior chamber and its anatomic structures appear normal, but drainage of the aqueous humor is impeded. In acute or chronic angle-closure glaucoma, the anterior chamber is shallow, the filtration angle is narrowed, and the iris may obstruct the trabecular meshwork at the entrance of the canal of Schlemm. Dilation of the pupil may push the root of the iris forward against the angle, and may produce pupillary block and thus precipitate an acute attack. Eyes with narrow anterior chamber angles are predisposed to acute angle-closure glaucoma attacks of various degrees of severity.

30

Secondary glaucoma is caused by any interference with the flow of aqueous humor from the posterior chamber into the anterior chamber and subsequently, into the canal of

Schlemm. Inflammatory disease of the anterior segment may prevent aqueous escape by causing complete posterior synechia in iris bombe and may plug the drainage channel with exudates. Other common causes are intraocular tumors, enlarged cataracts, central retinal vein occlusion, trauma to the eye, operative procedures and intraocular
5 hemorrhage.

Considering all types together, glaucoma occurs in about 2% of all persons over the age of 40 and may be asymptotic for years before progressing to rapid loss of vision. In cases where surgery is not indicated, topical beta-adrenoreceptor antagonists have
10 traditionally been the drugs of choice for treating glaucoma.

Prostaglandins were earlier regarded as potent ocular hypertensives; however, evidence accumulated in the last two decades shows that some prostaglandins are highly effective ocular hypotensive agents and are ideally suited for the long-term medical
15 management of glaucoma. (See, for example, Starr, M. S. Exp. Eye Res. 1971, 11, pp. 170-177; Bito, L. Z. Biological Protection with Prostaglandins Cohen, M. M., ed., Boca Raton, Fla. CRC Press Inc., 1985, pp. 231-252; and Bito, L. Z., Applied Pharmacology in the Medical Treatment of Glaucomas Drance, S. M. and Neufeld, A. H. eds., New York, Grune & Stratton, 1984, pp. 477-505). Such prostaglandins include PGF_{2α}, PGF_{1α}, PGE₂,
20 and certain lipid-soluble esters, such as C₁ to C₅ alkyl esters, e.g., 1-isopropyl ester, of such compounds.

Other uses of bimatoprost include use in hair growth, including scalp hair, eyelashes, and eyebrows. Bimatoprost has also shown promise in localized fat reduction
25 and inhibition of adipocyte differentiation.

Prostaglandins tend generally to occur in the form of oils or gums. Solid forms of some prostaglandins are known in the art. The solid forms may be prone to form change upon solid state manufacturing processes, such as granulation, tablet compression and hot
30 melt extrusion. For example, a solid form of a prostaglandin may take the form of an oil or gum during or after a granulation, tablet compression, or extrusion step.

It is known that many drug compounds exist in two or more crystalline forms, referred to as polymorphs. These polymorphs of the same molecule have identical

chemical properties but may exhibit different physical properties, such as melting point, solubility, hardness, etc.

5 Bimatoprost is known to occur in the solid form. US 2009/0163596 discloses bimatoprost crystalline Form I having a characteristic melting point range of 62-64°C. WO 2011/063276 A1 and US 8,629,185 B2 disclose crystalline bimatoprost polymorph II having an endothermic melting onset at about 70.9° C and a peak at 74.5° C in its DSC profile. WO 2012/164324 A1 discloses bimatoprost crystal Form II with a melting point range of 72-78°C. U.S. patent application serial number 14/136,914 discloses crystalline
10 forms of bimatoprost acid, with melting temperatures of 64.2°C (crystalline Form 1) and 66.4°C (crystalline Form 2), respectively. U.S. patent application serial number 14/136,914 also discloses that some bimatoprost polymorphs convert to an amorphous material after hot melt extrusion.

15 Preparation of amino acid salts of prostaglandin free acids are reported by DeLong et al. (see US 2010/0105775 A1). DeLong et al. teach that suitably, the salts have a melting point of at least about 35° C, or at least about 50° C.

20 When a drug compound exists in two or more crystalline forms, or polymorphs, the danger exists of less soluble polymorphic forms precipitating from a solution made from another more soluble but less stable form. The formation of crystals in an ophthalmic solution can cause serious injury to the eye. In addition, precipitation of the drug substance may cause an apparent reduction in potency and bioavailability of the product. Therefore it is important to formulate ocular solutions with drug concentrations
25 below the equilibrium solubility of the thermodynamically most stable polymorph of the drug.

A salt form of a prostaglandin may exhibit desirable solid state properties, such as high melting temperature and high crystallinity. Such properties may be advantageous
30 with respect to manufacturing, stability, and/or solubility. Such a salt form may tolerate solid dosage formulation processes such as granulation, tablet compression and hot melt extrusion.

There is a need for solid state prostaglandins, in particular bimatoprost, with desirable physical properties, including high melting temperature and high crystallinity, which may provide suitable stability, solubility, and tolerability to solid dosage formulation processes.

5

SUMMARY OF THE INVENTION

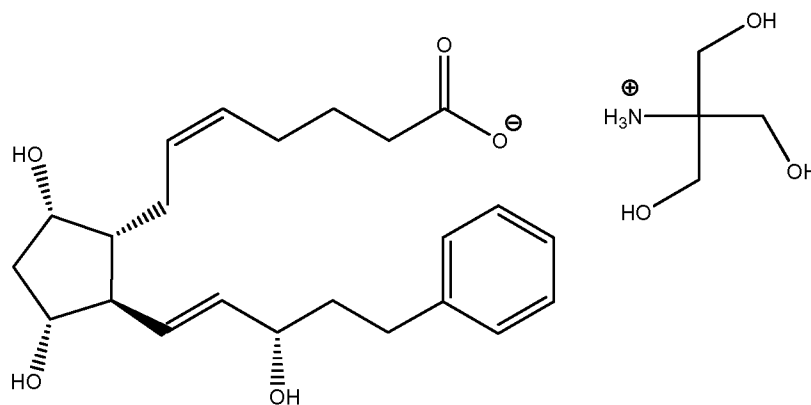
The present invention provides tromethamine salt of bimatoprost acid ((Z)-7-[3,5-Dihydroxy-2-((E)-3-hydroxy-5-phenyl-pent-1-enyl)-cyclopentyl]-hept-5-enoic acid) in its crystalline form (Form 1) and amorphous form. This compound may also be referred to as “tromethamine salt of bimatoprost acid,” “bimatoprost acid tromethamine salt” or “bimatoprost acid TRIS salt.” The invention crystalline Form 1 is useful for solid ocular implant formulations, gel formulations and topical ophthalmic compositions, such as topical ophthalmic solutions, microsuspensions and intraocular gel suspensions, which can be utilized in the treatment of various ocular conditions, such as, for example, ocular hypertension and glaucoma. In addition, bimatoprost acid tromethamine salt crystalline Form 1 is useful for solid or semisolid dosage formulations used to treat ocular hypertension and glaucoma. The crystalline Form 1 of tromethamine salt of bimatoprost acid may also be used in dermal solutions, lotions, creams, foams, ointments, gels, and suspensions, including gel suspension, micro-suspensions, or nano-suspension formulations, for example, for the treatment of hair growth (eyelash, eyebrow, scalp) and for localized fat reduction.

The invention amorphous form is useful for solid ocular implant formulations, gel formulations and topical ophthalmic compositions, such as topical ophthalmic solutions, microsuspensions and intraocular gel suspensions, which can be utilized in the treatment of various ocular conditions, such as, for example, ocular hypertension and glaucoma. In addition, bimatoprost acid tromethamine salt amorphous form is useful for solid or semisolid dosage formulations used to treat ocular hypertension and glaucoma. The amorphous form of tromethamine salt of bimatoprost acid may also be used in dermal solutions, lotions, creams, foams, ointments, gels, and suspensions, including gel suspension, micro-suspensions, or nano-suspension formulations, for example, for the treatment of hair growth (eyelash, eyebrow, scalp) and for localized fat reduction.

The present invention provides for pharmaceutical compositions comprising tromethamine salt of bimatoprost acid in crystalline Form 1, amorphous form, or mixtures thereof. The invention provides for pharmaceutical compositions comprising tromethamine salt of bimatoprost acid, wherein the compositions are prepared from the tromethamine salt of bimatoprost acid in crystalline Form 1, amorphous form, or mixtures thereof.

The invention provides for pharmaceutical compositions prepared from the tromethamine salt of bimatoprost acid in crystalline Form 1, wherein the composition is in the solid form. The invention also provides for pharmaceutical compositions prepared from the tromethamine salt of bimatoprost acid in crystalline Form 1, wherein the composition is not in the solid form. In some embodiments, the pharmaceutical composition may be a solution. It is understood that in embodiments of the invention reciting pharmaceutical compositions comprising tromethamine salt of bimatoprost acid in crystalline Form 1, wherein the composition is a solution, that the solution is prepared from tromethamine salt of bimatoprost acid in crystalline Form 1, and that the salt is dissolved in the solution.

