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(54) Titre : Crystalline N-acetyl neuraminic acid derivatives and processes for their preparation.

(57) Abrégé :

Acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in crystalline form, particularly in the form of a hydrate which exists as crystals having a low aspect ratio, or in the form of a hydrate which exists as crystals having a high aspect ratio.

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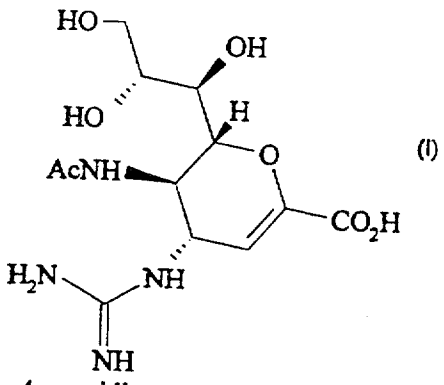
CRYSTALLINE N-ACETYL NEURAMINIC ACID DERIVATIVES AND PROCESSES FOR THEIR PREPARATION

The present invention relates to derivatives of N-acetyl neuraminic acid and their use in medicine. More particularly the invention is concerned with particular physical forms of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid (the 4-guanidino analogue of DANA; also known as 5-(acetylamino)-2,6 anhydro-3,4,5-trideoxy-4-guanidino-D-glycereo-D-galacto-non-2-enonic acid), pharmaceutical formulations thereof and their use in therapy.

PCT/AU91/00161 (publication no.WO91/16320) describes a number of derivatives of 5-acetamidino-2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (2,3,-dideoxy-2,3-didehydro-N-acetyl-neuraminic acid; DANA) including the 4-guanidino analogue of DANA. The 4-guanidino analogue of DANA is prepared by the reaction of the corresponding O-acyl protected 4-amino analogue of DANA by reaction with S-methylisourea followed by deprotection, purification by chromatography and freeze-drying.

The structure of the 4-guanidino analogue of DANA is shown below:



(I)

4-guanidino analogue of DANA

We have now found that the compound of formula (I) can be obtained in crystalline form.

There is thus provided in a first aspect of the invention 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in crystalline form.

We have further found that the compound of formula (I) may be obtained by crystallisation under certain conditions in the form of a crystalline hydrate (hereinafter Hydrate I). Hydrate I exists in the form of crystals having a low aspect ratio, for example, tabular crystals, which are favoured for pharmaceutical formulation because of their physical properties, e.g. good flow characteristics. The water content of Hydrate I is related to relative humidity (RH). Water uptake of Hydrate I varies from zero at RH of 0% up to 10% at RH of 90 - 100%.

The compound of formula (I) may also be crystallised in the form of a dihydrate (hereinafter Hydrate II). Hydrate II exists in the form of crystals having a high aspect ratio, for example, needle-shaped crystals. The water content of these crystals remains substantially constant over a broad relative humidity range (RH about 10 - 90%). The stable water content of Hydrate II represents an advantage of this crystalline form for use in pharmacy.

There is thus provided in a further aspect of the invention 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in the form of crystals having a low aspect ratio, such as tabular crystals.

In a further aspect there is provided 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in the form of crystals having a high aspect ratio, such as needle-shaped crystals.

Whilst tabular crystals are regarded as typical of Hydrate I and needle-shaped crystals are regarded as typical of Hydrate II, it will be appreciated that the possibility of either Hydrate I or Hydrate II existing in alternative crystal habits under certain circumstances cannot be excluded. It is to be understood that all such alternative crystal habits are within the scope of the present invention.

There is also provided 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in the form of crystals which have stable water content over a broad humidity range, for example RH 10 - 90%.

Hydrate I loses substantially all of its water of crystallisation at about 80-90°C. Decomposition occurs at 299°C.

Hydrate II loses one mole of water of crystallisation at about 84-90°C and a further mole of water of crystallisation by about 135-143°C.

In a yet further aspect the invention provides 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in the form of a crystalline hydrate which loses substantially all its water of crystallisation at 80-90°C.

In a yet further aspect the invention provides 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in the form of a crystalline hydrate which loses one mole of water of crystallisation at 84-90°C and a further mole of water of crystallisation at 135-143°C.

In a preferred aspect the invention provides 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in the form of Hydrate I as herein defined substantially free of Hydrate II as herein defined.

In a further preferred aspect the invention provides 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in the form of Hydrate II as herein defined substantially free of Hydrate I as herein defined.

