(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 2 807 178 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

(15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Claims EN 1

(48) Corrigendum issued on:
29.11.2017 Bulletin 2017/48

(45) Date of publication and mention of the grant of the patent:
17.05.2017 Bulletin 2017/20

(21) Application number: **13741502.2**

(22) Date of filing: **25.01.2013**

(51) Int Cl.:
C07H 19/167 *(2006.01)* ***A61K 31/7076*** *(2006.01)*
A61P 27/02 *(2006.01)* ***A61P 27/06*** *(2006.01)*

(86) International application number:
PCT/US2013/023166

(87) International publication number:
WO 2013/112850 (01.08.2013 Gazette 2013/31)

(54) **ANHYDROUS POLYMORPHS OF (2R,3S,4R,5R)-5-(6-(CYCLOPENTYLAMINO)-9H-PURIN-9-YL)-3,4-DIHYDROXYTETRAHYDROFURAN-2-YL) } METHYL NITRATE AND PROCESSES OF PREPARATION THEREOF**

WASSERFREIE POLYMORPHE VON (2R,3S,4R,5R)-5-(6-(CYCLOPENTYLAMINO)-9H-PURIN-9-YL)-3,4-DIHYDROXYTETRAHYDROFURAN-2-YL) } METHYLNITRAT UND VERFAHREN ZUR HERSTELLUNG DAVON

POLYMORPHES ANHYDRES DE NITRATE DE MÉTHYLE [(2R,3S,4R,5R)-5-(6-(CYCLOPENTYLAMINO)-9H-PURIN-9-YLE)-3,4-DIHYDROXYTETRAHYDROFURAN-2-YLE)]ET PROCESSUS DE PRÉPARATION DE CEUX-CI

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **26.01.2012 US 201261591037 P**

(43) Date of publication of application:
03.12.2014 Bulletin 2014/49

(60) Divisional application:
17170990.0 / 3 235 825

(73) Proprietor: **Inotek Pharmaceuticals Corporation**
Lexington, MA 02421 (US)

(72) Inventor: **MCVICAR, William, K.**
Sudbury, MA 01776 (US)

(74) Representative: **Soames, Candida Jane**
Maschio & Soames IP Limited
30 Carlton Crescent
Southampton SO15 2EW (GB)

(56) References cited:
WO-A1-2010/127210 **US-A1- 2009 062 314**
US-B2- 7 423 144

EP 2 807 178 B9

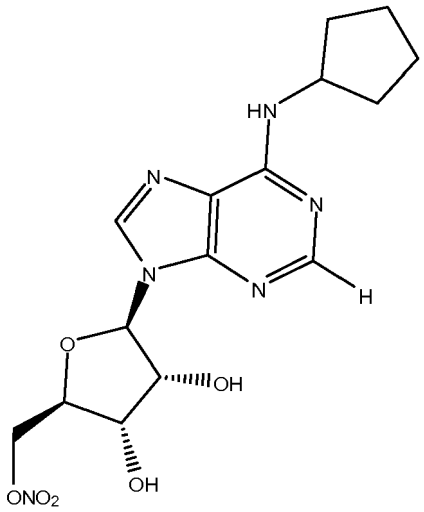
Description

FIELD OF THE INVENTION

[0001] The present invention provides a novel anhydrous polymorph form of [2R,3S,4R,5R)-5-(6-(cyclopentylamino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)] methyl nitrate (Compound A), pharmaceutical compositions containing the novel anhydrous polymorph form, and processes of preparation of the novel anhydrous polymorph form.

BACKGROUND OF THE INVENTION

[0002] Compound A is represented by the following structure



[(2R,3S,4R,5R)-5-(6-(cyclopentylamino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)] methyl nitrate,

[0003] Compound A is a selective adenosine A_1 receptor agonist and is of particular use in the treatment of elevated intra-ocular pressure as described in PCT/US2010/033112 (published as WO2010/127210).

[0004] Compound A can be prepared using the procedures described in US Patent No. 7,423,144, US 20090062314, and WO2010/127210.

[0005] Many pharmaceutical solids can exist in different physical forms. Polymorphism can be characterized as the ability of a drug substance to exist in two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice.

[0006] Polymorphs of a pharmaceutical solid can have different physical and solid state chemical properties. The most stable polymorphic form of a drug substance is often used because it has the lowest potential for conversion from one polymorphic form to another.

[0007] A particular crystalline form of a compound can have physical properties that differ from those of other polymorphic forms and such properties can influence the physico-chemical and pharmaceutical processing of the compound, particularly when the compound is prepared or used on a commercial scale. Such differences may alter the mechanical handling properties of the compound, such as dispersion in a blend of solid formulation excipients or within a suspension formulation. Polymorphs are also known in some cases to have different chemical stability profiles and different solubility of the solid material. As a result of these potential polymorph-specific physiochemical differences, the discovery of new polymorphic forms provides a new opportunity to improve the manufacturing or characteristics of a pharmaceutical end product.

[0008] Further, new polymorphic forms of a drug substance can display different melting point, hygroscopicity, stability, solubility and/or dissolution rate, crystallinity, crystal properties, and formulation handling characteristics, which are among the numerous properties that need to be considered in preparing medicament that can be effectively administered, they can materially impact the quality of a pharmaceutical product. Furthermore, regulatory agencies require a definitive knowledge, characterization and control of the polymorphic form of the active component in pharmaceutical dosage forms if it is in the solid state.

[0009] Compound A is under development by the Applicants for reducing intraocular pressure. The Applicants have found a number of polymorphs of Compound A that are useful for controlling certain desirable formulation properties. In particular two anhydrous forms have been identified, isolated and characterized.

SUMMARY OF INVENTION

[0010] Provided herein are Compound A in the form of anhydrous polymorph A2 as defined in claim 1, pharmaceutical compositions comprising the polymorph as defined in claim 7, and methods of preparation of the compound A of the invention as defined in claim 11. Preferred embodiments of the invention are defined in the dependent claims.

[0011] Compound A can exist as an alternative anhydrous polymorph A1 outside the scope of the present invention having the following crystal data,

$C_{15}H_{20}N_6O_6$;
Mr = 380.37;
Monoclinic crystal system;
$P2_1$ space group;
a = 5.546(2) Å;
b=7.107(2) Å;
c = 21.929(9) Å;
V = 858.8(5) $Å^3$, and
Z = 2.

[0012] The present invention provides Compound A in the form of anhydrous polymorph A2 having the following crystal data,

$C_{15}H_{20}N_6O_6$;
Mr = 380.37;
Orthorhombic crystal system;
$P2_12_12_1$ space group;
a = 5.51796(17) Å;
b = 7.14615(29) Å;
c = 42.9738(29) Å and
V = 1694.55(14) $Å^3$.

[0013] Thus, in the alternative, the present invention provides Compound A in the form of anhydrous polymorph A2 having substantially equivalent peaks at a reflection angle 2-theta as shown in Table 5.

[0014] The Compound A of the present invention is at least 75% free of other solid forms of Compound A.

[0015] In one embodiment there is provided Compound A as defined above that is at least 80% free of other solid forms of Compound A.

[0016] In one embodiment there is provided Compound A as defined above that is at least 90% free of other solid forms of Compound A.

[0017] In one embodiment there is provided Compound A as defined above that is at least 95% free of other solid forms of Compound A.

[0018] In one embodiment there is provided Compound A as defined above that is at least 99% free of other solid forms of Compound A.

[0019] In one embodiment there is provided Compound A as defined above that is 100% free of other solid forms of Compound A.

[0020] In another aspect there is provided a method of obtaining the polymorph A2, the method comprising the steps of taking Compound A in a liquid vehicle and heating up to about 40 degrees for at least 9 hours.

[0021] In one embodiment the Compound A is micronized and then added to an aqueous liquid vehicle. In one embodiment Compound A is micronized into particles with sizes less than 50 microns.

[0022] In one embodiment the method includes the step of heating to about 40 degrees C for 15 hours.

[0023] In one embodiment the liquid vehicle is adapted to provide an aqueous suspension of Compound A. In another embodiment the liquid vehicle includes a surfactant and a preservative. In one embodiment the surfactant is selected from polysorbate 80, polysorbate 60, polysorbate 40, polysorbate 20, polyoxyl 40 stearate, poloxamers, tyloxapol, POE 35 and castor oil. In one embodiment the preservative in selected from a quaternary ammonium salt, benzalkonium chloride, cetrimide, chlorobutanol, sorbic acid and boric acid.

[0024] In another aspect there is provided a pharmaceutical composition comprising Compound A in the form of anhydrous polymorph A2 as defined above and further comprising one or more pharmaceutically acceptable ingredients selected from the group consisting of carriers, excipients, diluents, additives, fillers, surfactants, binders, antimicrobial preservatives, viscosity enhancing agents, and buffers.

[0025] In one embodiment the pharmaceutical composition comprising polymorph A2 defined above is formulated for

ophthalmic administration.

[0026] In a further aspect, there is also provided a method of treating a subject in need of a selective adenosine A_1 agonist, the method comprising administering to a subject in need thereof a therapeutically effective amount of the Compound A in the form of anhydrous polymorph A2 defined above.

[0027] In a further aspect, there is also provided a method of reducing intraocular pressure in a subject, the method comprising topically administering to an eye of a subject in need thereof a therapeutically effective amount of the Compound A in the form of anhydrous polymorph A2 defined above.

[0028] The foregoing brief summary broadly describes the features and technical advantages of certain embodiments of the present invention. Further technical advantages will be described in the detailed description of the invention that follows. Novel features which are believed to be characteristic of the invention will be better understood from the detailed description of the invention when considered in connection with any accompanying figures and examples. However, the figures and examples provided herein are intended to help illustrate the invention or assist with developing an understanding of the invention, and are not intended to be definitions of the invention's scope.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029]

Figure 1: shows the molecular structure and atom numbering scheme for Compound A - Form A1.
Figure 2: shows the packing arrangement and H-bonds for Compound A - Form A1 crystals.
Figure 3: shows the molecular structure and atom numbering scheme for Compound A - Form A2
Figure 4: shows the packing arrangement and H-bonds for Compound A - Form A2 crystals.
Figure 5: shows an overlay in the x-ray powder spectra observed for the forms of Compound A described herein. The lower gray line represents the A1 form and the upper black line represents the form A2.
Figure 6: shows the superposition of molecules of Form A1 (black) and Form A2 (grey)
Figure 7: shows the XRPD data plot of conversion of polymorph form A1 to polymorph form A2 over time at 40 degrees C.

DETAILED DESCRIPTION OF THE INVENTION

[0030] Embodiments of the present invention provide Compound A in the form of anhydrous polymorph A2.

Definitions

[0031] Some chemical structures herein are depicted using bold and dashed lines to represent chemical bonds. These bold and dashed lines depict absolute stereochemistry. A bold line indicates that a substituent is above the plane of the carbon atom to which it is attached and a dashed line indicates that a substituent is below the plane of the carbon atom to which it is attached.

[0032] The term "effective amount" as used herein refers to an amount of a selective adenosine A1 agonist that is effective for: (i) treating or preventing elevated IOP; or (ii) reducing IOP in a human.

[0033] The term "subject" is intended to include organisms, e.g., prokaryotes and eukaryotes, which are capable of suffering from or afflicted with a disease, disorder or condition associated with elevated IOP. Examples of subjects include mammals, e.g., humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In certain embodiments, the subject is a human, e.g., a human suffering from, at risk of suffering from, or potentially capable of suffering from an increase in IOP. In another embodiment, the subject is a cell.

[0034] The term "treat," "treated," "treating" or "treatment" includes the diminishment or alleviation of at least one symptom associated or caused by the state, disorder or disease being treated. In certain embodiments, the treatment comprises the induction of elevated IOP, followed by the activation of the compound of the invention, which would in turn diminish or alleviate at least one symptom associated or caused by the elevated IOP. For example, treatment can be diminishment of one or several symptoms of a disorder or complete eradication of a disorder.

