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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 September 2014 (25.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/152996 A1

(51) International Patent Classification:
C07F 1/04 (2006.01) *C07D 487/04* (2006.01)

(21) International Application Number:
PCT/US2014/028571

(22) International Filing Date:
14 March 2014 (14.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/784,143 14 March 2013 (14.03.2013) US

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(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).

Published:

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

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) Title: CRYSTALLINE FORM OF A BETA-LACTAMASE INHIBITOR

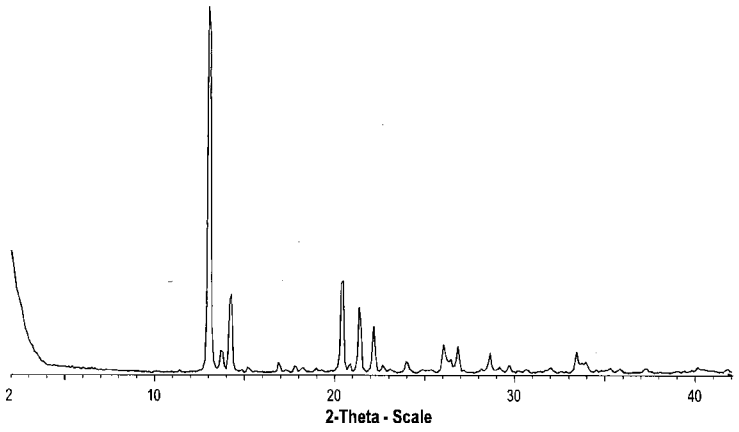

Fig. 1

(57) Abstract: This disclosure provides compositions containing solid forms of sodium (2S,5R)-2- (1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, and methods of manufacturing and using these compositions.

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CRYSTALLINE FORM OF A β-LACTAMASE INHIBITOR

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 61/784,143, filed March 14, 2013, the content of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

This disclosure relates to solid forms of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, and related compositions and methods.

BACKGROUND

The crystal state of a compound can be important when the compound is used for pharmaceutical purposes. Compared with an amorphous solid, the solid physical properties of a crystalline compound can change, which can affect its suitability for pharmaceutical use. For example, a particular crystalline compound can overcome the disadvantage of other solid forms of the compound that readily absorb moisture (high hygroscopicity). For an ionic drug substance, high hygroscopicity can diminish the drug product's stability profile by a host of mechanisms, as the drug substance may have a propensity to absorb water. Water that is absorbed from the environment (packaging materials, exposure to air, or in the case of formulated products, from other materials), can lead to degradation products and/or impurities in a drug product or add to the cost of manufacturing the drug product with acceptably low levels of water.

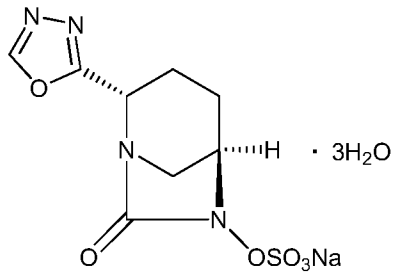
There is a need for solid forms of (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl hydrogen sulfate for use in drug substance and drug product development.

SUMMARY

Solid forms of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, and compositions comprising these solid forms, are

provided herein, in addition to various methods of preparing these compositions. A crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate solid form provided herein has advantageous characteristics that are beneficial to the preparation of various drug formulations. For example, the hydrate form of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is less hygroscopic compared to the amorphous form of this compound. These solid forms can have good stability in the process of preparation, packing, transportation and storage.

Sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, and solvates thereof, can be obtained in various solid forms. In one aspect, provided herein is a particularly preferred crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate solid designated herein as "Hydrate 1". Hydrate 1 is a trihydrate, and has the following structure:



In an embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees 2-Theta at angles of 13.09 ± 0.3°, 13.79 ± 0.3°, 14.29 ± 0.3°, 20.49 ± 0.3°, 21.46 ± 0.3°, 22.21 ± 0.3°, 26.10 ± 0.3°, 26.87 ± 0.3°, 28.65 ± 0.3°, and 33.46 ± 0.3°. Hydrate 1 can also be characterized by an X-ray powder diffraction pattern having peaks expressed in degrees 2-Theta at angles of 13.09 ± 0.3°, 14.29 ± 0.3°, 20.49 ± 0.3°, 21.46 ± 0.3°, and 22.21 ± 0.3°.

In one embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, and 21.5 ± 0.2°. In a particular embodiment, the X-ray powder diffraction pattern of Hydrate 1 has an additional peak expressed in degrees-2-theta at angle 22.2 ± 0.2°. In another embodiment, the X-ray powder diffraction pattern of Hydrate 1 has additional peaks expressed in degrees-2-theta at angles 13.8 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.6 ± 0.2°, and 33.5 ± 0.2°.

In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 13.8 ±

0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, 21.5 ± 0.2°, 22.2 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.7 ± 0.2°, and 33.5 ± 0.2°.The crystalline Hydrate 1 can be further characterized by a differential scanning calorimetry (DSC) thermogram having three main events; a dehydration (40°C-165°C) and two degradation/decomposition steps (onset at ~160°C and and 240°C, respectively). In another embodiment, Hydrate 1 is further characterized by a thermogravimetry (TGA) curve. Three distinct events observed by DSC are associated with weight losses by TGA. The endothermic first event occurs between 40°C-165°C, with a corresponding loss of 13.1% w/w loss on the TGA is attribute to loss of water of hydration. The other two events, an exothermic second event (165°C-240°C; 12.1% w/w) and an endothermic third event (240°C-330°C; 18.8%w/w) can be attributable to sample degradation or decomposition (Figure 3).

In one aspect, Hydrate 1 is obtained by a process comprising maintaining sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate in conditions effective to form Hydrate 1 from another solid form disclosed herein. In one example, Hydrate 1 can be formed by maintaining sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate at a temperature of 20°C – 30°C and a relative humidity ("RH") of about 40%-100% (e.g., 40% – 98% RH). In a particular embodiment, Hydrate 1 is obtained by a process comprising exposing amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate to conditions comprising a temperature of 25°C and a relative humidity of <60% (e.g., 40%-60% RH and 25 °C). In one embodiment, Hydrate 1 is obtained by a process comprising maintaining amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate in ambient conditions (e.g., 25 °C and about 40-100% RH, or about 40-60% RH).

Also provided herein is a method of making Hydrate 1 comprising the steps of:

(a) combining sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate and a solvent, such that a solution of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is formed; and

(b) combining an antisolvent with the solution, wherein the antisolvent is miscible with the solvent and wherein sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is partially or completely insoluble in the antisolvent, such that crystalline Hydrate 1 precipitates from the solution. In one embodiment, the solvent in step (a) of the method is water. In another embodiment, the antisolvent in step (b) of the

method is THF or acetonitrile.

Also provided herein is a crystalline form of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, which is referred to herein as Hydrate 2. In one embodiment, Hydrate 2 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 10.2 ± 0.2°, 16.2 ± 0.2°, 28.1 ± 0.2°, and 30.2 ± 0.2°. In a particular embodiment, the X-ray powder diffraction pattern of Hydrate 2 has additional peaks expressed in degrees-2-theta at angles 17.6 ± 0.2°, 20.8 ± 0.2°, and 22.9 ± 0.2°. In another embodiment, the X-ray powder diffraction pattern of Hydrate 2 has additional peaks expressed in degrees-2-theta at angles 21.1 ± 0.2°, 21.8 ± 0.2°, 31.6 ± 0.2°, and 33.4 ± 0.2°.

In still another embodiment, Hydrate 2 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 10.2 ± 0.2°, 16.2 ± 0.2°, 17.6 ± 0.2°, 20.8 ± 0.2°, 21.1 ± 0.2°, 21.8 ± 0.2°, 22.9 ± 0.2°, 28.1 ± 0.2°, 30.2 ± 0.2°, 31.6 ± 0.2°, and 33.4 ± 0.2°.

Also provided herein is a crystalline form of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, which is referred to herein as Hydrate 3. In one embodiment, Hydrate 3 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 6.7 ± 0.2°, 13.0 ± 0.2°, 16.5 ± 0.2°, and 25.0 ± 0.2°. In a particular embodiment, the X-ray powder diffraction pattern of Hydrate 3 has additional peaks expressed in degrees-2-theta at angles 6.1 ± 0.2°, 18.0 ± 0.2°, 21.6 ± 0.2°, and 22.0 ± 0.2°. In another embodiment, the X-ray powder diffraction pattern of Hydrate 3 has additional peaks expressed in degrees-2-theta at angles 4.3 ± 0.2°, 14.0 ± 0.2°, 14.2 ± 0.2°, 17.2 ± 0.2°, 19.8 ± 0.2°, 20.5 ± 0.2°, 21.4 ± 0.2°, and 22.4 ± 0.2°.

In still another embodiment, Hydrate 3 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 4.3 ± 0.2°, 6.1 ± 0.2°, 6.7 ± 0.2°, 13.0 ± 0.2°, 14.0 ± 0.2°, 14.2 ± 0.2°, 16.5 ± 0.2°, 17.2 ± 0.2°, 18.0 ± 0.2°, 19.8 ± 0.2°, 20.5 ± 0.2°, 21.4 ± 0.2°, 21.6 ± 0.2°, 22.0 ± 0.2°, 22.4 ± 0.2° and 25.0 ± 0.2°.

Also provided herein is a crystalline form of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, which is referred to herein as Hydrate 4. In one embodiment, Hydrate 4 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 6.5 ± 0.2°, 7.6 ± 0.2°, 8.3 ± 0.2°, 21.6 ± 0.2°, and 23.4 ± 0.2°. In a particular embodiment, the X-ray powder diffraction pattern of Hydrate 4 has additional peaks expressed in degrees-2-theta at angles 15.2 ± 0.2°, 15.8 ± 0.2°, 17.1 ± 0.2°,

18.0 ± 0.2°, 24.4 ± 0.2°, and 25.1 ± 0.2°. In another embodiment, the X-ray powder diffraction pattern of Hydrate 4 has additional peaks expressed in degrees-2-theta at angles 16.6 ± 0.2°, 18.3 ± 0.2°, 20.7 ± 0.2°, 21.2 ± 0.2°, and 30.2 ± 0.2°.

In still another embodiment, Hydrate 4 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 6.5 ± 0.2°, 7.6 ± 0.2°, 8.3 ± 0.2°, 15.2 ± 0.2°, 15.8 ± 0.2°, 16.6 ± 0.2°, 17.1 ± 0.2°, 18.0 ± 0.2°, 18.3 ± 0.2°, 20.7 ± 0.2°, 21.2 ± 0.2°, 21.6 ± 0.2°, 23.4 ± 0.2°, and 30.2 ± 0.2°.

Also provided herein is a crystalline form of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, which is referred to herein as Hydrate 5. In one embodiment, Hydrate 5 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 8.8 ± 0.2°, 15.8 ± 0.2°, 16.5 ± 0.2°, 17.9 ± 0.2°, 18.9 ± 0.2°, 19.9 ± 0.2°, 21.1 ± 0.2°, 22.6 ± 0.2°, and 23.9 ± 0.2°. In a particular embodiment, the X-ray powder diffraction pattern of Hydrate 5 has additional peaks expressed in degrees-2-theta at angles 7.8 ± 0.2°, 10.2 ± 0.2°, 16.2 ± 0.2°, 21.8 ± 0.2°, and 25.8 ± 0.2°. In another embodiment, the X-ray powder diffraction pattern of Hydrate 5 has additional peaks expressed in degrees-2-theta at angles 13.8 ± 0.2°, 14.9 ± 0.2°, 17.5 ± 0.2°, 18.4 ± 0.2°, 18.5 ± 0.2°, 20.6 ± 0.2°, 27.9 ± 0.2°, 29.1 ± 0.2°, 30.4 ± 0.2°, and 34.4 ± 0.2°.

In still another embodiment, Hydrate 5 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 7.8 ± 0.2°, 8.8 ± 0.2°, 10.2 ± 0.2°, 13.8 ± 0.2°, 14.9 ± 0.2°, 15.8 ± 0.2°, 16.2 ± 0.2°, 16.5 ± 0.2°, 17.5 ± 0.2°, 17.9 ± 0.2°, 18.4 ± 0.2°, 18.5 ± 0.2°, 18.9 ± 0.2°, 19.9 ± 0.2°, 20.6 ± 0.2°, 21.1 ± 0.2°, 21.8 ± 0.2°, 22.6 ± 0.2°, 23.9 ± 0.2°, 25.8 ± 0.2°, 27.9 ± 0.2°, 29.1 ± 0.2°, 30.4 ± 0.2°, and 34.4 ± 0.2°.

Also provided are pharmaceutical compositions comprising Hydrate 1 and a pharmaceutically acceptable carrier. In one embodiment the pharmaceutical composition is suitable for intravenous administration (e.g., by reconstituting the pharmaceutical composition comprising Hydrate 1 in a pharmaceutically acceptable liquid prior to intravenous administration). In another embodiment, provided herein is a unit dosage container (e.g., a vial) comprising Hydrate 1 in a fixed dose combination with a β-lactam antibiotic.

In another aspect, provided herein is a method for the treatment of bacterial infections in a subject, comprising administering to said mammal a therapeutically effective amount of Hydrate 1. In an embodiment of this method, Hydrate 1 can be characterized by an X-ray

powder diffraction pattern having peaks expressed in degrees 2-Theta at angles of 13.1 ± 0.3°, 13.8 ± 0.3°, 14.3 ± 0.3°, 20.5 ± 0.3°, 21.5 ± 0.3°, 22.2 ± 0.3°, 26.1 ± 0.3°, 26.9 ± 0.3°, 28.7 ± 0.3°, and 33.5 ± 0.3°. In another embodiment, Hydrate 1 can also be characterized by an X-ray powder diffraction pattern having peaks expressed in degrees 2-Theta at angles of 13.1 ± 0.3°, 14.3 ± 0.3°, 20.5 ± 0.3°, 21.5 ± 0.3°, and 22.2 ± 0.3°.

In one embodiment, the method further comprises reconstituting a combination of one or more of the hydrate forms provided herein and a beta-lactam antibiotic in an aqueous solvent. In an embodiment, a combination of Hydrate 1 and ceftolozane is reconstituted in an aqueous solvent (e.g., 0.9% aqueous sodium chloride saline), such that the resulting solution is suitable for infusion. The mixture can be reconstituted in saline and/or sterile water for injection. In another embodiment, provided herein is a method of treating a bacterial infection in a subject, comprising reconstituting a composition comprising Hydrate 1 and ceftolozane in an aqueous solvent, and intravenously administering the resulting composition to the subject.

In one embodiment of these methods, the bacterial infection is caused by bacteria selected from the group consisting of: *Staphylococcus aureus, Escherichia coli, Acinetobacter baumanii, Haemophilus influenzae, Klebsiella pneumonia, and Pseudomonas aeruginosa.* In another embodiment of these methods, the bacterial infection is selected from the group consisting of nosocomial pneumonia, complicated intra-abdominal infection and complicated urinary tract infection.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the X-ray powder diffraction pattern of Hydrate 1 of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate.

Figure 2 depicts the thermal stability of Hydrate 1 at 25°C at 40% relative humidity over 2 days.

Figure 3 depicts the differential scanning calorimetry (DSC) thermogram and the thermogravimetry curve of Hydrate 1.

Figure 4 depicts the DVS graph of amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate.

Figure 5 depicts the DVS graph of Hydrate 1.

Figure 6 depicts the X-ray powder diffraction patterns of a number of hydrate forms of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate identified in the polymorph studies described herein.

Figure 7 shows a diagram that summarizes the relative thermodynamic relationships between the different hydrated forms of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate.

Figure 8 shows the single crystal X-ray diffraction structure of Hydrate 1.

Figure 9 shows the single crystal X-ray diffraction structure of Hydrate 2.

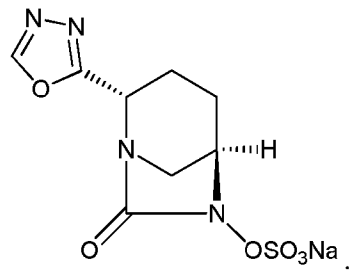
Figure 10 shows the single crystal X-ray diffraction structure of Hydrate 5.

DETAILED DESCRIPTION

Bacterial resistance to β -lactam antibiotics, especially in Gram-negative bacteria, is most commonly mediated by β-lactamases. β-lactamases are enzymes that catalyze the hydrolysis of the β-lactam ring, which inactivates the antibacterial activity of the β-lactam antibiotic and allows the bacteria to become resistant. Inhibition of the β-lactamase with a β-lactamase inhibitor (BLI) slows or prevents degradation of the β -lactam antibiotic and restores β -lactam antibiotic susceptibility to β-lactamase producing bacteria. Sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is an effective BLI.

Sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate can occur in an amorphous solid form or in a crystalline solid form. Crystalline solid forms of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate can exist in one or more unique polymorph forms, which may additionally comprise one or more equivalents of water (i.e., a hydrate of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate).

Sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate as represented by the structure below.



Accordingly, provided herein are hydrates of crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate. In particular, provided herein is "Hydrate 1", a particular hydrate of crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate.

In other embodiments, provided herein are "Hydrate 2", "Hydrate 3", "Hydrate 4", "Hydrate 5", "Hydrate 6", and "Hydrate 7", each of which is a particular hydrate of crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate.

Polymorphism

The ability of a substance to exist in more than one crystal form is defined as polymorphism; the different crystal forms of a particular substance are referred to as "polymorphs."

Polymorphic forms of a drug substance refer to crystalline and amorphous forms as well as solvate and hydrate forms (see, e.g., Guidance for industry, Q6A *Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances*, International Conference on Harmonisation (ICH), December 2000). Crystalline forms have different arrangements and/or conformations of the molecules in the crystal lattice. Solvates are crystal forms containing either stoichiometric or nonstoichiometric amounts of a solvent (see, e.g., SR Byrn, RR Pfeiffer, and JG Stowell. *Solid-State Chemistry of Drugs*. 2nd Edition, SSCI, Inc., West Lafayette, Indiana, 1999); if the incorporated solvent is water, the solvate is commonly known as a hydrate. In contrast, amorphous forms consist of disordered arrangements of molecules that do not possess a distinguishable crystal lattice. When a drug substance exists in polymorphic forms, it is said to exhibit polymorphism.

In general, polymorphism is affected by the ability of a molecule of a substance to change its conformation or to form different intermolecular or intra-molecular interactions, particularly hydrogen bonds, which is reflected in different atom arrangements in the crystal lattices of different polymorphs. In contrast, the overall external form of a substance is known as "morphology," which refers to the external shape of the crystal and the planes present, without reference to the internal structure. Crystals can display different morphology

based on different conditions, such as, for example, growth rate, stirring, and the presence of impurities.

Polymorphic forms of a drug substance can have different chemical and physical properties, including melting point, chemical reactivity, apparent solubility, dissolution rate, optical and mechanical properties, vapor pressure, and density. These properties can have a direct effect on the ability to process and/or manufacture the drug substance and the drug product, as well as on drug product stability, dissolution, and bioavailability. Thus, polymorphism also can affect the quality, safety, and efficacy of the drug product.

Access to different polymorphs of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is desirable for other reasons as well. One such reason is that different polymorphs of a compound (e.g., sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate) can incorporate different impurities, or chemical residues, upon crystallization. Certain polymorphs incorporate very little, or no, chemical residues. Accordingly, the formation of certain polymorph forms of a compound may result in purification of the compound.

Crystalline Hydrate 1 exhibits low hygroscopicity relative to amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl. Low hygroscopicity of a solid compound is desirable for several reasons. For example, compounds that are highly hygroscopic may be chemically unstable, or unsuitable for formulating as a drug product due to changes of the drug form's physical characteristics (e.g., bulk density, dissolution rate, etc.) that can occur if it is stored in settings with varying relative humidity. Also, hygroscopicity can impact large-scale manufacturing and handling of a compound. For example, it can be difficult to determine the true weight of a hygroscopic active agent when preparing a pharmaceutical composition comprising that agent.

Figure 4 depicts the DVS graph of amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate. In contrast, Figure 5 depicts the DVS graph of Hydrate 1. As can be seen, Hydrate 1 is significantly less hygroscopic than the amorphous form of this compound.

The Hydrate 1 has also demonstrated favorable stability. For example, Figure 2 depicts the thermal stability of Hydrate 1 at 25°C at 40% relative humidity over 2 days. As shown in this figure, there is little to no change to the Hydrate 1 crystal form over this time period.

Characterization of Polymorphs

There are a number of methods that can be used to characterize polymorphs of a drug substance (see, e.g., H Brittain. "Methods for the characterization of polymorphs and solvates." In HG Brittain (ed.) *Polymorphism in Pharmaceutical Solids*. Marcel Dekker, Inc., New York, 1999, pp. 227-278). Demonstration of a nonequivalent structure by single crystal X-ray diffraction is currently regarded as the definitive evidence of polymorphism. X-ray powder diffraction can also be used to provide unequivocal proof of polymorphism. Other methods, including microscopy, thermal analysis (e.g., differential scanning calorimetry, thermal gravimetric analysis, and hot-stage microscopy), and spectroscopy (e.g., infrared [IR], Raman, solid-state nuclear magnetic resonance [ssNMR]) also are helpful to further characterize polymorphic forms.

Accordingly, in certain embodiments, the compounds of the invention are identifiable on the basis of characteristic peaks in an X-ray powder diffraction analysis. X-ray powder diffraction, also referred to as XRPD, is a scientific technique using X-ray, neutron, or electron diffraction on powder, microcrystalline, or other solid materials for structural characterization of the materials.