By "substantially free" is meant containing less than 5% of the alternative hydrate, such as less than 2%, for example less than 1% of the alternative hydrate.

5-Acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid may be prepared in crystalline form by crystallisation of the compound from aqueous solution.

Each of Hydrate I and Hydrate II may be prepared substantially free from the alternative Hydrate by controlling the solution concentration and temperature at which crystallisation occurs.

In general, 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in the form of Hydrate I may be obtained by crystallisation of the compound from aqueous solution at a temperature greater than about 50°C, preferably 50-55°C.

In general, 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in the form of Hydrate II may be obtained by crystallisation of the compound from aqueous solution at a temperature below about 40°C, preferably about 20-30°C.

Crystallisation of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid from aqueous solution at a temperature in the range of about 40-50°C typically results in a mixture of tabular and needle-shaped crystals. Such mixtures are disfavoured for the preparation of pharmaceutical formulations because of the differing physical properties of Hydrate I and Hydrate II, in particular their flow properties.

Seeding of an aqueous solution of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid with crystals of Hydrate I or Hydrate II may lead to crystallisation of the seeded Hydrate. Preparation of Hydrate I or Hydrate II should therefore be conducted in the absence of seeds of the undesired Hydrate. Conversely, Hydrate I may be prepared by seeding an aqueous solution of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid with crystals of Hydrate I, and Hydrate II may be prepared by seeding an aqueous solution of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid with crystals of Hydrate II.

For the preparation of Hydrate II it is preferable to employ a relatively dilute aqueous solution, for example a solution of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in 15 - 30 volumes of water, for example 20 volumes of water. Hydrate I may conveniently be crystallised from a relatively concentrated aqueous solution, for example a solution of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in 12 - 20 volumes of water, such as 12 - 15 volumes of water.

We have found that Hydrate II may be converted into Hydrate I in aqueous suspension or saturated solution. Such interconversion may be effected by prolonged ageing of an aqueous suspension or saturated solution of Hydrate II, for example ageing for a period of days, for example more than 10 days, such as about 15 days. Alternatively, interconversion may be effected in the presence of a base, for example an organic base such as imidazole.

Recovery of either Hydrate I or Hydrate II from aqueous solution may be enhanced by the addition to the solution of a suitable counter-solvent. Suitable counter-solvents are water-miscible solvents in which the compound of formula (I) has poor solubility. Conveniently the counter-solvent will be a ketone, such as acetone, or an alkanol such as propan-2-ol. A preferred counter-solvent is acetone.

We have also found that addition of an aqueous solution of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-L-galacto-non-2-enopyranosonic acid to a similar volume of a counter-solvent as previously defined results in precipitation of Hydrate II. For example, addition of a solution of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in 12 - 15 volumes of water to 12 - 20 volumes of acetone gives crystals of Hydrate II.

The methods for the preparation of crystalline material, and in particular methods for the preparation of Hydrate I and Hydrate II, described herein constitute further aspects of the present invention.

5-Acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in crystalline form may be used as an antiviral agent as described in WO 91/16320, which is incorporated herein by reference.

5-Acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in crystalline form may be formulated as a pharmaceutical composition for use as an antiviral agent as described in WO 91/16320.

Preferred pharmaceutical formulations of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid include powder formulations and aqueous solutions or suspensions. Preparation of powder

formulations requires micronisation of the drug substance. The good flow properties of Hydrate I render it particularly suitable for micronisation. Hydrate II has adequate flow properties and also a particularly rapid dissolution rate in water. These properties render Hydrate II particular advantageous for the preparation of aqueous solutions/suspensions.

Hydrates I and II have been subjected to X-ray powder diffraction studies. Diffraction traces were obtained using a Seimens D-500 diffractometer and CuK_α radiation. X-Ray intensities were measured at 0.02° increments for 5 second intervals using a scintillation counter, between values of 5 and 55° 2θ. The d-spacings and line intensities obtained for Hydrate I and Hydrate II are shown in Tables I and II, respectively.