[0035] The term "about" or "substantially" usually means within 20%, more preferably within 10%, and most preferably still within 5% of a given value or range.

Methods of Preparation and Studies

Synthesis of Compound A

[0036] The following Scheme 1 shows the reaction scheme in the preparation of Compound A. The preparation of

Compound A is described in detail.

[0037] The quantities detailed are calculated for a production batch of approximately 40 gms of Compound A. The production described can be scaled up.

[0038] Step 1: 1 Liter of ethanol was charged into a reactor and stirred rapidly. 0.3 kg of 6-chloroadenosine and 0.267 kg of cyclopentylamine were added to the ethanol in the reactor. The reactor was heated to reflux for 2 hr, then cooled to 8 degrees C and kept under these conditions for 12 hours. The crystallized material was filtered from the mother liquid and the solid cake was washed with 0.33 L of ethanol to produced a wet cake. The wet cake was dried to obtain N6-cyclopentyladenosine (0.249 kg).

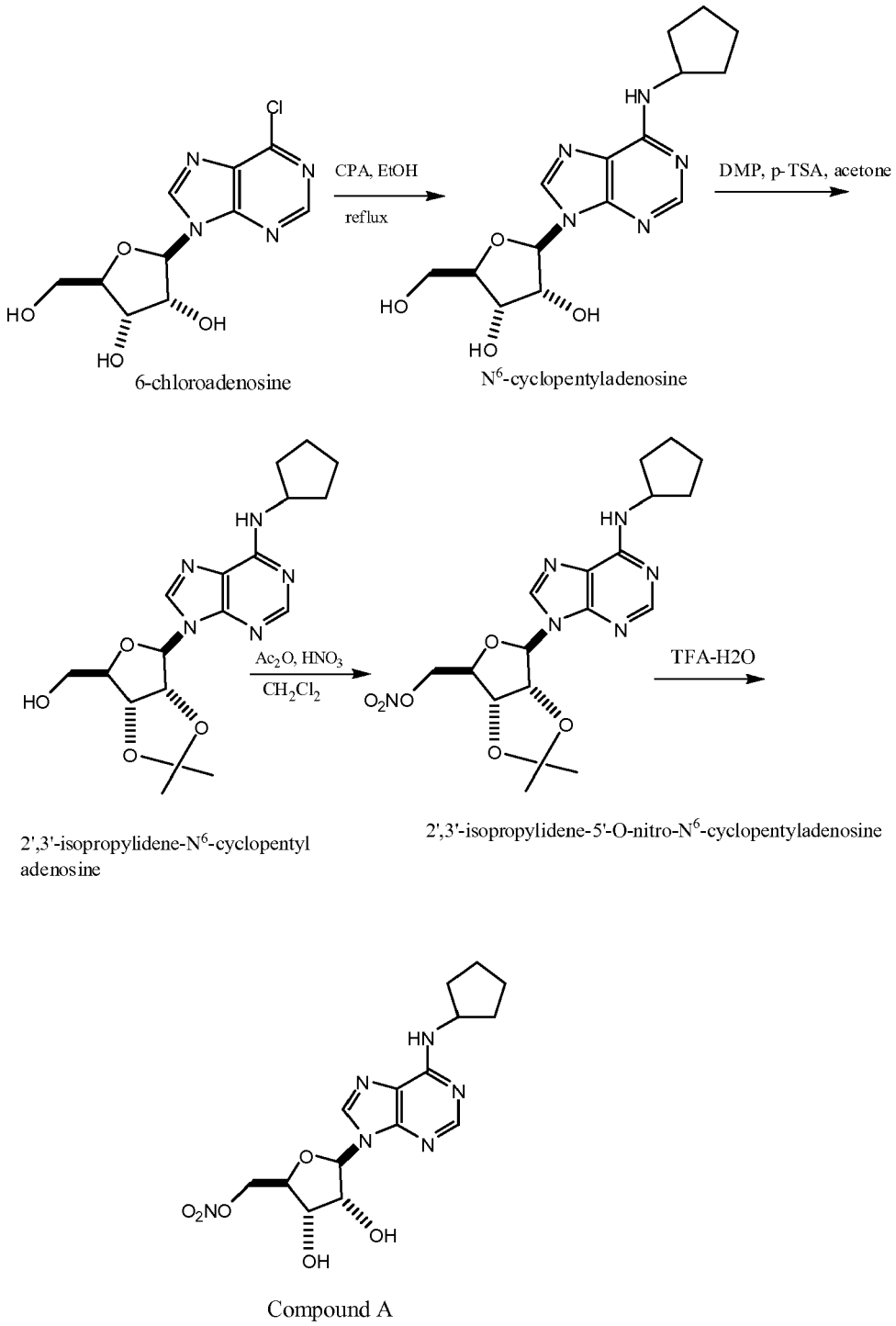
[0039] Step 2: Dimethoxypropane was used to protect the 2' and 3' hydroxyls on the sugar unit. 3.7 liters of acetone was charged into the reactor and was stirred rapidly. 0.249 kg of N6-cyclopentyladenosine; 0.386 kg of dimethoxypropane and 0.148 kg of p-toluenesulfonic acid were added to the acetone (3.7 L) in the reactor. The reactor was heated to 40 degrees C for 1.5 hours. The solvents were then removed by distillation under vacuum at 40 degrees C to prepare a dry crude material. 3.1 L of ethyl acetate were then added to the dry crude material obtained. The solution was then cooled to 6 degrees C and 0.5N NaOH solution was added by dripping until a pH of 8 was reached. This equated to approximately 1.55 L of NaOH solution. After the phase separation was complete, 0.78 L of saturated sodium chloride 20% solution was added to the organic phase. 0.78 L of saturation sodium chloride 20% solution was added again. The two phases were stirred for 30 minutes. The organic phase that was ethyl acetate based was separated and dried with 0.157 kg fo sodium sulfate and washed with 1 L of ethyl acetate. The solution was filtered and evaporated to an oil under vacuum at 55 degrees C. To the remaining oil 1.2 L of hexane and 0.3 L of ethyl acetate were added. The reaction mixture was heated to 55 degrees C for 3 hours and then the solution was cooled to 5 degrees C and maintained at this temperature for 12 hours. The solids were filtered and the resulting cake was washed with a 0.625 L of ethyl acetate:hexane (1:4) solution. After drying the solid 140 g of 2',3'-isopropylidene-N^6-cyclopentyl adenosine was obtained.

[0040] Step 3: Nitration of the 5' position of 2',3'-isopropylidene-N^6-cyclopentyl adenosine obtained in Step 2 was carried out with a nitric acid acetic anhydride mixture. 0.127 L of dichloromethane was charged into the reactor and stirred rapidly. 140 g of 2',3'-isopropylidene-N^6-cyclopentyl adenosine was added and the reaction solution was cooled to - 20 degrees C. 0.547 L of a solution composed of 0.127 L nitric acid 65% in 0.420 L of acetic anhydride was added at a rate that kept the reaction mixture below -15 degrees C - the temperature range of between -23 to -18 degrees C has been found to be the preferred target range. If the temperature increases, then impurities were found to be generated. The addition of the acid mixture took about 0.5 hr. The mixture was stirred for 20 minutes and then quenched into 0.35 L of cold saturated sodium bicarbonate solution. The pH was corrected to 7 by the addition of solid sodium bicarbonate to the aqueous later. The organic phase was separated and the aqueous layer extracted with 0.4L of dichloromethane. The organic phases were combined and washed with 0.6L of saturated sodium chloride solution. The organic phase containing 2',3'-isopropylidene-N^6-cyclopentyladenosine-5'-nitrate was then separated for use in Step 4 below.

[0041] Step 4: Because of its lability the protected 2',3'-isopropylidene-N^6-cyclopentyladenosine-5'-nitrate was hydrolyzed directly without purification. The solution from Step 3 was evaporated at 20 degrees C under vacuum to an oil. The oil was cooled to less than 2 degrees C. 1.95L of trifluoroacetic acid:water (3:1) solution was added. The reaction mixture was stirred for 0.5 hours and allowed to warm to room temperature while being stirred. After that, the sodium bicarbonate solution was prepared and cooled to less than 10 degrees C. The sodium bicarbonate solution was added to the reaction mixture to quench the reaction. The ethyl acetate was added to the reaction vessel and the pH was adjusted and the organic layer was worked up and dried with sodium sulfate. The resulting product solution was then dried several times with magnesium sulfate and the material stripper to form crude Compound A.

[0042] The crude compound A was then recrystallized from ethanol. The crude compound A material was dissolved in ethanol then concentrated to half volume to crystallize for 36 hours. After that the resulting product was isolated by filtration to provide Compound A. ^{1}H-NMR (DMSO-d_6): δ 1.49 - 1.58 (m, 4H), 1.66 - 1.72 (m, 2H), 1.89 - 1.94 (m, 2H), 4.12 - 4.17 (m, 1H), 4.28 - 4.33 (m, 1H), 4.48 (bs, 1H), 4.65 - 4.87 (m, 3H), 5.5 (d, J = 5.1 Hz, 1H), 5.63 (d, J = 5.7 Hz, 1H), 5.91 (d, J = 5.1 Hz, 1H), 7,75 (d, J = 7.5 Hz, 1H), 8.17 (bs, 1H), 8.30 (s, 1H); MS (ES^+): m/z 381.35 (M+ 1); Anal. Calculated for $C_{15}H_{20}N_6O_6$: C, 47.37; H, 5.30; N, 22.10; Found: C, 47.49; H, 5.12, N, 21.96.

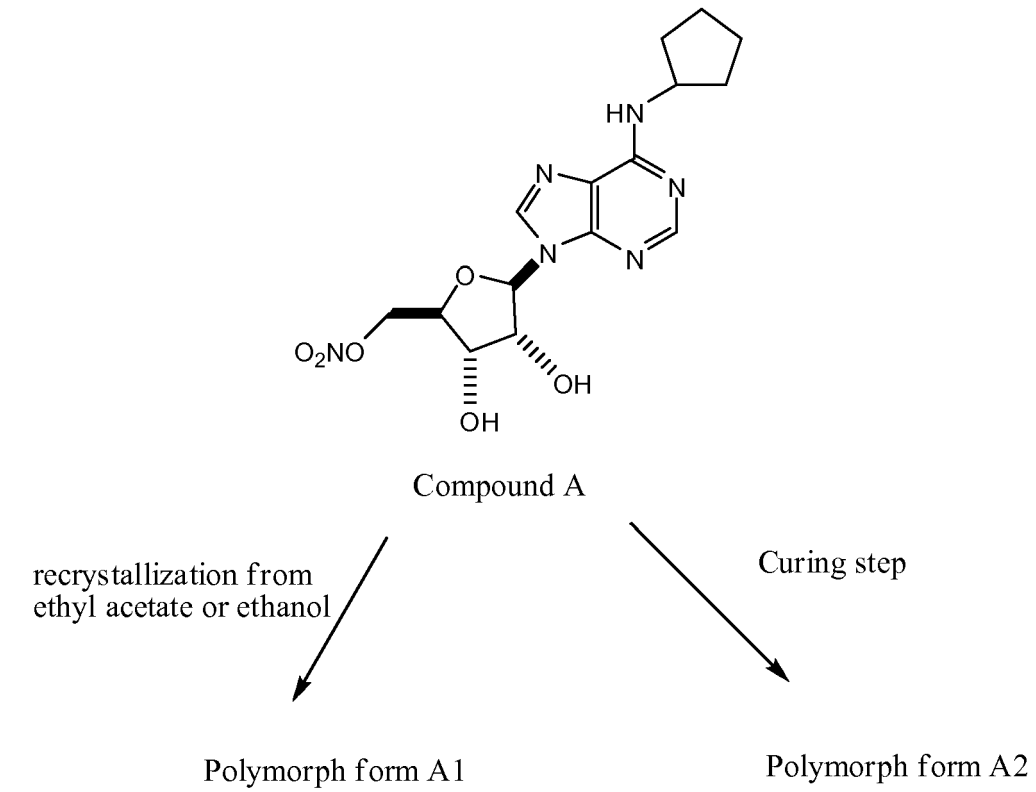
Scheme 1:



Compound A

Preparation of Polymorphs A1 and A2

[0043]



[0044] During the preparation of ophthalmic solutions of Compound A, variability was seen in particle growth size and stability. Because of the variability, efforts have been made to establish if one or more polymorphs could be isolated and purified in order to overcome the variability in particle size growth and stability.