One embodiment of crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is referred to herein as Hydrate 1. In one embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or more characteristic peaks expressed in degrees 2-Theta at angles selected from 13.1 ± 0.3°, 14.3 ± 0.3°, 20.5 ± 0.3°, 21.5 ± 0.3°, and 22.2 ± 0.3°. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or more peaks expressed in degrees 2-Theta at angles selected from 13.1 ± 0.3°, 14.3 ± 0.3°, 20.5 ± 0.3°, and 21.5 ± 0.3°. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or more peaks expressed in degrees 2-Theta at angles selected from 13.1 ± 0.3°, 14.3 ± 0.3°, 20.5 ± 0.3°, 21.5 ± 0.3°, and 22.2 ± 0.3°. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or more peaks expressed in degrees 2-Theta at angles selected from 13.1 ± 0.3°, 13.8 ± 0.3°, 14.3 ± 0.3°, 20.5 ± 0.3°, 21.5 ± 0.3°, 22.2 ± 0.3°, 26.1 ± 0.3°, 26.8 ± 0.3°, 28.7 ± 0.3°, and 33.5 ± 0.3°.

In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or more characteristic peaks expressed in degrees 2-Theta at angles selected from 13.1 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, 21.5 ± 0.2°, and 22.2 ± 0.2°. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or

more peaks expressed in degrees 2-Theta at angles selected from 13.1 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, and 21.5 ± 0.2°. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or more peaks expressed in degrees 2-Theta at angles selected from 13.1 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, 21.5 ± 0.2°, and 22.2 ± 0.2°. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or more peaks expressed in degrees 2-Theta at angles selected from 13.1 ± 0.2°, 13.8 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, 21.5 ± 0.2°, 22.2 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.7 ± 0.2°, and 33.5 ± 0.2°.

In yet another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, and 21.5 ± 0.2°. In a particular embodiment, the X-ray powder diffraction pattern of Hydrate 1 has an additional peak expressed in degrees-2-theta at angle 22.2 ± 0.2°. In another embodiment, the X-ray powder diffraction pattern of Hydrate 1 has additional peaks expressed in degrees-2-theta at angles 13.8 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.6 ± 0.2°, and 33.5 ± 0.2°.

In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 13.8 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, 21.5 ± 0.2°, 22.2 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.7 ± 0.2°, and 33.5 ± 0.2°. In yet another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having five or more peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 13.8 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, 21.5 ± 0.2°, 22.2 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.7 ± 0.2°, and 33.5 ± 0.2°. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having six or more peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 13.8 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, 21.5 ± 0.2°, 22.2 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.7 ± 0.2°, and 33.5 ± 0.2°.

In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or more characteristic peaks expressed in degrees 2-Theta at angles selected from 13.1, 14.3, 20.5, 21.5, and 22.2. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or more peaks expressed in degrees 2-Theta at angles selected from 13.1, 14.3, 20.5, and 21.5. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or more peaks expressed in degrees 2-Theta at angles selected from 13.1, 14.3, 20.5, 21.5, and 22.2. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having one or more peaks expressed in degrees 2-Theta at angles selected from 13.1, 13.8,

14.3, 20.5, 21.5, 22.2, 26.1, 26.9, 28.7, and 33.5. In one embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having peaks substantially in accordance with Figure 1. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having peaks substantially in accordance with Table 1.

The crystalline Hydrate 1 can be further characterized by a differential scanning calorimetry (DSC) thermogram having two main events; an endothermic event (dehydration; onselt ~65°C) and an exothermic event (degradation; onset ~187°C). In an embodiment, Hydrate 1 is further characterized by a thermogravimetric analysis (TGA) curve. Two distinct events observed by DSC are associated with weight losses by TGA. The endothermic first event occurs between 40°C-165°C, with a corresponding loss of 13.1% w/w loss on the TGA is attribute to dehydration. The other event, an exothermic second event (165°C-240°C; 12.1% w/w) can be attributable to sample degradation or decomposition (Figure 3).

In certain embodiments, Hydrate 1 may contain impurities. Non-limiting examples of impurities include undesired polymorph forms, or residual organic and inorganic molecules such as solvents, water or salts.

In another embodiment, Hydrate 1 is substantially free from impurities. In another embodiment, Hydrate 1 contains less than 10% by weight total impurities. In another embodiment, Hydrate 1 contains less than 5% by weight total impurities. In another embodiment, Hydrate 1 contains less than 1% by weight total impurities. In yet another embodiment, Hydrate 1 contains less than 0.1% by weight total impurities.

In certain embodiments, Hydrate 1 is a crystalline solid substantially free of amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate. As used herein, the term "substantially free of amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate" means that the compound contains no significant amount of amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate. In certain embodiments, at least about 95% by weight of crystalline Hydrate 1 is present. In still other embodiments of the invention, at least about 99% by weight of crystalline Hydrate 1 is present.

In another embodiment, Hydrate 1 is substantially free of Hydrates 2, 3, 4, 5, 6, or 7. As used herein, the term "substantially free of Hydrates 2, 3, 4, 5, 6, or 7" means that Hydrate 1 contains no significant amount of Hydrates 2, 3, 4, 5, 6, or 7. In certain embodiments, at

least about 95% by weight of crystalline Hydrate 1 is present. In still other embodiments of the invention, at least about 99% by weight of crystalline Hydrate 1 is present.

Another embodiment of crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is referred to herein as Hydrate 2. In one embodiment, Hydrate 2 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 10.2 ± 0.2°, 16.2 ± 0.2°, 28.1 ± 0.2°, and 30.2 ± 0.2°. In a particular embodiment, the X-ray powder diffraction pattern of Hydrate 2 has additional peaks expressed in degrees-2-theta at angles 17.6 ± 0.2°, 20.8 ± 0.2°, and 22.9 ± 0.2°. In another embodiment, the X-ray powder diffraction pattern of Hydrate 2 has additional peaks expressed in degrees-2-theta at angles 21.1 ± 0.2°, 21.8 ± 0.2°, 31.6 ± 0.2°, and 33.4 ± 0.2°.

In still another embodiment, Hydrate 2 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 10.2 ± 0.2°, 16.2 ± 0.2°, 17.6 ± 0.2°, 20.8 ± 0.2°, 21.1 ± 0.2°, 21.8 ± 0.2°, 22.9 ± 0.2°, 28.1 ± 0.2°, 30.2 ± 0.2°, 31.6 ± 0.2°, and 33.4 ± 0.2°. In yet another embodiment, Hydrate 2 is characterized by an X-ray powder diffraction pattern having five or more peaks expressed in degrees-2-theta at angles 10.2 ± 0.2°, 16.2 ± 0.2°, 17.6 ± 0.2°, 20.8 ± 0.2°, 21.1 ± 0.2°, 21.8 ± 0.2°, 22.9 ± 0.2°, 28.1 ± 0.2°, 30.2 ± 0.2°, 31.6 ± 0.2°, and 33.4 ± 0.2°. In another embodiment, Hydrate 2 is characterized by an X-ray powder diffraction pattern having six or more peaks expressed in degrees-2-theta at angles 10.2 ± 0.2°, 16.2 ± 0.2°, 17.6 ± 0.2°, 20.8 ± 0.2°, 21.1 ± 0.2°, 21.8 ± 0.2°, 22.9 ± 0.2°, 28.1 ± 0.2°, 30.2 ± 0.2°, 31.6 ± 0.2°, and 33.4 ± 0.2°.

Another embodiment of crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is referred to herein as Hydrate 3. In one embodiment, Hydrate 3 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 6.7 ± 0.2°, 13.0 ± 0.2°, 16.5 ± 0.2°, and 25.0 ± 0.2°. In a particular embodiment, the X-ray powder diffraction pattern of Hydrate 3 has additional peaks expressed in degrees-2-theta at angles 6.1 ± 0.2°, 18.0 ± 0.2°, 21.6 ± 0.2°, and 22.0 ± 0.2°. In another embodiment, the X-ray powder diffraction pattern of Hydrate 3 has additional peaks expressed in degrees-2-theta at angles 4.3 ± 0.2°, 14.0 ± 0.2°, 14.2 ± 0.2°, 17.2 ± 0.2°, 19.8 ± 0.2°, 20.5 ± 0.2°, 21.4 ± 0.2°, and 22.4 ± 0.2°.

In still another embodiment, Hydrate 3 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 4.3 ± 0.2°, 6.1 ± 0.2°, 6.7 ± 0.2°, 13.0 ± 0.2°, 14.0 ± 0.2°, 14.2 ± 0.2°, 16.5 ± 0.2°, 17.2 ± 0.2°, 18.0 ± 0.2°, 19.8 ± 0.2°, 20.5 ± 0.2°, 21.4 ± 0.2°, 21.6 ± 0.2°, 22.0 ± 0.2°, 22.4 ± 0.2° and 25.0 ± 0.2°. In

yet another embodiment, Hydrate 3 is characterized by an X-ray powder diffraction pattern having five or more peaks expressed in degrees-2-theta at angles 4.3 ± 0.2°, 6.1 ± 0.2°, 6.7 ± 0.2°, 13.0 ± 0.2°, 14.0 ± 0.2°, 14.2 ± 0.2°, 16.5 ± 0.2°, 17.2 ± 0.2°, 18.0 ± 0.2°, 19.8 ± 0.2°, 20.5 ± 0.2°, 21.4 ± 0.2°, 21.6 ± 0.2°, 22.0 ± 0.2°, 22.4 ± 0.2° and 25.0 ± 0.2°. In another embodiment, Hydrate 3 is characterized by an X-ray powder diffraction pattern having six or more peaks expressed in degrees-2-theta at angles 4.3 ± 0.2°, 6.1 ± 0.2°, 6.7 ± 0.2°, 13.0 ± 0.2°, 14.0 ± 0.2°, 14.2 ± 0.2°, 16.5 ± 0.2°, 17.2 ± 0.2°, 18.0 ± 0.2°, 19.8 ± 0.2°, 20.5 ± 0.2°, 21.4 ± 0.2°, 21.6 ± 0.2°, 22.0 ± 0.2°, 22.4 ± 0.2° and 25.0 ± 0.2°.

Another embodiment of crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is referred to herein as Hydrate 4. In one embodiment, Hydrate 4 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 6.5 ± 0.2°, 7.6 ± 0.2°, 8.3 ± 0.2°, 21.6 ± 0.2°, and 23.4 ± 0.2°. In a particular embodiment, the X-ray powder diffraction pattern of Hydrate 4 has additional peaks expressed in degrees-2-theta at angles 15.2 ± 0.2°, 15.8 ± 0.2°, 17.1 ± 0.2°, 18.0 ± 0.2°, 24.4 ± 0.2°, and 25.1 ± 0.2°. In another embodiment, the X-ray powder diffraction pattern of Hydrate 4 has additional peaks expressed in degrees-2-theta at angles 16.6 ± 0.2°, 18.3 ± 0.2°, 20.7 ± 0.2°, 21.2 ± 0.2°, and 30.2 ± 0.2°.

In still another embodiment, Hydrate 4 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 6.5 ± 0.2°, 7.6 ± 0.2°, 8.3 ± 0.2°, 15.2 ± 0.2°, 15.8 ± 0.2°, 16.6 ± 0.2°, 17.1 ± 0.2°, 18.0 ± 0.2°, 18.3 ± 0.2°, 20.7 ± 0.2°, 21.2 ± 0.2°, 21.6 ± 0.2°, 23.4 ± 0.2°, and 30.2 ± 0.2°. In yet another embodiment, Hydrate 4 is characterized by an X-ray powder diffraction pattern having five or more peaks expressed in degrees-2-theta at angles 6.5 ± 0.2°, 7.6 ± 0.2°, 8.3 ± 0.2°, 15.2 ± 0.2°, 15.8 ± 0.2°, 16.6 ± 0.2°, 17.1 ± 0.2°, 18.0 ± 0.2°, 18.3 ± 0.2°, 20.7 ± 0.2°, 21.2 ± 0.2°, 21.6 ± 0.2°, 23.4 ± 0.2°, and 30.2 ± 0.2°. In another embodiment, Hydrate 4 is characterized by an X-ray powder diffraction pattern having six or more peaks expressed in degrees-2-theta at angles 6.5 ± 0.2°, 7.6 ± 0.2°, 8.3 ± 0.2°, 15.2 ± 0.2°, 15.8 ± 0.2°, 16.6 ± 0.2°, 17.1 ± 0.2°, 18.0 ± 0.2°, 18.3 ± 0.2°, 20.7 ± 0.2°, 21.2 ± 0.2°, 21.6 ± 0.2°, 23.4 ± 0.2°, and 30.2 ± 0.2°.

Another embodiment of crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is referred to herein as Hydrate 5. In one embodiment, Hydrate 5 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 8.8 ± 0.2°, 15.8 ± 0.2°, 16.5 ± 0.2°, 17.9 ± 0.2°, 18.9 ± 0.2°, 19.9 ± 0.2°, 21.1 ± 0.2°, 22.6 ± 0.2°, and 23.9 ± 0.2°. In a particular embodiment, the X-

ray powder diffraction pattern of Hydrate 5 has additional peaks expressed in degrees-2-theta at angles 7.8 ± 0.2°, 10.2 ± 0.2°, 16.2 ± 0.2°, 21.8 ± 0.2°, and 25.8 ± 0.2°. In another embodiment, the X-ray powder diffraction pattern of Hydrate 5 has additional peaks expressed in degrees-2-theta at angles 13.8 ± 0.2°, 14.9 ± 0.2°, 17.5 ± 0.2°, 18.4 ± 0.2°, 18.5 ± 0.2°, 20.6 ± 0.2°, 27.9 ± 0.2°, 29.1 ± 0.2°, 30.4 ± 0.2°, and 34.4 ± 0.2°.

In still another embodiment, Hydrate 5 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 7.8 ± 0.2°, 8.8 ± 0.2°, 10.2 ± 0.2°, 13.8 ± 0.2°, 14.9 ± 0.2°, 15.8 ± 0.2°, 16.2 ± 0.2°, 16.5 ± 0.2°, 17.5 ± 0.2°, 17.9 ± 0.2°, 18.4 ± 0.2°, 18.5 ± 0.2°, 18.9 ± 0.2°, 19.9 ± 0.2°, 20.6 ± 0.2°, 21.1 ± 0.2°, 21.8 ± 0.2°, 22.6 ± 0.2°, 23.9 ± 0.2°, 25.8 ± 0.2°, 27.9 ± 0.2°, 29.1 ± 0.2°, 30.4 ± 0.2°, and 34.4 ± 0.2°. In another embodiment, Hydrate 5 is characterized by an X-ray powder diffraction pattern having five or more peaks expressed in degrees-2-theta at angles 7.8 ± 0.2°, 8.8 ± 0.2°, 10.2 ± 0.2°, 13.8 ± 0.2°, 14.9 ± 0.2°, 15.8 ± 0.2°, 16.2 ± 0.2°, 16.5 ± 0.2°, 17.5 ± 0.2°, 17.9 ± 0.2°, 18.4 ± 0.2°, 18.5 ± 0.2°, 18.9 ± 0.2°, 19.9 ± 0.2°, 20.6 ± 0.2°, 21.1 ± 0.2°, 21.8 ± 0.2°, 22.6 ± 0.2°, 23.9 ± 0.2°, 25.8 ± 0.2°, 27.9 ± 0.2°, 29.1 ± 0.2°, 30.4 ± 0.2°, and 34.4 ± 0.2°. In still another embodiment, Hydrate 5 is characterized by an X-ray powder diffraction pattern having six or more peaks expressed in degrees-2-theta at angles 7.8 ± 0.2°, 8.8 ± 0.2°, 10.2 ± 0.2°, 13.8 ± 0.2°, 14.9 ± 0.2°, 15.8 ± 0.2°, 16.2 ± 0.2°, 16.5 ± 0.2°, 17.5 ± 0.2°, 17.9 ± 0.2°, 18.4 ± 0.2°, 18.5 ± 0.2°, 18.9 ± 0.2°, 19.9 ± 0.2°, 20.6 ± 0.2°, 21.1 ± 0.2°, 21.8 ± 0.2°, 22.6 ± 0.2°, 23.9 ± 0.2°, 25.8 ± 0.2°, 27.9 ± 0.2°, 29.1 ± 0.2°, 30.4 ± 0.2°, and 34.4 ± 0.2°.

Processes and Methods

Provided herein is a method of making crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, comprising:

(1) combining sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate and a solvent, such that a solution of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is formed; and

(2) evaporating the solvent, such that crystalline sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is formed.

In another embodiment, provided herein is a method of making Hydrate 1, comprising:

(1) combining sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate and a solvent, such that a solution of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is formed; and

(2) evaporating the solvent, such that Hydrate 1 is formed.

In certain embodiments of these methods, the solvent is anisole, ethyl acetate, isopropyl acetate, methylisobutyl ketone, 2-propanol, dimethyl sulfoxide, t-butylmethyl ether, toluene, tetrahydrofuran, dichloromethane, acetonitrile, nitromethane, isopropyl alcohol, water, or mixtures thereof.

In step (1) of these methods, the sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate can be in an amorphous form. In one embodiment, an amorphous form of the compound is formed by combining sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate with a solvent, such as water, followed by freeze drying or lyophilization.

In certain embodiments, any one of the above methods is a method of making Hydrate 1 wherein the method further comprises: (3) drying the crystalline compound to form Hydrate 1.

Also provided herein is a method of making Hydrate 1 comprising:

(1) combining sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate and a solvent, such that a solution of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is formed; and

(2) combining an antisolvent with the solution, wherein the antisolvent is miscible with the solvent and wherein sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is partially or completely insoluble in the antisolvent, such that crystalline Hydrate 1 precipitates from the solution.

In certain embodiments of this solvent / antisolvent method, the solvent is water, and the antisolvent is THF or acetonitrile.

In one embodiment, the solvent in step (a) of the method is water. In another embodiment, the antisolvent in step (b) of the method is THF or acetonitrile.

As used herein, the term “antisolvent” generally comprises a solvent that can be used in crystallization of a compound; the antisolvent is miscible with a solution of the compound, but the compound itself is practically insoluble in the antisolvent.

In yet another embodiment, Hydrate 1 is obtained by a process comprising exposing amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl

sulfate to conditions comprising a temperature of 20° C – 30° C and a relative humidity of 40% – 98%. In a particular embodiment, Hydrate 1 is obtained by a process comprising exposing amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate to conditions comprising a temperature of 25° C and a relative humidity of <60%. In one embodiment, Hydrate 1 is obtained by a process comprising exposing amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate to ambient conditions.

In another embodiment, Hydrate 1 of (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, obtained by a process comprising exposing amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate to conditions comprising a temperature of 20° C – 45° C and a relative humidity of 40% – 98%.

In still another embodiment, Hydrate 1 is obtained by a process comprising exposing amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate to storage conditions comprising a temperature of 20° C – 30° C and a relative humidity of 40% – 98%. In a particular embodiment, Hydrate 1 is obtained by a process comprising exposing amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate to storage conditions comprising a temperature of 25° C and a relative humidity of less than 60%. In one embodiment, Hydrate 1 is obtained by a process comprising exposing amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate to ambient storage conditions.

In an embodiment, Hydrate 2 can be obtained by a process comprising exposing Hydrate 1 to conditions comprising a temperature of 25° C and a relative humidity of greater than 80%.

As used herein, the term “storage” refers to a period of time that is more than 1 hour, e.g, more than 2 hours, e.g., more than 3 hours, e.g., more than 4 hours, e.g., more than 5 hours, e.g., more than 6 hours, e.g., more than 7 hours, e.g., more than 8 hours, e.g., more than 9 hours, e.g., more than 10 hours, e.g., more than 24 hours. In addition, storage conditions preferably comprise a temperature of 20° C – 30° C.

As used herein, the term “ambient conditions” generally refers to a temperature of about 25° C and a relative humidity of about 40%.

Pharmaceutical Compositions Comprising Hydrate 1 and Use Thereof

Provided herein are pharmaceutical compositions or formulations comprising Hydrate 1. Also provided herein are pharmaceutical compositions or formulations comprising Hydrate 1 further comprising a β-lactam antibiotic.

In one embodiment, provided herein is a pharmaceutical composition Hydrate 1 and amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate. In another embodiment, provided herein is a pharmaceutical composition comprising Hydrate 1 and Hydrate 2. In yet another embodiment, provided herein is a pharmaceutical composition comprising Hydrate 1 and Hydrate 3. In still another embodiment, provided herein is a pharmaceutical composition comprising Hydrate 1 and Hydrate 4. In one embodiment, provided herein is a pharmaceutical composition comprising Hydrate 1 and Hydrate 5. The pharmaceutical compositions can further comprise a pharmaceutically acceptable carrier.

The pharmaceutical compositions can be formulated for oral, intravenous, intramuscular, subcutaneous or parenteral administration for the therapeutic or prophylactic treatment of diseases, such as bacterial infections. Preferably, the pharmaceutical composition is formulated for intravenous administration.

The pharmaceutical preparations disclosed herein may be prepared in accordance with standard procedures and are administered at dosages that are selected to reduce, prevent or eliminate infection (see, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA and Goodman and Gilman's "The Pharmaceutical Basis of Therapeutics," Pergamon Press, New York, NY, the contents of which are incorporated herein by reference, for a general description of the methods for administering various antimicrobial agents for human therapy).

The pharmaceutical compositions can comprise one or more of the compounds disclosed herein, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, in association with one or more nontoxic, pharmaceutically-acceptable carriers and/or diluents and/or adjuvants and/or excipients. In one embodiment, provided herein is a vial comprising Hydrate 1 in a fixed dose combination with a β-lactam antibiotic

As used herein, the phrase "pharmaceutically-acceptable carrier" refers to any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is

well known in the art. Non-limiting examples of carriers and excipients include corn starch or gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride and alginic acid. The compositions may contain croscarmellose sodium, microcrystalline cellulose, corn starch, sodium starch glycolate and alginic acid.

