

# Pseudopolymorphs of LB30870, a Direct Thrombin Inhibitor: One-Dimensional Solvent Channel Structures Explain Reversible Hydration/Dehydration

Jihye Lee,<sup>†,#</sup> Dongwook Kim,<sup>‡,#</sup> Jaehyeon Park,<sup>§</sup> Eunice EunKyeong Kim,<sup> $\perp$ </sup> Myoung Soo Lah,<sup>\*,‡</sup><sup> $\oplus$ </sup> and Aeri Kim<sup>\*,†</sup><sup> $\oplus$ </sup>

<sup>†</sup>College of Pharmacy, CHA University, 521 CHA Bio Complex, 335 Pangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do 13488, Korea

<sup>‡</sup>Department of Chemistry, Ulsan National Institute of Science and Technology, 50 Unist-gil, Ulju, Ulsan 44919, Korea <sup>§</sup>SRI Biosciences, 333 Ravenswood Avenue, Menlo Park, California 94025-3493, United States

Sit Diosciences, 555 Ravenswood Avenue, Menio Faik, Camorina 74025-5475, Oniced States

<sup>⊥</sup>Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 02792, Korea

**(5)** Supporting Information

**ABSTRACT:** Three different hydrates of LB30870, a new direct thrombin inhibitor, were identified during the screening of solid form, and their interconversion relationship and relative thermodynamic stabilities were investigated. Form I (hexahydrate) changes to Form II (dihydrate) or Form III (tetrahydrate) by dehydration, while Form II becomes Form I by hydration, and both Form II and Form III change to Form I in the aqueous slurry. Single crystals obtained from two different crystallization conditions, wet or air-dried, were found to be isostructural with a difference in the solvent channels, and based on the simulated and experimental powder X-ray diffraction patterns, the air-dried crystals are assigned as Form I and Form III, respectively. In all crystal structures, LB30870 is in a folded conformation forced by the presence of strong hydrogen bonds by two structural water molecules. The solvent channel formed can hold up to six additional hydration water sites per each LB30870, and the one-dimensional solvent channel



facilitates the interconversion among the hydrates and rapid conversion to Form I in water. Although all hydrate forms would not differ in oral bioavailability as Form I predominates in the aqueous phase, considering the stable water content at 40-75% relative humidity Form III would be the most suitable for further development.

# ■ INTRODUCTION

LB30870 is a new direct thrombin inhibitor with the chemical name 2-{[(1R)-2-((2S)-2{[ $({5-[amino(imino)methyl]}$ -2-thienyl}methyl)amino]carbonyl}-pyrrolidinyl)-1-benzhydryl-2-oxoethyl]amino}acetic acid (Figure 1a). It was proposed as a potential therapeutic agent for the prevention and treatment of thrombotic diseases based on its anticoagulation activities and oral bioavailability in preclinical animal species.<sup>1–3</sup> Pharmaco-kinetic properties of LB30870 in preclinical animal species were reported previously.<sup>3</sup> Solution formulations were used for preclinical studies and the first-in-human study of LB30870 prior to solid state characterization.<sup>4</sup> However, solid dosage forms are preferred for further development for commercialization. Identifying a suitable solid form is a prerequisite for solid dosage form development.

Pharmaceutical solids can exist in several subphases, such as polymorphs, solvates, hydrates, and cocrystals.<sup>5–7</sup> Different solid forms (at different free energy states) of the same molecule often show different physical properties including solubility, melting point, particle size, dissolution rate,



Figure 1. Chemical structure: (a) LB30870, (b) zwitterionic form.

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hygroscopicity, and others. Phase transitions such as polymorph interconversion, desolvation of solvates, formation of hydrate, and conversion of crystalline to amorphous form may occur during various chemical and pharmaceutical processes.<sup>8–13</sup> In particular, hydrates are frequently found and may be classified as stoichiometric or nonstoichiometric.14-16 Stoichiometric hydrates have well-defined water content with step-shaped isotherms and a different crystal structure than the anhydrous drug or other hydrates. On the other hand, nonstoichiometric hydrates are those with a continuously variable composition within a certain range, without any significant corresponding change in the crystal structure, except usually some anisotropic expansion of the crystalline network to accommodate the additional water molecules.<sup>14,17</sup> Therefore, an investigation of the dehydrated phase and the dehydration pathway is crucial to the development of a pharmaceutical product.<sup>8,18-20</sup> Investigation of hydration/dehydration behavior of nonstoichiometric hydrates could be challenging because of various mechanisms to accommodate water molecules. When a single crystal of X-ray diffraction analysis quality is available, it is the most reliable method for structure determination at the atomic level, which facilitates a thorough understanding of solid state chemistry of the solid form.<sup>21</sup> Solid state properties can have a dramatic impact on manufacturability, stability, quality control, and sometimes bioavailability of the commercial formulation.<sup>22,23</sup> Hence, it is desirable to choose the most suitable and stable solid form of the drug at the initial stages of drug development in order to minimize issues related to the solid form in a later stage of development.<sup>22-25</sup>