Crystallization Study:

[0045] The Compound A material used for crystallization experiments was taken from a CMC batch prepared substantially as described in steps 1 to 4 above, which was subsequently found to comprise a mixture of approximately 67 percent of form A1 and approximately 33 percent of Form A2.

Form A1 (Reference Example)

[0046] Several slow evaporations crystallisations as detailed in Table 1 below gave crystals using solvents ethyl acetate, isopropyl, acetate, MEK and 2-methoxyethanol that were used for establishing the crystal and molecular structure of Form A1 as shown in Figures 1 and 2. It has also been found that a second recrystallization from ethanol of Compound A obtained in step 4 above also yields a substantially pure form of polymorph A1. It is critical in the further recrystallization from ethanol that no moisture from the atmosphere be allowed to condense on the wet cake of compound A. This is because impurities have the potential to form in the presence of water. The preferred recrystallisation process from ethanol then dries the recrystallized compound in a freeze dryer at room temperature.

Table 1. Results of the slow evaporation crystallization experiments.

Solvents	*μl of solvents*	*Temperature*	*Crystals*
1,4-Dioxane	*400*	*RT*	*Form A1*
MEK♦	*400*	*RT*	*Too small*
Trifluoroethanol	*400*	*RT*	*Too small*
Ethyl Acetate♦	*400*	*RT*	*Form A1*
Isopropyl acetate♦	*400*	*RT*	*Form A1*
1,2-Dimethoxyethane	*400*	*RT*	*Glass*
2-Methoxyethanol	*400*	*RT*	*Form A1*

(continued)

Solvents	*μl of solvents*	*Temperature*	*Crystals*
3-Methyl-2-butanone♦	*400*	*RT*	*Form A1*
DMF	*400*	*RT*	*Glass*
Iso-propanol♦	*400*	*RT*	*Too small*
Ethanol/Water (80:20)♦	*400*	*RT*	*Too small*
Ethanol/Water (90:10)♦	*400*	*RT*	*Too small*

♦ To dissolve the material, the mixture was warmed up to 60 °C and kept at this temperature for approximately 30 min. Following, it was left for crystallization at room temperature (RT). MEK: Methylethyl ketone. DMF: Dimethylformamide.

[0047] Approximately 3-8 mg of Compound A was placed into 8 ml vials to which 400 μL of solvent as detailed in Table 1 was added. The experiments were carried out at room temperature. Each 8 ml vial was placed in a 20 ml vial that was then closed and a small hole was pierced in the cap of the 20 ml vials. The vials were left at room temperature. A single colorless crystal (plate shaped) of approximate size 0.35 x 0.25 x 0.05 mm was directly collected from the ethyl acetate solution and mounted on a goniometer. The measurements were performed at room temperature (296K). The final crystallographic data are as shown in Table 2 below:

Table 2: Crystal data and Structure refinement for Compound A - Form A1

Identification	Form A1
Empirical Formula	$C_{15}H_{20}N_6O_6$;
Formula Weight	Mr = 380.37;
Crystal System	Monoclinic crystal system;
Space Group	$P2_1$ space group;
Unit Cell Dimensions	a = 5.546(2) Å;
	b = 7.107(2) Å;
	c = 21.929(9) Å;
	V = 858.8(5) $Å^3$
[degrees]	96.501(8)
Z	2.
T[K]	296(2)
Å	0.71073
D_c [g/cm^3]	1.471
[mm^{-1}]	0.115
F(000)	400
Crystal size [mm^3]	0.35 x 0.25 x 0.05
Range of data collection [degrees]	3-27.4
Reflections collected	5868
Independent reflections	3315 [R_{int} = 0.0268]
Completeness to = 27.4 [%]	97.8
Max. and min. transmission	0.9942 and 0.9606
Data / restraints / parameters	3315 / 1 / 289
Goodness-of-fit on F^2	1.063
Final R indices[1>2(1)]	R1 = 0.0418, wR2 = 0.0970

(continued)

Identification	Form A1
R indices (all data)	R1 = 0.0556, wR2 = 0.1050
Absolute structure parameter	-0.1(12)
Extinction coefficient	0.081(8)

[0048] The single crystal measurements were performed on Nonius Kappa-CCD diffractometer equipped with Oxford Cryostream Liquid Nitrogen Cooler using MO K radiation. The data for form A1 was collected up to theta = 27.5° at 296K yielding 5868 reflections. Data reduction was performed using HKL Scalepack (Otwinowski & Minor 1997) and cell parameters were obtained using Denzo and Scalepak (Otwinowski & Minor 1997) from 2569 within theta range 1 to 27.5°. The structure was solved using direct methods by SHELXZ-97 (Sheldrick, G. M. 1997a).

[0049] In addition to the single x-ray crystallography data, powder diffraction data was also collected on a D8 Advance diffractometer using $CuK_{\alpha 1}$ radiation (1.54016 Å) with germanium monochromator at Room Temperature. The data were collected from 2.5 to 32.5° theta with 0.016° theta steps on solid state LynxEye detector. The sample was measured in an 8 mm long capillary with 0.5 mm diameter.

[0050] Crystalline anhydrous polymorph form A1 is preferably characterized by a PXRD spectra having peaks at about 17.5, 20.5, 21.2, 22.7, 24.8, 33.2 and 42.1 + 0.2 degrees 2 theta.

[0051] In Table 3 the intensity, 2 theta and D spacing are listed together with the HKL indices. Because intensity as well as 2 theta values are dependent on the radiation used, therefore the D spacing was implemented. The radiation used was $CuK_{\alpha 1}$.2

Table 3. HKL, 2 theta, D spacing and intensity from the powder diffraction of Form A1 ($P2_1$)

h	*k*	*l*	*D spacing*	*2θ*	*Intensity*
0	0	1	21.757	4.058	3.070(29)
0	0	2	10.878	8.121	1.910(36)
0	0	3	7.252	12.194	0.623(59)
0	1	1	6.745	13.115	0.025(65)
0	1	2	5.943	14.895	2.323(93)
-1	0	1	5.498	16.109	3.19(30)
1	0	0	5.480	16.162	6.84(33)
0	0	4	5.439	16.283	0.91(15)
-1	0	2	5.192	17.064	1.06(15)
1	0	1	5.147	17.214	4.07(16)
0	1	3	5.072	17.472	11.87(17)
-1	0	3	4.697	18.878	0.92(18)
1	0	2	4.642	19.104	16.40(23)
0	0	5	4.351	20.393	0.5(17)
1	-1	-1	4.346	20.420	20.7(26)
1	1	0	4.337	20.462	19.5(15)
0	1	4	4.317	20.559	10.14(40)
1	-1	-2	4.190	21.187	42.01(46)
1	1	1	4.166	21.309	7.14(92)
-1	0	4	4.160	21.342	1.29(81)
1	0	3	4.106	21.624	1.29(24)
1	-1	-3	3.916	22.686	77.44(52)
1	1	2	3.884	22.876	12.02(34)
0	1	5	3.709	23.971	2.41(28)
-1	0	5	3.664	24.270	0.03(28)
0	0	6	3.626	24.530	1.18(60)
1	0	4	3.617	24.590	5.78(63)
1	-1	-4	3.589	24.791	22.15(38)

(continued)

h	*k*	*l*	*D spacing*	2θ	*Intensity*
1	1	3	3.554	25.035	5.20(97)
0	2	0	3.547	25.082	14.93(93)
0	2	1	3.501	25.419	9.96(33)
0	2	2	3.373	26.405	0.01(32)
1	-1	-5	3.256	27.371	1.19(38)
-1	0	6	3.238	27.525	0.76(70)
0	1	6	3.229	27.604	2.8(13)
1	1	4	3.223	27.658	12.60(99)
1	0	5	3.198	27.873	0.26(46)
0	2	3	3.187	27.977	0.30(44)
0	0	7	3.108	28.699	0.65(36)
1	-2	-1	2.981	29.953	14.3(20)
1	2	0	2.978	29.982	0.2(25)
0	2	4	2.971	30.050	5.21(90)
1	-1	-6	2.946	30.318	7.63(44)
1	-2	-2	2.929	30.494	1.64(66)
1	2	1	2.921	30.581	0.0(11)
1	1	5	2.916	30.638	2.36(86)
-1	0	7	2.881	31.021	5.24(41)
1	0	6	2.848	31.390	2.6(62)
0	1	7	2.847	31.397	0.1(62)
1	-2	-3	2.831	31.580	11.04(53)
1	2	2	2.819	31.720	3.23(48)
-2	0	1	2.766	32.335	1.54(44)
0	2	5	2.750	32.539	4.6(62)
-2	0	2	2.749	32.548	1.3(64)
2	0	0	2.740	32.657	1.45(63)
0	0	8	2.720	32.908	0.37(43)
1	-2	-4	2.699	33.163	18.54(59)
-2	0	3	2.689	33.286	0.53(97)
1	2	3	2.684	33.350	0.65(92)
2	0	1	2.673	33.500	2.6(11)
1	-1	-7	2.669	33.550	0.12(97)
1	1	6	2.643	33.894	0.46(44)
-2	0	4	2.596	34.521	1.31(47)
-1	0	8	2.583	34.701	0.04(83)
2	-1	-1	2.577	34.778	1.6(15)
2	0	2	2.574	34.832	0.3(12)
2	-1	-2	2.563	34.978	1.06(92)
2	1	0	2.556	35.081	0(15)
1	0	7	2.556	35.086	3(15)
1	-2	-5	2.549	35.182	9.2(11)
0	1	8	2.539	35.316	2.7(16)
0	2	6	2.536	35.369	2.3(26)
1	2	4	2.533	35.412	4.6(16)
2	-1	-3	2.515	35.673	7.10(49)
2	1	1	2.501	35.874	0.56(48)
-2	0	5	2.479	36.208	0.01(47)
2	1	5	2.089	43.279	5.9(61)
1	-2	-8	2.088	43.295	0.3(65)

(continued)

h	*k*	*l*	*D spacing*	*2θ*	*Intensity*
2	2	2	2.083	43.403	2.0(33)
0	1	10	2.080	43.471	2(110)
-2	0	8	2.080	43.474	1(120)
0	3	5	2.078	43.519	0.7(59)
1	2	7	2.074	43.615	9.8(13)
1	-3	-4	2.056	44.008	9.4(15)
2	0	6	2.053	44.070	0.0(17)
1	3	3	2.049	44.156	2.0(11)
1	-1	-10	2.036	44.452	5.63(92)
2	-2	-5	2.032	44.555	7.3(10)
1	1	9	2.019	44.850	2.3(18)
2	2	3	2.018	44.889	0.0(21)
0	2	9	1.998	45.362	0(670)
2	-1	-8	1.996	45.403	70(970)