Tablet binders that can be included are acacia, methylcellulose, sodium carboxymethylcellulose, polyvinylpyrrolidone (Povidone), hydroxypropyl methylcellulose, sucrose, starch and ethylcellulose.

Lubricants that can be used include magnesium stearate or other metallic stearates, stearic acid, silicone fluid, talc, waxes, oils and colloidal silica.

Flavoring agents such as peppermint, oil of wintergreen, cherry flavoring or the like can also be used. It may also be desirable to add a coloring agent to make the dosage form more aesthetic in appearance or to help identify the product.

For oral or parenteral administration, compounds of the present invention preferably in conjunction with a β-lactam antibiotic, can be mixed with conventional pharmaceutical carriers and excipients and used in the form of tablets, capsules, elixirs, suspensions, syrups, wafers and the like. The compositions comprising a compound of this invention may contain from about 0.1% to about 99% by weight of the active compound, such as from about 10% to about 30%.

For oral use, solid formulations such as tablets and capsules are useful. Sustained release or enterically coated preparations may also be devised. For pediatric and geriatric applications, one embodiment provides suspensions, syrups and chewable tablets. For oral administration, the pharmaceutical compositions are in the form of, for example, a tablet, capsule, suspension or liquid.

The pharmaceutical compositions may be made in the form of a dosage unit containing a therapeutically-effective amount of the active ingredient. Examples of such dosage units are tablets and capsules. For therapeutic purposes, the tablets and capsules which can contain, in addition to the active ingredient, conventional carriers such as binding agents, fillers, lubricants, disintegrants, or acceptable wetting agents. Oral liquid preparations generally are in the form of aqueous or oily solutions, suspensions, emulsions, syrups or elixirs.

For intravenous (IV) use, the pharmaceutical composition, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, can be dissolved or suspended in any of the commonly used intravenous fluids and administered by infusion. Intravenous fluids include,

without limitation, physiological saline or Ringer's solution. Intravenous administration may be accomplished by using, without limitation, syringe, mini-pump or intravenous line.

Pharmaceutical compositions of this invention for parenteral injection comprise pharmaceutically-acceptable aqueous or non-aqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use.

Injectable depot forms can be made by forming microencapsulating matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations can also be prepared by entrapping the drug in liposomes or microemulsions, which are compatible with body tissues.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions, which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

For intramuscular preparations, a sterile formulation of compounds, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, or suitable soluble salt forms thereof, for example hydrochloride salts, can be dissolved and administered in a pharmaceutical diluent such as Water-for-Injection (WFI), physiological saline or 5% glucose. A suitable insoluble form of the compound may be prepared and administered as a suspension in an aqueous base or a pharmaceutically acceptable oil base, e.g., an ester of a long chain fatty acid such as ethyl oleate.

A dose of an intravenous, intramuscular, or parental formulation of compounds, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, may be administered as a bolus or by slow infusion. A bolus is a dose that is administered in less than 30 minutes. In one embodiment, a bolus is administered in less than 15 or less than 10 minutes. In another embodiment, a bolus is administered in less than 5 minutes. In yet another embodiment, a bolus is administered in one minute or less. An infusion is a dose that is administered at a rate of 30 minutes or greater. In one embodiment, the infusion is one hour or greater. In another embodiment, the infusion is substantially constant.

For topical use the pharmaceutical compositions, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, can also be prepared in suitable forms to be applied to the skin, or

mucus membranes of the nose and throat, and can take the form of creams, ointments, liquid sprays or inhalants, lozenges, or throat paints. Such topical formulations further can include chemical compounds such as dimethylsulfoxide (DMSO) to facilitate surface penetration of the active ingredient.

For application to the eyes or ears, the pharmaceutical composition can be presented in liquid or semi-liquid form formulated in hydrophobic or hydrophilic bases as ointments, creams, lotions, paints or powders.

For rectal administration, the pharmaceutical compositions, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, can be administered in the form of suppositories admixed with conventional carriers such as cocoa butter, polyethylene glycol or a suppository wax or other glyceride that are solid at room temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Alternatively, the pharmaceutical compositions can be in powder form for reconstitution in the appropriate pharmaceutically acceptable carrier at the time of delivery. In another embodiment, the unit dosage form of compounds, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, can be a solution of one or more compounds, or salts thereof, in a suitable diluent, in sterile hermetically sealed ampoules or sterile syringes. The concentration of the compounds, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, in the unit dosage may vary, e.g. from about 1 percent to about 50 percent, depending on the compound used and its solubility and the dose desired by the physician. If the compositions contain dosage units, each dosage unit can contain from 1-500 mg of the active material. For adult human treatment, the dosage employed can range from 5 mg to 10 g, per day, depending on the route and frequency of administration.

The pharmaceutical compositions disclosed herein can be placed in a pharmaceutically acceptable carrier and are delivered to a recipient subject (e.g., a human) in accordance with known methods of drug delivery. In general, the methods of delivering the pharmaceutical compositions *in vivo* utilize art-recognized protocols for delivering the agent with the only substantial procedural modification being the substitution of the compounds of the present invention for the drugs in the art-recognized protocols. Likewise, methods for using the claimed compositions for treating cells in culture, for example, to eliminate or reduce the level of bacterial contamination of a cell culture, utilize art-recognized protocols for treating cell cultures with antibacterial agent(s) with the only substantial procedural

modification being the substitution of the compounds of the present invention, preferably in combination with a β-lactam antibiotic for the drugs in the art-recognized protocols.

As used herein, the phrases "therapeutically-effective dose" and "therapeutically-effective amount" refer to an amount of a compound that prevents the onset, alleviates the symptoms, stops the progression of a bacterial infection, or results in another desired biological outcome such as, e.g., improved clinical signs or reduced/elevated levels of lymphocytes and/or antibodies. The term "treating" or "treatment" is defined as administering, to a subject, a therapeutically-effective amount of one or more compounds both to prevent the occurrence of an infection and to control or eliminate an infection. Those in need of treatment may include individuals already having a particular medical disease as well as those at risk for the disease (*i.e.*, those who are likely to ultimately acquire the disorder).

The term "subject," as used herein, refers to a mammal, a plant, a lower animal, or a cell culture. In one embodiment, a subject is a human or other animal patient in need of antibacterial treatment.

The term "administering "or "administration" and the like, refers to providing the Hydrate 1 to the subject in need of treatment. Preferably the subject is a mammal, more preferably a human.

Provided herein are liquid pharmaceutical compositions comprising Hydrate 1, wherein the composition is suitable for intravenous administration. The present invention also comprises administering Hydrate 1 in conjunction with a β-lactam antibiotic. When Hydrate 1 is administered in conjunction with a β-lactam antibiotic, Hydrate 1 and the β-lactam antibiotic can be administered at the same time or different times. When Hydrate 1 and the β-lactam antibiotic are administered at the same time, they can be administered as a single composition or pharmaceutical composition or they can be administered separately. It is understood that when Hydrate 1 is administered in conjunction with a β-lactam antibiotic, that the active agents can be administered in a single combination or in multiple combinations. For example, when administered by IV, Hydrate 1 can be dissolved or suspended in any of the commonly used intravenous fluids and administered by infusion, then a β-lactam antibiotic can be dissolved or suspended in any of the commonly used intravenous fluids and administered by infusion. Conversely the β-lactam antibiotic can be dissolved or suspended in any of the commonly used intravenous fluids and administered by infusion, then Hydrate 1 can be dissolved or suspended in any of the commonly used intravenous fluids and

administered by infusion. Alternatively, a pharmaceutical composition comprising Hydrate 1 and a β-lactam antibiotic can be dissolved or suspended in any of the commonly used intravenous fluids and administered by infusion.

In one embodiment of the invention, is provided a method of treating or preventing a bacterial infection comprising administering to a subject in need thereof a therapeutically-effective amount of the pharmaceutical composition comprising Hydrate 1 and a β-lactam antibiotic.

In one embodiment of the invention, is provided a method of treating or preventing a bacterial infection comprising administering to a subject in need thereof, a therapeutically-effective amount of a β-lactam antibiotic in conjunction with Hydrate 1.

In one embodiment of the invention, is provided a method of treating or preventing a bacterial infection in a subject comprising the steps of

a. administering to the subject Hydrate 1; and

b. administering a therapeutically-effective amount of a β-lactam antibiotic.

In one embodiment of the invention, is provided a method of treating or preventing a bacterial infection in a subject comprising the steps of

a. administering a therapeutically-effective amount of a β-lactam antibiotic; and

b. administering to the subject Hydrate 1.

In one embodiment, Hydrate 1, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, can be used to treat a subject having a bacterial infection in which the infection is caused or exacerbated by any type of bacteria, such as Gram-negative bacteria. In one aspect of the invention, the bacterial infection is caused by β -lactam resistant bacteria. In one aspect the bacterial infection is caused by β -lactamase producing bacteria. In another aspect the bacterial infection is caused by class A, class C or class D β -lactamase producing bacteria. In another aspect the bacterial infection is caused by class A β -lactamase producing bacteria. In another aspect the infection is caused by class C β-lactamase producing bacteria. In still another aspect the infection is caused by class D β -lactamase producing bacteria. In still another aspect the infection is caused by KPC β-lactamase producing bacteria. In still another aspect the infection is caused by OXA β-lactamase producing bacteria.

Representative Gram-negative pathogens known to express β-lactamases include, but are not limited to *Acinetobacter* spp. (including *Acinetobacter baumannii*), *Citrobacter* spp.,

Escherichia spp. (including *Escherichia coli*), *Haemophilus influenzae*, *Morganella morganii*, *Pseudomonas aeruginosa*, *Klebsiella* spp. (including *Klebsiella pneumoniae*), *Enterobacter* spp. (including *Enterobacter cloacae and Enterobacter aerogenes*), *Pasteurella* spp., *Proteus* spp. (including *Proteus mirabilis*), *Serratia* spp. (including *Serratia marcescens*), and *Providencia* spp. Bacterial infections can be caused or exacerbated by Gram-negative bacteria including strains which express β-lactamases that may confer resistance to penicillins, cephalosporins, monobactams and/or carbapenems. The co-administration of a novel BLIs that inhibits these β-lactamases with a β-lactam antibiotic could be used to treat infections caused by β-lactam resistant bacteria.

In one aspect of the invention the infection is caused by a β-lactamase producing bacteria selected from *Acinetobacter* spp, *Citrobacter* spp, *Escherichia coli, Enterobacter cloacae*), *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Proteus mirabilis, Serratia marcescens,* and *Klebsiella pneumoniae*,

β-Lactam antibiotics that may be co-administered with Hydrate 1 include, but are not limited to cephalosporin, carbapenem, monobactam, penem and penicillin classes of antibiotics.

In one embodiment of the invention, the β-lactam antibiotic is a cephalosporin. Examples of cephalosporins include, but are not limited to, Cefacetrile (cephacetrile), Cefadroxil (cefadroxyl), Cefalexin (cephalexin), Cefaloglycin (cephaloglycin), Cefalonium (cephalonium), Cefaloridine (cephaloradine), Cefalotin (cephalothin), Cefapirin (cephapirin), Cefatrizine,Cefazaflur, Cefazedone, Cefazolin (cephazolin), Cefradine (cephradine), Cefroxadine, Ceftezole, Cefaclor, Cefamandole, Cefmetazole, Cefonicid, Cefotetan, Cefoxitin, Cefprozil (cefproxil), Cefuroxime, Cefuzonam, Cefcapene, Cefdaloxime, Cefdinir, Cefditoren, Cefetamet, Cefixime, Cefmenoxime, Cefodizime, Cefotaxime, Cefpimizole, Cefpodoxime, Cefteram, Ceftibuten, Ceftiofur, Ceftiolene, Ceftizoxime, Ceftriaxone, Cefoperazone, Ceftazidime, Cefclidine, Cefepime, Cefluprenam, Cefoselis, Cefozopran, Cefpirome, Cefquinome, Cefaclomezine, Cefaloram, Cefaparole, Cefcanel, Cefedrolor, Cefempidone, Cefetrizole, Cefivitril, Cefmatilen, Cefmepidium, Cefovecin, Cefoxazole, Cefrotil, Cefsumide, Ceftaroline, Ceftioxide, Cefuracetime, cefbuperazone, cefminox, ceforanide, cefotiam, cefpiramide, cefsulodin, ceftobiprole latamoxef, loracarbef and Ceftolozane. In one embodiment the cephalosporin is Ceftolozane or Ceftazidime.

In another embodiment, the cephalosporin is ceftolozane. In one embodiment, provided herein is a combination therapy comprising Hydrate 1 and ceftolozane.

In one embodiment of the invention, the β-lactam antibiotic is a carbapenen. Examples of carbapenem antibiotics include, but are not limited to, Imipenem, Imipenem/Cilastatin, Biapenem, Doripenem, Meropenem, Ertapenem and Panipenem. In one embodiment the Carbapenem is Imipenem/Cilastatin or Meropenem.

In one embodiment of the invention, the β-lactam antibiotic is a monobactam. Examples of monobactam antibiotics include, but are not limited to Aztreonam, Tigemonam, Carumonam, BAL30072 and Nocardicin A.

In one embodiment of the invention, the β-lactam antibiotic is a penem. In one embodiment of the invention, the β-lactam antibiotic is a penicillin. Examples of penicillin antibiotics include, but are not limited to Amoxicillin, Ampicillin, Azlocillin, Mezlocillin, Apalcillin, Hetacillin, Becampicillin, Carbenicillin, Sulbenicillin, Ticarcillin, Piperacillin, Azlocillin, Mecillinam, Pivmecillinam, Methicillin, Ciclacillin, Talampicillin, Aspoxicillin, Oxacillin, Cloxacillin, Dicloxacillin, Flucloxacillin, Nafcillin and Pivampicillin.

In another aspect, provided herein is a method for the treatment of a bacterial infection in a subject, comprising administering to said mammal a therapeutically effective amount of Hydrate 1 (e.g., by reconstiting a pharmaceutical composition comprising Hydrate 1 for subsequent intravenous delivery to a patient in need thereof). In an embodiment of this method, Hydrate 1 can be characterized by an X-ray powder diffraction pattern having peaks expressed in degrees 2-Theta at angles of 13.1 ± 0.3°, 13.8 ± 0.3°, 14.3 ± 0.3°, 20.5 ± 0.3°, 21.5 ± 0.3°, 22.2 ± 0.3°, 26.1 ± 0.3°, 26.9 ± 0.3°, 28.7 ± 0.3°, and 33.5 ± 0.3°. In another embodiment, Hydrate 1 can also be characterized by an X-ray powder diffraction pattern having peaks expressed in degrees 2-Theta at angles of 13.1 ± 0.3°, 14.3 ± 0.3°, 20.5 ± 0.3°, 21.5 ± 0.3°, and 22.2 ± 0.3°.

In one embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, and 21.5 ± 0.2°. In a particular embodiment, the X-ray powder diffraction pattern of Hydrate 1 has an additional peak expressed in degrees-2-theta at angle 22.2 ± 0.2°. In another embodiment, the X-ray powder diffraction pattern of Hydrate 1 has additional peaks expressed in degrees-2-theta at angles 13.8 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.6 ± 0.2°, and 33.5 ± 0.2°.

In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 13.8 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, 21.5 ± 0.2°, 22.2 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.7 ± 0.2°, and 33.5 ± 0.2°. In yet another embodiment, Hydrate 1 is characterized by an X-ray powder

diffraction pattern having five or more peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 13.8 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, 21.5 ± 0.2°, 22.2 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.7 ± 0.2°, and 33.5 ± 0.2°. In another embodiment, Hydrate 1 is characterized by an X-ray powder diffraction pattern having six or more peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 13.8 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, 21.5 ± 0.2°, 22.2 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.7 ± 0.2°, and 33.5 ± 0.2°.

In one embodiment, the methods provided herein further comprise reconstituting a combination of one or more of the hydrate forms provided herein and a beta-lactam antibiotic in an aqueous solvent. In an embodiment, a combination of Hydrate 1 and ceftolozane is reconstituted in an aqueous solvent, such that the resulting solution is suitable for infusion. The mixture can be reconstituted in saline and/or sterile water for injection. In another embodiment, provided herein is a method of treating a bacterial infection in a subject, comprising reconstituting a composition comprising Hydrate 1 and ceftolozane in an aqueous solvent, and intravenously administering the resulting composition to the subject. In an embodiment, the composition comprising Hydrate 1 and ceftolozane is in a solid form.

The pharmaceutical compositions, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, can be used to treat a bacterial infection of any organ or tissue in the body caused by β-lactam resistant bacteria, preferably, Gram-negative β-lactam resistant bacteria. These organs or tissue include, without limitation, skeletal muscle, skin, bloodstream, kidneys, heart, lung and bone. For example, a pharmaceutical composition comprising at least Hydrate 1, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, can be administered to a subject to treat, without limitation, skin and soft tissue infections (e.g., complex skin infections), bacteremia, intra-abdominal infections and urinary tract infections (e.g., cUTI). In addition, Hydrate 1 may be used to treat community acquired respiratory infections, including, without limitation, otitis media, sinusitis, chronic bronchitis and pneumonia (including community-acquired pneumonia, hospital-acquired pneumonia and ventilator associated pneumonia), including pneumonia caused by drug-resistant *Pseudomonas aeruginosa* . Hydrate 1, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, can be administered to a subject to treat mixed infections that comprise different types of Gram-negative bacteria, or which comprise both Gram-positive and Gram-negative bacteria. These types of infections include intra-abdominal infections and obstetrical/gynecological infections. Hydrate 1, preferably in conjunction with a β-lactam antibiotic, may also be administered to a subject to treat an infection including, without

limitation, endocarditis, nephritis, septic arthritis, intra-abdominal sepsis, bone and joint infections and osteomyelitis. Hydrate 1, preferably in conjunction with a β-lactam antibiotic, or pharmaceutical compositions thereof, may also be directly injected or administered into an abscess, ventricle or joint. Pharmaceutical compositions administered as an aerosol for the treatment of pneumonia or other lung-based infections. In one embodiment, the aerosol delivery vehicle is an anhydrous, liquid or dry powder inhaler.

Actual dosage levels of active ingredients in the pharmaceutical compositions of Hydrate 1, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, may be varied so as to obtain a therapeutically-effective amount of the active compound(s) to achieve the desired therapeutic response for a particular patient, compositions, and mode of administration. The effective amount can be determined as described herein. The selected dosage level will depend upon the activity of the particular compound, the route of administration, the severity of the condition being treated, and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. In one embodiment, the data obtained from the assays can be used in formulating a range of dosage for use in humans. It will be understood by one of skill in the art that the when the composition comprises Hydrate 1 and a β-lactam antibiotic, both Hydrate 1 and the β-lactam antibiotic are active compounds.

The method comprises administering to the subject an effective dose of Hydrate 1, preferably in conjunction with a β lactam antibiotic. An effective dose of Hydrate 1 is generally between 125 mg/day to 2000 mg/day. In one embodiment, an effective dose is from about 0.1 to about 100 mg/kg of Hydrate 1. In one embodiment, the dose is from about 0.1 to about 50 mg/kg of Hydrate 1. In another embodiment, the dose is from about 1 to about 25 mg/kg of Hydrate 1

Hydrate 1, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, may be administered according to this method until the bacterial infection is eradicated or reduced. In one embodiment, Hydrate 1, preferably a compound of Formula in conjunction with a β-lactam antibiotic, are administered for a period of time from 3 days to 6 months. In another embodiment, Hydrate 1, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, are administered for 7 to 56 days. In another embodiment, Hydrate 1, preferably a compound of Hydrate 1 in conjunction with a β-lactam antibiotic, are administered for 7 to 28 days. In a

further embodiment, Hydrate 1, preferably Hydrate 1 in conjunction with a β-lactam antibiotic, are administered for 7 to 14 days.

Other embodiments provided herein include:

A pharmaceutical composition comprising Hydrate 1 and at least 1 β-lactam antibiotic or a pharmaceutically acceptable salt thereof.

A pharmaceutical composition comprising Hydrate 1 and at least one cephalosporin antibiotic or a pharmaceutically acceptable salt thereof.

A pharmaceutical composition comprising Hydrate 1 and Ceftolozane antibiotic or a pharmaceutically acceptable salt thereof.

A pharmaceutical composition comprising Hydrate 1 and at least one carbapenem antibiotic or a pharmaceutically acceptable salt thereof.

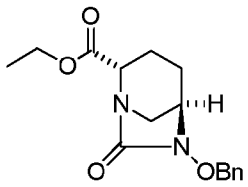
A pharmaceutical composition comprising Hydrate 1 and at least one monobactam antibiotic or a pharmaceutically acceptable salt thereof.

Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

Examples

Example 1: Preparation of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate

*Synthesis of (2S,5R)-ethyl 6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate (Intermediate Compound **1**)*



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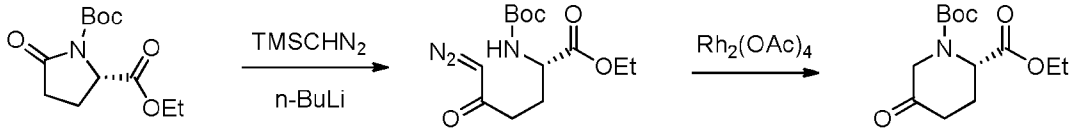
Step 1: Synthesis of (S)-1-tert-butyl 2-ethyl 5-oxopiperidine-1,2-dicarboxylate

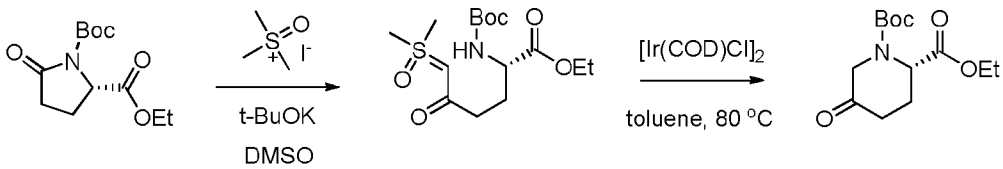
Method A:



n-BuLi (600 mL, 1.5 mol) was added dropwise to a solution of $TMSCHN_2$ (690 mL, 1.38 mol) in dry THF (3 L) at -78 °C, and the mixture was stirred at -78 °C for 30 minutes. The mixture was then transferred to a solution of (*S*)-1-tert-butyl 2-ethyl 5-oxopyrrolidine-1,2-dicarboxylate (300 g, 1.17 mol) in dry THF (3 L) via cannula, and the mixture was stirred at -78 °C for 30 minutes. The reaction mixture was then quenched with sat. NH_4Cl solution, and extracted with DCM (3x). The combined organic layer was concentrated under reduced pressure and the crude product was purified by silica gel column chromatography (3:1 petroleum ether:EtOAc) to afford (*S*)-ethyl 2-((tert-butoxycarbonyl)amino)-6-diazo-5-oxohexanoate (262 g, 75 %) as a yellow solid.

A solution of (*S*)-ethyl 2-((tert-butoxycarbonyl)amino)-6-diazo-5-oxohexanoate (350 g, 1.18 mol) in DCM (1500 mL) was added to a solution of $Rh_2(OAc)_4$ (3.5 g, 7.9 mmol) in DCM (750 mL) at 0 °C. The reaction was then stirred at 20 °C overnight and then concentrated in vacuum. The crude sample was purified by silica gel column chromatography (5:1 petroleum ether/EtOAc) to afford (*S*)-1-tert-butyl 2-ethyl 5-oxopiperidine-1,2-dicarboxylate (175.9 g, 55%) as a yellow oil.

Method B:

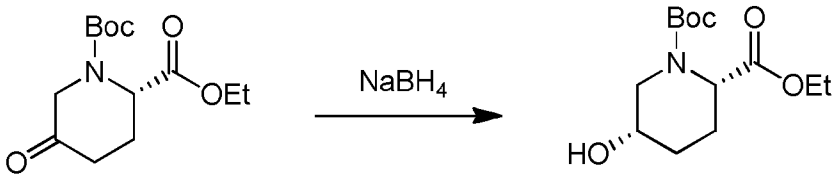


t-BuOK (330 g, 2.9 mol) was added to a solution of trimethylsulfoxonium iodide (750 g, 3.5mol) in dry DMSO (3 L) and the mixture was stirred at rt for 1 h. (*S*)-1-tert-Butyl 2-ethyl 5-oxopyrrolidine-1,2-dicarboxylate (900 g, 3.5 mol) was added and the mixture was stirred at rt for 2-3 hrs. Water was added to quench the reaction and the mixture was extracted with EtOAc (5x). The combined organic layer was concentrated in vacuum and the crude sample was purified by silica gel column chromatography (1:1petroleum ether/EtOAc

then 1:10 MeOH/DCM) to afford sulfoxonium ylide intermediate (977 g, 80%) as a white solid.

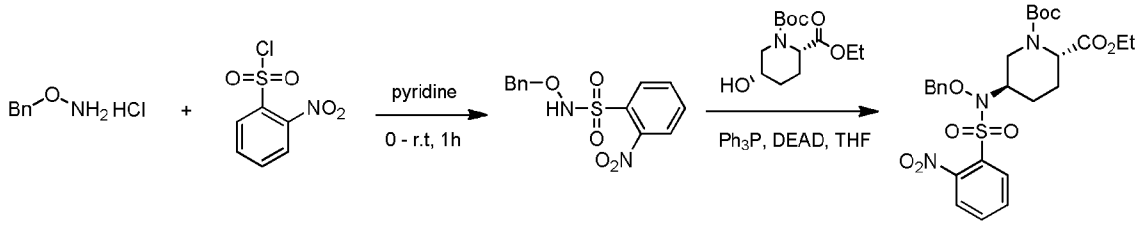
A solution of sulfoxonium ylide intermediate (156 g, 0.446 mol) and $[Ir(COD)Cl]_2$ (3 g, 4.46 mmol) in toluene(4 L) was degassed by bubbling nitrogen through the solution for 10 minutes. The reaction mixture was heated to 80-90 °C for 2-3 hrs and then cooled to 20 °C. Then toluene was concentrated under reduced pressure, the residue was purified by silica gel column chromatography (10:1 to 3:1gradient petroleum ether/EtOA) to afford (*S*)-1-tert-butyl 2-ethyl 5-oxopiperidine-1,2-dicarboxylate (140 g, 57.8%) as a yellow oil.

Step 2: Synthesis of (2S,5S)-1-tert-butyl 2-ethyl 5-hydroxypiperidine-1,2-dicarboxylate



$NaBH_4$ (36 g, 1.0 mol) was added in portions to a solution of (*S*)-1-tert-butyl 2-ethyl 5-oxopiperidine-1,2-dicarboxylate (250 g, 0.92 mol) in EtOH (1500 mL) at -40 °C. The reaction mixture was then stirred at -40 °C for 0.5 hr then quenched with 10% HOAc solution. After diluting with water, the mixture was extracted with DCM (3x). The combined organic layer was concentrated in vacuum and purified by silica gel column chromatography (1:1 petroleum ether/EtOAc) to afford (2*S*,5*S*)-1-tert-butyl 2-ethyl 5-hydroxypiperidine-1,2-dicarboxylate (205 g, 80%) as a yellow oil.

Step 3: Synthesis of (2S,5R)-1-tert-butyl 2-ethyl 5-(N-(benzyloxy)-2-nitrophenylsulfonamido)piperidine-1,2-dicarboxylate

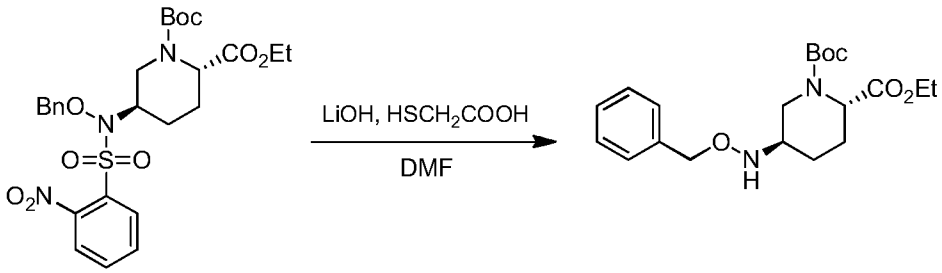


A solution of 2-nitrobenzene-1-sulfonyl chloride (500 g, 2.26 mol) in pyridine (1500 mL) was added dropwise to a solution of *O*-benzylhydroxylamine hydrochloride (400 g, 2.51 mol) in pyridine (1500 mL) at 0 °C. The reaction mixture was then stirred at 20 °C overnight.

The mixture was concentrated in vacuum, diluted with DCM and washed with HCl (10%) three times. The combined organic layer was concentrated under reduced pressure and re-crystallized with DCM to afford *N*-(benzyloxy)-2-nitrobenzenesulfonamide (485 g, 62.6%) as a yellow solid.

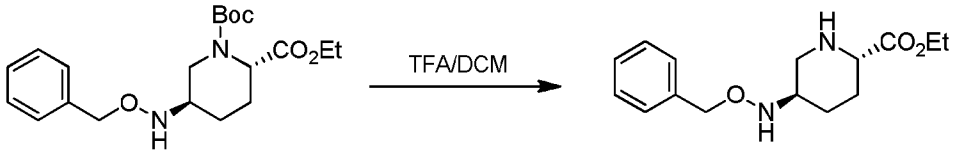
To a solution of *N*-(benzyloxy)-2-nitrobenzenesulfonamide (212 g, 0.69 mol) in THF (1000 mL) was added (2*S*,5*S*)-1-tert-butyl 2-ethyl 5-hydroxypiperidine-1,2-dicarboxylate (171 g, 0.63 mol) and PPh_3 (275 g, 1.05 mol), followed by dropwise addition of a solution of DEAD (195 g, 1.12 mol) in THF (500 mL). The mixture was then stirred at 20 °C overnight. The reaction mixture was then concentrated in vacuum and purified by silica gel column chromatography (3:1 petroleum ether/EtOAc) to afford (2*S*,5*R*)-1-tert-butyl 2-ethyl 5-(*N*-(benzyloxy)-2-nitrophenylsulfonamido)piperidine-1,2-dicarboxylate (283.8 g, 80%) as a yellow oil.

Step 4: Synthesis of (2S,5R)-1-tert-butyl 2-ethyl 5-((benzyloxy)amino)piperidine-1,2-dicarboxylate



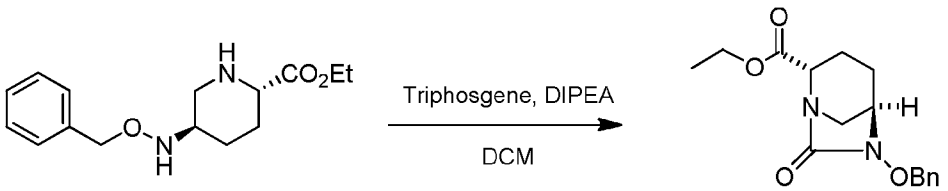
$LiOH{\cdot}H_2O$ (95 g, 2.3 mol) and 2-mercaptoacetic acid (124 g, 1.3 mol) were added to a solution of (2*S*,5*R*)-1-tert-butyl 2-ethyl 5-(*N*-(benzyloxy)-2-nitrophenylsulfonamido)piperidine-1,2-dicarboxylate (251 g, 0.45 mol) in DMF (1200 mL) . The reaction mixture was then stirred at 20 °C overnight. The reaction mixture was diluted with water and extracted with EtOAc (3x). The combined organic layer was washed with brine (3x), concentrated under reduced pressure and purified by silica gel column chromatography (3:1 petroleum ether/EtOAc) to afford (2*S*,5*R*)-1-tert-butyl 2-ethyl 5-((benzyloxy)amino)piperidine-1,2-dicarboxylate (122.9 g, 85%) as a yellow solid.

Step 5: Synthesis of (2S,5R)-ethyl 5-((benzyloxy)amino)piperidine-2-carboxylate



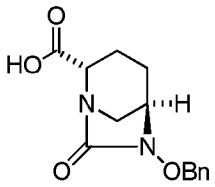
TFA (600 mL) was added to a solution of (2*S*,5*R*)-1-tert-butyl 2-ethyl 5-((benzyloxy)amino)piperidine-1,2-dicarboxylate (263 g, 0.7 mol) in DCM (600 mL) at 20 °C. The mixture was stirred at rt overnight and then concentrated in vacuum. The crude product was adjusted to pH 10 with sat. $NaHCO_3$ solution, and then extracted with DCM three times. The combined organic layer was concentrated in vacuum and purified by silica gel column chromatography (20:1 DCM/MeOH) to afford (2*S*,5*R*)-ethyl 5-((benzyloxy)amino)piperidine-2-carboxylate (184.9 g, 95%) as a yellow oil.

Step 6: Synthesis of (2S,5R)-ethyl 6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate

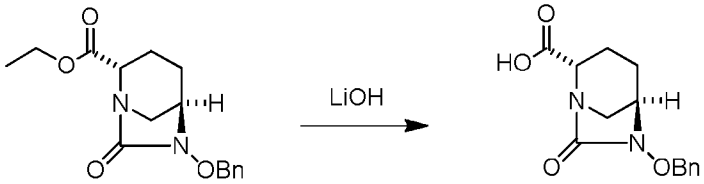


Triphosgene (21.3 g, 72 mmol) was added in portions to a solution of (2*S*,5*R*)-ethyl 5-((benzyloxy)amino)piperidine-2-carboxylate (50 g, 0.18 mol) and DIPEA (128 mL, 0.72 mol) in DCM (2000 mL) at 0 °C. After stirring at 20 °C overnight, the reaction mixture was washed with H_3PO_4 (10%), sat. $NaHCO_3$ and saturated NaCl. The combined organic layer was concentrated under reduced pressure and purified by silica gel column chromatography (3:1 petroleum ether/EtOAc) to afford (2*S*,5*R*)-ethyl 6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate (27.4 g, 50%) as a yellow solid. ^1H NMR (400Mz, $CDCl_3$): δ 7.43-7.36 (m, 5H), 5.06 (d, *J* = 11.4 Hz, 1H), 4.90 (d, *J* = 11.4 Hz, 1H), 4.24 (q, *J* = 7.1 Hz, 2H), 4.11-4.08 (m, 1H), 3.32-3.31 (m, 1H), 3.08-3.05 (m, 1H), 2.93 (d, *J* = 11.9 Hz, 1H), 2.14-2.05 (m, 2H), 2.05-2.00 (m, 1H), 1.71-1.63 (m, 1H), 1.29 (t, *J* = 7.1 Hz, 3H).

*Synthesis of (2S,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylic acid (Intermediate Compound **2a**)*

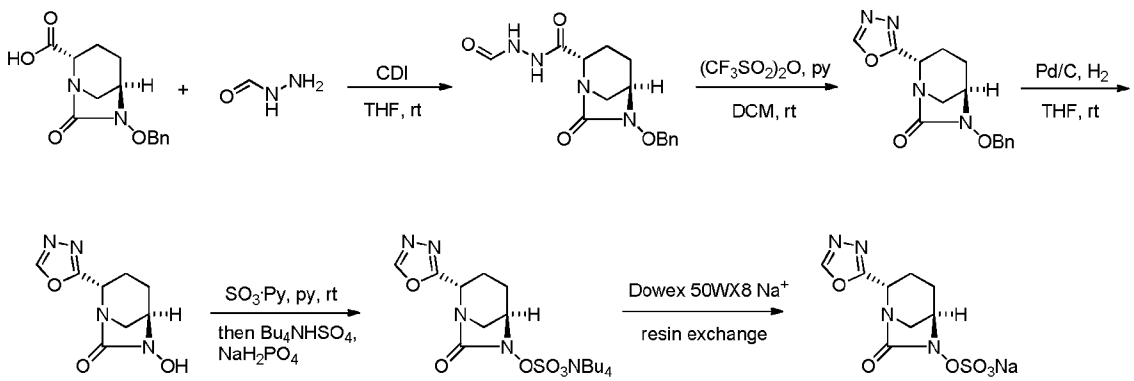


2a



LiOH (1.2 g, 29.6 mmol) was added to a solution of (2*S*,5*R*)-ethyl 6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylate (9 g, 29.6 mmol) in THF/H_2O (3:1, 240 mL). The mixture was then stirred at rt overnight. The reaction mixture was washed with EtOAc twice, then the aqueous solution was adjusted pH 2-3 with 1N HCl. The resulting mixture was extracted with DCM three times, and the combined organic layer was dried over saturated Na_2SO_4 and concentrated under reduced pressure to provide (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylic acid (7.0 g, 77.7%), which was directly used in the next step without further purification. ESI-MS (EI^+, m/z): 277.31. ^1H NMR (300 MHz, $CDCl_3$) δ 7.49-7.29 (m, 5H), 5.06 (d, *J* = 11.4 Hz, 1H), 4.91 (d, *J* = 11.4 Hz, 1H), 4.15-4.10 (m, 1H), 3.36-3.34 (m, 1H), 3.15-3.11 (m, 1H), 2.83 (d, *J* = 11.8 Hz, 1H), 2.32-2.15 (m, 1H), 2.11-2.01 (m, 2H), 1.74-1.56 (m, 1H).

Synthesis of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate



Step 1: 1,1'-Carbonyldiimidazole (5.8 g, 36.2 mmol) was added to a solution of (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylic acid (5.0 g, 18.1 mmol) in dry THF (200 mL) at 0 °C. The reaction mixture was stirred at rt for 3 hrs. Formohydrazide (5.4 g, 90.5 mmol) was added in one portion, and the reaction mixture was stirred for additional 3 hrs. The mixture was then diluted with brine and exacted with EtOAc (3x). The combined organic layer was washed with Saturated sodium chloride (2x), dried over Na_2SO_4, and concentrated to afford crude (2*S*,5*R*)-6-(benzyloxy)-*N'*-formyl-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carbohydrazide (~11 g), which was directly used in the next step. ESI-MS (EI^+, *m/z*): 319.1 $[M+H]^+$.

Step 2: To a solution of (2*S*,5*R*)-6-(benzyloxy)-*N'*-formyl-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carbohydrazide (11 g) in dry DCM (200 mL) at -10 °C was added pyridine (28 mL), followed by dropwise addition of $(CF_3SO_2)_2O$ (28 mL). The reaction mixture was allowed to warm to rt and was stirred for 3 hrs. The reaction mixture was then cooled to -10 °C and quenched with sat. $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted with EtOAc (3x). The combined organic layer was dried over Na_2SO_4, concentrated and purified by silica gel column chromatography (gradient elution 1:3 to 2:1 EtOAc/hexanes) to give (2*S*,5*R*)-6-(benzyloxy)-2-(1,3,4-oxadiazol-2-yl)-1,6-diazabicyclo[3.2.1]octan-7-one (4.6 g, 86% for two steps) as a slight yellow solid. ESI-MS (EI^+, *m/z*): 301.0 $[M+H]^+$.

Step 3: To a solution of (2*S*,5*R*)-6-(benzyloxy)-2-(1,3,4-oxadiazol-2-yl)-1,6-diazabicyclo[3.2.1]octan-7-one (4.6 g, 15.3 mmol) in THF (150 mL) was added 10% Pd/C (1 g). The mixture was stirred under H_2 atmosphere at rt for 3 hrs. The reaction mixture was then filtered and concentrated to afford (2*S*,5*R*)-6-hydroxy-2-(1,3,4-oxadiazol-2-yl)-1,6-diazabicyclo[3.2.1]octan-7-one (2.9 g, 91 %), which was used directly in the next step. ESI-MS (EI^+, *m/z*): 211.1 $[M+H]^+$.

Step 4: To a solution of (2*S*,5*R*)-6-hydroxy-2-(1,3,4-oxadiazol-2-yl)-1,6-diazabicyclo[3.2.1]octan-7-one (2.9 g, 13.8 mmol) in dry pyridine (60 mL) was added $SO_3 \cdot Py$ (11.0 g, 69.0 mmol). The reaction mixture was stirred at rt for 8 hrs and then concentrated under vacuum. The residue was re-dissolved in aqueous NaH_2PO_4 (1.5 M, 100 mL) then tetrabutylammonium hydrogensulphate (5.88 g, 17.3 mmol) was added. The mixture was stirred at rt for 20 minutes, then extracted with EtOAc (4x). The combined organic layer was dried and concentrated and the residue was purified by silica gel column chromatography (gradient elution 10:1 to 2:1 DCM/acetone) to afford tetrabutylammonium

(2*S*,5*R*)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (4.1 g, 97%) as a white solid. ESI-MS (EI$^-$, *m/z*): 289.0 [M-H]$^-$. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.48 (s, 1H), 4.75 (d, J = 6.5 Hz, 1H), 4.40 (br s, 1H), 3.34-3.26 (m, 9H), 2.82 (d, J = 12.0 Hz, 1H), 2.37-2.25 (m, 3H), 2.06-1.98 (m, 1H), 1.71-1.65 (m, 8H), 1.49-1.42 (m, 8H), 1.01 (t, J = 7.5 Hz, 12H).

Step 5: Resin Exchange: Tetrabutylammonium (2*S*, 5*R*)-2-(1, 3, 4-oxadiazol-2-yl)-7-oxo-1, 6-diaza-bicyclo[3.2.1]octan-6-yl sulfate (4.1 g, 7.72 mmol) was dissolved in a minimum amount of HPLC grade water (~ 40 mL) and passed through a column of 80 g of DOWEX 50WX 8 Na^+ resin (the resin was prewashed with >4 L of HPLC grade water) and eluted with HPLC grade water to afford sodium (2*S*,5*R*)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (2.2 g, 91%) as a white solid after lyophilization. ESI-MS (EI$^+$, *m/z*): 291.2 [M+H]$^+$. ^{1}H NMR (300 MHz, D_2O) δ 8.92 (s, 1H), 4.84 (d, J = 6.7 Hz, 1H), 4.20 (br s, 1H), 3.25-3.16 (m, 1H), 2.92 (d, J = 12.3 Hz, 1H), 2.41-2.26 (m, 1H), 2.26-2.11 (m, 2H), 2.04-1.89 (m, 1H).

Example 2: Procedure for Manufacturing Hydrate 1

Method 1

Sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate lyophilized powder (30 g) was dissolved in 60 mL of water to obtain clear yellowish solution. The aqueous solution was slowly dripped into 900 mL of tetrahydrofuran (THF) while stirring at 300-350 rpm, ambient temperature. The slurry was stirred for additional 1 hour upon completion of charging the aqueous solution. After smooth filtration with a 350-mL fine fritted funnel, the wet cake was washed with 200-mL of THF and further dried overnight at room temperature under -74 cmHg vacuum to afford crystalline material Hydrate 1 (29.6 g, 99.0% purity).

Method 2

Sequentially charged 1.10 kg of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate lyophilized powder, 2.12 kg of water-for-injection (WFI), and 1.94 kg of THF to a 5-L reactor. The mixture was stirred at 150-200 rpm, ambient temperature for 15 min to obtain a clear yellowish solution which was further transferred to a 50-L Chemglass jacketed reactor equipped with two staged impellers. Crystallization was observed almost immediately after further addition of 34.56 kg THF in vacuo to the reactor at ca. 5.8 kg/min while stirring at 92-95 rpm.