In one embodiment, there is provided tromethamine salt of bimatoprost acid ((*Z*)-7-[3,5-Dihydroxy-2-((*E*)-3-hydroxy-5-phenyl-pent-1-enyl)-cyclopentyl]-hept-5-enoic acid), having the structure:



wherein the salt is in crystalline Form 1, having X-ray powder diffraction (XRPD) peaks at the diffraction angles of about: 6.8°, 10.2°, 13.4°, 16.1°, 17.3°, 18.0°, 19.1°, 19.4°, 20.0°, 20.4°, 21.1°, 22.9°, 23.3°, 24.5°, 25.2° and 33.7 ° of 2-theta (Cu, K-alpha1 radiation with wavelength 1.5406 Å).

In another embodiment, there is provided tromethamine salt of bimatoprost acid of crystalline Form 1 having XRPD peaks at the diffraction angles of: 6.80°, 10.18°, 13.35°, 16.10°, 17.30°, 17.99°, 19.05°, 19.35°, 19.95°, 20.40°, 21.11°, 22.85°, 23.25°, 24.46°, 25.22° and 33.73 ° of 2-theta (Cu, K-alpha1 radiation with wavelength 1.5406 Å).

In another embodiment, there is provided tromethamine salt of bimatoprost acid of crystalline Form 1 having the XRPD pattern as shown in Figure 6.

In another embodiment, there is provided tromethamine salt of bimatoprost acid of crystalline Form 1 having a melting enthalpy from about 103.6 J/g to about 119.9 J/g.

In another embodiment, there is provided tromethamine salt of bimatoprost acid of crystalline Form 1 having a melting temperature within the range of about 104-110°C.

In another embodiment, there is provided tromethamine salt of bimatoprost acid of crystalline Form 1 having a melting temperature of about 105 °C.

In another embodiment, there is provided tromethamine salt of bimatoprost acid of crystalline Form 1 having a melting temperature of about 105.6 °C.

In another embodiment, there is provided tromethamine salt of bimatoprost acid of crystalline Form 1 having a melting temperature of 105.6 °C.

In another embodiment, there is provided tromethamine salt of bimatoprost acid in amorphous form having a glass transition temperature (Tg) of about 29 °C.

In another embodiment of the invention, there are provided methods for the preparation of tromethamine salt of bimatoprost acid of crystalline Form 1. Such methods include, for example, lyophilization, crystallization in solvent or solvent mixtures by maturation, slurry, cooling, evaporation, and anti-solvent precipitation.

In another embodiment of the invention, there are provided methods for the preparation of tromethamine salt of bimatoprost acid in amorphous form. Such methods include, for example, freeze drying, rotary evaporation, and quenching the melt.

- 5 In another embodiment of the invention, there is provided a pharmaceutical composition comprising a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline Form 1.

- 10 In another embodiment, there is provided a pharmaceutical composition comprising a therapeutically effective amount of tromethamine salt of bimatoprost acid in amorphous form.

- 15 In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in crystalline Form 1 is in a pharmaceutically acceptable carrier.

- 20 In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in amorphous form is in a pharmaceutically acceptable carrier.

- 25 In another embodiment, there is provided a pharmaceutical composition comprising tromethamine salt of bimatoprost acid in crystalline Form 1 as a solid dosage form.

- 30 In another embodiment, there is provided a pharmaceutical composition comprising tromethamine salt of bimatoprost acid in amorphous form as a solid dosage form.

- 35 In another embodiment, there is provided a pharmaceutical composition comprising tromethamine salt of bimatoprost acid in crystalline Form 1 as a semi-solid dosage form.

In another embodiment, there is provided a pharmaceutical composition comprising tromethamine salt of bimatoprost acid in amorphous form as a semi-solid dosage form.

5 In another embodiment, there is provided a pharmaceutical composition including a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline Form 1, which is a topical formulation for ophthalmic or dermal administration. In some embodiments, the topical formulation is an aqueous or non-aqueous solution.

10 In another embodiment, there is provided a pharmaceutical composition including a therapeutically effective amount of tromethamine salt of bimatoprost acid in amorphous form, which is a topical formulation for ophthalmic or dermal administration. In some embodiments, the topical formulation is an aqueous or non-aqueous solution.

15 In one embodiment of the invention, there is provided a pharmaceutical composition comprising a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline Form 1 in a pharmaceutically acceptable carrier, such as an ophthalmically acceptable carrier. In a particular embodiment, the composition is suitable for ophthalmic administration. In another embodiment, the composition is suitable for
20 dermal administration.

 In another embodiment of the invention, there is provided a pharmaceutical composition comprising a therapeutically effective amount of tromethamine salt of bimatoprost acid in amorphous form in a pharmaceutically acceptable carrier, such as an
25 ophthalmically acceptable carrier. In a particular embodiment, the composition is suitable for ophthalmic administration. In another embodiment, the composition is suitable for dermal administration.

 In another embodiment, there is provided a pharmaceutical composition including
30 a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline Form 1, which is a dermal formulation. The dermal formulation may be an aqueous or non-aqueous solution, a lotion, an emulsion, a foam, a gel, a cream, an ointment, or a suspension, such as a gel-based micro-suspension or nano-suspension.

In another embodiment, there is provided a pharmaceutical composition including a therapeutically effective amount of tromethamine salt of bimatoprost acid in amorphous form, which is a dermal formulation. The dermal formulation may be an aqueous or non-aqueous solution, a lotion, an emulsion, a foam, a gel, a cream, an ointment, or a
5 suspension, such as a gel-based micro-suspension or nano-suspension.

In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in crystalline Form 1 is in a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is an aqueous solution.
10 In some embodiments, the bimatoprost acid in crystalline Form 1 is present in a concentration of 0.01% w/v to 0.1% w/v. In another embodiment, the pharmaceutical composition in crystalline Form 1 is present in a concentration of 0.03% w/v. In yet another embodiment, the tromethamine salt of bimatoprost acid in crystalline Form 1 is at a concentration selected from 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2,
15 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 to 1.0% w/v.

In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in amorphous form is in a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is an aqueous solution.
20 In some embodiments, the bimatoprost acid in amorphous form is present in a concentration of 0.01% w/v to 0.1% w/v. In another embodiment, the pharmaceutical composition in amorphous form is present in a concentration of 0.03% w/v. In yet another embodiment, the tromethamine salt of bimatoprost acid amorphous form is at a concentration selected from 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2,
25 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 to 1.0% w/v.

In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in crystalline Form 1 is in a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is selected from the
30 group consisting of ophthalmically acceptable diluents, buffers, hydrochloric acid, sodium hydroxide, preservatives, stabilizers, tonicity adjusters, viscosity-enhancing agents, chelating agents, surfactants and/or solubilizers and combinations thereof.

In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in amorphous form is in a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is selected from the group consisting of ophthalmically acceptable diluents, buffers, hydrochloric acid, sodium hydroxide, preservatives, stabilizers, tonicity adjustors, viscosity-enhancing agents, chelating agents, surfactants and/or solubilizers and combinations thereof.

In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in crystalline Form 1 is in a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is a gel dosage form.

In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in amorphous form is in a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is a gel dosage form.

In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in crystalline Form 1 is in a pharmaceutically acceptable carrier, wherein the composition is a suspension, a dermal solution, or a lotion.

In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in amorphous form is in a pharmaceutically acceptable carrier, wherein the composition is a suspension, a dermal solution, or a lotion.

In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in crystalline Form 1 is in a pharmaceutically acceptable carrier, wherein the composition is a gel suspension, a gel-based micro-suspension, or a nano-suspension.

In another embodiment, there is provided a pharmaceutical composition wherein the tromethamine salt of bimatoprost acid in amorphous form is in a pharmaceutically acceptable carrier, wherein the composition is a gel suspension, a gel-based micro-suspension, or a nano-suspension.

In another embodiment, there is provided a pharmaceutical composition comprising a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline Form 1, wherein the composition is used for localized fat reduction.

- 5 In another embodiment, there is provided a pharmaceutical composition comprising a therapeutically effective amount of tromethamine salt of bimatoprost acid in amorphous form, wherein the composition is used for localized fat reduction.

- 10 In another embodiment, there is provided a pharmaceutical composition comprising a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline Form 1, wherein the composition is used to promote hair growth.

- 15 In another embodiment, there is provided a pharmaceutical composition comprising a therapeutically effective amount of tromethamine salt of bimatoprost acid in amorphous form, wherein the composition is used to promote hair growth.

- 20 In another embodiment, there is provided a pharmaceutical composition comprising a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline Form 1, wherein the composition is used to treat elevated intraocular pressure or glaucoma.

- 25 In another embodiment, there is provided a pharmaceutical composition comprising a therapeutically effective amount of tromethamine salt of bimatoprost acid in amorphous form, wherein the composition is used to treat elevated intraocular pressure or glaucoma.

- 30 In another embodiment, there is provided methods for treating ocular hypertension or glaucoma. Such methods can be performed, for example, by administering to a subject in need thereof a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline Form 1 in an ophthalmically acceptable carrier. In some embodiments, the carrier is an emulsion, suspension, or solution. In a particular embodiment, the solution is a topical ophthalmic solution administered as eye drops.

In another embodiment, there is provided methods for treating ocular hypertension or glaucoma. Such methods can be performed, for example, by administering to a subject in need thereof a therapeutically effective amount of tromethamine salt of bimatoprost acid in amorphous form in an ophthalmically acceptable carrier. In some embodiments, the carrier is an emulsion, suspension, or solution. In a particular embodiment, the solution is a topical ophthalmic solution administered as eye drops.