TABLE I

d(A)	I(%)
10.06	30.25
6.77	69.81
6.63	89.61
6.35	12.69
6.05	54.56
5.38	25.11
5.05	98.58
4.61	12.58
4.42	100.00
4.31	8.28
4.17	11.67
3.98	75.00
3.90	52.61
3.77	20.33
3.69	36.17
3.48	26.53
3.41	53.25
3.37	17.61
3.16	18.39
3.02	31.08

2.98	9.25
2.92	6.28
2.87	13.58
2.82	10.78
2.78	6.78
2.74	18.03
2.69	15.33
2.65	6.25
2.63	6.44
2.59	11.44
2.49	14.31
2.45	18.81
2.41	8.64
2.35	11.36
2.19	5.42
2.13	12.25
2.11	6.56
2.02	8.33
1.98	5.47

TABLE II

d(A)	I(%)
16.88	66.34
10.38	50.60
9.50	16.08
8.47	40.46
7.12	100.00
5.84	11.78
5.33	18.83
5.21	33.99
4.78	12.94
4.57	75.81
4.32	16.37
4.25	18.49

4.14	43.26
3.96	10.33
3.76	22.11
3.64	25.16
3.57	37.04
3.52	15.69
3.40	16.85
3.34	21.20
3.17	13.52
3.13	17.04
3.06	7.48
2.94	10.19
2.92	8.45
2.86	9.17
2.76	9.56
2.72	9.22
2.67	6.81
2.64	8.06
2.60	5.46
2.58	6.52
2.51	5.31
2.49	6.66
2.45	5.55
2.43	5.89
2.39	15.93
2.38	10.38
2.31	8.40
2.22	5.94
2.16	5.36
2.11	6.28
2.03	7.24
1.91	6.57

The following examples illustrate the invention but are not intended as a limitation thereof. All temperatures are in °C.

Example 1

Preparation of Hydrate I

A mixture of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid (5.0g) and water (60ml) was heated at 100° to give a clear solution. The solution was cooled during 30 min to 55° and maintained at between 55° and 50° during 4h, to give a crystalline suspension. Acetone (80ml) was added during 90 min, the temperature being maintained between 48 and 55°. The resultant slurry was stirred 1 h, the temperature being allowed to fall to ca. 20°, and the suspension was allowed to stand 17h at ambient temperature. The product was collected by vacuum filtration and the filter bed was washed with 4:1 acetone/water (2 x 10ml) then with acetone (10ml). The product was air dried at ambient temperature and humidity to give Hydrate I (tabular crystals) (4.5g).

PMR (D_2O) 2.04 (3H,s), 3.67 (2H,m), 4.23 (1H,m), 4.42 (2H,m), 5.63 (1H, d, J, 2.5 Hz)

IR (Nujol)	3248, 3338, 3253; NH, OH 1692, 1666, 1646, 1619, 1575; CO (CH_3CONH, CO_2), CN
Water content	8.4% w/w; calculated for $C_{12}H_{20}N_4O_7.1.7H_2O$

Example 2

Preparation of Hydrate I

A mixture of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid (15.0g) and water (180ml) was heated at 100° to give a clear solution. The solution was clarified by vacuum filtration through a filter paper then cooled to ca. 55° and maintained at between 55° and 50° during 4h, allowing crystallisation to become established. Acetone (210ml) was added with stirring, during 2h, the temperature being maintained at 48-55°. The resultant suspension was stirred and cooled to 30°and was then allowed to stand at ambient temperature 17h. The solid was filtered and the product was washed with 4:1 acetone/water (2x30ml) and then acetone (30ml). The solid was air dried at ambient temperature and humidity to give Hydrate I (tabular crystals) (12.0g).

Characterisation as above.

Example 3

Preparation of Hydrate II

A mixture of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid (10.0g) and water (100ml) was heated to 95°. The resultant solution was clarified by vacuum filtration. The solution was then cooled to 30° and acetone (250ml) was added during 5 min stirring. The resultant thick white suspension was allowed to stand at ambient temperature 20 h. The solid was collected by vacuum filtration and was washed with 4:1 acetone/water (2x20ml) then with acetone (20ml). The solid was dried in a vacuum oven at 35° for 24h and then equilibrated with atmospheric moisture at ambient temperature and humidity to give Hydrate II (needle-shaped crystals) (8.16g).

Characterisation as above.

Water content 10.6% w/w; calculated for $C_{12}H_{20}N_4O_7.2H_2O$ 9.8% w/w.

Example 4

Preparation of Hydrate II

A mixture of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid (10.0g) and water (400ml) was heated to 20° for 2h. The resultant solution was clarified by vacuum filtration. Acetone (110ml) was added and solid began to crystallise. The resultant suspension was stirred for 2.5h at 20°. The solid was collected by vacuum filtration and was washed with 4:1 v/v acetone/water (2x20ml) then with acetone (20ml). The solid was dried in a vacuum oven at 30° and then equilibrated with atmospheric moisture at ambient temperature and humidity to give Hydrate II (needle-shaped crystals) (7.7g)

Characterisation as above.