Form A2

[0052] None of the crystallization trials or techniques attempted, including (i) slow evaporation of solvent, (ii) vapor diffusion of non polar solvent into liquid solution of Compound A and (iii) polar solvent and temperature controlled crystallization with slow cooling rate; yielded suitable crystals of Form A2 for single crystal analysis. In some experiments, such as, for example, in the temperature controlled crystallizations using various mixtures of ethanol/water, very thin needles were obtained. In most of the cases the crystals seemed to be twinned crystals, however none of these crystals gave enough reflections to obtain proper cell parameters. These crystals were however used to attempt X-ray powder diffraction. Therefore the X-ray Powder Diffraction Pattern (XRPD) was obtained and attempts were then made for solving the structure of the Form A2 from the powder data. The first step was to obtain the proper unit cell. After several trials, two possible cell settings were obtained. Both were orthorhombic although with different Bravais face centering. One of these cells was a face centred cell C, while the other was primitive P. Based on the fact that the cell C could be transformed into a smaller one, namely P, the latter was refined and attempts to solve the structure with this configuration setting were made. Also, with the P cell the asymmetric unit was reduced to 1 molecule with C it concerned 2 symmetry independent molecules. For the cell refinement the Pawley fit was used. A Pawley fit based on the high resolution X-ray diffraction pattern was used to check the purity of the sample. The main purpose of the Pawley fit is to refine cell parameters from the complete pattern. In the Pawley method, profiles are analytical, their width is constrained to follow a Caglioti law with the three refinable parameters U, V, W as defined in most of the Rietveld-derived software. The software used for calculation in this project was Topas with following criteria of fit:

$Y_{o,m}$ and $Y_{c,m}$ are the observed and calculated data, respectively at data point m.
M the number of data points,
P the number of parameters,
W_m the weighting given to data point m which for counting statistics is given by $w_m = 1/\sigma (Y_{o,m})^2$ where $\sigma (Y_{o,m})$ is the error in $Y_{o,m}$

$$R_{\exp} = \sqrt{\frac{M-P}{\sum w_m Y_{o,m}^2}} \; ; \quad R_{wp} = \sqrt{\frac{\sum w_m \left(Y_{o,m} - Y_{c,m}\right)^2}{\sum w_m Y_{o,m}^2}} \; ; \quad R_p = \sqrt{\frac{\sum \left|Y_{o,m} - Y_{c,m}\right|}{\sum Y_{o,m}}}$$

$$GOF = chi^2 = \frac{R_{wp}}{R_{\exp}} = \sqrt{\frac{\sum w_m \left(Y_{o,m} - Y_{c,m}\right)^2}{M-P}}$$

Table 4: Parameters of the Pawley fit for Compound A - Form A2

Identification	Form A2
T[K]	293(2)
Å	1.54056
Crystal System	Orthorhombic crystal system;
Space Group	$P2_12_12_1$ space group;
Unit Cell Dimensions	a = 5.51796(17) Å;
	b = 7.14615(29) Å;
	c = 42.9738(29) Å;
	V = 1694.55(14) $Å^3$
Capillary size	0.5 x 0.8
Range for data collection	2-22.5
R_{exp}	1.52
R_{wp}	2.64
Rp	1.91
R_{Bragg}	7.8
GOF	1.74

[0053] For the structure solution, the Topas 3.0 software was employed (Bruker-AXS, 2005) using simulated annealing method. The model was built on the Z-matrix and several torsion angles were set as free variables. The obtained model was not refined except for the unit cell. The H-atoms were included based on geometry and H-Bond scheme. Figure 3 shows the molecular structure of Form 2 of Compound A and Figure 4 shows the crystal packing and the H-bond scheme.

[0054] XRPD patterns were obtained using a high-throughput XRPD set-up. The plates were mounted on a Bruker GADDS diffractometer equipped with a Hi-Star area detector. The XRPD platform was calibrated using Silver Behenate for the long d-spacings and Corundum for the short d-spacings.

[0055] Data collection was carried out at room temperature using monochromatic CuKa radiation in the 2-theta region between 1.5 degrees and 41.5 degrees, which is the most distinctive part of the XRPD pattern between the polymorph forms. The diffraction pattern of each well was collected in 2 theta ranges (1.5 degrees ≤ 2 theta ≤ 21.5 degrees for the first frame, and 19.5 degrees ≤ 2 theta ≤ 41.5 degrees for the second) with an exposure time of 30 seconds for each frame. No background subtraction or curve smoothing was applied to the XRPD patterns. The carrier material used during XRPD analysis was transparent to X-rays and contributed only slightly to the background.

[0056] Crystalline anhydrous polymorph form A2 is preferably characterized by PXRD spectra having peaks at about 16.9, 18.1, 19.1, 20.8, 21.3, 22.0, 22.8, 23.8, 24.9, 25.0, 29.1, 29.8, 34.2 and 35.8 + 0.2 degress 2 theta.

[0057] In Table 5 the intensity, 2 theta and D spacing are listed together with the HKL indices. Because intensity as well as 2 theta values are dependent on the radiation used , therefore the D spacing was implemented. The radiation used was $CuK_{\alpha 1}$.2

Table 5. HKL, 2 theta, D spacing and intensity from the powder diffraction of Form A2 ($P2_12_12_1$)

h	***k***	***l***	***D spacing***	***2θ***	***Intensity***
0	0	2	21.487	4.109	3.341(28)
0	0	4	10.743	8.223	2.277(38)
0	0	6	7.162	12.348	0.690(57)
0	1	1	7.049	12.547	0.802(58)
0	1	2	6.781	13.045	0.032(57)
0	1	3	6.395	13.837	1.088(68)
0	1	4	5.950	14.877	2.330(82)
0	1	5	5.495	16.117	2.50(22)
1	0	1	5.473	16.182	16.69(24)

(continued)

h	*k*	*l*	*D spacing*	*2θ*	*Intensity*
0	0	8	5.372	16.489	0.31(12)
1	0	2	5.345	16.574	0.02(12)
1	0	3	5.149	17.207	0.78(11)
0	1	6	5.059	17.517	9.55(14)
1	0	4	4.908	18.058	15.49(17)
0	1	7	4.657	19.043	2.50(33)
1	0	5	4.643	19.098	18.42(35)
1	0	6	4.371	20.300	0.0(12)
1	1	0	4.367	20.317	14.0(13)
1	1	1	4.345	20.423	21.46(33)
0	0	10	4.297	20.652	4.4(20)
0	1	8	4.294	20.669	14.2(23)
1	1	2	4.280	20.737	34.73(53)
1	1	3	4.178	21.251	62.18(36)
1	0	7	4.104	21.637	3.26(21)
1	1	4	4.046	21.951	64.81(39)
0	1	9	3.970	22.375	3.18(21)
1	1	5	3.894	22.821	67.15(41)
1	0	8	3.849	23.089	0.02(22)
1	1	6	3.729	23.844	23.77(31)
0	1	10	3.683	24.147	1.11(24)
1	0	9	3.611	24.636	5.44(27)
0	0	12	3.581	24.843	1.32(64)
0	2	0	3.573	24.900	0.0(10)
0	2	1	3.561	24.987	16.0(37)
1	1	7	3.559	25.001	57.1(34)
0	2	2	3.525	25.247	7.78(28)
0	2	3	3.467	25.675	0.11(25)
0	1	11	3.428	25.972	0.02(26)
0	2	4	3.390	26.264	0(1200)
1	0	10	3.390	26.264	0(1200)
1	1	8	3.389	26.278	13(11)
0	2	5	3.299	27.003	0.24(27)
1	1	9	3.223	27.658	6.71(31)
0	1	12	3.202	27.843	4.61(77)
0	2	6	3.197	27.882	0.02(89)
1	0	11	3.188	27.961	0.02(42)
0	2	7	3.088	28.889	3.68(33)
0	0	14	3.070	29.067	0.02(57)
1	1	10	3.063	29.129	13.39(58)
1	0	12	3.004	29.716	0.3(17)
0	1	13	3.000	29.754	0.3(90)
1	2	0	2.999	29.765	4.9(81)
1	2	1	2.992	29.839	23.29(88)
0	2	8	2.975	30.012	0.81(68)
1	2	2	2.970	30.060	4.79(66)
1	2	3	2.936	30.426	0.16(34)
1	1	11	2.912	30.680	1.09(34)
1	2	4	2.889	30.931	2.18(35)
0	2	9	2.861	31.241	3.31(36)

(continued)

h	*k*	*l*	*D spacing*	*2θ*	*Intensity*
1	0	13	2.836	31.524	2.60(83)
1	2	5	2.832	31.569	13.87(87)
0	1	14	2.820	31.700	0.94(41)
1	1	12	2.769	32.301	1.3(12)
1	2	6	2.766	32.335	12.9(14)
2	0	0	2.759	32.425	2.17(88)
2	0	1	2.753	32.493	3.93(89)
0	2	10	2.747	32.564	2.65(63)
2	0	2	2.737	32.698	1.03(41)
2	0	3	2.709	33.037	0.47(39)
1	2	7	2.695	33.219	13.32(50)
0	0	16	2.686	33.333	0.44(92)
1	0	14	2.682	33.376	0.02(88)
2	0	4	2.672	33.507	1.62(43)
0	1	15	2.659	33.677	0.31(39)
0	2	11	2.637	33.974	0.0(33)
1	1	13	2.636	33.985	1.5(34)
2	0	5	2.627	34.103	3.97(60)
1	2	8	2.619	34.214	14.87(53)
2	0	6	2.575	34.818	0.3(46)
2	1	0	2.574	34.829	2.2(52)
2	1	1	2.569	34.893	4.43(92)
2	1	2	2.556	35.086	4.65(43)
1	0	15	2.543	35.270	0.0(11)
1	2	9	2.540	35.312	11.6(13)
2	1	3	2.533	35.405	0.15(98)
0	2	12	2.529	35.461	5.87(80)
2	0	7	2.517	35.648	0.0(15)
0	1	16	2.514	35.683	0.0(25)
1	1	14	2.511	35.724	2.5(15)
2	1	4	2.503	35.847	15.03(56)
2	1	5	2.466	36.409	3.57(54)
1	2	10	2.459	36.504	1.95(65)
2	0	8	2.454	36.585	0.02(56)
0	2	13	2.427	37.018	2.26(64)
2	1	6	2.422	37.086	1.62(68)
1	0	16	2.415	37.201	1.13(49)
1	1	15	2.396	37.514	2.28(58)
2	0	9	2.389	37.623	4.3(25)
0	0	18	2.387	37.646	0.0(30)
0	1	17	2.383	37.716	0.6(17)
1	2	11	2.379	37.785	8.3(82)
0	3	1	2.378	37.795	2.2(80)
2	1	7	2.374	37.873	2.95(98)
0	3	2	2.368	37.974	1.72(55)
0	3	3	2.350	38.273	0.02(46)
0	2	14	2.328	38.639	6.6(11)
0	3	4	2.326	38.687	2.9(18)
2	0	10	2.322	38.754	0.9(66)
2	1	8	2.321	38.764	1.8(59)

(continued)

h	*k*	*l*	*D spacing*	*2θ*	*Intensity*
1	2	12	2.299	39.146	1.3(32)
1	0	17	2.298	39.167	0.0(43)
0	3	5	2.296	39.214	11.8(17)
1	1	16	2.288	39.351	2.64(54)
2	1	9	2.266	39.753	5.2(23)
0	1	18	2.264	39.775	0.0(26)
0	3	6	2.260	39.850	1.52(90)
2	0	11	2.254	39.973	4.10(57)
0	2	15	2.235	40.318	1.37(55)
1	2	13	2.221	40.582	0.5(43)
0	3	7	2.221	40.591	4.1(43)
2	1	10	2.208	40.835	0.02(60)
1	0	18	2.191	41.165	0.0(15)
1	1	17	2.188	41.230	3(14)
1	3	0	2.187	41.246	1(25)
2	0	12	2.186	41.274	2(24)
1	3	1	2.184	41.302	6(49)
2	2	0	2.184	41.310	0(40)
2	2	1	2.181	41.366	8.8(38)
0	3	8	2.178	41.433	2.1(33)
1	3	2	2.176	41.469	11.6(29)
2	2	2	2.173	41.533	2.8(12)
1	3	3	2.162	41.747	11.1(12)
2	2	3	2.159	41.810	7.1(18)
0	1	19	2.156	41.860	2.7(15)
2	1	11	2.149	42.003	5.0(99)
0	0	20	2.149	42.016	0(14)
0	2	16	2.147	42.052	0.9(81)
1	2	14	2.145	42.087	2.4(56)
1	3	4	2.143	42.132	3.6(29)
2	2	4	2.140	42.195	6.3(12)
0	3	9	2.132	42.370	5.30(72)
1	3	5	2.119	42.624	2.3(32)
2	0	13	2.118	42.651	0.2(49)
2	2	5	2.116	42.686	6.8(25)
1	1	18	2.095	43.148	1.4(22)
1	0	19	2.093	43.194	0.2(81)
1	3	6	2.092	43.219	3(11)
2	1	12	2.090	43.254	6.0(89)
2	2	6	2.089	43.280	2.5(44)
0	3	10	2.083	43.398	0.10(84)
1	2	15	2.072	43.657	6.91(88)
0	2	17	2.064	43.835	0.0(11)
1	3	7	2.060	43.913	5.9(22)
0	1	20	2.058	43.968	4(18)
2	2	7	2.057	43.974	0(17)
2	0	14	2.052	44.098	0.99(86)
0	3	11	2.034	44.512	1.2(11)
2	1	13	2.031	44.581	6.0(12)
1	3	8	2.026	44.704	10.2(16)