After stirring for 2.5 hours, the slurry was filtered through a HDPE table-top Buchner vacuum funnel within one hour. The cake was then washed with a pre-prepared mixture of 4.65 kg THF and 0.2 kg WFI water, smoothed out and applied to vacuum for 30 min. The collected wet cake (2.0 kg) was further dried at room temperature under -74 cmHg vacuum for 35 hours with periodically stirring to afford Hydrate 1 (1.09 kg, 99.5% purity, 6% Na, 15% water by KF, 1 ppm THF).

Method 3

Campaign Equipment

5 L glass jacketed, stirred reactor with two staged impellers (5" OD high viscosity [lower] and 4" OD 60° [upper])

50 L glass jacketed, stirred reactor with two staged impellers (5" OD high viscosity [lower] and 4" OD 60° [upper])

Continuous filtration system consisting of: a HDPE table-top Buchner funnel 18" ID; HDPE 20" OD top with hose barb on top and Y-fitting underneath; 18" flange gasket sandwiched between Buchner and top; HDPE tubing with 0.6" ID and 0.75" OD; HDPE NPT to hose barb fittings; 20L vacuum filter flask

To effect continuous filtration, the slurry is gravity-fed from the bottom of the 50L reactor to the filter top while the filtrate is piped to the vacuum filter flask

Material Requirements

Provided below is the way to calculate required material masses based on the input of anhydrous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate ("A"). For instance, if A = 1.00 kg, then the amount of WFI water (water for injection) required is 1.00*1.93 = 1.93 kg.

Material requirements

Item	Ratio
Anhydrous Na Salt	**A**
THF (solution)	1.77
WFI water (solution)	1.93
THF (antisolvent)	31.69
THF (wash)	4.28
WFI water (wash)	0.18

Feed solution preparation

1	Charge to 5L reactor 1.10 kg of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-

	oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate
2	Charge to 5L reactor 2.12 kg of WFI water **Note:** sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate and water were charged in alternating portions because of the very low bulk density of this compound
3	Charge to 5L reactor 1.94 kg of tetrahydrofuran
4	Stir system at 150 – 200 rpm until a clear solution is obtained (approx. 15 – 20 min.)

Recrystallization, Filtration, and Wash

1	Charge to 50L reactor 5.10 kg of feed solution (98.8% of original system) **Note**: The feed solution was a deep, golden-yellow color
2	Set reactor to stir at 92 - 95 rpm
3	Charge by vacuum 34.56 kg of tetrahydrofuran. **Note:** Addition rate targeted at 2.2 kg/min, but actual addition rate was ~5.8 kg/min. Addition by vacuum was difficult to control precisely since a minimum amount of vacuum was needed to provide sufficient pressure head to get liquid out of the drum. **Note:** Crystals are observed almost immediately after addition begins. **Note**: Some THF seen condensing on condenser below point where vacuum was drawn. **Note**: This step represents the maximum volume of approximately 47 L.
4	Let stir for 2.5 hours
5	Filter product. Vacuum downstream of filter flask was opened first, then the bottom valve was opened to feed slurry by gravity to filter. **Note**: ~300 mL of solvent was collected in cold trap downstream of filter flask **Note**: The mother liquors were a light yellow color **Note:** The filtration was fast and smooth.

5	Wash cake with 4.84 kg wash solvent. Smooth out cake, then draw vacuum to filter. **Note:** Wash solvent = 4.65 kg tetrahydrofuran + 0.20 kg WFI water; prepared in advance **Note**: The amount of water in the wash solvent should be no more than 5% of the total wash mass
6	The isolated mass was 1.082 kg of Hydrate 1 in 88.0% molar yield

Example 3: Polymorph Studies

Described herein are polymorphism studies carried out on sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate. A number of different conditions, including a diverse range of solvent systems and temperature profiles, were used during this investigation. In summary, seven different hydrated crystalline forms were identified for this salt.

Solid state characterisation was performed on five of the seven forms, together with an assessment of stability relationships between the different hydrates. The remaining forms could not be isolated due to their metastable nature.

Based on these findings, it was concluded that Hydrate 1 is the most stable crystalline form at ambient conditions (25 °C and 40 %RH) (see Figure 2).

X-Ray Powder Diffraction (XRPD)

Bruker AXS D8 Advance

X-Ray Powder Diffraction patterns were collected on a Bruker D8 diffractometer using Cu Kα radiation (40 kV, 40 mA), θ - 2θ goniometer, and divergence of V4 and receiving slits, a Ge monochromator and a Lynxeye detector. The instrument is performance checked using a certified Corundum standard (NIST 1976). The software used for data collection was Diffrac *Plus* XRD Commander v2.6.1 and the data were analysed 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 using powder as received. The sample was gently packed into a cavity cut into 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 °2θ; step size: 0.05 °2θ; collection time: 0.5 s/step.

Differential Scanning Calorimetry (DSC)

DSC data were collected on a Mettler DSC 823E equipped with a 34 position auto-sampler. The instrument was calibrated for energy and temperature using certified indium. Typically 0.5-3 mg of each sample, in a pin-holed aluminium pan, was heated at 10 °C/min from 25 °C to 300 °C. A nitrogen purge at 50 ml/min was maintained over the sample.

The instrument control and data analysis software was STARe v9.20.

Thermo-Gravimetric Analysis (TGA)

TGA data were collected on a Mettler TGA/SDTA 851e equipped with a 34 position auto-sampler. The instrument was temperature calibrated using certified indium. Typically 5-30 mg of each sample was loaded onto a pre-weighed aluminium crucible and was heated at 10 °C/min from ambient temperature to 350 °C. A nitrogen purge at 50 ml/min was maintained over the sample.

The instrument control and data analysis software was STARe v9.20.

Figure 3 depicts the differential scanning calorimetry (DSC) thermogram and the thermogravimetry curve of Hydrate 1.

Gravimetric Vapour Sorption (GVS)

Sorption isotherms were obtained using a SMS DVS Intrinsic moisture sorption analyser, controlled by DVS Intrinsic Control software v1.0.1.2 (or v 1.0.1.3). The sample temperature was maintained at 25 °C by the instrument controls. The humidity was controlled by mixing streams of dry and wet nitrogen, with a total flow rate of 200 ml/min The relative humidity was measured by a calibrated Rotronic probe (dynamic range of 1.0 – 100 %RH), located near the sample. The weight change, (mass relaxation) of the sample as a function of %RH was constantly monitored by the microbalance (accuracy ±0.005 mg).

Typically 5 – 20 mg of sample was placed in a tared mesh stainless steel basket under ambient conditions. The sample was loaded and unloaded at 40 %RH and 25 °C (typical room conditions). A moisture sorption isotherm was performed as outlined below (2 scans giving 1 complete cycle). The standard isotherm was performed at 25 °C at 10 %RH

intervals over a 0 – 90 %RH range. Data analysis was undertaken in Microsoft Excel using DVS Analysis Suite v6.2 (or 6.1 or 6.0).

Method Parameters for SMS DVS Intrinsic Experiments

Parameters	Values
Adsorption - Scan 1	40 - 90
Desorption / Adsorption - Scan 2	90 - 0, 0 - 40
Intervals (%RH)	10
Number of Scans	2
Flow rate (ml/min)	200
Temperature (°C)	25
Stability (°C/min)	0.2
Sorption Time (hours)	6 hour time out

The sample was recovered after completion of the isotherm and re-analysed by XRPD.

Figure 4 depicts the DVS graph of amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate. In contrast, Figure 5 depicts the DVS graph of Hydrate 1. As can be seen, Hydrate 1 is significantly less hygroscopic than the amorphous form of this compound.

Polymorphism Studies

Screen Procedure and Results

A wide range of methodologies was used in an attempt to fully evaluate the polymorphic landscape of the sodium salt. These include traditional crystallisation, slow evaporation, heat/cool cycles and suspension/equilibration techniques. Slurry ripening or slurry maturation increases the possibility of generating metastable forms in accordance with the Ostwald rule of stages (Ostwald, W. (1897). "Studien über die Bildung und Umwandlung fester Körper. 1. Abhandlung: Übersättigung und Überkaltung". Zeitschrift für Physikalische Chemie 22: 289–330).

Procedure 1: Sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate (~ 40 mg) was dissolved in 400 µL of water and the resulting clear solution was freeze-dried to generate amorphous material (a control sample was analysed by XRPD to ensure that the freeze dry material was completely amorphous).

Different solvent systems were then added to the amorphous material in 5 vol. portions until a clear solution was obtained or 50 vol. had been used. Slurries were split in two sets; the first one was stirred at 50 °C (a) while the second one was stirred at 4 °C (b) for 3 days. Clear solutions were also split in two sets, the first one was allowed to slowly evaporate (c) and the second one was placed at 4 °C (d). All residual solids were filtered, air dried and analysed by XRPD. The results are summarized in Table 5.

Procedure 2: Amorphous material (~ 40 mg) was generated as in Procedure 1. Different solvents systems (2 mL) were added and the resulting slurries were subjected to heat/cool cycles 25/50 °C (two hours at each temperature and ramps of 0.5 °C/min) for 3 days. All residual solids were filtered, air dried and analysed by XRPD. The results are summarized in Table 5.

Procedure 3: A few experiments were repeated following Procedure 1a, with samples being analyzed after 3h, 24h, and 48h. The final product was filtered after 120h. The solids isolated were analysed by XRPD. The results are summarized in Table 6.

Procedure 4: Amorphous material (~ 60 mg) was generated as in Procedure 1. The amorphous material was suspended in different solvent systems (3 mL) at 50 °C. A sample was withdrawn after 3h and 24h. The final product was filtered after 48h and analysed by XRPD. The solids isolated were re-analysed again by XRPD after few days at ambient conditions (~25 °C and ~40 %RH). The results are summarized in Table 6.

Procedure 5: Amorphous material (~ 100 mg) was generated as in Procedure 1. The amorphous material was split in two portions (~50 mg each) and suspended in IPA/water (98:2) (2 mL). The first set of experiments (a) was subjected to heat/cool cycles as described in Procedure 2 and the second set of experiments (b) was stirred at 50 °C. A sample was withdrawn after 3h and 24h. The final product was filtered after 48h, dried under nitrogen current and analysed by XRPD. The results are summarized in Table 6.

Table 5 shows the results of the initial screen where the crystalline forms were identified and denoted Hydrate 1, Hydrate 2, Hydrate 3, Hydrate 4, Hydrate 5 and Hydrate 7.

Table 5 Results from Polymorphism Screen (Procedures 1 and 2)

Solvent	Procedure	Observation	Result
Heptane	1a	Suspension	Mainly Amorphous
Heptane	1b	Gum	N/A
Cumene	1a	Suspension	Mainly Amorphous
Cumene	1b	Gel	N/A

Anisole	1a	Suspension	Hydrate 1
Anisole	1b	Suspension	Hydrate 1
Ethyl Acetate	1a	Suspension	Hydrate 1 + extra peaks
Ethyl Acetate	1b	Suspension	Hydrate 1
Isopropyl Acetate	1a	Suspension	Hydrate 1
Isopropyl Acetate	1b	Suspension	Hydrate 1
Methylisobutyl Ketone	1a	Suspension	Hydrate 1
Methylisobutyl Ketone	1b	Suspension	Hydrate 1
2-Propanol	1a	Suspension	Hydrate 1
2-Propanol	1b	Suspension	Hydrate 1
Methylethyl Ketone	1a	Suspension	Hydrate 1
Methylethyl Ketone	1b	Suspension	Hydrate 1
Acetone	1c	Powder	Hydrate 1
Acetone	1d	Clear solution	N/A
Dimethyl Sulfoxide	1c	Gum	N/A
Dimethyl Sulfoxide	1a	Suspension	Hydrate 1
t-Butylmethyl Ether	1b	Suspension	Hydrate 1
1-4-Dioxane	1a	Suspension	Mainly amorphous
Toluene	1a	Slurry	Hydrate 1
Toluene	1b	Gel	N/A
Tetralin	1a	Slurry	Mainly amorphous
Tetralin	1b	Gel	N/A
1-2-Dimethoxyethane	1a	Slurry	Hydrate 3
Tetrahydrofuran	1a	Slurry	Hydrate 1 + Hydrate 3
Tetrahydrofuran	1b	Slurry	Hydrate 1
Dichloromethane	1a	Slurry	Hydrate 1 + Hydrate 4
Dichloromethane	1b	Slurry	Hydrate 1
DMF	1c	Gel	N/A
DMF	1d	Clear solution	N/A
Acetonitrile	1c	Powder	Hydrate 1
Acetonitrile	1b	Slurry	Hydrate 1
Nitromethane	1a	Slurry	Hydrate 1 + extra peaks
Ethylene glycol	1a	Slurry	Pattern 1
Water:THF (2:98)	1a	Oil	N/A
Water:THF (2:98)	1b	Slurry	Hydrate 1
Water:IPA (2:98)	1a	Slurry	Hydrate 1 + Hydrate 7
Water:IPA (2:98)	1b	Slurry	Hydrate 1

Water:Acetone (2:98)	1c	Powder	Hydrate 1
Water:Acetone (2:98)	1d	Big crystals	Hydrate 1
THF	2	Slurry	Hydrate 1
DCM	2	Slurry	Hydrate 1
Nitromethane	2	Slurry	Hydrate 1
Acetone	2	Slurry	Hydrate 1
IPA	2	Slurry	Hydrate 1
IPA/water (95:5)	2	Slurry	Hydrate 1
IPA/water (90:10)	2	Slurry	Hydrate 1
IPA/water (80:20)	2	Clear solution	Hydrate 2
Ethyl Acetate	2	Slurry	Hydrate 1 + extra peaks
IPA/water (99.5:0.5)	2	Slurry	Hydrate 1
IPA/water (99:1)	2	Slurry	Hydrate 1
IPA/water (98:2)	2	Slurry	Hydrate 5

Table 6 shows the results from experiments in which samples were taken at different times to identify new metastable forms; crystalline Hydrate 6 was identified during these experiments.

Table 6 Results from Polymorphism Screen (Procedures 3, 4 and 5)

Solvent	**Vol.**	**Procedure**	**3 h**	**24 h**	**48 h**	**120 h**
EtOAc	50	3	H1	H1 + H5	H1	H1
DME	50	3	H1	H1 + H5	Amorphous	Amorphous
DCM	50	3	H1	H1 + H4	H1 + H4	H1
Nitromethane	50	3	H1 + H5	Amorphous	N/A	H1
IPA	50	3	H1 + H5	H1 + H5	H1 + H5	H1
IPA/H_2O (98.5:1.5)	50	3	H1 + H5	H1 + H5	H5 + extra peaks	H1
IPA/ H_2O (98:2)	50	3	H1 + H5	H1 + H5	H1 + H5	H1
IPA/ H_2O (97.5:2.5)	50	3	H1 + H5	H1 + H5	H5 + H1	H1
Acetone/ H_2O (98.5:1.5)	25	3	H1	H1 + H5	H1 + H5	H1

Acetone/ H_2O (98:2)	25	3	*Clear solution obtained, no further analyses carried out.*			
Acetone/ H_2O (97.5:2.5)	25	3	*Clear solution obtained, no further analyses carried out.*			
IPA	50	4	H1 + H4	H3	H5	N/A
IPA/ H_2O (98:2)	50	4	H5 + H6	H6 + H5	H6 + H1	N/A
DME	50	4	H4	H4	H4	N/A
DCM	50	4	H4	H4	H4 + H1	N/A
IPA/ H_2O (98:2)	40	5a	H5 + H1	H5 + H1	H5	N/A
IPA/ H_2O (98:2)	40	5b	H5 + H1	H5 + H1	H5	N/A

Table 7 shows the re-analyses of some samples after storage at ambient conditions. From these results can be observed that Hydrate 4 is metastable and transforms to Hydrate 1; Hydrate 3 and Hydrate 6 are also metastable and transform to Hydrate 5 or Hydrate 1 at ambient conditions.

Table 7 Results from the Re-analysis of Samples

XRPD	**Conditions**	**XRPD**
H1 + H4	48 h a.c.	H1
H5 + H6	48 h a.c.	H5 +H1
H4	48 h a.c.	H1 + extra peaks
H4	48 h a.c.	H1
H3	24 h a.c.	H3 + H5
H6 + H5	24 h a.c.	H5 + H6
H4	24 h a.c.	H5
H4	24 h a.c.	H1 + H5
H5	72 h a.c.	H5
H6 + H1	72 h a.c.	H6 + H1
H4	72 h a.c.	H5
H4 + H1	72 h a.c.	H4 + H5 + H1

H: Hydrate; a.c.: ambient conditions.

Drying Experimens

In order to understand the minimum amount of water required to obtain pure Hydrate

1 material, with no contamination from other hydrated forms, a series of experiments were carried out to investigate the effect of the water activity in the hydration level of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate. Sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate was placed at the vacuum oven over the weekend at 70 °C and 5 mBar. The resulting amorphous material was suspended in different solvent systems and stirred at 25 °C for 24 hours. The solids were then filtered and analysed by XRPD (see Table 1 for details).

Table 1 Water Activity (a_w) Results

Na Salt	Solvent	% water	mL	Water activity	XRPD
40	THF	0.0	1.0	0	Mainly amorphous
40	THF	0.2	1.0	0.11	Hydrate 5
40	THF	0.4	1.0	0.20	Hydrate 5
20	THF	0.6	0.5	0.28	Hydrate 5
20	THF	0.8	0.5	0.35	Hydrate 5 + Hydrate 1

Pure Hydrate 5 was observed in a range of 0.1-0.3 of water activity. Above this threshold, Hydrate 1 started appearing. Based on these results (a_w >0.35), it is possible to estimate the minimum ratio of water required to achieve the formation of Hydrate 1 in other solvents such as IPA (>2.5%), acetone (>1.8%) or ethanol (>4.3%).

Summary

Table 2 Summary of Forms shows the correspondence between each crystalline form and the name that was tentatively used during the study, and reported throughout the update meetings, together with a description of the nature of the crystalline form.

Table 2 Summary of Forms

Name	Description
Hydrate 1	Stable tri-hydrate
Hydrate 2	Metastable, hexa-hydrate
Hydrate 3	Metastable, suspected mono-hydrate
Hydrate 4	Metastable, suspected hemi-hydrate (maybe lower stoichiometry)
Hydrate 5	Metastable, di-hydrate

Hydrate 6	Metastable, suspected mono-hydrate, only isolated as a mixture with Hydrate 5
Hydrate 7	Metastable, only isolated as a mixture with Hydrate 1, undefined

Figure 6 shows XRPD spectra of the various polymorph forms of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate.

Hydrate 1 is the most stable crystalline form of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate at ambient conditions (25 °C and 40 %RH) (see Figure 2) compared to the other hydrates described herein.

The diagram on Figure 7 summarizes the relative thermodynamic relationships between the different hydrated forms of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate.

XRPD Scanning Data of Hydrate 1 (Figure 1)

2-Theta Angle	**Intensity %**		**2-Theta Angle**	**Intensity %**
11.38	1.2		25.41	1.3
13.09	100.0		26.10	8.1
13.79	6.4		26.87	7.6
14.29	21.6		28.20	1.6
15.29	1.9		28.65	5.9
16.99	3.2		29.17	2.0
17.39	1.3		29.78	2.3
17.89	2.2		30.21	1.0
18.29	1.7		30.67	1.4
18.94	1.7		31.98	1.8
20.49	25.4		33.46	6.2
21.46	18.1		33.97	3.5

22.21	12.9		35.30	1.7
22.73	2.4		35.87	1.6
23.19	1.5		37.34	1.7
24.09	3.5		40.28	2.1
24.94	1.3		41.77	1.6

XRPD Scanning Data of Hydrates 2, 3, 4, and 5 (Figure 6)

Hydrate 2	
Angle 2-Theta °	Intensity Count %
10.16	100.0
10.75	9.5
11.51	8.6
12.85	10.7
13.37	6.9
13.89	11.0
16.23	89.7
17.00	9.3
17.62	38.0
18.79	17.7
19.23	9.4
19.78	10.7
20.04	10.1
20.53	6.1
20.80	34.3
21.08	25.0
21.43	17.8
21.79	26.0
22.91	36.5
23.26	14.2
23.65	6.9
24.39	5.9
25.02	16.3
25.39	11.2
25.73	10.1
26.00	7.4
26.47	11.9
27.22	5.1
27.51	14.0

Hydrate 3	
Angle 2-Theta °	Intensity Count %
4.30	23.6
6.13	42.5
6.72	100.0
8.07	11.4
12.64	10.9
12.98	52.0
13.95	23.6
14.19	20.5
15.05	11.1
15.67	17.2
16.48	51.8
17.19	24.1
18.03	42.9
18.66	40.6
18.95	23.2
19.36	12.2
19.82	20.9
20.46	26.8
21.12	16.7
21.43	27.7
21.62	31.9
22.01	30.0
22.39	23.2
22.85	11.7
23.17	15.4
23.78	15.0
24.20	14.0
24.43	17.3
24.99	54.8

Hydrate 4	
Angle 2-Theta °	Intensity Count %
6.54	100.0
7.64	43.9
8.34	44.4
10.08	17.2
11.95	17.0
13.14	9.6
14.30	12.1
14.91	18.2
15.21	37.4
15.39	33.8
15.80	36.7
16.63	22.7
17.08	30.2
17.67	22.9
17.97	32.8
18.26	24.2
19.16	14.1
19.74	17.3
20.27	15.1
20.69	21.7
21.19	21.7
21.63	62.4
22.65	13.7
22.94	19.1
23.38	85.0
23.89	15.3
24.36	39.9
25.12	32.8
26.34	12.8

Hydrate 5	
Angle 2-Theta °	Intensity Count %
7.81	46.3
8.80	71.5
10.20	52.0
11.88	14.4
13.79	27.9
14.94	28.6
15.76	58.0
16.18	50.3
16.50	62.4
16.94	13.1
17.47	27.2
17.88	100.0
18.35	26.3
18.53	27.5
18.92	80.0
19.89	89.9
20.44	14.3
20.65	27.3
21.14	64.6
21.60	19.5
21.79	34.6
22.14	8.7
22.64	68.0
22.89	17.4
23.28	6.7
23.94	57.9
24.40	8.6
24.97	12.9
25.32	4.5

Hydrate 2	
Angle	Intensity
2-Theta °	Count %
27.82	7.7
28.11	61.7
28.86	10.1
29.52	8.7
29.92	7.3
30.18	59.5
31.02	9.2
31.62	22.2
32.22	18.9
33.04	18.4
33.45	24.3

Hydrate 3	
Angle	Intensity
2-Theta °	Count %
25.76	15.7
26.95	14.1
27.85	10.1
28.18	12.2
29.25	10.1
30.48	14.0

Hydrate 4	
Angle	Intensity
2-Theta °	Count %
27.04	17.1
27.69	17.4
28.60	19.2
29.21	11.5
30.16	23.8
33.68	13.4

Hydrate 5	
Angle	Intensity
2-Theta °	Count %
25.79	33.0
26.45	12.6
27.07	12.8
27.32	8.3
27.86	25.4
28.38	16.8
29.13	26.8
29.53	12.5
29.79	12.7
30.12	14.9
30.39	24.3
30.90	12.6
31.28	12.3
32.38	21.1
32.70	12.6
33.08	5.8
33.78	9.3
34.04	11.1
34.35	28.3
35.04	15.0
35.36	7.1
35.63	9.8
36.21	19.8
36.96	6.7
37.34	11.7
37.83	4.8
38.44	8.2
38.95	5.8
39.39	5.5
39.72	6.9
40.83	12.5

Example 4: Single Crystal Experiments

Data were collected on a Bruker-Nonius Kappa CCD diffractometer equipped with an Oxford Cryosystems Cryostream cooling device. Structures were solved using either the SIR-97, SHELXS or SHELXD programs and refined with the SHELXL program as part of the Bruker AXS SHELXTL suite. Unless otherwise stated, hydrogen atoms attached to carbon were placed geometrically and allowed to refine with a riding isotropic displacement

parameter. Hydrogen atoms attached to a heteroatom were located in a difference Fourier synthesis and were allowed to refine freely with an isotropic displacement parameter.