Water content 11.1% w/w calculated for $C_{12}H_{20}N_4O_7.2H_2O$ 9.8 w/w.

Example 5

Preparation of Hydrate II

A mixture of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-eno-pyranosonic acid (50g) and water (1150ml) was heated to 75°. The resultant solution was clarified by vacuum filtration, washing through with water

(100ml). The solution was then cooled to 7° over 1h and acetone (500ml) was added. The resultant solution was stirred slowly for 0.5h, during which time solid began to crystallise. Acetone (750ml) was then added to the suspension over 2h, maintaining the temperature at 5 - 10°. The solid was collected by vacuum filtration and washed with 4 : 1 v/v acetone/water (2 x 100ml) then with acetone (100ml). The solid was air-dried at ambient temperature and humidity to give Hydrate II (needle-shaped crystals) (46.0g).

Water content 9.8% w/w; calculated for $C_{12}H_{20}N_4O_7.2H_2O$ 9.8% w/w.

XRD: consistent with Hydrate II (>99%).

Example 6

Precipitation of Hydrate II

A mixture of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-eno-pyranosonic acid (50g) and water (600ml) was heated to 100°. The resultant clear hot solution was added over 8 minutes to acetone (700ml) stirred rapidly at ambient temperature, causing the temperature of the mixture to rise from 20° to 56° and precipitation of solid. The resultant suspension was allowed to cool to 20° with stirring, then the solid was collected by vacuum filtration and washed with 4 : 1 acetone/water (2 x 100ml) then with acetone (100ml). The solid was air-dried at ambient temperature and humidity to give Hydrate II (needle-shaped crystals) (47.9g).

Water content 10.0 w/w; calculated for $C_{12}H_{20}N_4O_7.2H_2O$ 9.8% w/w.

XRD: consistent with Hydrate II (>99%).

Example 7

Crystallisation of Hydrate I from Water

A mixture of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-eno-pyranosonic acid (35.7g) and water (350ml) was heated at 95° to give a clear solution. This was adjusted to pH7.0 (from pH6.4) with aqueous acetic acid (100µl, 10%v/v). The resulting solution was allowed to cool to ambient temperature with stirring, to give a crystalline suspension. The product was

collected by vacuum filtration, then vacuum-dried at ambient temperature to give Hydrate I (tabular crystals) (28.9g).

IR consistent with Hydrate I

Example 8

Preparation of Hydrate II by Seeding

A mixture of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-eno-pyranosonic acid (30.0g) and water (540ml) was heated at 75° to give a solution. The resultant solution was clarified by vacuum filtration, washing through with water (54ml). The solution was heated to 100°, cooled to 40°, then seeded with Hydrate II (0.3g). Crystallisation occurred as the temperature was further reduced to ambient temperature. The resultant slurry was stirred for 1h at ambient temperature, cooled to 5°, then acetone (600ml) was added over 1.5h. The solid was collected by vacuum filtration and washed with 4:1v/v acetone/water (2x60ml) then with acetone (60ml). The solid was air-dried at ambient temperature and humidity to give Hydrate II (needle-shaped crystals) (26.5g).

XRD 90-95% Hydrate II (5-10% Hydrate I)

Example 9

Interconversion of Hydrate II to Hydrate I by Ageing

A mixture of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-eno-pyranosonic acid (10.0g) and water (200ml) was heated at 100° to give a solution. The resultant solution was rapidly cooled to 30°, seeded with Hydrate II (0.05g) then left unstirred overnight. Light-microscopy showed exclusively the characteristic needles of Hydrate II. The suspension was aged unstirred at ambient temperature for 11 days (when light-microscopy showed the presence of some crystals of Hydrate I), then stirred for 3 days. Acetone (200ml) was added, and the slurry was stirred for 1h. The solid was collected by vacuum filtration and washed with 4:1v/v acetone/water (2 x 20ml) then with acetone (20ml). The solid was air-dried at ambient temperature and humidity to give Hydrate I (tabular crystals) (9.0g).

XRD: Consistent with Hydrate I >99%)

Example 10

Interconversion of Hydrate II to Hydrate I using Base

A suspension of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid (5.0g, Hydrate II) in water (30ml), containing imidazole (2.96g) was stirred and heated at 30° for 40h. The remaining solid was collected by vacuum filtration and washed with water (2 x 1ml, 2 x 5ml) then air-dried at ambient temperature and humidity to give Hydrate I (tabular crystals) (3.98g).