(continued)

h	*k*	*l*	*D spacing*	*2θ*	*Intensity*
2	2	8	2.023	44.764	8.8(23)
1	1	19	2.008	45.105	0(38)
1	0	20	2.002	45.252	0(2700)
1	2	16	2.001	45.286	0(4300)

Controlling the formation of the form of polymorph

[0058] It has been found that the formation of the particular polymorphic form can be controlled. As described above the Form A1 can be obtained predominantly via recrystallization from ethanol or under slow evaporation conditions. It has also been established that ripening or curing of Compound A particles suspended in an aqueous suspension formulated for ocular delivery at 40 degrees C for a relatively short period of time formed polymorph form A2 from polymorph form A1. The aqueous suspension samples were kept at 40 degrees C for up to 108 hours and monitored by particle size measurement, XRPD and microphotography. Particle size measurements showed that average sizes increased significantly over 15 hours. Thereafter, sizes remained effectively constant to 108 hours. XRPD analyses indicated a change in polymorph content from about 74% A1 to 26 % A2 at time zero to 0% A1 to 100 % A2 at 108 hours at 40 degrees C. Figure 7 shows the conversion of form A1 to A2 over time. Also, habit changes coupled with A2 growth were reflected in the XRPD patterns and could be monitored by a difference in selected peak intensities from planes within the crystal lattice perpendicular to the c axis that change in intensity as the habit of the crystal changes. The intensity differences changed up to 9 hours and remained constant thereafter indicating that the habit changes were completed during this time. Microphotographs showed blade or platelike crystal habits of particles in suspension.

[0059] When the aqueous suspension ocular formulation containing Compound A in the A1 polymorph is stored at 2-8°C, a temperature required to limit decomposition of Compound A over long term storage, the habit of the suspended particles changes slowly over a period of 6 to 12 months. During this time the small irregular particles of suspended drug change to rod-like habits, with many particles having a length along the longest dimension over 100 microns. These changes make it much more difficult to resuspend Compound A particles by sonication and shaking in order to form a homogeneous suspension for dosing.

[0060] The conversion of the A1 form to the A2 form has been found to limit any further changes to particle habit, size or polymorph content when the aqueous suspension, which is suitable for ocular delivery of the drug, is stored over a 6 month period at either 5°C or 25°C. Also, the cured aqueous suspension is more easily resuspended by shaking, a favourable characteristic for suspension formulations for ocular drug delivery.

[0061] The particle size analyses were performed on a Cilas 1180 Particle Size Analyzer. The parameters used were liquid mode, sample refractive index = 1.62 (determined using Cargille immersion oils), liquid refractive index = 1.333 (value for water), 30 second measurement, 180 rpm stirring, 120 rpm pump circulation, no sonication, 5 repeat measurements.

Formulation Example

[0062] A batch of sterile material of Compound A was prepared as described above under the "Synthesis of Compound A". The resulting Compound A material was then sterilized with gamma irradiation at up to 40 kGray and then formulated into the following aqueous formulations:

Aqueous Formulation	
Ingredient	%, W/V
Compound A	0.152 - 0.76
Sodium CMC	0.7
Benzalkonium Chloride	0.01
Polysorbate 80	0.3
Citric Acid Monohydrate	0.152 (7mM)
NaOH/HCl	pH 5.1 ±0.1
NaCl	q.s. to 270-330 mOsm
Purified Water	q.s. to 100.00

[0063] Various concentrations of Compound A formulation lots were prepared from 0.152, 0.30, 0.61, 0.91, 2.42, 0.46,

0.76 %, W/V to provide for the ability to deliver different levels of Compound A per drop of formulation. For example one drop of the 0.152 %, W/V of compound A would deliver 50 mcg per drop, 0.30 %, W/V would deliver 100 mcg per drop, right through to 0.76 % W/V delivering 250 mcg per drop. The formulation lots were then heated to undergo the curing step and convert the A1 polymorph form of Compound A to the A2 polymorph form of Compound A. The curing step was undertaken by placing the formulation lots at 40 degrees C for 48 hours and then reverting the formulations lots to the desired longer term storage conditions for stability studies.

[0064] Two of the formulation lots, namely 0.46 % W/V of Compound A and a 0.76 % W/V were studied for long term stability and particle size growth at 5 degrees Celsius and 25 degrees Celsius for 6 months. Two of the formulation lots, namely 0.46 % W/V of Compound A and a 0.76 % W/V were studied for long term stability and particle size growth at 5 degrees Celsius for 18 months. The results are tabulated below in Table 6.

Table 6

Formulation	Time (months)	Impurities	pH	Particle Size Distribution (microns)
0.46% at 5°C	0	1%	5.1	X_{10} = 1.746 X_{50} = 6.992 X_{90} = 14.087
0.46% at 5°C	1	1%	5.0	X_{10} = 0.907 X_{50} = 6.285 X_{90} = 13.485
0.46% at 5°C	3	1%	5.0	X_{10} = 1.792 X_{50} = 7.082 X_{90} = 14.356
0.46% at 5°C	6	1%	5.1	X_{10} = 1.777 X_{50} = 6.939 X_{90} = 13.698
0.46% at 5°C	12	1%	5.1	X_{10} = 1.398 X_{50} = 6.679 X_{90} = 13.396
0.46% at 5°C	18	1%	5.1	X_{10} = 1.666 X_{50} = 6.882 X_{90} = 13.074
0.46% at 25°C	0	1%	5.1	X_{10} = 1.746 X_{50} = 6.416 X_{90} = 13.698
0.46% at 25°C/60%RH	1	1%	5.0	X_{10} = 1.036 X_{50} = 6.416 X_{90} = 13.698
0.46% at 25°C/60%RH	3	3%	5.1	X_{10} = 1.656 X_{50} = 6.705 X_{90} = 12.805
0.46% at 25°C/60%RH	6	4%	5.0	X_{10} = 1.809 X_{50} = 6.741 X_{90} = 12.380
0.76% at 5°C	0	1%	5.1	X_{10} = 1.524 X_{50} = 6.773 X_{90} = 12.778
0.76% at 5°C	1	1%	5.1	X_{10} = 1.115 X_{50} = 6.456 X_{90} = 12.944

(continued)

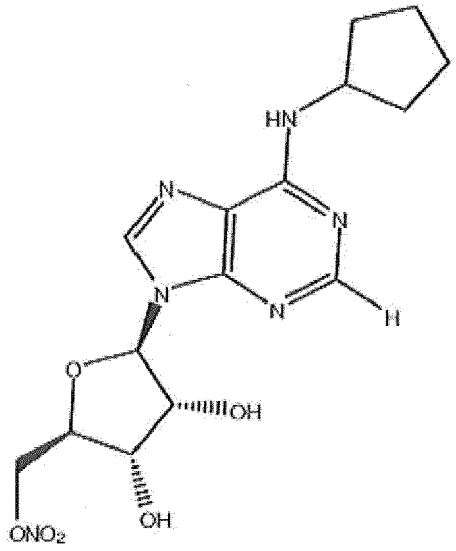
Formulation	Time (months)	Impurities	pH	Particle Size Distribution (microns)
0.76% at 5°C	3	1%	5.1	X_{10} = 1.455 X_{50} = 6.745 X_{90} = 13.104
0.76% at 5°C	6	1%	5.1	X_{10} = 1.541 X_{50} = 6.638 X_{90} = 11.833
0.76% at 5°C	12	1%	5.1	X_{10} = 1.407 X_{50} = 6.635 X_{90} = 12.314
0.76% at 5°C	18	1%	5.1	X_{10} = 1.611 X_{50} = 6.840 X_{90} = 12.672
0.76% at 25°C/60%RH	0	1%	5.1	X_{10} = 1.524 X_{50} = 6.773 X_{90} = 12.778
0.76% at 25°C/60%RH	1	1%	5.1	X_{10} = 1.056 X_{50} = 6.107 X_{90} = 11.551
0.76% at 25°C/60%RH	3	2%	5.1	X_{10} = 1.446 X_{50} = 6.691 X_{90} = 12.724
0.76% at 25°C/60%RH	6	3%	5.1	X_{10} = 1.619 X_{50} = 6.292 X_{90} = 10.240

[0065] It can be seen from the results in Table 6 that the particle size distributions of the two formulation lots are stable over the time under the conditions tested. The results also show that the levels of impurities and pH remain stable for the formulations at 5 degrees Celsius over 18 months, while there is a slow increase in the impurities for the formulations held at 25 degrees Celsius over 6 months.

[0066] The present invention and its embodiments have been described in detail. However, the scope of the present invention is not intended to be limited to the particular embodiments described in the specification. Various modifications, substitutions, and variations can be made to the disclosed material without departing from the scope of the accompanying claims.

Claims

1. Compound A having the structure:



in the form of crystalline anhydrous polymorph A2 having the following crystal data,

(i) $C_{15}H_{20}N_6O_6$;

Mr=380.37;
Orthorhombic crystal system;
$P2_12_12_1$ space group;
a = 5.51796(17) Å;
b = 7.14615(29) Å;
c = 42.9738(29) Å and
V = 1694.55(14) $Å^3$; or

(ii) having substantially equivalent peaks in powder X-ray diffraction with $CuK_{\alpha 1}$ radiation at a reflection angle 2-theta as shown in Table 5; or
(iii) having powder X-ray diffraction with $CuK_{\alpha 1}$ radiation spectra having peaks at about 16.9, 8.1, 19.1, 20.8, 21.3, 22.0, 22.8, 23.8, 24.9, 25.0, 29.1, 29.8, 34.2 and 35.8 + 0.2 degrees 2 theta,
characterized in that the compound A is at least 75% free from other solid forms of Compound A.

2. The Compound A of claim 1 that is at least 80% free of other solid forms of Compound A.

3. The Compound A of claim 1 that is at least 90% free of other solid forms of Compound A.

4. The Compound A of claim 1 that is at least 95% free of other solid forms of Compound A.

5. The Compound A of claim 1 that is at least 99% free of other solid forms of compound A.

6. The Compound A of claim 1 that is 100% free of other solid forms of compound A.

7. A pharmaceutical composition comprising the Compound A of any of claims 1 to 6 and further comprising one or more pharmaceutically acceptable ingredients selected from the group consisting of carriers, excipients, diluents, additives, fillers, surfactants, binders, antimicrobial preservatives, viscosity enhancing agents, and buffers.

8. The pharmaceutical composition of claim 7 that is formulated for ophthalmic administration.

9. The pharmaceutical composition of claim 8 that is formulated as follows:

Ingredient	%, W/V
Compound A	0.152 - 0.76
Sodium CMC	0.7
Benzalkonium Chloride	0.01
Polysorbate 80	0.3
Citric Acid Monohydrate	0.152 (7mM)
NaOH/HCl	pH 5.1 ±0.1
NaCl	q.s. to 270-330 mOsm; and
Purified Water	q.s. to 100.00.