In this study, we used various approaches such as moisture sorption/desorption, slurry conversion, Karl Fischer titration, powder X-ray diffraction (PXRD), dynamic vapor sorption (DVS), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and single crystal X-ray diffraction to investigate the hydrates of LB30870 and establish their relative thermodynamic stability and interconversion relationships among them.

# EXPERIMENTAL SECTION

**Materials.** LB30870 was obtained from lab synthesis or from pilot synthesis at LG Life Sciences Ltd. (Daejeon, Korea). Analytical grade solvents were purchased from Merck KGaA (Germany) and used without further purification.

**Methods.** Conversion by Moisture Sorption and Desorption. About 50 mg of LB30870 was placed in open vials in desiccators and dried over  $P_2O_5$  under a vacuum overnight, and the weight change was recorded. Desiccators pre-equilibrated with saturated solutions of various salts to obtain ranges of relative humidity (RH) such as MgCl<sub>2</sub> (33% RH), Mg(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O (53% RH), NaNO<sub>2</sub> (64% RH), NaCl (75% RH), or KNO<sub>3</sub> (93% RH) were used for subsequent water sorption/desorption test at  $25 \pm 1 \, {}^{\circ}C.^{26,27}$  After being dried over  $P_2O_5$ under a vacuum, the sample vials were transferred consecutively from lower to higher RH, one step a day to obtain the sorption isotherm. Equilibrium moisture content (EMC) values on a dry weight basis were calculated from the weight gain at each RH.<sup>28</sup> After completion of sorption isotherm at 93% RH, vials were then transferred back again to lower RH for desorption isotherm. Appearance and the weight change were recorded before transferring sample vials at each step. Solid form change was monitored by PXRD.

Conversion in Aqueous Slurry. LB30870 samples were suspended in water, and the slurry was stirred vigorously with a magnetic stirrer in closed vials. Samples were collected at given time points, and their PXRD was taken while still wet after air-drying or drying for 1 day at ambient temperature. After additional stirring for 24 h, remaining suspension was filtered, and the solid was dried under a vacuum for 3 days and characterized by PXRD.

Karl Fischer Water Titration. Samples with an approximate water content of 1000  $\mu$ g were placed in prelabeled 1 mL vials. Sample vials were then transferred into the glovebox in which the Karl Fischer moisture titrator (DL37 KF Coulometer, Mettler Toledo) was located. When the RH within the glovebox reached 15% after overnight dry nitrogen purging, the water content of each sample at each time point was measured in triplicates. HYDRANAL-Coulomat A and C were used as solvents, and they were exchanged with fresh ones before titration.

*PXRD*. PXRD data were collected using Rigaku Geigerflex D/max– IIIC diffractometer (Cu K $\alpha$ ,  $\lambda$  = 1.5418 Å) with a step size 0.03° in 2 $\theta$  angle and using a Bruker D2 Phaser automated diffractometer (Cu K $\alpha$ ,  $\lambda$  = 1.5418 Å) with a step size of 0.02° in 2 $\theta$  angle. Simulated PXRD patterns were calculated with Materials Studio software<sup>29</sup> using the structural model from single crystal data.

Dynamic Vapor Sorption (DVS). Vapor sorption and desorption isotherms were measured with a Dynamic Vapor Sorption Advantage (Surface Measurement Systems Ltd., UK) at 25 °C. Approximately 11–14 mg of LB30870 (Form II, obtained by vacuum drying Form I) was placed directly into the sample cup. The first step of the DVS experiment involved drying the material at 25 °C, 2–3% RH for 25 min. The RH was increased in increments of 5% up to 90–95%, and the water sorption was monitored. For desorption, the RH was decreased in a similar manner to accomplish a sorption/desorption cycle. The equilibrium condition was set to the total mass change less than 0.015% within 20 min and with a maximum dwell time of 120 min.