In another embodiment, there is provided a method for treating ocular hypertension comprising administering to a subject in need thereof a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline Form 1 in an ophthalmically acceptable carrier. In a particular embodiment of the method, the ophthalmically acceptable carrier is selected from the group consisting of ophthalmically acceptable diluents, buffers, hydrochloric acid, sodium hydroxide, preservatives, stabilizers, tonicity adjusters, viscosity-enhancing agents, chelating agents, surfactants and/or solubilizers and combinations thereof. In yet another embodiment of the method, the tromethamine salt of bimatoprost acid in crystalline Form 1 is dosed at least once a day. In another embodiment, Form 1 is dosed once a day.

In another embodiment, there is provided a method for treating ocular hypertension comprising administering to a subject in need thereof a therapeutically effective amount of tromethamine salt of bimatoprost acid in amorphous form in an ophthalmically acceptable carrier. In a particular embodiment of the method, the ophthalmically acceptable carrier is selected from the group consisting of ophthalmically acceptable diluents, buffers, hydrochloric acid, sodium hydroxide, preservatives, stabilizers, tonicity adjusters, viscosity-enhancing agents, chelating agents, surfactants and/or solubilizers and combinations thereof. In yet another embodiment of the method, the tromethamine salt of bimatoprost acid in crystalline Form 1 is dosed at least once a day. In another embodiment, Form 1 is dosed once a day.

30

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts DSC data for Bimatoprost Acid (crystalline anhydrous) (top trace) compared to the Tromethamine Salt of Bimatoprost Acid (crystalline Form 1) (bottom

trace), showing a melting temperature for the salt at about 40°C higher than the melting temperature of the free acid.

Fig. 2 depicts DSC/TGA analyses of Tromethamine Salt of Bimatoprost Acid (Form 1), showing that the tromethamine salt of bimatoprost acid melted at about 107.2 °C (bottom trace) followed by a weight loss at a temperature greater than the melting temperature of the salt (top trace).

Fig. 3 depicts XRPD patterns of Tromethamine Salt of Bimatoprost Acid (Form 1) before (bottom trace) and after (top trace) drying process.

Fig. 4 shows the ¹H NMR of Tromethamine Salt of Bimatoprost Acid (Form 1, dissolved in DMSO-d₆). The absence of the ¹H NMR peak for the -COOH proton at about 12 ppm indicates formation of the salt.

Fig. 5 shows the pH-solubility of Tromethamine Salt of Bimatoprost Acid (Form 1): the intrinsic solubility is 1.385 mg/mL at pH 2 (solubility of the free acid). Solubility of the salt at pH above 6.5 is measured as higher than 250 mg/mL (≥ 25 w/v%).

Fig. 6 shows the XRPD pattern of Tromethamine Salt of Bimatoprost Acid (Form 1) including peak positions (2-theta) for the Characteristic peaks. The data were generated by using Cu K-alpha1 radiation with wavelength 1.5406 Å.

Fig. 7 shows the XRPD pattern and microscopy of amorphous Tromethamine Salt of Bimatoprost Acid with its characteristic halo.

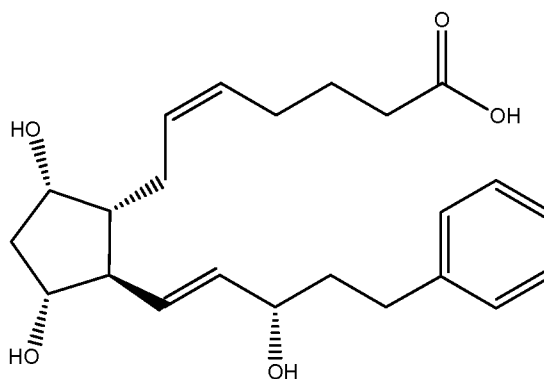
Fig. 8 shows Modulated differential scanning calorimetry (MDSC) of Amorphous Tromethamine Salt of Bimatoprost Acid. Reversed Heat Flow data (the lower curve) is indicating a glass transition temperature for the amorphous form at 29 °C (midpoint of the baseline shift).

DETAILED DESCRIPTION OF THE INVENTION

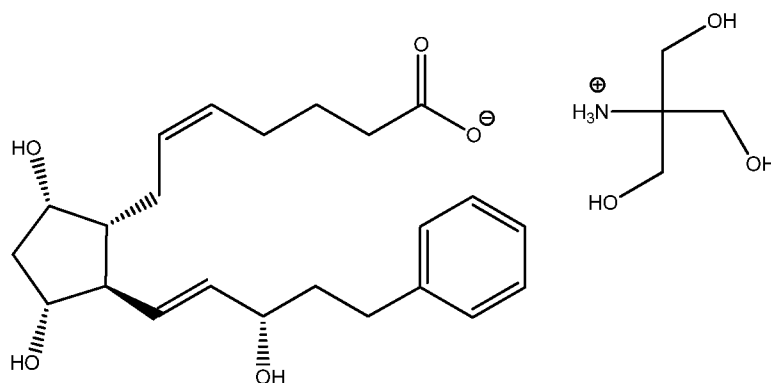
It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the

invention claimed. As used herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, “or” means “and/or” unless stated otherwise. Furthermore, use of the term “including” as well as other forms, such as “includes,” and “included,” is not limiting. The section headings used herein are for
 5 organizational purposes only and are not to be construed as limiting the subject matter described.

It is to be understood that “(Z)-7-[3,5-Dihydroxy-2-((E)-3-hydroxy-5-phenyl-pent-1-enyl)-cyclopentyl]-hept-5-enoic acid” and “bimatoprost acid” refer to the same
 10 compound and may be used interchangeably throughout. Bimatoprost acid has the following structure:



In addition, “tromethamine salt of (Z)-7-[3,5-Dihydroxy-2-((E)-3-hydroxy-5-phenyl-pent-1-enyl)-cyclopentyl]-hept-5-enoic acid” and “tromethamine salt of
 15 bimatoprost acid,” “bimatoprost acid tromethamine salt” and “bimatoprost acid TRIS salt” refer to the same compound and may be used interchangeably throughout. Tromethamine salt of bimatoprost acid has the structure:



20

In addition, it is to be understood that “crystalline form” and “polymorphic form” and (“Form 1”) may be used interchangeably throughout the specification.

5 Unless specific definitions are provided, the nomenclatures utilized in connection with, and the laboratory procedures and techniques of analytical chemistry, synthetic organic and inorganic chemistry described herein are those known in the art. Standard chemical symbols are used interchangeably with the full names represented by such symbols. Thus, for example, the terms “hydrogen” and “H” are understood to have
10 identical meaning. Standard techniques may be used for chemical syntheses, chemical analyses, and formulation.

The present invention provides tromethamine salt of bimatoprost acid in crystalline Form 1.

15

Tromethamine salt of bimatoprost acid crystalline Form 1 was characterized using X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), ion chromatography and ¹H NMR. The crystalline Form 1 of tromethamine salt of bimatoprost acid exhibits a distinct XRPD spectrum, which is set forth in Figure 6. The
20 pattern has characteristic peaks observed at 6.80°, 10.18°, 13.35°, 16.10°, 17.30°, 17.99°, 19.05°, 19.35°, 19.95°, 20.40°, 21.11°, 22.85°, 23.25°, 24.46°, 25.22° and 33.73 ° of 2-theta (Cu, K-alpha1 radiation with wavelength 1.5406 Å).

Tromethamine salt of bimatoprost acid of crystalline Form 1 has advantages over
25 the crystalline free acid of bimatoprost, such as a significantly higher melting temperature (~40 °C) (see Figure 1). This makes the tromethamine salt of bimatoprost acid of crystalline Form 1 desirable for hot melt extrusion and process manufacturing of implants and solid dosage forms, because the form does not change under these conditions. The salt (Form 1) has shown feasibility for extrusion. The salt (Form 1) was shown to be
30 physically stable for > 3 months at 25 °C/60% relative humidity (RH) in open container closure, and > 3 months at 40 °C/75% RH in closed container closure. Furthermore, the tromethamine salt (Form 1) has a faster dissolution rate than the free acid, and a high solubility (e.g., ≥250 mg/mL at pH 6.5; see Figure 5).

Tromethamine salt of bimatoprost acid may be prepared in crystalline Form 1 by using methods described herein. For example, tromethamine solution (1 equiv) is added to bimatoprost free acid (1 equiv), followed by the addition of water to dissolve the material. The resulting solution is rapidly frozen and freeze dried to obtain the

5 tromethamine salt of bimatoprost acid as a lyophilized solid. After lyophilization, the salt may be in crystalline Form 1, amorphous form, or an amorphous/crystalline mixture. The lyophilized salt is then crystallized from an organic solvent, such as ethyl acetate, by subjecting the lyophilized solid sample in the organic solvent to a series of heat/cool cycles. The heat/cool cycles may include heating to about 50°C at about 5°C/min,

10 holding at about 50°C for about 30 minutes, followed by cooling at about 0°C at about 0.1°C/min, and holding at about 0°C for about 1 hour under constant stirring. The recovered material (crystalline Form 1) is analyzed by XRPD.

In another example, tromethamine salt of bimatoprost acid in crystalline Form 1 is

15 prepared by reacting bimatoprost free acid and tromethamine base in methanol, followed by evaporation of the methanol to dryness. Then, isopropyl alcohol is added, and the mixture is heated to about 40°C. A solid is formed after about 30 minutes. Then isopropylacetate is added at about 40°C, followed by cooling to about 20°C and stirring for about 3 hours. The mixture is filtered to recover the tromethamine salt of bimatoprost

20 acid in crystalline Form 1.