Samples of Hydrates 1, 2, and 5 were submitted for single crystal X-ray diffraction studies, the results of which are shown in Tables 1 to 3.

Hydrate 1

Table 1 Single Crystal Structure of Hydrate 1

Molecular formula	C8H9N4NaO6S·3H2O				
Molecular weight	366.29				
Crystal system	Orthorhombic				
Space group	*P2(1)2(1)2(1)*	*a*	13.5194(2)Å,	α	90°,
		b	15.48040(10)Å,	β	90°,
		c	21.0792(2)Å,	γ	90°
V	4411.58(8)Å^3				
Z	12				
D_c	1.654g.cm^{-1}				
μ	2.793mm^{-1}				
Source, λ	Mo-K(alpha), 1.54178Å				
F(000)	2280				
T	100(2)K				
Crystal	Colourless prism, 0.5 x 0.2 x 0.1mm				
Data truncated to	0.80 Å				
θmax	74.49°				
Completeness	99.9%				
Reflections	23885				
Unique reflections	8990				
Rint	0.0386				

The structure solution was obtained by direct methods, full-matrix least-squares refinement on F^2 with weighting $w^{-1} = \sigma^2(F0^2) + (0.0580P)^2 + (0.0428P)$, where $P = (F_0^2+^2F_c^2)/3$, anisotropic displacement parameters. Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm, absolute structure parameter = -0.013(8). Final $wR^2 = \{\Sigma[w(F_0^2-F_c^2)2]/\Sigma[w(F_0^2)^2]^{1/2}\} = 0.0802$ for all data, conventional $R_1 = 0.0311$ on F values of 8810 reflections with $F_0 > 4\sigma(F_0)$, S = 1.038 for all data and 702 parameters. Final Δ/σ(max) 0.002, Δ/σ(mean), 0.000. Final difference map between +0.410 and -0.350 e Å-3.

Figure 8 shows a view of a molecule of Hydrate 1 the crystal structure showing the numbering scheme employed. Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level.

Hydrate 2

Table 2 Single Crystal Structure of Hydrate 2

Molecular	C8H9N4NaO6S·6H2O		
Molecular	420.34		
Crystal	Orthorhombic		
Space group	*P2(1)2(1)2(1)*	6.69670(	
		15.9704(	
		16.9529(	
V	1813.10(7)Å^3		
Z	4		
D_c	1.54g.cm^{-1}		
μ	2.467mm^{-1}		
Source, A	Mo-K(alpha), 1.54178Å		
F(000)	880		
T	100(2)K		
Crystal	Colourless plate, 0.5 x 0.3 x 0.03mm		
Data	0.80 Å		
θ_{max}	74.38°		
Completenes	99.4%		
Reflections	8077		
Unique	3691		
R_{int}	0.0464		

The structure solution was obtained by direct methods, full-matrix least-squares refinement on F ^{2} with weighting $w^{-1} = \sigma^2(F_0^2) + (0.0891P)^2 + (0.0000P)$, where $P = (F_0^2+2F_c^2)/3$, anisotropic displacement parameters, Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. Final $wR^2 = \{\Sigma[w(F_0^2-F_c^2)^2]/\Sigma[w(F_0^2)^2]^{1/2}\} = 0.1192$ for all data, conventional $R_1 = 0.0424$ on F values of 3518 reflections with $F_0 > 4\sigma(F_0)$, S = 1.042 for all data and 295 parameters. Final Δ/σ(max) 0.000, Δ/σ(mean), 0.000. Final difference map between +0.443 and -0.375 e Å^{-3}.

Figure 9 shows a view of a molecule of Hydrate 2 the crystal structure showing the numbering scheme employed. Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level. The inter-molecular hydrogen bonds are shown as dashed lines. Hydrogen atoms are displayed with an arbitrarily small radius.

Hydrate 5

Table 3 Single Crystal Structure of Hydrate 5

Molecular formula	C8H9N4NaO6S·2H2O

Molecular weight	348.27				
Crystal system	Orthorhombic				
Space group	*P2(1)2(1)2(1)*	*a*	6.1694(2)Å,	α	90°,
		b	11.8215(4)Å,	β	90°,
		c	38.0836(10)Å,	γ	90°
V	2777.50(15)Å^3				
Z	8				
D_c	1.666g.cm^{-1}				
μ	2.869mm^{-1}				
Source, A	Mo-K(alpha), 1.54178Å				
F(000)	1440				
T	100(1)K				
Crystal	Colourless lath, 0.45 x 0.05 x 0.03mm				
Data truncated to	0.80 Å				
θmax	74.48°				
Completeness	99.3%				
Reflections	13548				
Unique reflections	5661				
Rint	0.0493				

The structure solution was obtained by direct methods, full-matrix least-squares refinement on F 2 with weighting $w^{-1} = \sigma^2(F_0^2) + (0.0669P)^2 + (1.2775P)$, where $P = (F_0^2+2F_c^2)/3$, anisotropic displacement parameters. Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. Final $wR^2 = \{\Sigma[w(F_0^2-F_c^2)^2]/\Sigma[w(F_0^2)^2]^{1/2}\} = 0.1246$ for all data, conventional $R_1 = 0.0473$ on F values of 5057 reflections with $F_0 > 4\sigma(F_0)$, S = 1.021 for all data and 429 parameters. Final Δ/σ(max) 0.001, Δ/σ(mean), 0.000. Final difference map between +0.589 and -0.427 e Å^{-3}.

Figure 10 shows a view of a molecule of Hydrate 5 the crystal structure showing the numbering scheme employed. Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level. The inter-molecular hydrogen bonds are shown as dashed lines. Hydrogen atoms are displayed with an arbitrarily small radius.

Example 5: Standard BLI Potentiation MIC Assay

The ability of compounds to potentiate the activity of β-lactams was demonstrated by determining the minimum inhibitory concentrations (MIC) of β-lactam and BLI compound combinations against various β-lactamase producing bacterial strains using the broth microdilution method. The experimental protocol was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines with modifications as described below (CLSI guidelines can be derived from the CLSI document M07-A9 published in January

2012: "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition").

To prepare for MIC testing, frozen glycerol stocks of clinical isolates (*Klebsiella pneumoniae, Eschericia coli, Enterobacter spp, Citrobacter spp,* or *Pseudomonas aeruginosa*) were used to streak for isolated colonies on rich, non-selective, tryptic soy agar containing 5% sheep's blood (TSAB). Frozen glycerol stocks of laboratory engineered, isogenic *E. coli* strains, which contain cloned β-lactamase expressing plasmids were used to streak for isolated colonies on rich, selective LB agar supplemented with 25 μg/mL tetracycline to maintain the plasmid. All strains were incubated at 37°C for 18-24 hrs.

On the day of testing, primary cultures were started by scraping off 5-10 colonies from the TSAB plates containing clinical strains or the tetracycline supplemented LB plates containing engineered strains. The clinical strain material was suspended in ~5 mL of cation adjusted Mueller Hinton Broth (CAMHB) in 14 mL culture tubes. The engineered strain material was suspended in CAMHB (supplemented with 25 μg/mL tetracycline) in 14 mL culture tubes. All strains were incubated at 37°C with aeration (200 rpm) for ~2 hrs until the optical density at 600 nm (OD600) was ≥0.1.

The two compound components of the assay were each diluted in CAMHB and added to the 96-well broth microdilution assay plates. 50 μL of the β-lactam was added to each well of the assay plate in 2-fold dilutions with final concentrations ranging from 128 to 0.13 μg/mL. 25 μL of the BLI compound was added to all wells in the broth microdilution plates at a final concentration of 4 μg/mL. Inoculum cultures were prepared by standardizing the primary cultures to OD600 = 0.1 and then adding 20 μL of the adjusted primary culture per 1 mL CAMHB for clinical strains or CAMHB (supplemented with tetracycline at 100 μg/mL) for engineered strains, so that the final inoculum density was ~10^5 colony forming units per milliliter. Diluted inoculum cultures were used to inoculate 25 μL per well in 96-well broth microdilution assay plates. The final volume of each well was 100 μL and contained a β-lactam at different concentrations, a BLIcompound at 4 μg/mL concentration, the bacterial culture at an OD600 of approximately 0.001 and when necessary tetracycline at 25 μg/mL.

Plates were incubated for 18-20 hours at 37°C with aeration (200 rpm). Following incubation, growth was confirmed visually placing plates over a viewing apparatus (stand with a mirror underneath) and then OD600 was measured using a SpectraMax 340PC384 plate reader (Molecular Devices, Sunnyvale, CA). Growth was defined as turbidity that could

be detected with the naked eye or achieving minimum OD600 of 0.1. MIC values were defined as the lowest concentration producing no visible turbidity.

MIC values of -Hydrate 1 are shown in Table A.

Example 6: Synergy MIC (sMIC) Assay

The synergy MIC (sMIC) assay determines the concentration of the BLI required to potentiate the activity of a fixed concentration of a β-lactam antibiotic against β-lactamase producing bacterial strains. The experimental protocol was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines with modifications as described below (CLSI guidelines can be derived from the CLSI document M07-A9 published in January 2012: "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition"). The assay is set-up by serially diluting the BLI across 11 of the 12 wells in each row of a 96-well broth microdilution assay plate, adding the β-lactam at a fixed concentration to all wells in the assay plate, inoculating the assay plate with bacterial strains, and determining the lowest concentration of BLI required to inhibit overnight bacterial growth. Bacterial growth in the 12th well of the assay plate, which contains the β-lactam at a fixed concentration but does not contain any BLI, demonstrates that the bacterial strains are resistant to the β-lactam antibiotic (e.g ceftolozane) at the fixed concentration of 4 μg/mL.

To prepare for MIC testing, frozen glycerol stocks of clinical isolates (*Klebsiella pneumoniae, Eschericia coli, Enterobacter spp, Citrobacter spp,* or *Pseudomonas aeruginosa*) were used to streak for isolated colonies on rich, non-selective, tryptic soy agar containing 5% sheep's blood (TSAB). Frozen glycerol stocks of laboratory engineered, isogenic *E. coli* strains, which contain cloned β-lactamase expressing plasmids were used to streak for isolated colonies on rich, selective LB agar supplemented with 25 μg/mL tetracycline to maintain the plasmid. All strains were incubated at 37°C for 18-24 hrs.

On the day of testing, primary cultures were started by scraping off 5-10 colonies from the TSAB plates containing clinical strains or the tetracycline supplemented LB plates containing engineered strains. The clinical strain material was suspended in ~5 mL of cation adjusted Mueller Hinton Broth (CAMHB) in 14 mL culture tubes. The engineered strain material was suspended in CAMHB (supplemented with tetracycline at 25 μg/mL) in 14 mL culture tubes. All strains were incubated at 37°C with aeration (200 rpm) for ~2 hrs until the OD600 was ≥0.1.

The two compound components of the assay were each prepared in CAMHB and added to the 96-well broth microdilution assay plates. 50 µL of the BLI was added to each well of the assay plate in 2-fold dilutions with final concentrations ranging from 128 to 0.13 µg/mL. 25 µL of the β-lactam was added to all wells in the broth microdilution plates at a final concentration of 4 µg/mL. Inoculum cultures were prepared by standardizing the primary cultures to OD600 = 0.1 and then adding 20 µL of the adjusted primary culture per 1 mL CAMHB for clinical strains or CAMHB (supplemented with tetracycline at 100 µg/mL) for isogenic strains, so that the final inoculum density was ~10^5 colony forming units per milliliter. Diluted inoculum cultures were used to inoculate 25 µL per well in 96-well broth microdilution assay plates. The final volume of each well was 100 µL and contained a BLI at different concentrations, a β-lactam at 4 µg/mL concentration, the bacterial culture at an OD600 of approximately 0.001 and when necessary tetracycline at 25 ug/mL.

Interpreting the sMIC data:

Plates were incubated for 18-20 hours at 37°C with aeration (200 rpm). Following incubation, growth was confirmed visually placing plates over a viewing apparatus (stand with a mirror underneath) and then OD600 was measured using a SpectraMax 340PC384 plate reader (Molecular Devices, Sunnyvale, CA). Growth was defined as turbidity that could be detected with the naked eye or achieving minimum OD600 of 0.1. sMIC values were defined as the lowest concentration producing no visible turbidity.

The sMIC values represent the amount of BLI required to potentiate the activity of 4 µg/ml of CXA-101 (Ceftolozane) or ceftazidime to inhibit the growth of the β-lactamase producing bacteria.

sMIC values of Hydrate 1 are shown inTable B.

Example 7: Inhibition Kinetics

Inhibition or inactivation of KPC-2 by test inhibitors was assessed using 100 µM nitrocefin (NCF) as a reporter substrate. Assays were performed in 1x PBS pH 7.4, 0.1 mg/ml BSA, in 96-well half area plates, 50 µl reaction volume. NCF was dissolved in DMSO and diluted in assay buffer. Test inhibitors were dissolved in water or DMSO and serially diluted in the assay with final concentrations between 2000 – 0.195 µM.

The enzyme activity in the presence of varying concentrations of test inhibitor was determined by monitoring the hydrolysis of NCF spectrophotometrically at 486 nm, for 5

minutes, 25°C, using a SpectraMax Plus384 microplate reader with SoftMax Pro software (Molecular Devices). Data analysis was performed using GraphPad Prism (GraphPad Software, Inc.).

Progress curves were fit to a first-order rate decay equation (Eq. 1) to determine $k_{observed}$ (k_{obs}).

k_{obs} vs. inhibitor concentration [I] curves were then fit to Eq.2 to determine the inhibitor dissociation constant (K) and the first order rate constant of enzyme inactivation at infinite inhibitor concentration (k_{inact}). Kinetics results from the test of Hydrate 1 against the KPC-2 β-lactamase showed 134 – 163 $mM^{-1}s^{-1}$ (Kinact/K $mM^{-1}s^{-1}$).

Eq. 1

$$Y_t=V_0*(1-e^{(-kobs*t)})/k_{obs}$$

Where Y is the absorbance at time t, V_0 is the uninhibited enzyme velocity, k_{obs} is the observed rate constant of the enzyme inactivation.

Eq. 2

$$k_{obs}=k_{inact}*[I]/([I] + K(1 + S/K_m))$$

Where S is the NCF concentration, K_m is the KPC-2 K_m for NCF.

Table A: Standard BLI Potentiation MIC Assay Against a Panel of Isogenic and Clinical Strains Expressing β-Lactamases

Strain #	β-Lactamase	Bkgd	No BLI	Hydrate 1
Eco.2806	KPC-2	isogenic	E	A
Pae.2808	KPC-2	clinical	E	C
Kpn.2478	KPC-2, TEM+	clinical	E	C
Kpn.2490	KPC-3, SHV+, TEM+	clinical	E	A
Kpn.2783	CTX-M-15, SHV+, TEM+	clinical	E	A
Kpn.571	TEM-26	clinical	D	A
Pae.2885	AmpC	clinical	B	A
Cfr.568	AmpC	clinical	E	B
Ecl.569	AmpC	clinical	E	A
Kpn.2914	KPC-2, SHV+	clinical	D	B
Kpn.2913	KPC-2, SHV+	clinical	D	A
Kpn.2917	KPC-2, SHV+	clinical	D	A
Kpn.2918	KPC-3, SHV+, TEM+	clinical	E	B
Kpn.2909	KPC-3, SHV+, TEM+	clinical	E	B
Eco.2711	KPC	clinical	D	A
Eco.2781	KPC-2, TEM+	clinical	C	A
Kpn.2926	CTX-M-15, OXA-48	clinical	E	B
Pae.2757	AmpC over-expn	clinical	C	B
Pae.2863	AmpC de-repress	clinical	C	B
Eco.2843	DHA-1	isogenic	E	A
Eco.2491	CMY-2	clinical	D	A
Eco.2902	Aba-ADC-33	isogenic	E	B
Eco.2840	KPC-4	isogenic	E	B

Eco.2845	OXA-15	isogenic	E	B
MIC90			E	B
MIC50			E	B

A=0.25-0.5 µg/mL; B=1-2 µg/mL; C=4-8 µg/mL; D=16-32 µg/mL; E>64 µg/mL

Table B: Synergy MIC (sMIC) Against a Panel of Isogenic and Clinical Strains Expressing β-lactamases

β-Lactamase	**Bkgd**	**Sp**	**β-Lactam (4 µg/mL)**	**Hydrate 1**
none	isogenic	Eco	none	F
KPC-2	isogenic	Eco	CXA-101	AA
OXA-15	isogenic	Eco	CXA-101	B
CTX-M-15	isogenic	Eco	CXA-101	A
SHV-12	isogenic	Eco	CXA-101	B
P99	isogenic	Eco	CXA-101	AA
KPC-2	clinical	Kpn	CXA-101	C
KPC-2	clinical	Pae	CXA-101	C

AA=< 0.25µg/mL; A = 0.25-0.5 µg/mL; B = 1-2 µg/mL; C = 4-8 µg/mL; D = 16-32 µg/mL; E = 64µg/mL; F =≥128µg/mL

CLAIMS

1. Hydrate 1 of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, and 21.5 ± 0.2°.

2. Hydrate 1 of claim 1, wherein the X-ray powder diffraction pattern further comprises a peak expressed in degrees-2-theta at angle 22.2 ± 0.2°.

3. Hydrate 1 of claim 2, wherein the X-ray powder diffraction pattern further comprises peaks expressed in degrees-2-theta at angles 13.8 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.7 ± 0.2°, and 33.5 ± 0.2°.

4. Hydrate 1 of claim 1, wherein Hydrate 1 is characterized by an X-ray powder diffraction pattern having four or more peaks expressed in degrees-2-theta at angles 13.1 ± 0.2°, 13.8 ± 0.2°, 14.3 ± 0.2°, 20.5 ± 0.2°, 21.5 ± 0.2°, 22.2 ± 0.2°, 26.1 ± 0.2°, 26.9 ± 0.2°, 28.7 ± 0.2°, and 33.5 ± 0.2°.

5. Solid forms of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate selected from the group consisting of:

a. Hydrate 2 characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 10.2 ± 0.2°, 16.2 ± 0.2°, 28.1 ± 0.2°, and 30.2 ± 0.2°;

b. Hydrate 3 characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 6.7 ± 0.2°, 13.0 ± 0.2°, 16.5 ± 0.2°, and 25.0 ± 0.2°;

c. Hydrate 4 characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 6.5 ± 0.2°, 7.6 ± 0.2°, 8.3 ± 0.2°, 21.6 ± 0.2°, and 23.4 ± 0.2°; and

d. Hydrate 5 characterized by an X-ray powder diffraction pattern having peaks expressed in degrees-2-theta at angles 8.8 ± 0.2°, 15.8 ± 0.2°, 16.5 ± 0.2°, 17.9 ± 0.2°, 18.9 ± 0.2°, 19.9 ± 0.2°, 21.1 ± 0.2°, 22.6 ± 0.2°, and 23.9 ± 0.2°.

6. A pharmaceutical composition comprising sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-

7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate in Hydrate 1 form and obtained by a process comprising exposing amorphous sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate to conditions comprising a temperature of 20° C – 30° C and a relative humidity of 40% – 98%.