Thermal Analysis. TGA was performed on a TA Instruments Q50 system (New Castle, DE, USA) in open alumina pans. Sample sizes ranged from 4 to 6 mg and the heating rate was 5 °C/min over the temperature range of 30-250 °C, under a nitrogen purge of 50 mL/min. DSC experiments were carried out using a TA Instruments Q20 system in crimped alumina pans with a pinhole under a nitrogen purge of 50 mL/min. The sample size was in the range of 3-5 mg and the heating rate was 5 °C/min over the temperature range of 25-250 °C.

Preparation of LB30870 Single Crystals. Two representative crystallization conditions which yielded good single crystals are as follows: First, LB30870 has dissolved in 1:3 methanol/water solution at 1 mg/mL by heating at 70-80 °C. After the solution cooled down to ambient temperature with the cap closed, the vial was left open for 3 d allowing the solvent to evaporate slowly. Single crystals obtained from this condition were designated as Form Ia. Air-dried single crystals of Form Ia were obtained by exposing the single crystals of Form Ia at ambient condition for 1 d and designated as Form Ia-airdried. In the second condition, LB30870 was dissolved in 3:1 methanol/water solution at 0.2 mg/mL at ambient temperature. The vial was left open at room temperature for 3 d allowing the solvent to evaporate slowly. Single crystals obtained from this condition were designated as Form Ib. Air-dried single crystals of Form Ib were obtained by exposing the single crystals of Form Ib at ambient condition for 12 h and designated as Form Ib-air-dried.

Single Crystal X-ray Structural Analysis. Single crystals of Form Ia and Form Ia-air-dried were coated with paratone-N oil, and the diffraction data were measured at 100 and 298 K, respectively, with synchrotron radiation ( $\lambda$  = 0.650 and 0.660 Å) on an ADSC Quantum-210 detector at 2D SMC with a silicon(111) double crystal monochromator at the Pohang Accelerator Laboratory, Korea. The PAL BL2D-SMDC Program<sup>30</sup> was used for data collection (detector distance is 63 mm, omega scan;  $\Delta \omega = 2^{\circ}$ , an exposure time is 0.5 s per frame), and HKL3000sm (Ver. 703r)<sup>31</sup> was used for cell refinement, reduction and absorption corrections. Single crystals of Form Ib and Form Ib-air-dried were coated with paratone-N oil, and the diffraction data were measured at 173 K with Mo K $\alpha$  radiation on an X-ray diffraction camera system using an imaging plate equipped with a graphite crystal incident beam monochromator. The RapidAuto software<sup>32</sup> was used for data collection and data processing. All the structures were solved by the direct method and refined by full-matrix least-squares calculation with the SHELX software package.<sup>33</sup> Details

of data collection, structure determination, and structure analysis are available in Supporting Information.

#### RESULTS AND DISCUSSION

Conversion among Various Hydrates by Moisture Sorption and Desorption. Form I, a hexahydrate, was used as synthesized.<sup>1,2</sup> Form II was obtained by vacuum drying Form I in a desiccator with  $P_2O_5$  and corresponds to a dihydrate. Form III, a tetrahydrate, was obtained while constructing the sorption curve of Form II at RH between 53 and 75%. Each solid form was assigned its name based on the differences in the PXRD patterns (Figure 3).

The water content of Form I by Karl Fischer titration was 17.1%, corresponding to a hexahydrate. Form I did not show any change in appearance even at 93% RH, but its solid form conversion during desorption and subsequent sorption was confirmed by PXRD pattern changes. It lost about 12% of its original weight after drying with a vacuum over  $P_2O_5$  (Table 1).

Table 1. Hydrate Conversion of Form I/II/III by Sorption/ Desorption

relative humidity	% <sup>a</sup>	Form <sup>b</sup>
	111.9 <sup>c</sup>	Ι
0	$100^d$	II
33	104.5	II + N.W. <sup><math>e</math></sup>
53	105.9	III
64	106	III
75	106.2	III
93	117.3	$I + N.W.^{e}$
75	113.8	Ι
64	112.7	Ι
53	112.3	Ι
33	105.2	III
0	93.6	IIa + $A^f$

"Sample weight after equilibrium for 1 day at each RH relative to the weight after vacuum-drying over  $P_2O_5$ ." Hydrate form assigned based on the water content by weight change and PXRD patterns (Figure 3). "Initial sample weight percent relative to the weight after vacuum-drying over  $P_2O_5$ ." Sample weight normalized to 100% after vacuum-drying over  $P_2O_5$ ." N.W.: nonstoichiometric water." II a + A: another crystal + amorphous form.