Pharmaceutical compositions may be prepared by combining a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline Form 1 according to the invention, with pharmaceutically acceptable excipients, including excipients

25 suitable for ophthalmic or dermal administration, and by preparation of unit dosage forms suitable for topical ocular or dermal use. The therapeutically efficient amount typically is between about 0.0001 and about 5% (w/v), preferably about 0.001 to about 1.0% (w/v) in liquid formulations, or preferably about 0.01 to about 0.1% w/v and 0.01% w/v to about 0.03% w/v. An “effective amount” is an amount sufficient to accomplish a stated purpose

30 (e.g., achieve the effect for which it is administered, treat a disease, reduce one or more symptoms of a disease or improve the condition for hair growth). An example of an “effective amount” is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which can be referred to as a “therapeutically effective amount.” A “reduction” of a symptom or symptoms (and

grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). The actual amount effective for a particular application will depend, *inter alia*, on the condition being treated.

- 5 The present invention provides tromethamine salt of bimatoprost acid in amorphous form.

Tromethamine salt of bimatoprost acid may be prepared in amorphous form by using methods described herein, including lyophilization, rotary evaporation, or
10 preferably, by quenching the melt. For example, after preparing the tromethamine salt of bimatoprost acid by any of the methods described herein, including the methods described for the preparation of crystalline Form 1, the solid salt may then be heated up in a vacuum oven at about 120 °C, and the temperature maintained for about 10 minutes to melt the product. The samples are quenched in air and then cooled to about -20 °C (freezer) to
15 obtain an amorphous glass.

Pharmaceutical compositions may be prepared by combining a therapeutically effective amount of tromethamine salt of bimatoprost acid in amorphous form according to the invention, with pharmaceutically acceptable excipients, including excipients
20 suitable for ophthalmic or dermal administration, and by preparation of unit dosage forms suitable for topical ocular or dermal use. The therapeutically efficient amount typically is between about 0.0001 and about 5% (w/v), preferably about 0.001 to about 1.0% (w/v) in liquid formulations, or preferably about 0.01 to about 0.1% w/v and 0.01% w/v to about 0.03% w/v. An “effective amount” is an amount sufficient to accomplish a stated purpose
25 (e.g., achieve the effect for which it is administered, treat a disease, reduce one or more symptoms of a disease or improve the condition for hair growth). An example of an “effective amount” is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which can be referred to as a “therapeutically effective amount.” A “reduction” of a symptom or symptoms (and
30 grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). The actual amount effective for a particular application will depend, *inter alia*, on the condition being treated.

EXAMPLES

Example 1. Salt screening studies of bimatoprost free acid, and method of making Crystalline Form 1 of Bimatoprost Tromethamine Salt

5 The tromethamine salt of bimatoprost acid was prepared during salt screening studies on bimatoprost free acid, where basic counter ions (both organic and inorganic) were selected based on their pKa values and suitability for pharmaceutical use.

 The following salt formation techniques were used for salt screening on
10 bimatoprost acid:

a) Lyophilization: Salts were prepared via lyophilization of aqueous solutions of bimatoprost free acid (10 mg bimatoprost acid per vial, diluted to 100 μ L) and selected bases (1 molar equivalents). Bases which were supplied as liquids
15 were added neat whereas bases which were supplied as powders were added as 1M stock solutions as described in Table 1. The solutions were rapidly frozen using dry ice in order to avoid nucleation occurring prior to lyophilization. The resulting gums or solids were analysed by ^1H NMR to confirm salt formation.

20 b) Salt Formation via Direct Solvent Crystallization: For bases which were insoluble in water, the following procedure was employed. Experiments were conducted by directly adding the base (1 molar equivalent) to the bimatoprost free acid (10 mg per vial) in the crystallization solvent (100 μ l). Bases which
25 were supplied in liquid form were used directly whereas bases which were supplied as solids were added as 1M aqueous solutions. Any gums which resulted were matured for up to 10 days, and any solutions obtained were stored at 5°C. If no solid resulted, a drop of methanol was added to the gums to try and aid dissolution at 50°C, anti-solvent was added to solutions and all
30 experiments were matured again for a further two days. Salt formation was confirmed by ^1H NMR.

c) Salt Preparation Via Ion Exchange: A number of the bases were supplied as salts, therefore, ion exchange experiments with the sodium salt were carried

out. The sodium salt was initially prepared by lyophilization. The sodium salt was then dissolved in a suitable organic solvent, specifically, IPA for the calcium and magnesium salts; dichloromethane (DCM) for tridodecylmethylammonium bromide, tetraoctylammonium bromide, and 1- α -phosphatidylethanolamine; and chloroform for tetraoctadecylammonium bromide. The desired base (0.5-1 molar equivalents) was added as a stock solution, again in a suitable organic solvent (1M solution in methanol for calcium and magnesium chloride, minimum amount of DCM and a minimum amount of chloroform). In the case of the calcium and magnesium salt experiments, sodium chloride was precipitated and removed by filtration. The solvent was then allowed to evaporate to dryness leaving a gum. Equal volumes (0.5ml) of water and diethyl ether were added and the solution shaken. Any solid formed was isolated and analysed by XRPD. The sodium chloride and sodium bromide was removed from the quaternary amine experiments by washing with water to give two layers. The aqueous layer was removed and the organic layer allowed to evaporate to dryness. The resulting gums and solids were analyzed by ^1H NMR and XRPD.

d) Salt Formation Via Grinding: An attempt was also made to generate salts by grinding. In these experiments, 25 mg of the bimatoprost free acid and 1 molar equivalent of the base were placed in a stainless steel milling container along with a stainless steel ball and shaken horizontally for 1 minute at 25Hz. Any solids collected were analyzed by XRPD.

Table 1 provides a summary of the bases and salt forming techniques used in small scale salt screening studies on bimatoprost acid, and the observed physical forms for the resulting solids. The majority of the counter ions resulted in crude materials that appeared as oils and gums. Surprisingly, when tromethamine was used as the base, a crystalline solid was obtained.

Table 1. Bases and Salt Preparation Techniques.

Base (Source of Basic Counter Ion)	pKa	Addition Type	Salt Forming Technique	Solid Crystalline Salt Obtained
Tromethamine	8.07	Isopropylacetate (iPrOAc)	Lyophilization	Yes

Potassium	14.00	2.56M solution of KOEt in EtOH	Lyophilization	No
Sodium	14.00	2.68M solution of NaOEt in EtOH	Lyophilization	No
Arginine	13.20	1M aq. solution	Lyophilization	No
Calcium	12.70	1M solution of CaCl ₂ in MeOH	Ion exchange	Partially crystalline, no stoichiometry
Magnesium	11.40	1M solution of MgCl ₂ in MeOH	Ion exchange	No
Choline	11.00	50% aq. Solution	Lyophilization	No
Ethylenediamine	10.09	Neat	Lyophilization	No
Benzathine	9.99	Neat	Direct crystallization	No
Piperazine	9.82	1M aq. Solution	Lyophilization	No
2-diethylamino ethanol	9.58	Neat	Lyophilization	No
Hydroxyethyl pyrrolidine	9.44	Neat	Lyophilization	No
Ammonium hydroxide	9.27	Neat	Lyophilization	No
Dimethylamino ethanol	8.83	Neat	Lyophilization	No
N-ethylglucamine	8.03	1M aq. Solution	Lyophilization	No
N-methylglucamine	8.03	1M aq. Solution	Lyophilization	No
Hydroxyethylmorpholine	7.39	Neat	Lyophilization	No
Citrulline	7.00	1M aq. Solution	Direct crystallization	No
Benethamine	4.46	Neat	Direct crystallization	No
Tridodecylmethylammonium chloride	-	Minimum amount of DCM	Ion exchange	No
Tetraoctylammonium bromide	-	Minimum amount of DCM	Ion exchange	No
Tetraoctadecylammonium bromide	-	Minimum amount of CHCl ₃	Ion exchange	No
L- α -Phosphatidylethanolamine, dioleoyl	-	Minimum amount of DCM	Direct crystallization	No

The tromethamine salt of bimatoprost acid obtained as described above was analyzed by XRPD, DSC, and ¹H NMR. The melting temperature of the tromethamine salt (105.6°C) was significantly higher than melting temperature of the free acid (66.4°C), as determined by DSC (Figure 1). The formation of the tromethamine salt was confirmed by ¹H-NMR and ion chromatography, which confirmed the structure of the molecule and a 1:1 ratio of the acid and the counter ion. It was concluded that bimatoprost acid tromethamine salt was a pure crystalline salt suitable for pharmaceutical development.