IR consistent with Hydrate I

Example 11

Preparation of Hydrate I by Seeding

A mixture of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-eno-pyranosonic acid (5.0g) and water (60ml) was heated at 100° to give a solution. The resultant solution was clarified by vacuum filtration. The resultant solution was cooled to about 50°, seeded with Hydrate I, left unstirred at 50-55° for 1h, then stirred for 1h at 50-55°. Acetone (70ml) was added whilst a temperature of 48 - 55° was maintained. The slurry was stirred for 1h at 50 - 55°, then aged unstirred overnight at ambient temperature. The solid was collected by vacuum filtration and washed with 4:1v/v acetone/water (2 x 10ml) then with acetone (2 x 10ml). The solid was vacuum-dried, then allowed to re-equilibrate at ambient temperature and humidity to give Hydrate I (tabular crystals) (3.8g).

XRD: Consistant with Hydrate I (>99%)

CLAIMS

1. 5-Acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in crystalline form.

2. 5-Acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in the form of crystals having a low aspect ratio.

3. The crystalline form as claimed in Claim 2 wherein the crystals are tabular.

4. The crystalline form as claimed in any one of Claims 1 to 3 wherein substantially all water of crystallisation is lost at about 80 to 90°C.

5. The crystalline form as claimed in any one of Claims 1 to 4 for which the X-ray data are as shown in Table I.

6. 5-Acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in the form of crystals having a high aspect ratio.

7. The crystalline form as claimed in Claim 6 wherein the crystals are needle-shaped.

8. The crystalline form as claimed in any one of Claims 1, 6 or 7 wherein water content is stable over a broad range of relative humidity.

9. The crystalline form as claimed in any one of Claims 1, 6 to 8 wherein one mole of water of crystallisation is lost at about 84-90°C and a further mole of water of crystallisation is lost by about 135-143°C.

10. The crystalline form as claimed in any one of Claims 1, 6 to 9 for which the X-ray data are as shown in Table II.

11. The crystalline form claimed in any one of Claims 2 to 5 substantially free of the crystalline form claimed in any one of Claims 6 to 10.

12. The crystalline form claimed in any one of Claims 6 to 10 substantially free of the crystalline form claimed in any one of Claims 2 to 5.

13. A method for the preparation of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in crystalline form, which method comprises crystallisation of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid from aqueous solution.

14. A method as claimed in Claim 13 for the preparation of the crystalline form as claimed in any one of Claims 2 to 5.

15. A method as claimed in Claim 14 wherein the temperature of the aqueous solution is greater than about 50°C.

16. A method as claimed in Claim 15 wherein the temperature of the aqueous solution is in the range 50 to 55°C.

17. A method as claimed in any one of Claims 14 to 16 wherein the aqueous solution is seeded with crystals of the crystalline form as claimed in any one of Claims 2 to 5.

18. A method as claimed in Claim 13 for the preparation of the crystalline form as claimed in any one of Claims 6 to 10.

19. A method as claimed in Claim 18 wherein the temperature of the aqueous solution is less than about 40°C.

20 A method as claimed in Claim 19 wherein the temperature of the aqueous solution is in the range 20 to 30°C.

21. A method as claimed in any one of Claims 18 to 20 wherein the aqueous solution is seeded with crystals of the crystalline form as claimed in any one of Claims 6 to 10.

22. A method as claimed in any one of Claims 13 to 21 comprising addition of a counter solvent to the aqueous solution.

23. A method as claimed in Claim 22 wherein the counter solvent is a ketone or an alkanol.

24. A method as claimed in Claim 23 wherein the counter solvent is acetone.

25. A method for the preparation of the crystalline form as claimed in any one of Claims 2 to 5, which method comprises interconversion of the crystalline form as claimed in any one of Claims 6 to 10.

26. The method as claimed in Claim 25 wherein interconversion is effected by ageing of the aqueous solution.

27. The method as claimed in Claim 25 wherein the interconversion is effected by addition of a base to the aqueous solution.

28. A method for the preparation of the crystalline form as claimed in any one of Claims 6 to 10, which process comprises addition of an aqueous solution of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid to a similar volume of a counter solvent.

29. The method as claimed in Claim 28 wherein the counter solvent is acetone.

30. A pharmaceutical formulation comprising 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid in crystalline form and a pharmaceutically acceptable carrier therefor.

31. A pharmaceutical formulation as claimed in Claim 30 in the form of a powder.

32. A pharmaceutical formulation as claimed in Claim 30 in the form of an aqueous solution or suspension.