The resulting solid form with the water content of 6% by Karl Fischer titration was assigned as a dihydrate and named Form II. During the sorption process, the PXRD pattern of Form II did not change up to 33% RH despite its weight increase. The result implies that the weight increase was due to nonstoichiometric water sorption without solid form change. The weight increase at RH between 53% and 75% was about 6% without significant fluctuation (Table 1). The PXRD patterns at this RH range were different from those of Form I or Form II, and therefore this new solid form was designated as Form III, a tetrahydrate. It returned to Form I at 93% RH with a weight increase of 17.3% of dried weight. It should be noted that the exact number of water molecules of Form I based solely on the weight change during sorption/desorption is ambiguous: The weight increase at 93% RH may include not only the hydration water molecules but also the nonstoichiometric water molecules in the one-dimensional porous channel described below in the single crystal X-ray structures. During desorption between 75% and 53% RH, there was about 5% decrease in weight without a change in the PXRD pattern. These weight

changes during sorption/desorption are illustrated in Figure 2. Relatively little weight change over 53-75% RH during



Figure 2. Sorption and desorption profiles depending on solid forms of LB30870 (Form I).

sorption and desorption implies that Form III is a stoichiometric hydrate in this RH range. The solid form changes during desorption and sorption were further confirmed by additional PXRD measurements as shown in Figure 4. When



Figure 3. PXRD patterns of various solid forms of LB30870.



Figure 4. Moisture-dependent PXRD measurements of LB30870.

Form I was vacuum-dried at 40  $^{\circ}$ C, it was transformed to Form II after 2 h. However, the PXRD changed with some amorphous character after continuing vacuum-drying overnight at 40  $^{\circ}$ C, indicating that Form II is not a stable form. After resorption of this sample at 93% RH for 12 h, the PXRD pattern returned to that of Form I.

**Conversion in Aqueous Slurry.** When suspended as an aqueous slurry, all solid forms turn into Form I after 5 min based on their PXRD taken while they were still wet after air-

drying. After the same samples were dried under ambient conditions for 1 day, the PXRD pattern of Form I changed to that of Form III. And then after vacuum drying for 3 days under ambient temperature, it transformed to Form II.

**Dynamic Vapor Sorption (DVS).** DVS of LB30870 was also investigated to further understand its hydration/ dehydration and identify a stable hydrate. Figure 5 displays



Figure 5. Dynamic vapor sorption data for LB30870 Form II.

the DVS data collected with Form II as the initial solid form. The overall DVS profile is similar to the sorption/desorption profile obtained by the desiccator method shown in Figure 2. The adsorption up to 40% RH with about 6% weight increase indicates the appearance of a tetrahydrate which shows less than 1% weight change up to 70% RH. A hexahydrate (Form I) with two additional water molecules appeared after further adsorption at 90% RH. The desorption profile of DVS showed a weight loss of 6% at 30% RH after a gradual weight decrease from 90% RH, suggesting the solid form change of Form I to Form III at 30% RH. DVS provided additional information on the existence of Form III between 30 and 5% RH during desorption, which was not available from the desiccator method. The subsequent loss of mass below 5% RH indicates transformation to Form II.

Thermal Analysis. TGA measurements provide information about the thermal stability of hydrate forms because the onset temperature of the water loss reflects the hydration forces. In general, less stable forms show dehydration starting at a low and broad temperature range. More stable hydrate forms, as in the case of hydrates involving crystal lattice water, show a water loss signal with a discontinuity at well-defined temperatures.<sup>34</sup> TGA profiles of three hydrate forms of LB30870 differ significantly: Three steps in weight decrease were observed for Form I, two steps for Form III, and one step but with a slight inflection in the middle for Form II (Figure 6a). The first derivative curves shown in broken lines in Figure 6a clearly show different steps of dehydration for these hydrates. The early dehydration steps would be due to evolving water molecules in the solvent channel described below in the single crystal structures, while the later dehydration step would be from the lattice water with stronger binding. The weight decreases up to ~110 °C during the TGA correspond to the total number of water molecules of these hydrates determined by KF water titration. The initial rapid decrease of weight observed in Form I must be due to two additional channel water molecules which are not present in Form III. The last