In a first scale up procedure, the preparation and crystallization of the tromethamine salt of bimatoprost acid (Form 1) was conducted on about 100 mg scale. To bimatoprost free acid (100 mg) was added 1 equivalent of base (tromethamine) as a 1M aqueous solution (254μl). Water (2 ml) was added to dissolve the material and then

the solution was rapidly frozen and freeze dried to obtain the tromethamine salt as a lyophilized solid. The salt was then crystallized from ethyl acetate as follows. The lyophilized solid sample in ethyl acetate was subjected to initial heat/cool cycles using an Anachem React-Array. The sample was then subjected to three heat/cool cycles by using the following procedure: heating to 50°C at 5°C/min, holding at 50°C for 30 minutes, followed by cooling to 0°C at 0.1°C/min, holding at 0°C for 1 hour under constant stirring at 500rpm. The recovered material (crystalline form 1) was analyzed by XRPD (Figure 6), ¹H NMR, thermal analysis, and gravimetric vapor sorption (GVS) analysis, as summarized in Table 2.

10

Table 2. Characterization of Tromethamine Salt of Bimatoprost Acid (Form 1)

Technique	Results
XRPD	Crystalline Form 1 (Figure 6)
¹ H NMR	Consistent with structure Stoichiometry 1.0:1.0 (Parent:Counter ion)
Thermal Analysis	TGA: Loss of 1.0 % w/w from 40 °C to 150 °C (Process solvent). Loss of 25.4 % w/w from 150 °C to 350 °C (possible salt dissociation, theoretical value for the loss of tromethamine: 23.7 % w/w). DSC: melting onset at 104-110 °C (ΔH _f = 103 to 119 J/g).
GVS analysis	90% RH, no deliquescence, no hydrate formed.

15 In another scale-up procedure, tromethamine salt of bimatoprost acid (crystalline Form 1) was prepared at multi-gram scale by using the following procedure.

Bimatoprost free acid was reacted with tromethamine base in methanol, followed

by the evaporation of the methanol to dryness. Then isopropyl alcohol (IPA) was added, and the mixture was heated to 40°C. No seeding was required. A solid was formed within 30 minutes. Then, isopropylacetate (iPrOAc) was added at 40°C, then cooled to 20°C and stirred for 3 hours, and then filtered to recover the tromethamine salt of bimatoprost acid (Form 1) in ~85 %. The XRPD pattern of the recovered salt was consistent with crystalline Form 1 shown in Figure 6, confirming that the multigram scale-up procedure generated crystalline Form 1.

When stored at or above 80% RH, tromethamine salt of bimatoprost acid behaves hygroscopically, with significant moisture uptake observed. Without being bound by any particular theory, the significant uptake of moisture may be a result of the large specific surface area of the material. On desorption, the adsorbed water was rapidly lost with no equilibrium established. At the end of the gravimetric vapor sorption (GVS) cycle, at 40% RH, the overall sample mass increased by 5 wt%. XRPD re-analysis of the sample post GVS analysis showed no change in the crystalline Form 1 (Figure 3). Thermal analyses by TGA and DSC showed that the tromethamine salt of bimatoprost acid melted at about 107.2 °C followed by a weight loss (Fig. 2).

Example 2. Method of Making the Amorphous Form of Tromethamine Salt of Bimatoprost Acid

Attempts were made to prepare amorphous tromethamine salt of bimatoprost acid by freeze drying (using a mixture of tert-butanol / water (50/50 v/v) as solvent), rotary evaporation, and by quenching the melt. Quenching the melt was selected as a suitable method for the preparation of the amorphous material, followed by characterization.

In one experiment, Tromethamine Salt of Bimatoprost acid (prepared as described in Example 1) was heated in a vacuum oven at 120 °C, the temperature was maintained for 10 minutes to melt the product. The samples were quenched in air and then cooled to -20 °C (freezer) to obtain an amorphous glass. The glass transition (T_g) was determined as about 29 °C by modulated differential scanning calorimetry (MDSC).

Characterization of Amorphous Tromethamine Salt of Bimatoprost Acid:

The characterization of the prepared amorphous material is summarized in Table 3. The broad X-ray powder diffraction pattern obtained from the amorphous material

- showed a continuous intensity distribution indicative of an amorphous form, consisting of a non-crystalline phase material (see Fig. 7). The ^1H NMR spectrum was consistent with the expected structure, therefore, no measurable degradation or dissociation of the produced salt was observed. The glass transition of the amorphous material of
- 5 Bimatoprost Acid Tromethamine Salt was determined as approximately 29 °C by the modulated differential calorimetry (MDSC); no re-crystallization was observed on further heating up to 100 °C.

10

Table 3. Characterization of Amorphous Bimatoprost Acid Tromethamine Salt

Compound	Bimatoprost Acid Tromethamine Salt
Preparation Method	Quenching the melt
XRPD	Amorphous
^1H -NMR	Consistent with structure. Stoichiometry 1.0:1.0 (Parent:Counterion)
MDSC	Tg at about 29°C

15

I. Instrument and Methodology Details

A. X-Ray Powder Diffraction (XRPD)

- 20 X-Ray Powder Diffraction patterns were collected on a Bruker D8 diffractometer using Cu and anode (40 kV, 40 mA) as the X-ray source that generates $\text{K}\alpha_1$ radiation with a wavelength of 1.5406 Å, theta/2-theta goniometer, and divergence of V4 and receiving slits, a Ge monochromator and a Lynxeye detector. The instrument was performance checked using a certified Corundum Standard (NIST 1976). The software used for data
- 25 collection was Diffrac Plus XRD Commander v2.6.1 and the data were analyzed and presented using Diffrac Plus EVA v13.0.0.2 or v15.0.0.0.

Samples were run under ambient conditions as flat plate specimens. The solid sample was gently packed into a cavity cut into a polished, zero-background (510) silicon

wafer. The sample was rotated in its own plane during analysis. The details of the data collection are:

- Angular range: 2 to 42 °(2-theta);
- Step size: 0.05 °(2-theta); and,
- 5 • Collection time: 0.5 sec./step.

B. Nuclear Magnetic Resonance (NMR):

¹H NMR spectra were collected on a Bruker 400MHz instrument equipped with an auto-sampler and controlled by a DRX400 console. Automated experiments were acquired using ICON NMR v4.0.4 running with Topspin v1.3 using the standard Bruker loaded experiments. For non-routine spectroscopy, data were acquired through the use of Topspin alone. Samples were prepared in DMSO-d₆, unless otherwise stated. Off-line analysis was carried out using Topspin v1.3 or ACD SpecManager v12.5.

15

C. Differential Scanning Calorimetry (DSC):

DSC data were collected on a TA Instruments Q2000 equipped with a 50 position autosampler. The calibration for thermal capacity was carried out using sapphire and the calibration for energy and temperature was carried out using certified indium. Typically 3 to 3.5 mg of each sample, in an aluminum pan, was heated at 10 °C/min from 25 °C to 130 °C. A purge of dry nitrogen at 50 ml/min was maintained over the sample.

20

Modulated temperature DSC ("MDSC") was carried out using an underlying heating rate of 2 °C/min and temperature modulation parameters of ± 0.318 °C (amplitude) every 60 seconds (period). The instrument control software was Advantage for Q Series v2.8.0.394 and Thermal Advantage v5.2.6 and the data were analyzed using Universal Analysis v4.7A or v4.4A.

25

30 D. Thermo-Gravimetric Analysis (TGA):

TGA data were collected on a Mettler TGA/SDTA 851e equipped with a 34-position autosampler. The instrument was temperature calibrated using certified indium. Typically, 5 to 30 mg of each sample was loaded onto a pre-weighed aluminum crucible and was heated at 10 °C/min from ambient temperature to 350 °C. A nitrogen

35

purge at 50 mL/min was maintained over the sample. The instrument control and data analysis software was STARe v9.20.

E. Ion Chromatography (IC):

5

Data were collected on a Metrohm 761 Compact IC using IC Net software v2.3. Accurately weighed samples were prepared as stock solutions in an appropriate dissolving solution and diluted appropriately prior to testing. Quantification was achieved by comparison with standard solutions of known concentration of the ion being analysed.

10

Table 4. IC Method Parameters for Cation Chromatography

Type of method	Cation exchange
Column	Metrosep C 2 – 250 (4.0 x 250 mm)
Column Temperature (°C)	Ambient
Injection volume (µl)	20
Detection	Conductivity detector
Flow Rate (ml/min)	1.0
Eluent	4.0 mM Tartaric acid, 0.75 mM Dipicolinic acid in 5% aqueous acetone

F. pH-Solubility Determination:

15

The pH solubility profile for tromethamine salt of bimatoprost acid of Form 1 was determined by suspending a sufficient amount of the compound in aqueous media, then placing on a shaker at 25°C, 750rpm for 48hrs. The pH was checked, and adjusted if necessary, at intervals of 1, 5, 23, 28 and 47hrs. An aliquot of sample was taken off at 24hrs and the final pH was recorded. The suspension was then filtered through a glass fiber C filter. The filtrate was then diluted by an appropriate factor. This was repeated at 20 48 hours. Quantitation was by HPLC with reference to 6 standard solutions of concentrations between 0.3873-0.0008 mg/ml dissolved in 66:21:13 H₂O:ACN:MeOH. The solubility vs. pH plot (Fig. 5) was fit to the theoretical relationship presented Henderson-Hasselbalch equation: $S = S_o (1 + 10^{(pH - pK_a)})$ for the free acid by using the 25 measured pK_a of 4.76 for bimatoprost acid.

G. Ophthalmic Preparations:

For ophthalmic application, preferably solutions are prepared using a physiological saline solution as a major vehicle. The pH of such ophthalmic solutions should preferably
5 be maintained between 4.5 and 8.0 with an appropriate buffer system, a neutral pH being preferred but not essential. The formulations may also contain conventional, pharmaceutically acceptable preservatives, stabilizers and surfactants.