10. A composition comprising the Compound A as defined in any of claims 1 to 6 for use in a method of reducing intraocular pressure in a subject, the method comprising topically administering to an eye of a subject in need thereof a therapeutically effective amount of the Compound A as defined in any of claims 1 to 6.

11. A method of obtaining the Compound A according to any of claims 1 to 6, the method comprising the steps of suspending Compound A in an aqueous liquid vehicle and heating to about 40 degrees for at least 9 hours; or preferably at least 15 hours.

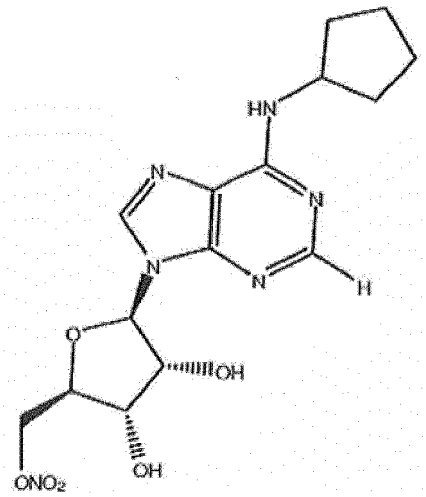
12. The method as claimed in claim 11 wherein the Compound A is micronized, preferably into particle sizes less than 50 microns, and then added to the liquid vehicle, which liquid vehicle is preferably adapted to provide an aqueous suspension of Compound A.

13. The method as claimed in claim 12, wherein the liquid vehicle includes a surfactant and a preservative.

14. The method as claimed in claim 13, wherein the surfactant is selected from polysorbate 80, polysorbate 60, polysorbate 40, polysorbate 20, polyoxyl 40 stearate, poloxamers, tyloxapol, POE 35 and castor oil and wherein the preservative is selected from a quaternary ammonium salt, benzalkonium chloride, cetrimide, chlorobutanol, sorbic acid and boric acid.

Patentansprüche

1. Verbindung A der Struktur:



in der Form kristallinen wasserfreien polymorphen Form A2 mit den folgenden Kristalldaten,

(i) $C_{15}H_{20}N_6O_6$;

Mr = 380,37;

Orthorhombisches Kristallsystem;
Raumgruppe $P2_12_12_1$;
a = 5,51796(17) Å;
b = 7,14615(29) Å;
c = 42,9738(29) Å und
V = 1694,55(14) $Å^3$; oder

(ii) mit im Wesentlichen äquivalenten Peaks in einer Pulverröntgendiffraktometrie mit $CuK_{\alpha 1}$-Strahlung bei einem Reflexionswinkel 2-Theta wie in Tabelle 5 gezeigt; oder
(iii) mit einer Pulverröntgendiffraktometrie mit $CuK_{\alpha 1}$-Strahlungsspektren mit Peaks bei ungefähr 16,9, 18,1, 19,1, 20,8, 21,3, 22,0, 22,8, 23,8, 24,9, 25,0, 29,1, 29,8, 34,2 und 35,8 + 0,2 Grad 2 Theta,

dadurch gekennzeichnet, dass Verbindung A wenigstens zu 75 % frei von weiteren festen Formen der Verbindung A ist.

2. Verbindung A nach Anspruch 1, welche wenigstens zu 80 % frei von weiteren festen Formen der Verbindung A ist.

3. Verbindung A nach Anspruch 1, welche wenigstens zu 90 % frei von weiteren festen Formen der Verbindung A ist.

4. Verbindung A nach Anspruch 1, welche wenigstens zu 95 % frei von weiteren festen Formen der Verbindung A ist.

5. Verbindung A nach Anspruch 1, welche wenigstens zu 99 % frei von weiteren festen Formen der Verbindung A ist.

6. Verbindung A nach Anspruch 1, welche zu 100 % frei von weiteren festen Formen der Verbindung A ist.

7. Pharmazeutische Zusammensetzung umfassend Verbindung A nach einem der Ansprüche 1 bis 6 und ferner umfassend einen oder mehrere pharmazeutisch verträgliche Bestandteile ausgewählt aus der Gruppe bestehend aus Trägern, Hilfsstoffen, Verdünnungsmitteln, Additiven, Füllstoffen, Tensiden, Bindemitteln, antimikrobiellen Konservierungsmitteln, viskositätsverbesserenden Mitteln und Puffern.

8. Pharmazeutische Zusammensetzung nach Anspruch 7, welche zur ophthalmischen Verabreichung formuliert ist.

9. Pharmazeutische Zusammensetzung nach Anspruch 8, welche wie folgt formuliert ist:

Bestandteil	%, W/V
Verbindung A	0,152 - 0,76
Natrium CMC	0,7
Benzalkoniumchlorid	0,01
Polysorbat 80	0,3
Zitronensäuremonohydrat	0,152 (7 mM)
NaOH/HCl	pH 5,1 $\pm$ 0,1
NaCl	q.s. für 270 - 330 mOsm; und
Gereinigtes Wasser	q.s. für 100,00.

10. Zusammensetzung umfassend Verbindung A nach einem der Ansprüche 1 bis 6 zur Verwendung in einem Verfahren zum Verringern des Augeninnendrucks in einem Subjekt, wobei das Verfahren ein topisches Verabreichen an einem Auge eines Subjekts, welches dessen bedarf, in einer therapeutisch wirksamen Menge der Verbindung A nach einem der Ansprüche 1 bis 6 umfasst.

11. Verfahren zum Erhalten der Verbindung A nach einem der Ansprüche 1 bis 6, wobei das Verfahren die Schritte des Suspendierens der Verbindung A in einem wässrigen flüssigen Vehikel und Erwärmen auf ungefähr 40 °C für wenigstens 9 Stunden; oder bevorzugt wenigstens 15 Stunden umfasst.

12. Verfahren nach Anspruch 11, wobei Verbindung A mikronisiert wird, bevorzugt in Partikelgrößen kleiner als 50 Mikron, und anschließend zu dem flüssigen Vehikel hinzugefügt wird, wobei das flüssige Vehikel bevorzugt ausgelegt

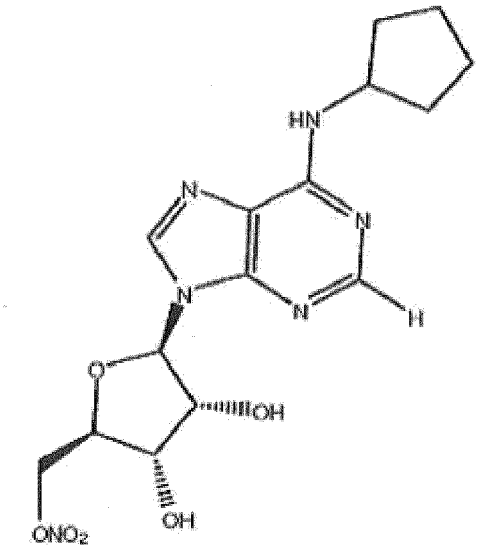
ist, um eine wässrige Suspension der Verbindung A bereitzustellen.

13. Verfahren nach Anspruch 12, wobei das flüssige Vehikel ein Tensid und ein Konservierungsmittel umfasst.

14. Verfahren nach Anspruch 13, wobei das Tensid ausgewählt ist aus Polysorbat 80, Polysorbat 60, Polysorbat 40, Polysorbat 20, Polyoxyl-40-Stearat, Poloxameren, Tyloxapol, POE 35 und Rizinusöl und wobei das Konservierungsmittel ausgewählt ist aus quartären Ammoniumsalzen, Benzalkoniumchorid, Cetrimid, Chlorbutanol, Sorbinsure und Borsäure.

Revendications

1. Composé A possédant la structure :



sous la forme du polymorphe cristallin anhydre A2 possédant les données cristallographiques suivantes :

(i) $C_{15}H_{20}N_6O_6$;

Mr = 380,37 ;
système cristallin orthorhombique ;
groupe d'espace $P2_12_12_1$;
a = 5,51796(17) Å ;
b = 7,14615(29) Å ;
c = 42,9738(29) Å ; et
V = 1694,55(14) $Å^3$; ou

(ii) possédant des pics essentiellement équivalents dans le diagramme de diffraction des rayons X sur poudre avec un rayonnement $CuK_{\alpha 1}$ à un angle de réflexion 2-thêta tel que représenté dans le tableau 5 ; ou
(iii) possédant un diagramme de diffraction des rayons X sur poudre avec des spectres de rayonnement $CuK_{\alpha 1}$ possédant des pics à environ 16,9, 18,1, 19,1, 20,8, 21,3, 22,0, 22,8, 23,8, 24,9, 25,0, 29,1, 29,8, 34,2 et 35,8 + 0,2 degré 2-thêta ;

caractérisé en ce que le composé A est exempt à concurrence d'au moins 75 % d'autres formes solides du composé A.

2. Composé A selon la revendication 1, qui est exempt à concurrence d'au moins 80 % d'autres formes solides du composé A.

3. Composé A selon la revendication 1, qui est exempt à concurrence d'au moins 90 % d'autres formes solides du

composé A.

4. Composé A selon la revendication 1, qui est exempt à concurrence d'au moins 95 % d'autres formes solides du composé A.

5. Composé A selon la revendication 1, qui est exempt à concurrence d'au moins 99 % d'autres formes solides du composé A.

6. Composé A selon la revendication 1, qui est exempt à concurrence d'au moins 100 % d'autres formes solides du composé A.

7. Composition pharmaceutique comprenant le composé A selon l'une quelconque des revendications 1 à 6, et comprenant en outre un ou plusieurs ingrédients pharmaceutiquement acceptables choisis parmi le groupe constitué par des supports, des excipients, des diluants, des additifs, des matières de charge, des agents tensioactifs, des liants, des conservateurs antimicrobiens, des agents augmentant la viscosité et des tampons.

8. Composition pharmaceutique selon la revendication 7, qui est formulée pour une administration ophtalmique.

9. Composition pharmaceutique selon la revendication 8, qui est formulée comme suit :

Ingrédients	%, en poids/volume
Composé A	0,152 - 0,76
Carboxyméthylcellulose de sodium	0,7
Chlorure de benzalkonium	0,01
Polysorbate 80	0,3
Monohydrate de l'acide citrique	0,152 (7 mM)
NaOH/HCl	pH 5,1 $\pm$ 0,1
NaCl	qsp 270-330 mOsm ; et
Eau purifiée	qsp 100,00.

10. Composition comprenant le composé A selon l'une quelconque des revendications 1 à 6, pour son utilisation dans un procédé de réduction de la pression intraoculaire dans un sujet, le procédé comprenant l'administration par voie topique à un oeil d'un sujet qui en manifeste le besoin d'une quantité thérapeutiquement efficace du composé A tel que défini dans l'une quelconque des revendications 1 à 6.

11. Procédé pour l'obtention du composé A selon l'une quelconque des revendications 1 à 6, le procédé comprenant les étapes de mise en suspension du composé A dans un véhicule aqueux liquide et de chauffage jusqu'à environ 40 degrés pendant au moins 9 heures, ou de préférence au moins 15 heures.

12. Procédé selon la revendication 11, dans lequel le composé A est micronisé, de préférence pour obtenir des granulométries inférieures à 50 microns, et est ensuite ajouté au véhicule liquide, ledit véhicule liquide étant de préférence conçu pour procurer une suspension aqueuse du composé A.

13. Procédé selon la revendication 12, dans lequel le véhicule liquide comprend un agent tensioactif et un conservateur.

14. Procédé selon la revendication 13, dans lequel l'agent tensioactif est choisi parmi le polysorbate 80, le polysorbate 60, le polysorbate 40, le polysorbate 20, le stéarate de polyoxyéthylène 40, des poloxamères, le tyloxapol, le POE 35 et l'huile de ricin, et dans lequel le conservateur est choisi parmi un sel d'ammonium quaternaire, le chlorure de benzalkonium, le cétrimide, le chlorobutanol, l'acide sorbique et l'acide borique.