**Figure 6.** (a) TGA curves (solid lines) and their corresponding first derivatives (broken lines) (b) DSC traces for LB30870 Form I, II, and III.

steps for Form I and Form III are associated with a 2.8 and 3.2% weight decrease, corresponding to approximately one water molecule, which suggests that there is one particular water molecule with strong binding energy in the crystal lattice of Form I or Form III. The DSC profiles of these hydrates show the same melting peaks with the onset at  $\sim$ 190 °C after various dehydration endothermal peaks (Figure 6b). Three distinct endotherms in Form I represent dehydration peaks of water molecules with different binding energies. Form III also showed two dehvdration endotherms, but it is not as distinguishable as that of Form I. The first and second endotherms at low temperature are from loosely bound water molecules with different binding energies. The third endotherms above 100 °C are from more tightly bound water molecules which can be associated with the dehydration of one water molecule observed as the third or second weight decrease step in TGA profiles of Form I or Form III. TGA of Form II shows dehydration at a lower temperature than those of Form I or Form III, while the endotherm of Form II in DSC was similar to the second endotherm of Form III. It could be that the water molecules in Form II are sensitive to the condition of thermal analysis compared to the hydration water molecules in the crystal lattice of Form I or Form III. Thermal analysis data are elaborated further along with their single crystal structures below in the section Relative Stability of LB30870 Solid Forms.

**Descriptions of LB30870 Crystal Structures.** Form Ia and Ib, and their air-dried crystals all belong to the same space group,  $P2_12_12_1$ . Crystal data and structure refinement are summarized in Table 2, and details on the single crystal diffraction analyses and structure descriptions are provided in

# Table 2. Crystal Data and Structure Refinement of LB30870 Solid Forms

compound	Form Ia	Form Ia-air-dried (Form I)	Form Ib	Form Ib-air-dried (Form III)
empirical formula	$C_{29}H_{45.20}N_5O_{10.10}S$	$C_{28}H_{42.62}N_5O_{9.81}S$	$C_{28}H_{45}N_5O_{11}S$	C <sub>28</sub> H <sub>39</sub> N <sub>5</sub> O <sub>8</sub> S
formula weight	657.56	638.27	659.75	605.70
temperature (K)	100(2)	298(2)	173(2)	173(2)
wavelength (Å)	0.650	0.660	0.71073	0.71073
crystal system	orthorhombic	orthorhombic	orthorhombic	orthorhombic
space group	$P2_{1}2_{1}2_{1}$	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	$P2_{1}2_{1}2_{1}$	$P2_{1}2_{1}2_{1}$
unit cell dimensions	a = 10.845(2) Å	a = 10.683(2) Å	a = 10.989(2) Å	a = 9.6077(19) Å
	b = 12.767(3) Å	b = 12.969(3) Å	b = 12.300(3) Å	b = 13.438(3) Å
	c = 23.970(5) Å	c = 24.365(5) Å	c = 24.858(5) Å	c = 23.935(5) Å
	$\alpha=\beta=\gamma=90^\circ$	$\alpha = \beta = \gamma = 90^{\circ}$	$\alpha=\beta=\gamma=90^\circ$	$\alpha = \beta = \gamma = 90^{\circ}$
volume (Å <sup>3</sup> )	3318.8(12)	3375.7(12)	3360.0(12)	3090.3(11)
Ζ	4	4	4	4
density (calculated) (Mg/m <sup>3</sup> )	1.316	1.256	1.304	1.302
absorption coefficient (mm <sup>-1</sup> )	0.126	0.126	0.159	0.160
F(000)	1404	1360	1408	1288
crystal size/mm <sup>3</sup>	$0.093 \times 0.073 \times 0.067$	$0.054 \times 0.052 \times 0.035$	$0.080 \times 0.080 \times 0.050$	$0.180 \times 0.140 \times 0.090$
theta range for data collection (°)	1.653-33.369	1.552-32.311	3.079-27.480	3.032-27.428
index ranges	$-18 \le h \le 18$	$-15 \le h \le 15$	$-14 \le h \le 14$	$-11 \le h \le 12$
	$-20 \le k \le 20$	$-20 \le k \le 19$	$-15 \le k \le 15$	$-17 \le k \le 17$
	$-36 \le l \le 36$	$-36 \le l \le 36$	$-32 \le l \le 32$	$-31 \le l \le 31$
reflections collected	45000	40333	32514	28624
independent reflections	14236	11961	7675	7051
	[R(int) = 0.0674]	[R(int) = 0.0739]	[R(int) = 0.2188]	[R(int) = 0.1928]
completeness to theta/ $^{\circ}$	99.90%	100.00%	99.70%	99.70%
refinement method	full-matrix least-squares on	$F^2$		
	full-matrix least-squares on	$F^2$		
data/restraints/parameters	14236/9/464	11961/34/445	7675/8/478	7051/8/421
goodness-of-fit on $F^2$	1.047	0.904	0.965	1.005
final R indices $[I > 2\sigma(I)]$	$R_1 = 0.0482$	$R_1 = 0.0566$	$R_1 = 0.0751$	$R_1 = 0.0844$
	$wR_2 = 0.1155$	$wR_2 = 0.1491$	$wR_2 = 0.0873$	$wR_2 = 0.1119$
R indices (all data)	$R_1 = 0.0773$	$R_1 = 0.0999$	$R_1 = 0.1769$	$R_1 = 0.1768$
	$wR_2 = 0.1304$	$wR_2 = 0.1671$	$wR_2 = 0.1089$	$wR_2 = 0.1342$
absolute structure parameter	-0.04(3)	-0.02(3)	0.5	0.13(9)
extinction coefficient	0.031(2)		0.0035(6)	
largest diff. peak and hole/e·Å $^{\!\!-3}$	0.485 and -0.703	0.364 and -0.286	0.258 and -0.274	0.260 and -0.228