Preferred preservatives that may be used in the pharmaceutical compositions of the
10 present invention include, but are not limited to, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric acetate and phenylmercuric nitrate. Likewise, various preferred vehicles may be used in the ophthalmic preparations of the present invention. These vehicles include, but are not limited to, polyvinyl alcohol, povidone, hydroxypropyl methyl cellulose, poloxamers, carboxymethyl cellulose, hydroxyethyl cellulose
15 cyclodextrin and purified water. The pharmaceutical compositions of the present invention may include a surfactant; however, surfactants are not necessary as surfactants are added to formulations to dissolve or increase solubility of the drug in formulation vehicles. In case of tromethamine salt, the solubility is high enough (~25 w/v% at pH 6.8) so that no surfactant is needed.

20

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

25 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. Acids or bases may be used to adjust the pH of these formulations as needed.

30 In a similar vein, an ophthalmically acceptable antioxidant for use in the present invention includes, but is not limited to, sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene.

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

5 The ingredients are usually used in the following amounts:

Ingredient Amount (% w/w) active ingredient about 0.001-5 preservative, 0-0.10 vehicle, 0-40 tonicity adjustor, 0-10 buffer, 0.01-10 pH adjustor, q.s. pH 4.5-7.5, antioxidant as needed, surfactant as needed, purified water as needed to make 100%.

10

The actual dose of the active compounds of the present invention depends on the specific compound, and on the condition to be treated; the selection of the appropriate dose is well within the knowledge of the skilled artisan.

15 The ophthalmic formulations of the present invention are conveniently packaged in forms suitable for metered application, such as in containers equipped with a dropper, to facilitate application to the eye. Containers suitable for drop-wise application are usually made of suitable inert, non-toxic plastic material, and generally contain between about 0.5 and about 15 ml solution. One package may contain one or more unit doses.

20

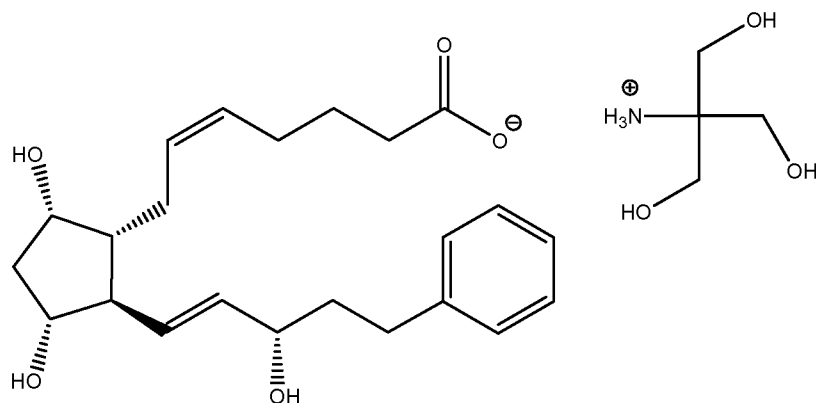
Especially preservative-free solutions are often formulated in non-resealable containers containing up to about ten, preferably up to about five units doses, where a typical unit dose is from one to about 8 drops, preferably one to about 3 drops. The volume of one drop usually is about 20-35 μ l.

25

While this invention has been described with respect to these specific examples, it is understood that other modifications and variations are possible without departing from the spirit of the invention.

WHAT IS CLAIMED IS:

1. Tromethamine salt of bimatoprost acid ((*Z*)-7-[3,5-Dihydroxy-2-((*E*)-3-hydroxy-5-phenyl-pent-1-enyl)-cyclopentyl]-hept-5-enoic acid), having the structure:



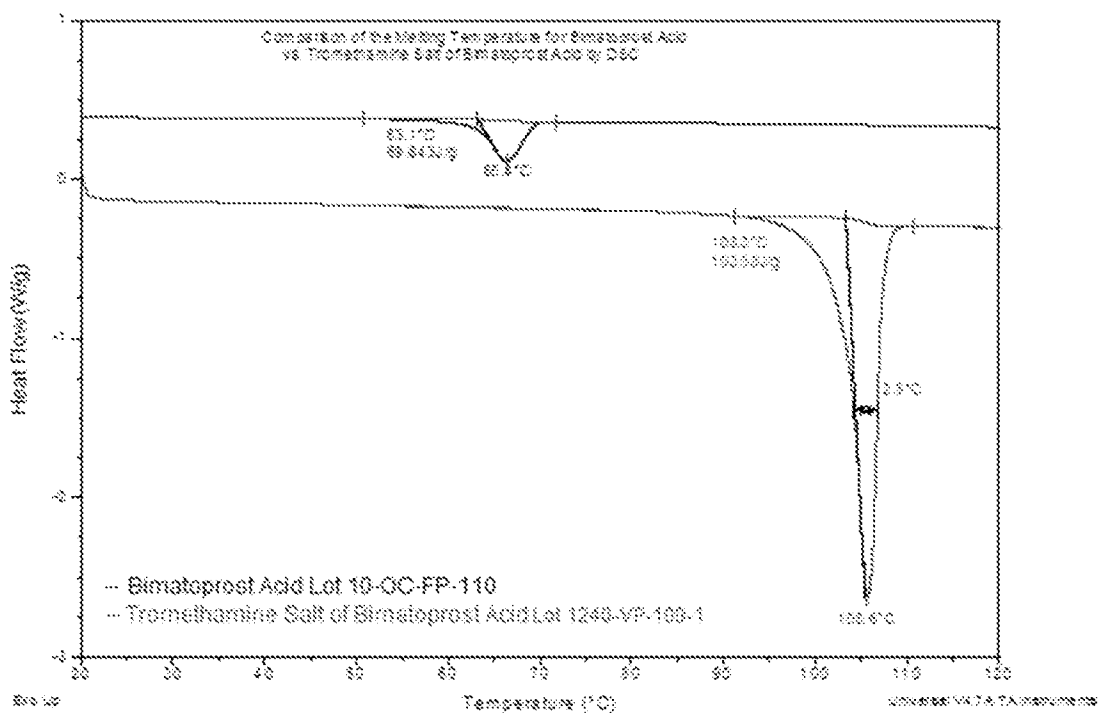
wherein the salt is in crystalline Form 1, having an X-ray powder diffraction pattern with peaks at 6.80°, 10.18°, 13.35°, 16.10°, 17.30°, 17.99°, 19.05°, 19.35°, 19.95°, 20.40°, 21.11°, 22.85°, 23.25°, 24.46°, 25.22° and 33.73 ° of 2-theta (Cu, K-alpha1 radiation with wavelength 1.5406 Å).

2. The crystalline Form 1 of claim 1 having the X-ray powder diffraction pattern as shown in Figure 6.
3. The crystalline form of claim 1 having a melting enthalpy from about 103.6 J/g to about 119.9 J/g.
4. The crystalline form of claim 1 having a melting temperature within the range of about 104-110°C .
5. The crystalline form of claim 1 having a melting temperature of about 105 °C.
6. The crystalline form of claim 1 having a melting temperature of about 105.6 °C.
7. A pharmaceutical composition comprising a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline Form 1.

8. The pharmaceutical composition of claim 7 wherein the tromethamine salt of bimatoprost acid in crystalline Form 1 is in a pharmaceutically acceptable carrier.
9. The pharmaceutical composition of claim 7, wherein the pharmaceutical composition is a suspension, solution, foam, gel, cream, ointment, emulsion, or lotion.
10. The pharmaceutical composition of claim 9, wherein the suspension is a gel suspension, a gel-based micro-suspension, or a nano-suspension.
11. The pharmaceutical composition of claim 7, wherein the pharmaceutical composition is a solid dosage form.
12. The pharmaceutical composition of claim 7 for the treatment of elevated intraocular pressure, glaucoma, or localized fat reduction, or for promotion of hair growth.
13. A method for treating ocular hypertension comprising administering to a subject in need thereof a therapeutically effective amount of tromethamine salt of bimatoprost acid in crystalline form in an ophthalmically acceptable carrier.
14. Use of tromethamine salt of bimatoprost acid crystalline Form 1 of claim 1 for the manufacture of a medicament for the treatment of elevated intraocular pressure, glaucoma, or localized fat reduction, or for promotion of hair growth.