7. A method of making Hydrate 1 of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate, the method comprising the steps of:

(a) combining sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate and a solvent, such that a solution of sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is formed; and

(b) combining an antisolvent with the solution, wherein the antisolvent is miscible with the solvent and wherein sodium (2S,5R)-2-(1,3,4-oxadiazol-2-yl)-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate is partially or completely insoluble in the antisolvent, such that crystalline Hydrate 1 precipitates from the solution.

8. The method of claim 7, wherein the solvent in step (a) of the method is water.

9. The method of claim 11, wherein the antisolvent in step (b) of the method is THF or acetonitrile.

10. A pharmaceutical composition comprising the Hydrate 1 of claim 1, and a pharmaceutically acceptable carrier.

11. The pharmaceutical composition of claim 10, wherein Hydrate 1 is reconstituted in a pharmaceutically acceptable liquid.

12. The pharmaceutical composition of claim 11, wherein the pharmaceutical composition is suitable for intravenous administration.

13. A pharmaceutical composition comprising the Hydrate 1 of claim 1 and at least one β-lactam antibiotic.

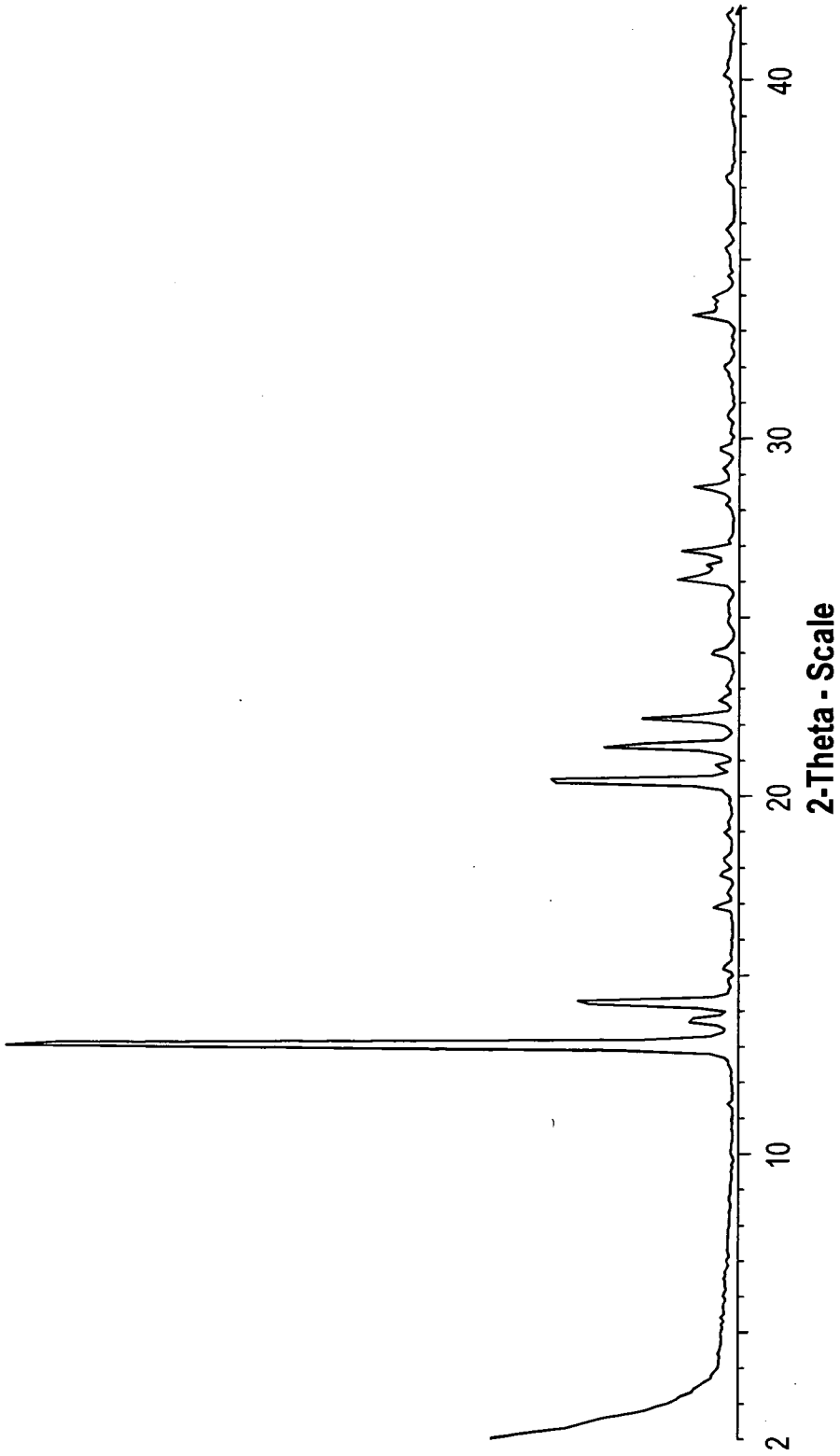


Fig. 1

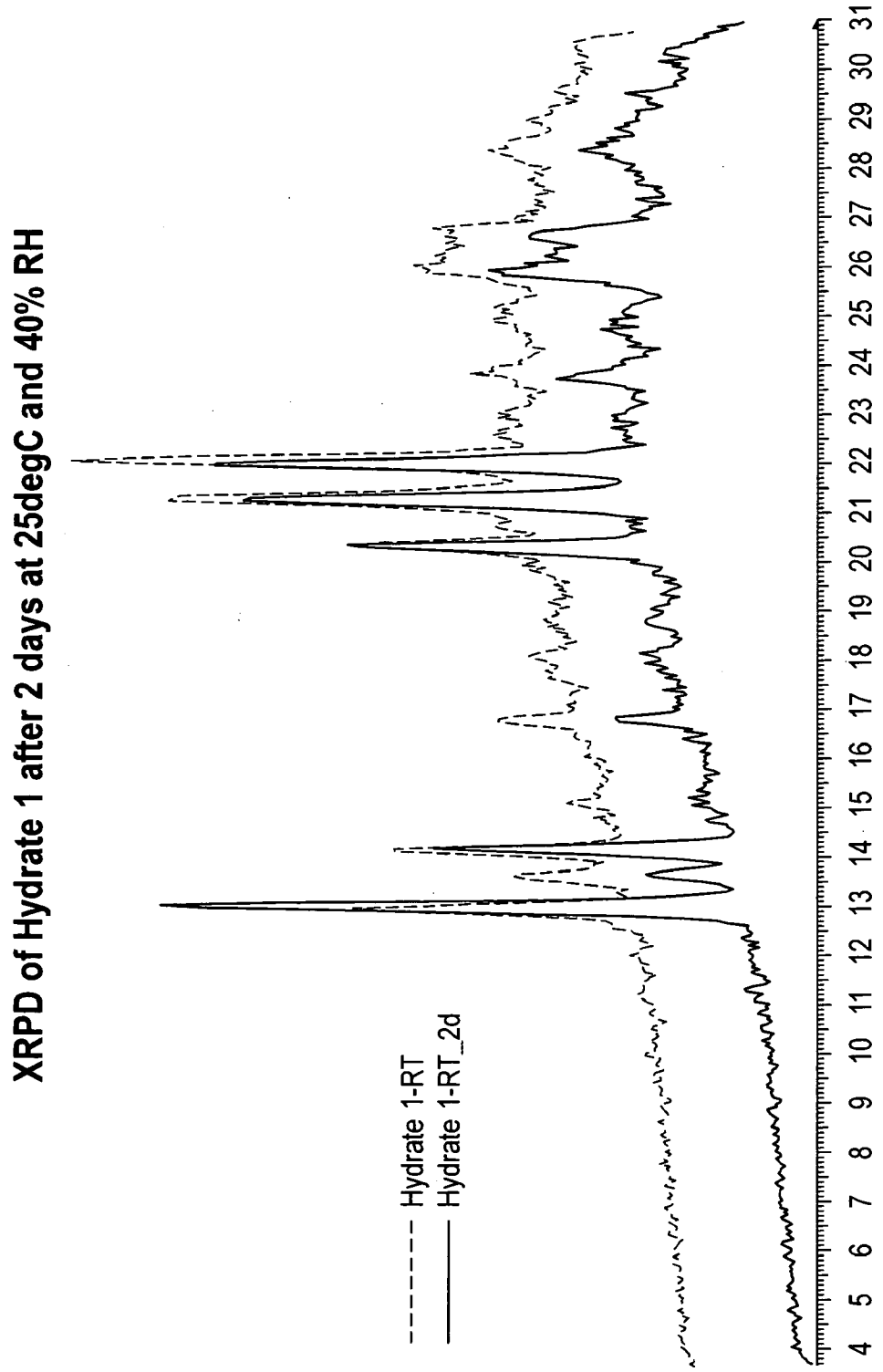

XRPD of Hydrate 1 after 2 days at 25degC and 40% RH
Hydrate 1-RT
Hydrate 1-RT_2d
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
2-Theta - Scale

Fig. 2

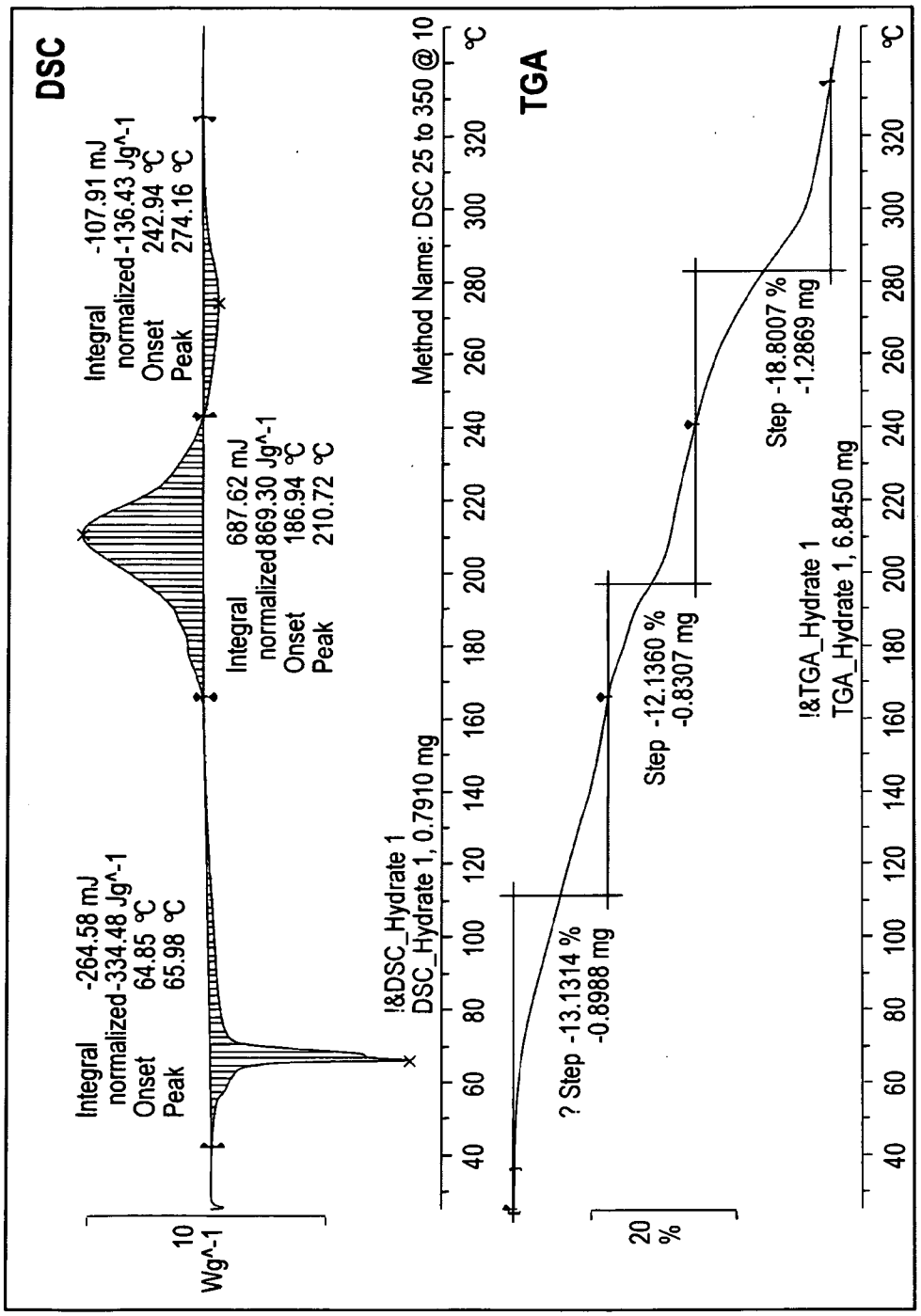

DSC
Integral -264.58 mJ
normalized -334.48 Jg^-1
Onset 64.85 °C
Peak 65.98 °C
Integral 687.62 mJ
normalized 869.30 Jg^-1
Onset 186.94 °C
Peak 210.72 °C
Integral -107.91 mJ
normalized -136.43 Jg^-1
Onset 242.94 °C
Peak 274.16 °C
10
Wg^-1
!&DSC_Hydrate 1
DSC_Hydrate 1, 0.7910 mg
Method Name: DSC 25 to 350 @ 10
TGA
? Step -13.1314 %
-0.8988 mg
Step -12.1360 %
-0.8307 mg
Step -18.8007 %
-1.2869 mg
!&TGA_Hydrate 1
TGA_Hydrate 1, 6.8450 mg
20
%
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 °C
STARe SW 9.20

Fig. 3

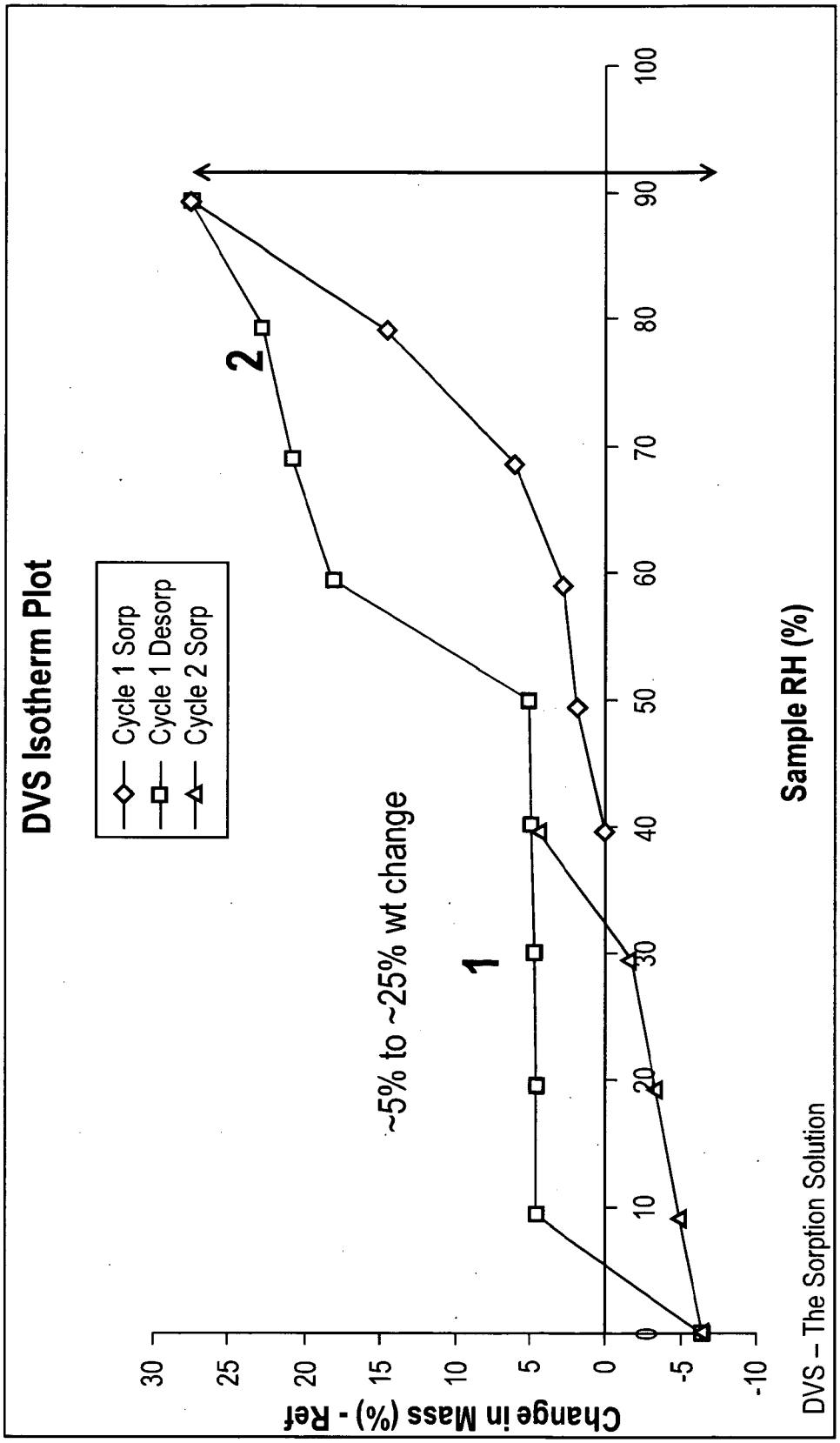

DVS Isotherm Plot
Cycle 1 Sorp
Cycle 1 Desorp
Cycle 2 Sorp
~5% to ~25% wt change
1
2
Change in Mass (%) - Ref
Sample RH (%)
DVS – The Sorption Solution
30
25
20
15
10
5
0
-5
-10
0
10
20
30
40
50
60
70
80
90
100

Fig. 4

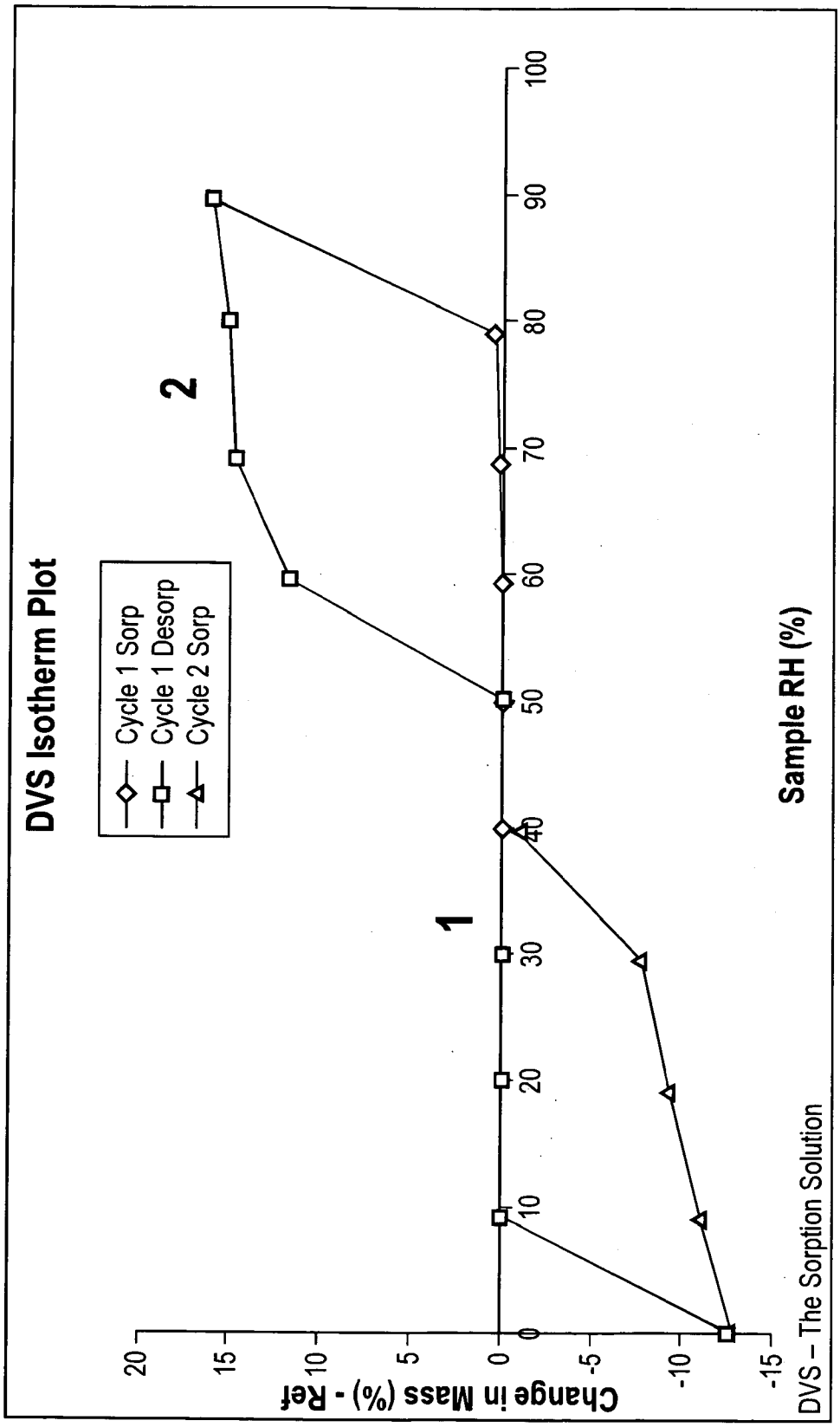

DVS Isotherm Plot
Cycle 1 Sorp
Cycle 1 Desorp
Cycle 2 Sorp
1
2
Change in Mass (%) - Ref
Sample RH (%)
DVS – The Sorption Solution
20
15
10
5
0
-5
-10
-15
0
10
20
30
40
50
60
70
80
90
100