the Supporting Information. The structure analysis reveals that the LB30870 molecule is in its zwitterionic form in all four crystal structures with a deprotonated carboxylate and a protonated amino(imino)methyl residues (Figure 1b). The LB30870 molecule in all the crystal structures is in the same folded conformation forced by the presence of strong hydrogen bonds with two structural water molecules (Figures 7 and S1– S3, and Table 3). However, the total solvent contents as well as the subsequent hydrogen bonding network are slightly different from one another in the four crystal forms.

In Form Ia, intermolecular hydrogen bonds between the LB30870 molecules (Figure 8a and Table 3) lead to the formation of one-dimensional zigzag chains (Figure 8b,c). The one-dimensional chains are further interconnected via other hydrogen bonds to form a three-dimensional hydrogen bonding network structure with one-dimensional solvent channels (Figure 9a and Table 3), where the hydrogen bonding interactions are mediated by one of the water molecules involved in the folded conformation of LB30870 molecule (O2W) (pink dashed lines in Figure 9a). The solvent channels are filled with one methanol molecule and ~3.1 additional water molecules at four different sites per LB30870 molecule in addition to the two water molecules involved in the folded conformation of the folded conformation of the folded conformation of the channels are filled with one methanol molecule and ~3.1 additional water molecules at four different sites per LB30870 molecule in addition to the two water molecules involved in the folded conformation of the LB30870 molecule (Figure 9b,c).



**Figure 7.** A ball-and-stick diagram of the LB30870 with two structural water molecules in hydrogen bonding interactions in Form Ia. The hydrogen bonding interactions are represented as the cyan dashed line.

Since Form Ia contains a methanol molecule in the solvent channel and is not stable at ambient condition, Form Ia-airdried was prepared by exposing Form Ia at ambient condition for 1 day. The crystal packing structure of Form Ia-air-dried was