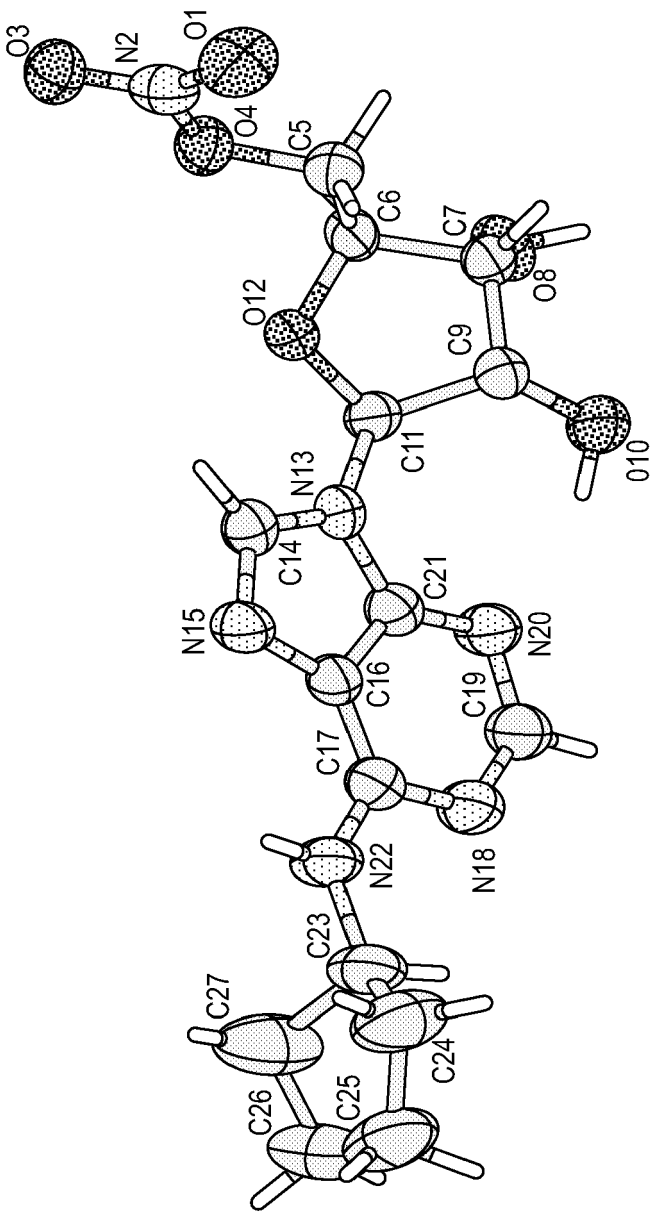
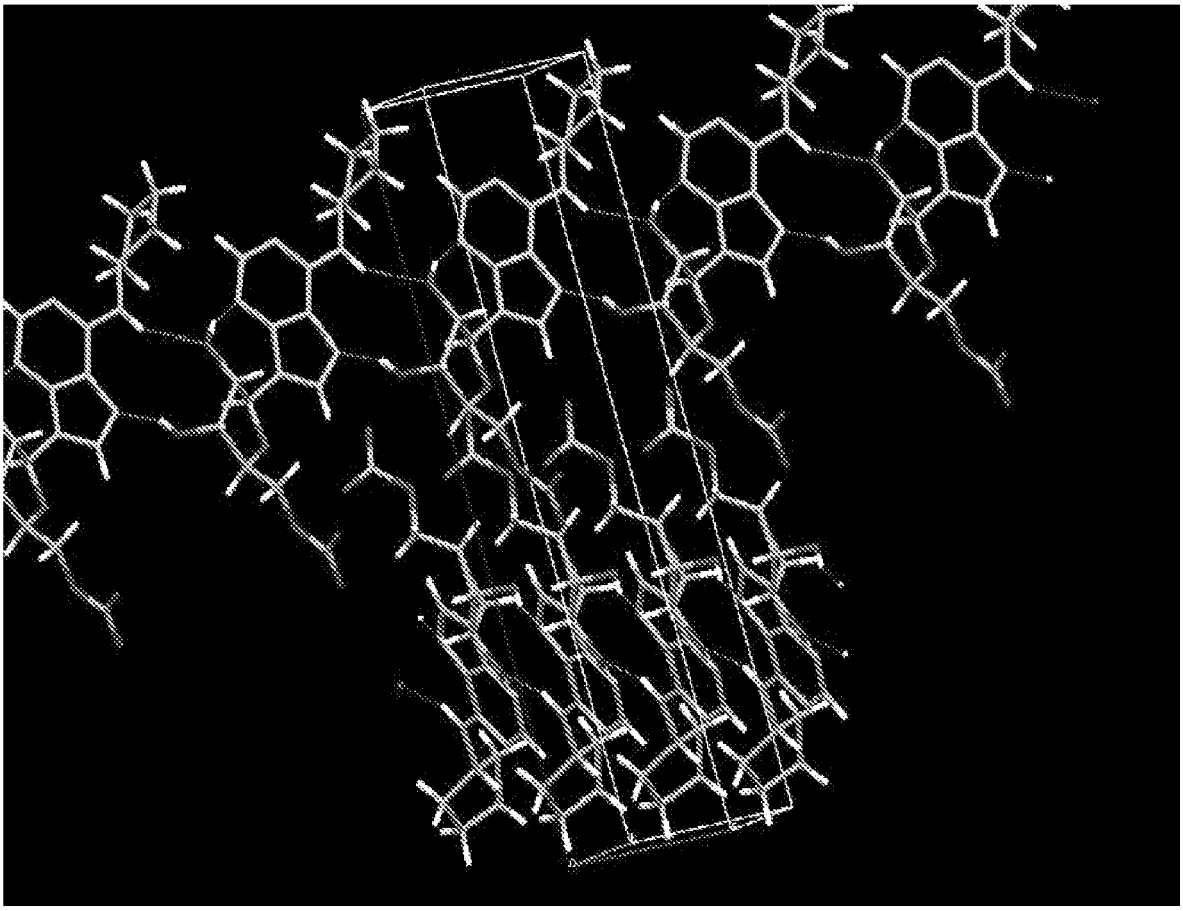