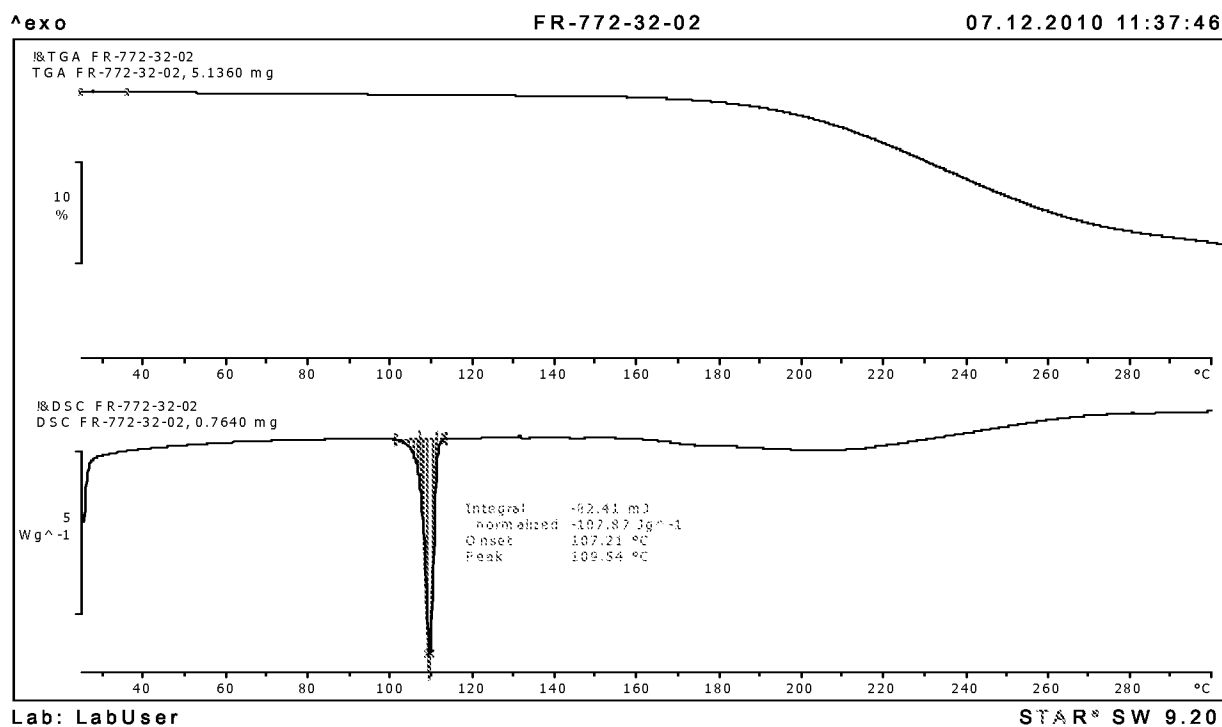
1/8

Figure 1: DSC data for Bimatoprost Acid (crystalline anhydrous) (top trace) compared to the Tromethamine Salt of Bimatoprost Acid (crystalline Form 1) (bottom trace), showing a melting temperature for the salt at about 40°C higher than the melting temperature of the free acid.



2/8

Figure 2: DSC/TGA analyses of Tromethamine Salt of Bimatoprost Acid (Form 1) show that the tromethamine salt of bimatoprost acid melted at about 107.2 °C (bottom trace) followed by a weight loss at a temperature greater than the melting temperature of the salt (top trace).



3/8

Figure 3: XRPD patterns of Tromethamine Salt of Bimatoprost Acid (Form 1) before (bottom trace) and after (top trace) drying process.

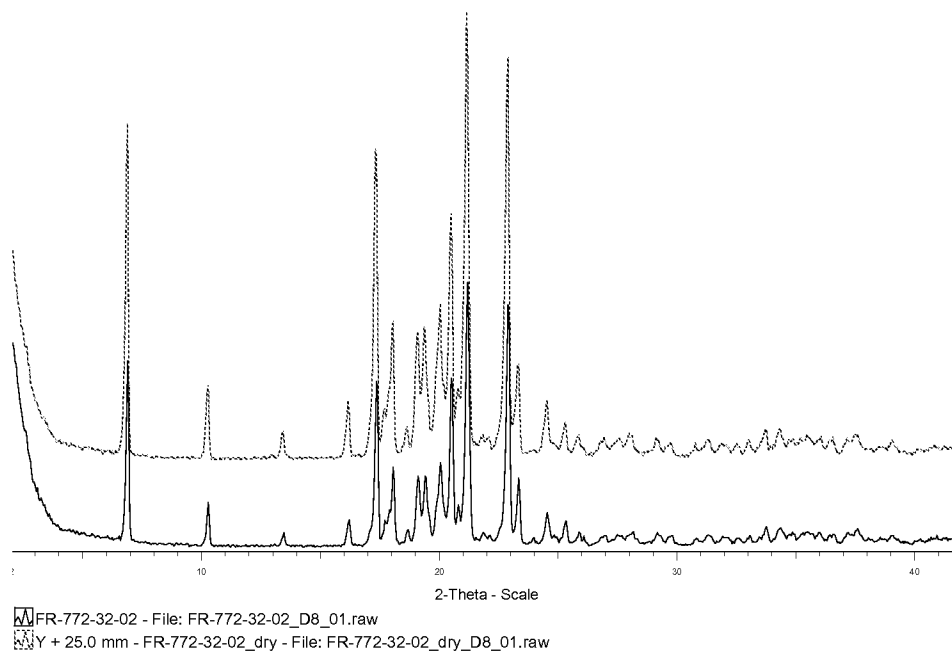


Figure 4: ^1H NMR of Tromethamine Salt of Bimatoprost Acid (Form 1 dissolved in DMSO-d_6). The absence of ^1H NMR peak for the $-\text{COOH}$ proton at about 12 ppm indicates formation of the salt.

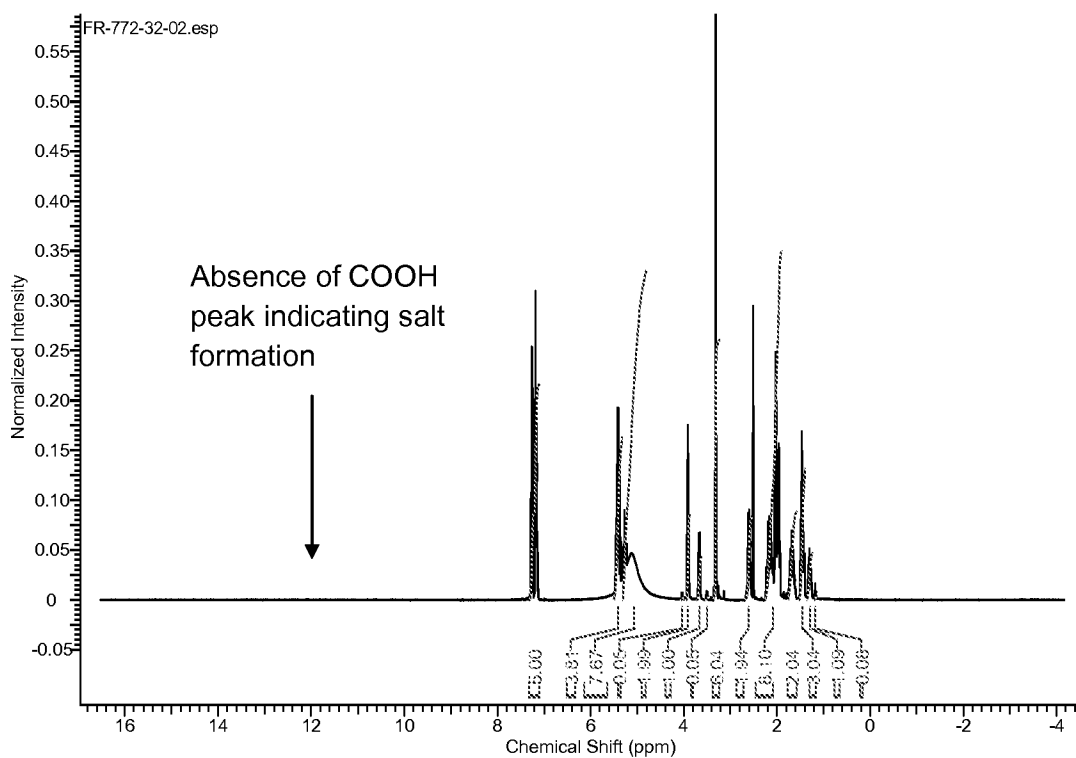
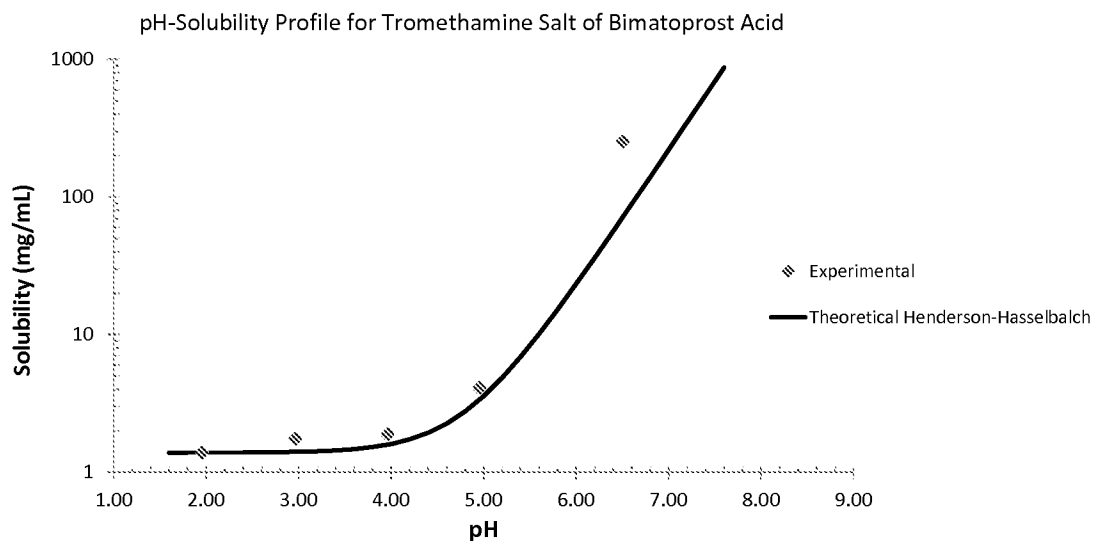


Figure 5: pH-Solubility of Tromethamine Salt of Bimatoprost Acid (Form 1): the intrinsic solubility is 1.385 mg/mL at pH 2 (solubility of the free acid). Solubility of the salt at pH above 6.5 is measured as higher than 250 mg/mL (≥ 25 w/v%).