Fig. 5

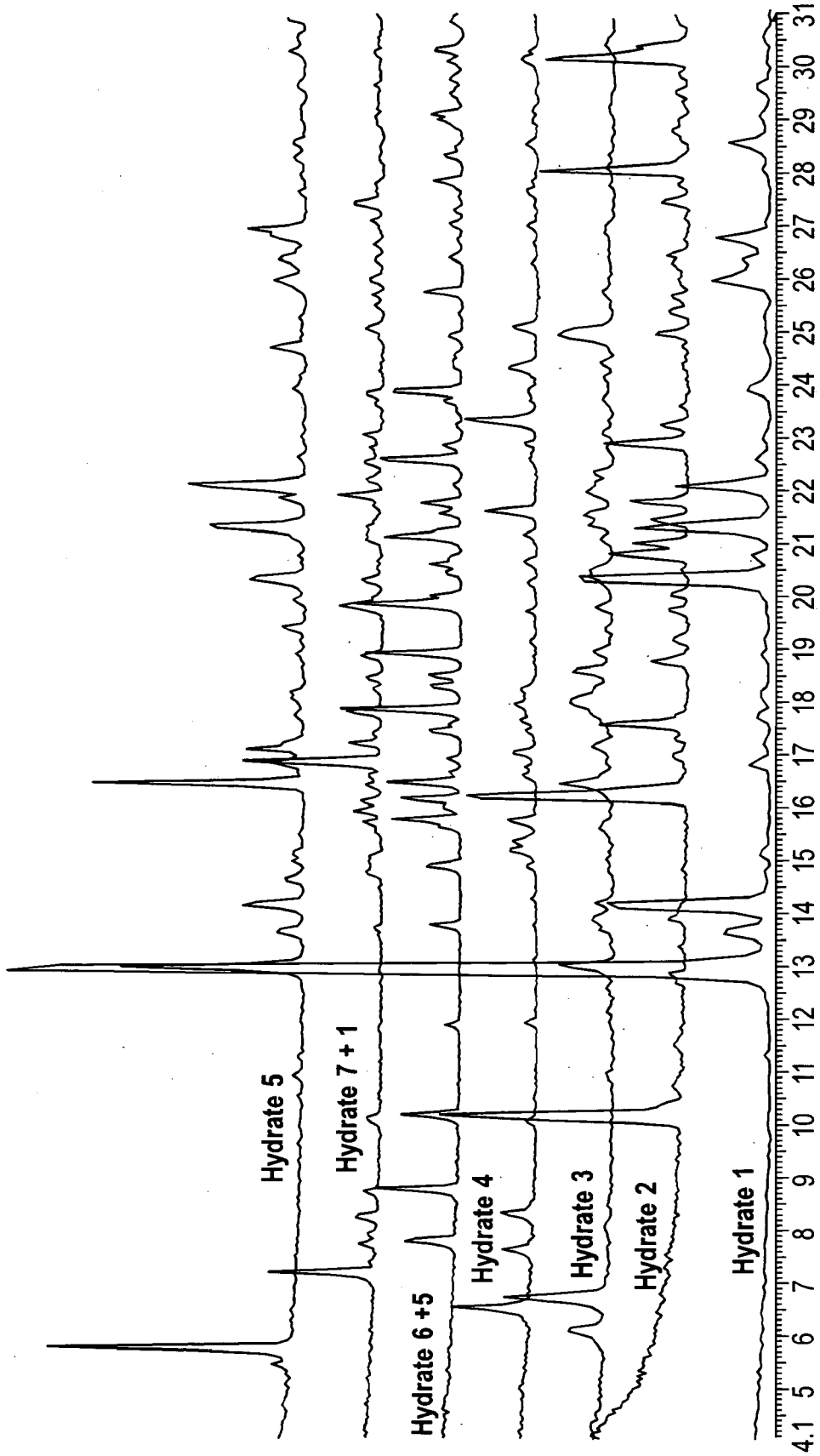
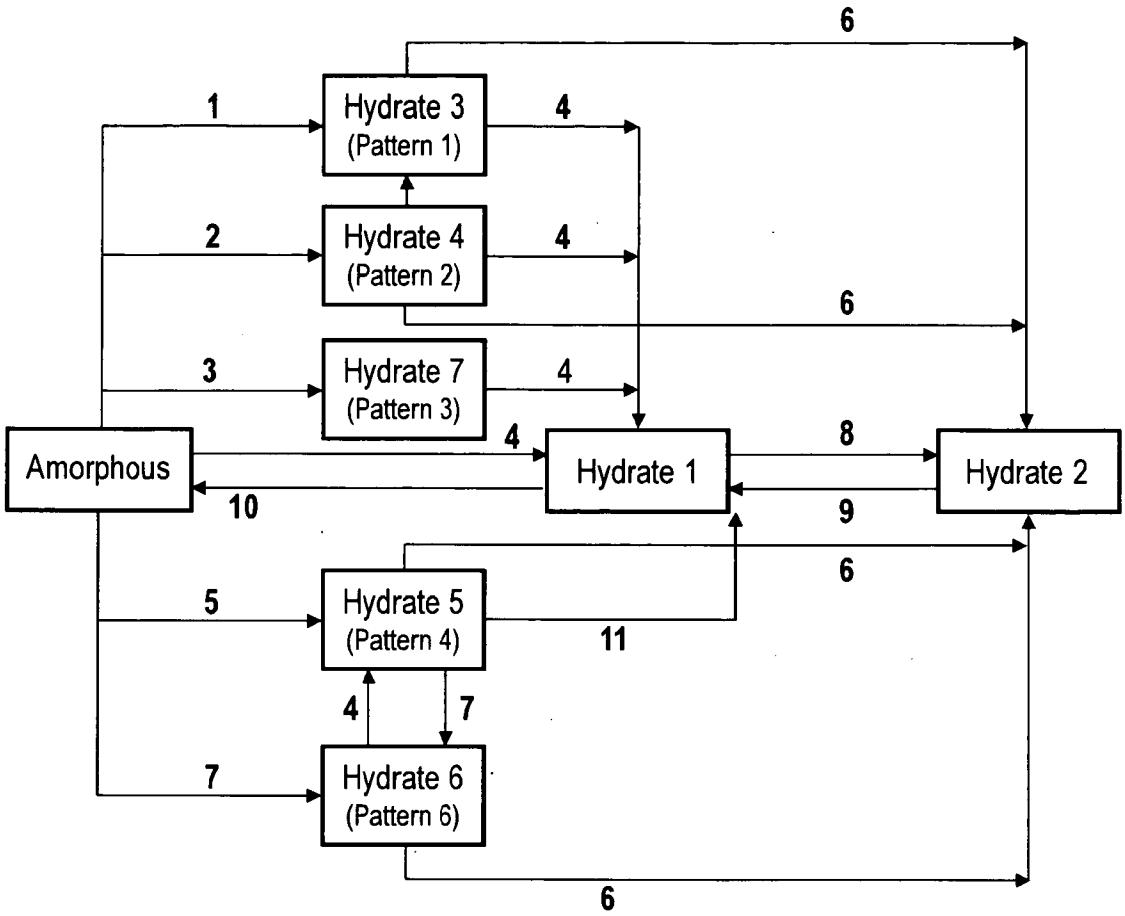
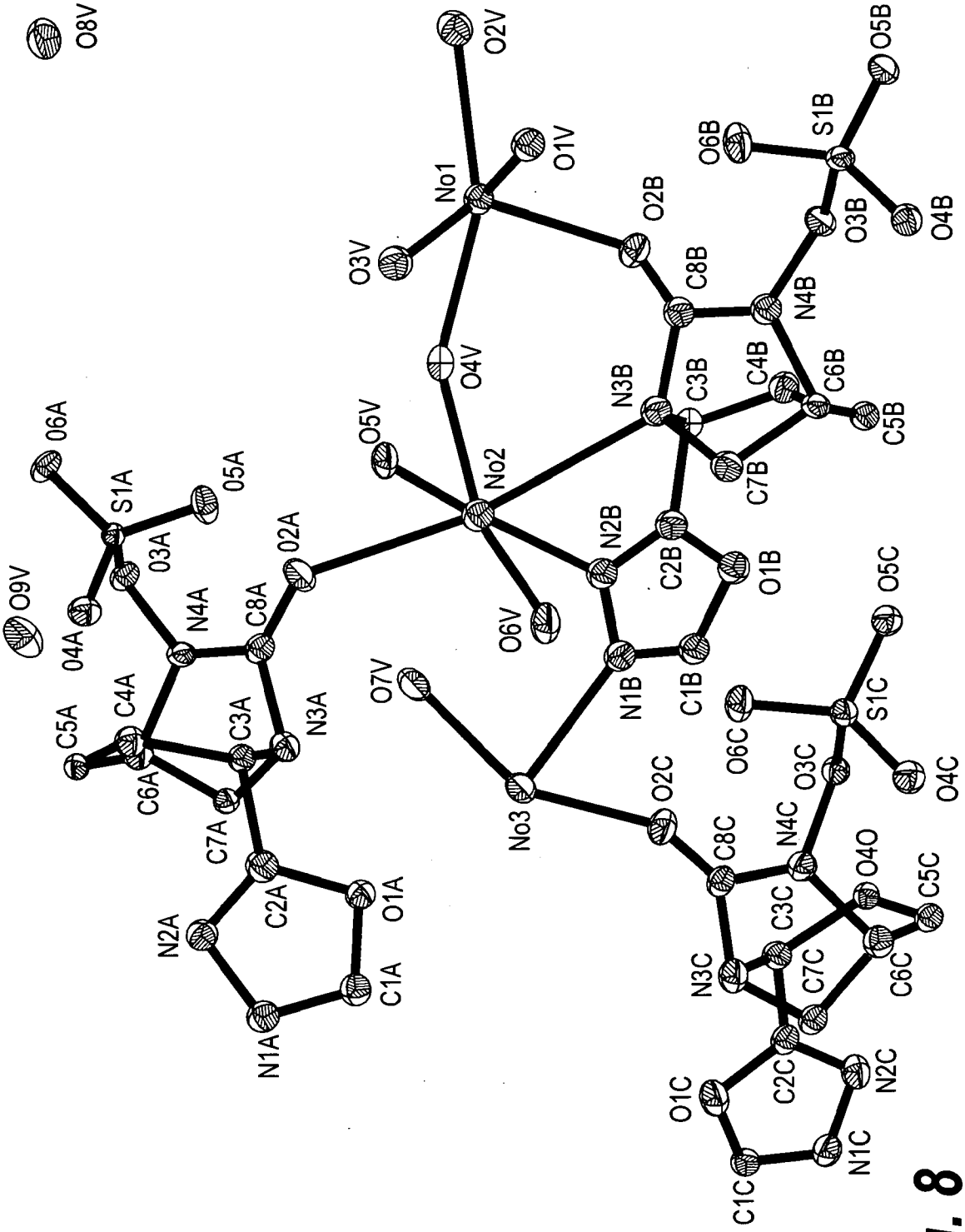

Hydrate 5
Hydrate 7 + 1
Hydrate 6 +5
Hydrate 4
Hydrate 3
Hydrate 2
Hydrate 1
4.1 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
2-Theta - Scale

Fig. 6



1. 1, 2-Dimethoxyethane at 50°C for 3 days
2. DCM at 50°C for 24 hours
3. IPA/water (98:2) at 50°C for 3 days
4. Ambient conditions
5. IPA/water (98:2) cycling 25- 50°C for 3 days
6. Storage at 25°C and 96% RH
7. IPA/water (98:2) at 50°C for 3 days
8. Storage at 25° C and RH > 80%
9. Storage at 25° C and RH < 60%
10. Storage at 60° C for 24 hours
11. Storage at 40° C and 75% RH

Fig. 7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O2V
O5B
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O6B
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O1V
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O4V
C4B
C6B
N3B
C3B
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C5B
06A
No2
C7B
S1A
05A
N2B
02A
03A
C2B
O1B
O9V
O6V
O5C
N4A
C8A
04A
C4A
O7V
N1B
C1B
S1C
C5A
O6C
N3A
C3A
O3C
C6A
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No3
O2C
C7A
C8C
N4C
O4O
C2A
O1A
C5C
N2A
C3C
C1A
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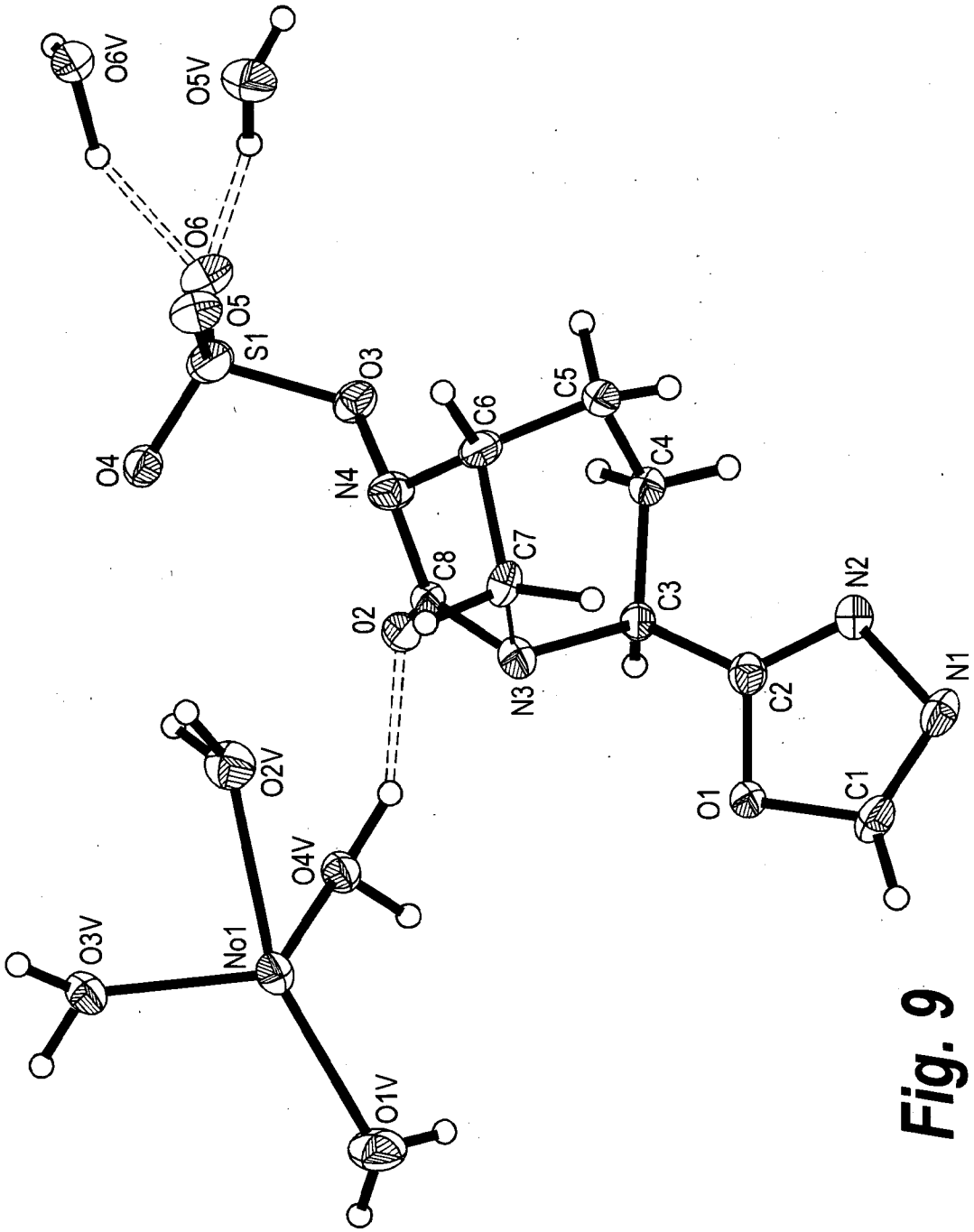
C6C
O1C
C2C
N2C
N1A
N1C
C1C

Fig. 8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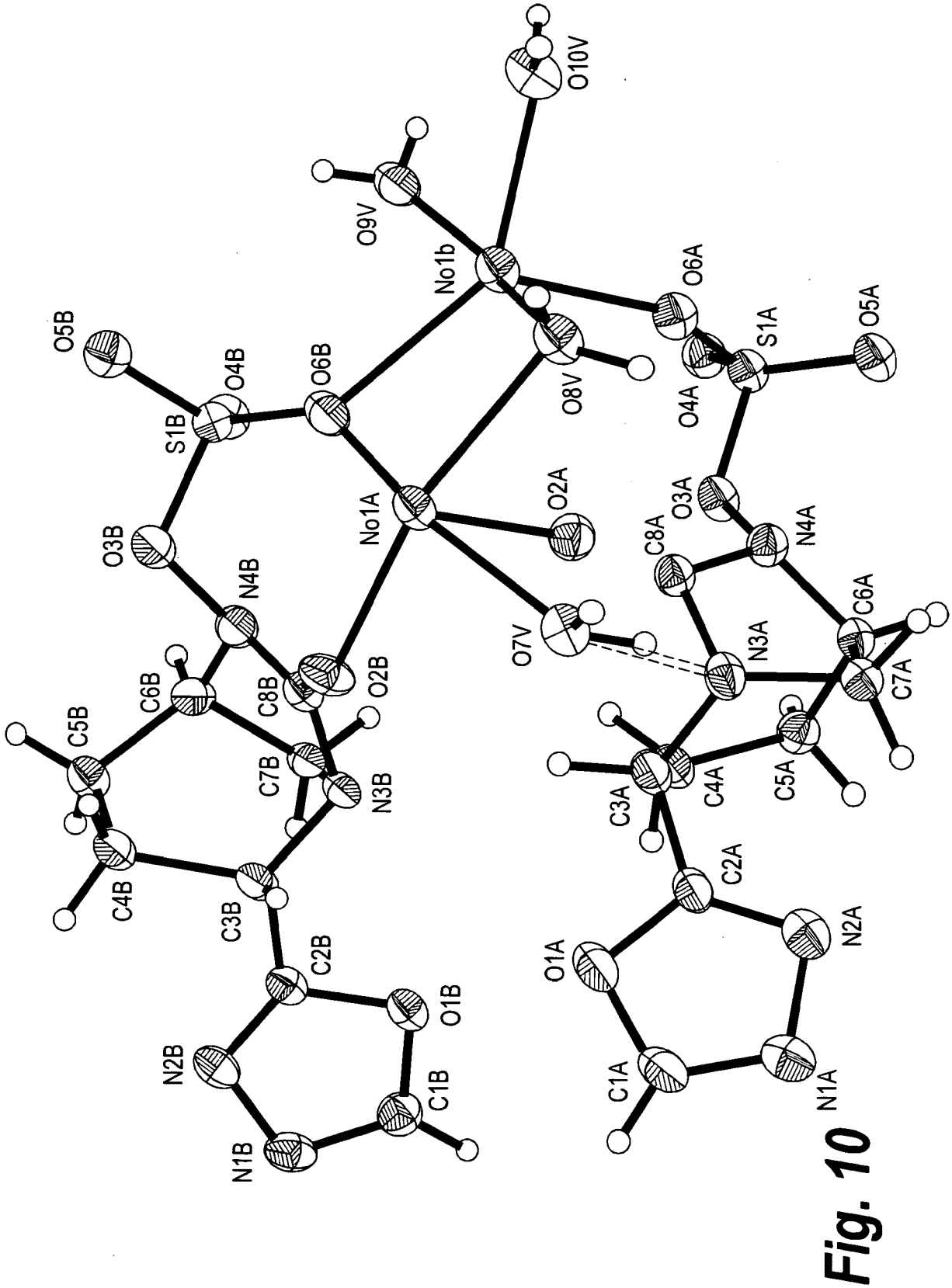
O5V
O6
O5
S1
O3
O4
N4
C6
C5
C4
C8
C7
C3
N2
O2
N3
C2
N1
O1
C1
O2V
O4V
O3V
No1
O1V

Fig. 9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O9V
No1b
O6A
O5B
O4B
O6B
S1B
O8V
S1A
O5A
O4A
O2A
No1A
O3A
O3B
C8A
N4A
N4B
O7V
C6A
C6B
C8B
O2B
N3A
C7A
C5B
C7B
N3B
C3A
C4A
C5A
C4B
C3B
C2A
C2B
O1A
N2A
O1B
N2B
C1A
C1B
N1A
N1B

Fig. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/028571

A. CLASSIFICATION OF SUBJECT MATTER

C07F 1/04(2006.01)i, C07D 487/04(2006.01)i

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)
C07F 1/04; C07D 487/08; C07D 471/08; A61K 31/439; A61K 31/4545; A01N 43/00; C07D 487/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: -OXADIAZOLE, THIADIAZOLE, BETA-LACTAMASE INHIBITORS.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2011-0294777 A1 (BLIZZARD TIMOTHY A. et al.) 01 December 2011 See claim 1.	1-13
A	US 2011-0046102 A1 (LEDOUSSAL BENOIT et al.) 24 February 2011 See abstract.	1-13
A	WO 2010-118361 A1 (SOPHARMIA, INC. et al.) 14 October 2010 See claims.	1-13
PX	WO 2013-149121 A1 (CUBIST PHARMACEUTICALS, INC.) 03 October 2013 See page 22.	1-13

☐ 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	Date of mailing of the international search report
25 July 2014 (25.07.2014)	**25 July 2014 (25.07.2014)**

Name and mailing address of the ISA/KR
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Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2014/028571

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		KR 10-2010-0130176 A	10/12/2010
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		RU 2445314 C9	10/04/2013
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