O3
N2
O1
O4
C5
C6
C7
O8
O12
C9
C11
O10
N13
C14
C21
N15
N20
C16
C19
C17
N22
N18
C23
C27
C24
C26
C25

Fig. 1

Figure 2



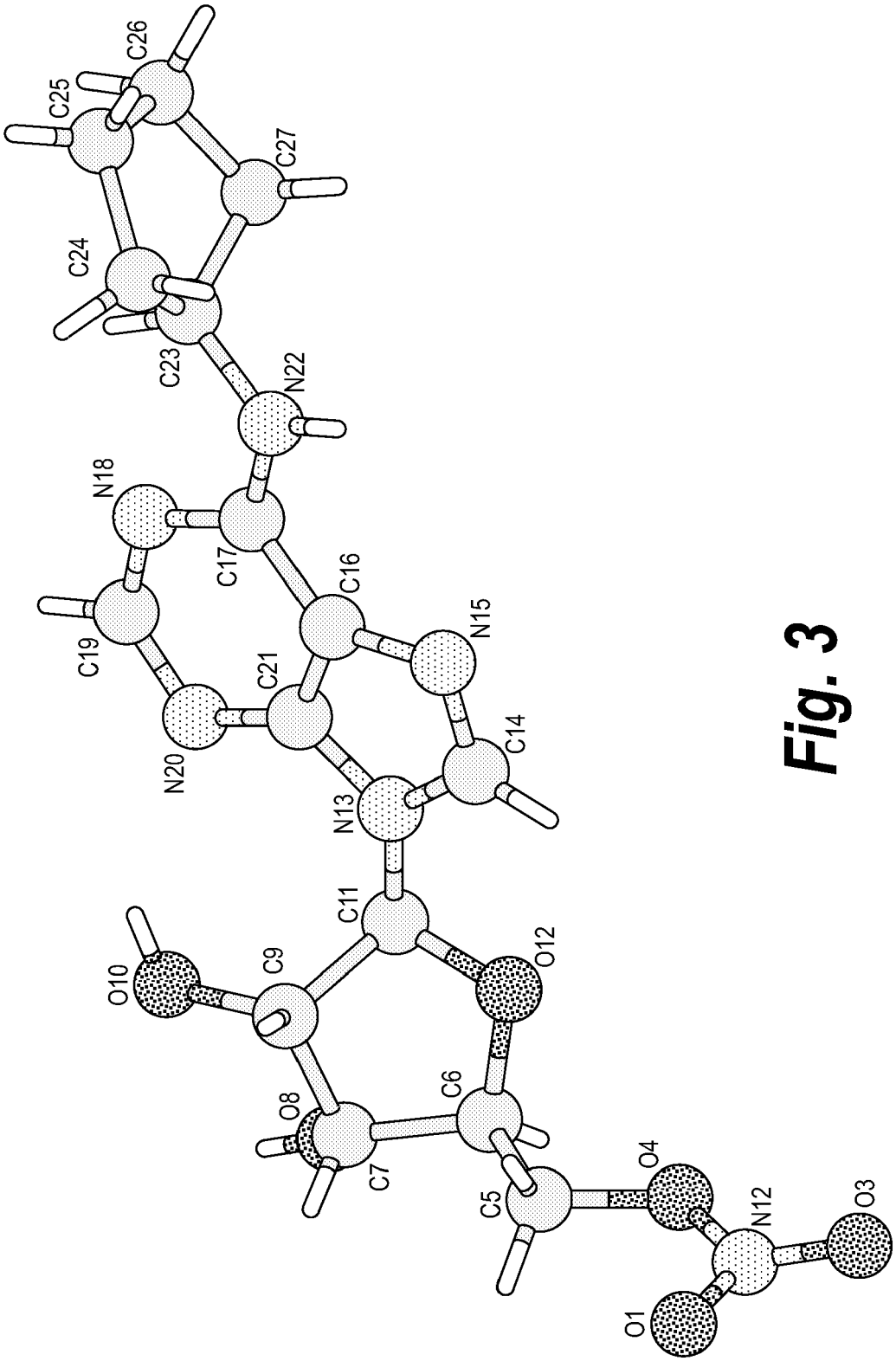

C26
C25
C27
C24
C23
N22
N18
C17
C16
N15
C19
C21
C14
N20
N13
C11
O12
O10
C9
C6
O8
C7
O4
C5
N12
O3
O1

Fig. 3

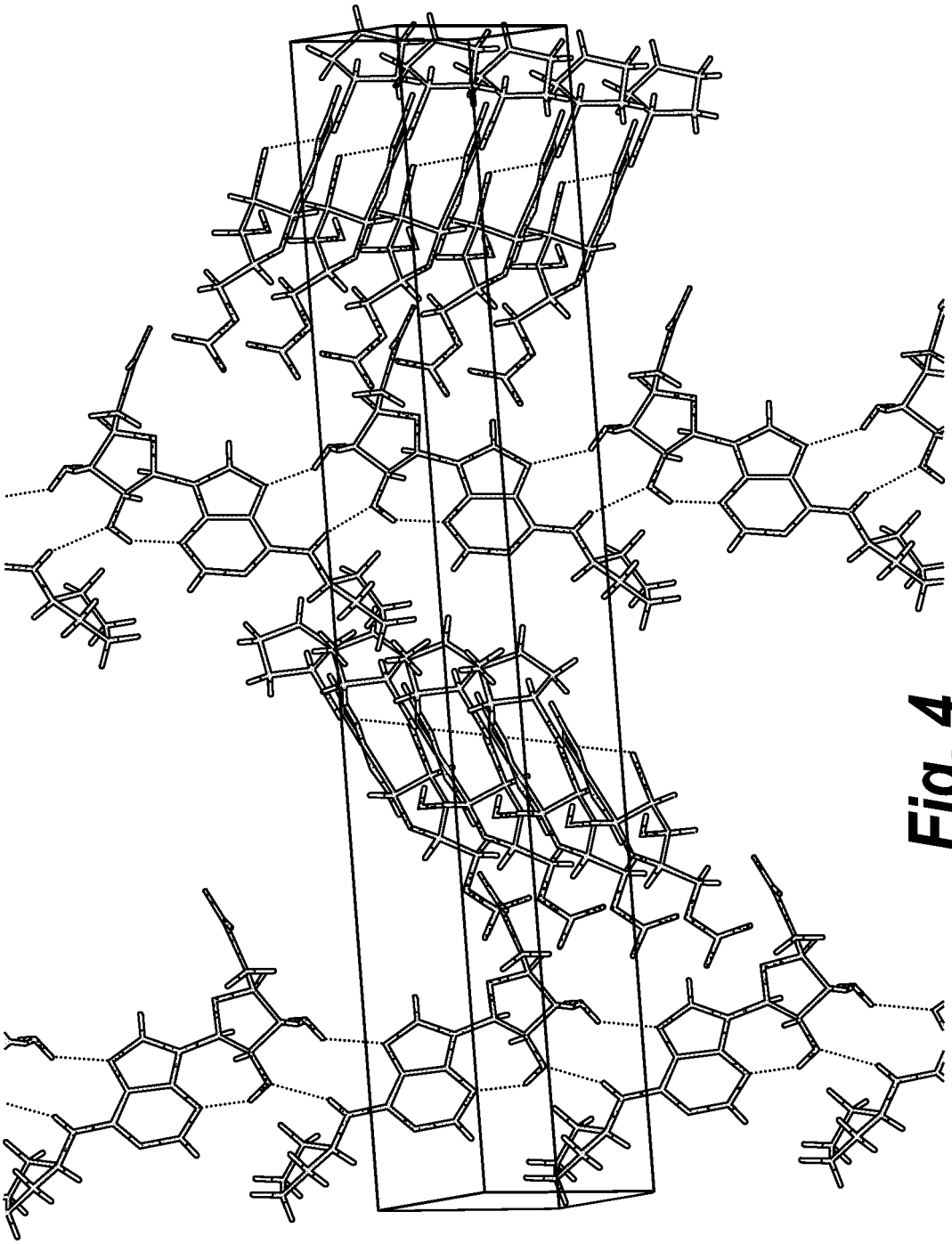


Fig. 4

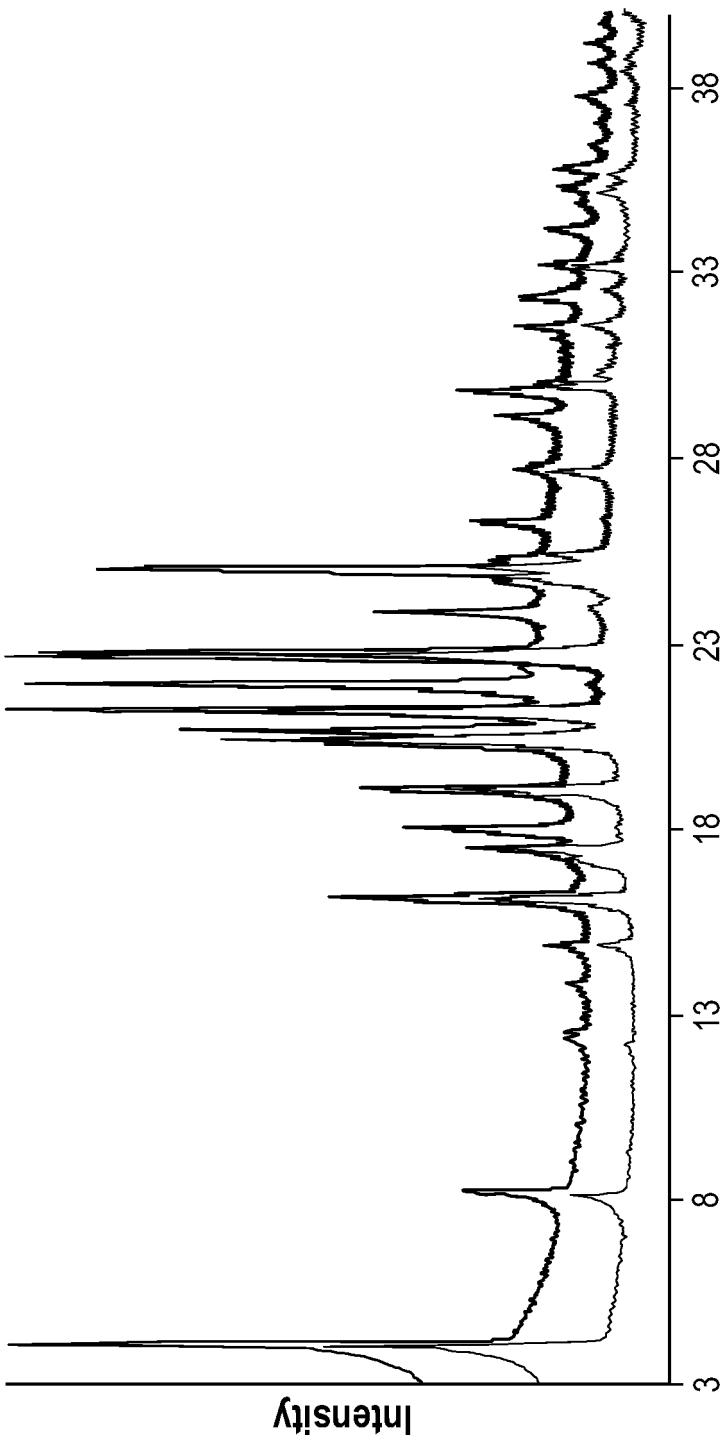


Fig. 5

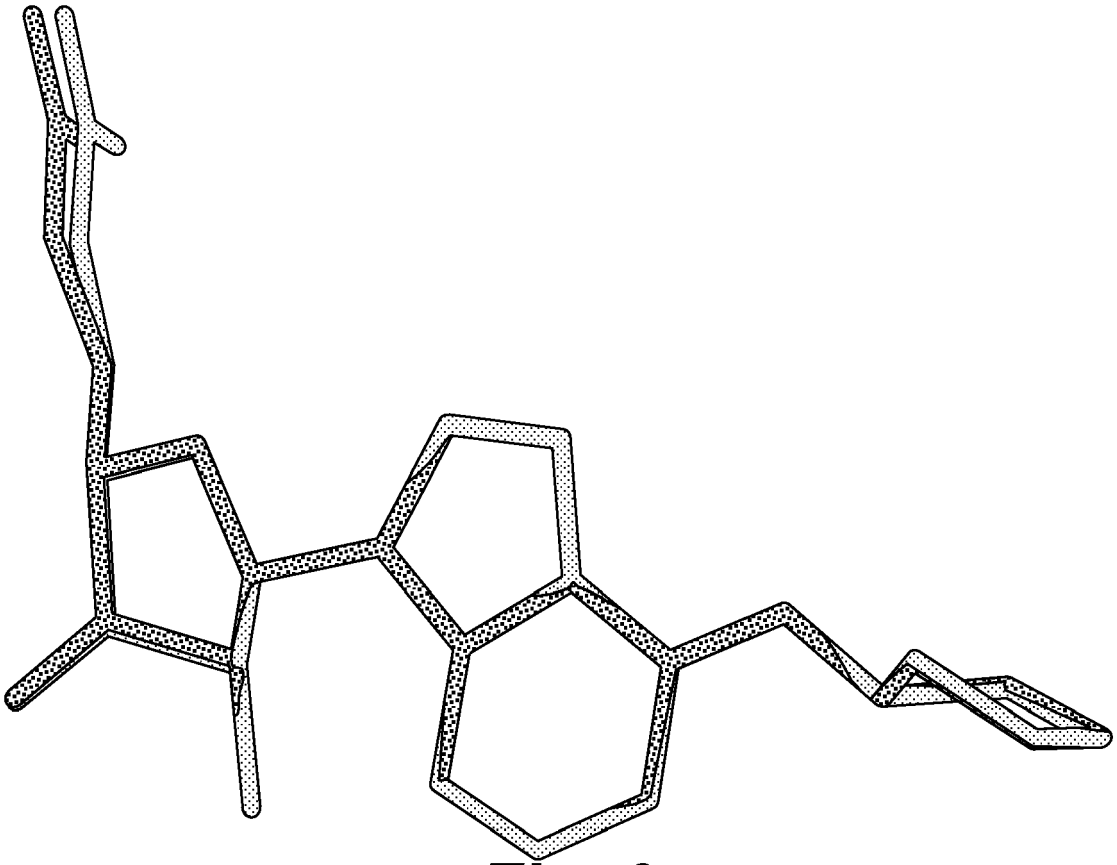


Fig. 6

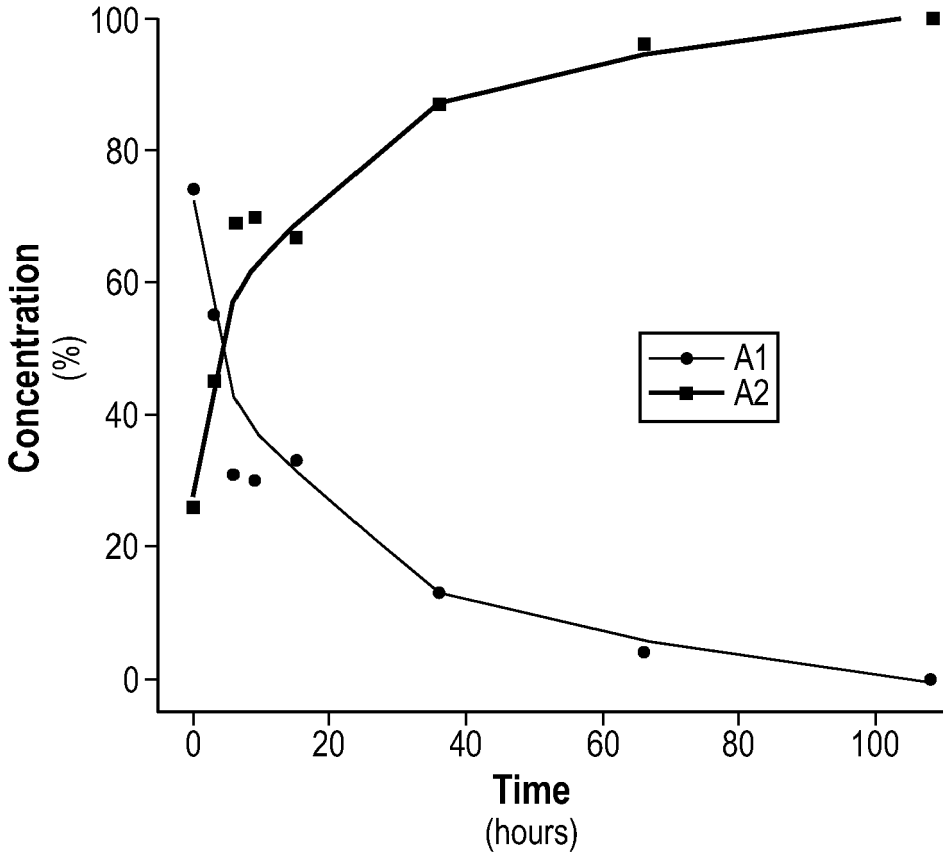

100
80
60
40
20
0
Concentration
(%)
0
20
40
60
80
100
Time
(hours)
A1
A2

Fig. 7

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 2010033112 W **[0003]**
- WO 2010127210 A **[0003] [0004]**
- US 7423144 B **[0004]**
- US 20090062314 A **[0004]**