6/8

Figure 6: XRPD pattern of Tromethamine Salt of Bimatoprost Acid (Form 1) including peak positions (2-theta) for the Characteristic peaks. The data were generated by using Cu K-alpha1 radiation with wavelength 1.5406 Å.

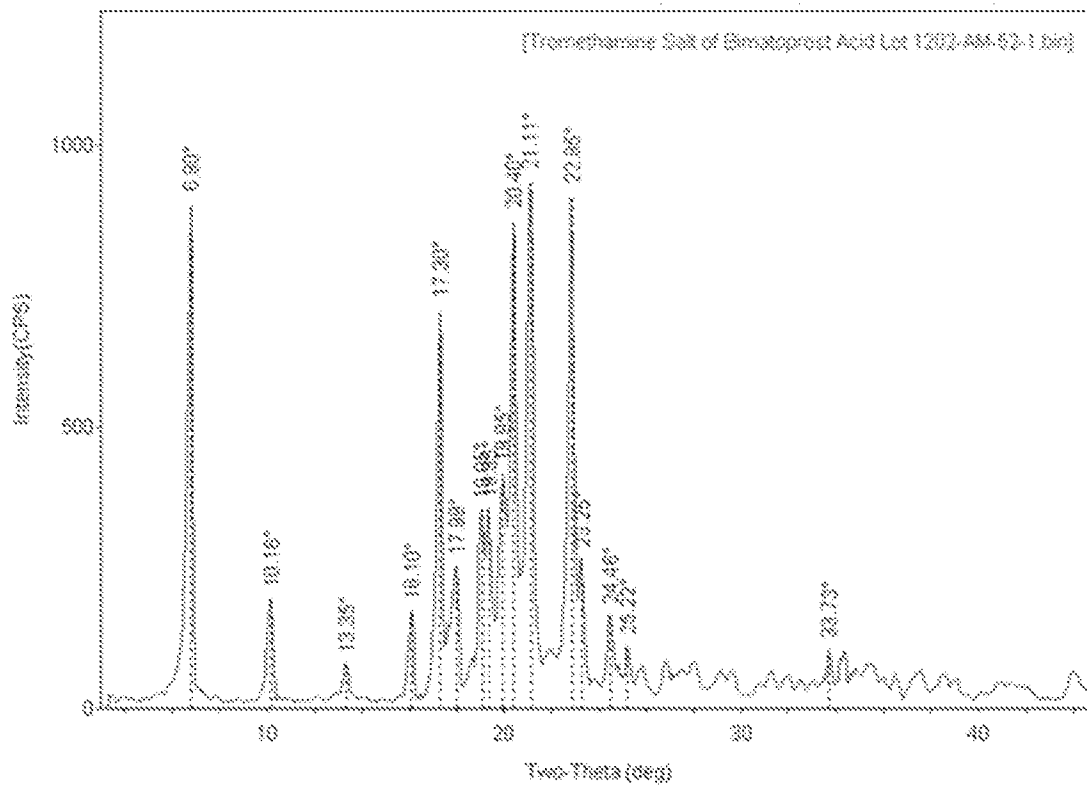


Figure 7: XRPD pattern and microscopy of amorphous Tromethamine Salt of Bimatoprost Acid with its characteristic halo.

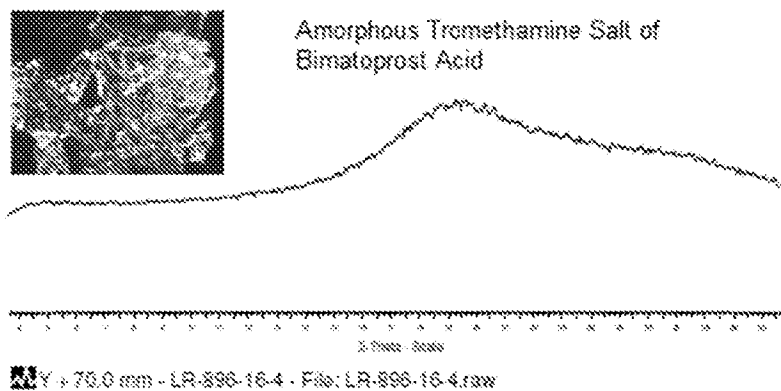
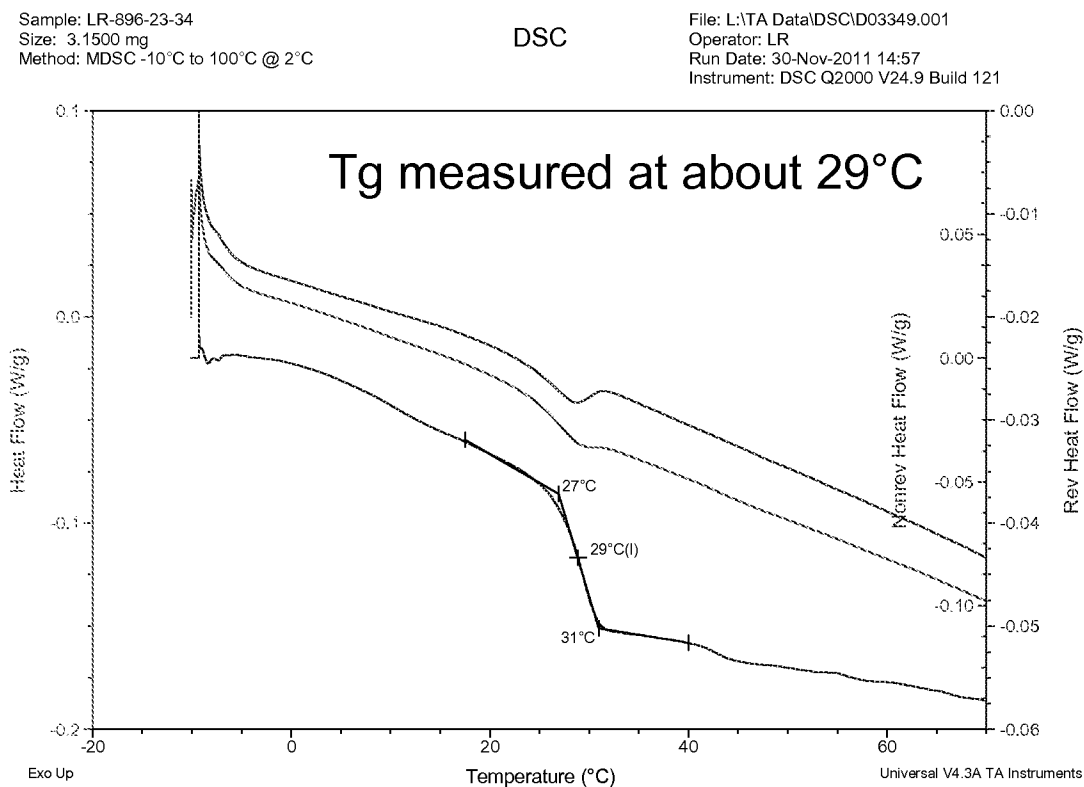


Figure 8: Modulated differential scanning calorimetry (MDSC) of Amorphous Tromethamine Salt of Bimatoprost Acid. Reversed Heat Flow data (the lower curve) is indicating a glass transition temperature for the amorphous form at 29 °C (midpoint of the baseline shift).



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/044658

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07C59/54 C07C215/10 A61K31/192 A61P27/06
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)

C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where 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.
X	WO 2012/164324 A1 (CHINOIN ZRT [HU]; HAVASI GABOR [HU]; KISS TIBOR [HU]; HORTOBAGYI IREN) 6 December 2012 (2012-12-06) cited in the application page 20, line 21 - page 21, line 6; figures IX, X	1-14
X	----- US 2010/105775 A1 (DELONG MITCHELL A [US] ET AL) 29 April 2010 (2010-04-29) cited in the application claim 1; examples 1-74	1-14
E	----- WO 2014/106204 A1 (ALLERGAN INC) 3 July 2014 (2014-07-03) claim 3; figure 3 -----	1-14

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

☒ 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

16 February 2015

Date of mailing of the international search report

25/02/2015

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Matés Valdivielso, J

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2014/044658

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012164324 A1	06-12-2012	CA 2837261 A1 CN 103649047 A EP 2723714 A1 JP 2014527026 A TW 201311629 A US 2014135503 A1 WO 2012164324 A1	06-12-2012 19-03-2014 30-04-2014 09-10-2014 16-03-2013 15-05-2014 06-12-2012
US 2010105775 A1	29-04-2010	US 2010105775 A1 US 2014031423 A1	29-04-2010 30-01-2014
WO 2014106204 A1	03-07-2014	US 2014187637 A1 WO 2014106204 A1	03-07-2014 03-07-2014