# Table 3. Hydrogen Bonds in the Crystal Structures

Form	Form Ia			Form Ia-air-dried (Form I)			Form Ib		Form Ib-air-dried (Form III)			
D–H···A	$d(H \cdots A)$	$d(D \cdots A)$	∠(DHA)	d(H···A)	$d(D \cdots A)$	∠(DHA)	$d(H \cdots A)$	$d(D \cdots A)$	∠(DHA)	$d(H \cdots A)$	$d(D \cdots A)$	∠(DHA)
Hydrogen Bondings for Folded Conformation												
N(1)-H(1B)… O(1W)	2.00(1)	2.832(3)	177(4)	2.13	2.952(5)	159	1.93(6)	2.924(7)	165(4)	2.10(2)	2.945(8)	168(6)
O(1W)-H(2W)… O(2)	1.97(2)	2.857(2)	177(4)	1.97(4)	2.828(3)	156(6)	1.77(6)	2.755(5)	175(6)	1.94(3)	2.787(6)	175(8)
N(3)-H(3N)… O(2W)	2.14(3)	2.924(3)	153(3)	2.13	2.932(4)	155	2.09(5)	2.869(7)	158(6)	2.16(6)	2.950(7)	143(5)
O(2W)-H(4W)… O(3)	2.01(2)	2.842(3)	159(3)	1.94(3)	2.821(4)	175(6)	2.11(6)	2.850(6)	173(8)	1.96(3)	2.793(6)	165(7)
Intermolecular Hydroge	n Bondings	for One-Dim	ensional Zig	zag Chain								
N(1)-H(1A)-O(4)	2.09(2)	2.929(2)	162(3)	2.1	2.896(4)	152.9	2.17(5)	2.959(6)	153(5)	1.95(6)	2.876(7)	172(5)
$N(2)-H(2A)\cdots N(5)$	2.28(3)	2.899(2)	141(3)	2.17	2.904(3)	143.4	2.27(5)	2.909(7)	135(5)	2.10(6)	2.864(8)	151(6)
N(2)-H(2A)-O(4)	2.53(3)	3.152(3)	141(3)	2.38	3.101(4)	141.4	2.30(5)	3.024(7)	147(5)			
Interchain Hydrogen Bo	ondings for '	Three-Dimen	sional Netwo	ork								
O(1W)-H(1W)… O(1)				2.36(5)	3.049(4)	135(5)	2.13(6)	2.867(5)	166(7)	2.01(4)	2.811(7)	157(8)
O(2W)-H(3W)… O(1)	1.96(2)	2.774(2)	176(4)	2.02(3)	2.833(4)	168(6)	1.90(7)	2.826(6)	170(6)	2.02(3)	2.879(8)	166(6)
Other Hydrogen Bondi	ngs via an A	dditional Co	nserved Wate	er Molecule	at One-Dime	nsional Solve	nt Channel					
N(2)-H(2B)… O(3W)	2.19(3)	2.834(3)	150(3)	1.97	2.795(6)	159.3	1.92(6)	2.876(7)	169(5)	2.01(6)	2.878(8)	165(6)
O(1W)-H(1W)… O(3W)	2.08(3)	2.863(3)	152(4)									
O(3W)-H(5W)… O(1W)				2.28(6)	3.001(6)	139(8)	2.01(6)	2.866(6)	163(6)	2.01(4)	2.847(7)	161(7)
O(3W)-H(6W)… O(3)										1.97(4)	2.782(7)	157(7)



Figure 8. LB30870 molecules in Form Ia interconnected via (a) two different types of intermolecular hydrogen bonding interactions. The onedimensional zigzag chain viewed: (b) *a*-axis and (c) *b*-axis. The hydrogen bonding interactions involved in the folded conformation of LB30870 molecule are represented in a cyan dashed line, and the intermolecular hydrogen bonding interactions are represented in a green dashed line.

similar to that of Form Ia (Figure S1). The only difference was the solvent molecules in the one-dimensional solvent channels (Figures 9b,c and S1b,c). Water molecules appeared to replace the methanol molecule in the solvent channel of Form Ia during drying. One can speculate that metastable Form Ia, after desolvation in air, takes up water molecules in air to minimize free volume in the crystal.<sup>14–17,34–36</sup> Form Ia-air-dried contains ~4 additional water molecules in the solvent channel at six different sites in addition to the two conserved structural water molecules involved in the folded conformation of the LB30870 molecule. The altered solvent environment in the solvent channel led to slightly different hydrogen bonding interactions. Both structural water molecules (O1W and O2W) participated in interchain hydrogen bonding interactions to form a threedimensional hydrogen bonding network structure (Table 3).

Form Ib was also similar to Form Ia (Figure S2). Again, the difference was the solvent contents in the one-dimensional solvent channels. Despite the fact that they were obtained from the solution with higher methanol content, no methanol was observed in the solvent channel, but additional  $\sim$ 5 water molecules in six different sites could be placed in the solvent channels in addition to the two conserved structural water



**Figure 9.** Packing profiles of Form Ia: (a) One-dimensional zigzag chains with the intermolecular hydrogen bonding interactions represented in a green dashed line and the interchain hydrogen bonding interactions in a pink dashed line. (b, c) in a ball-and-stick model, LB30870 molecules in green and the additional solvent molecules in red-and-gray balls and brown sticks.

molecules involved in the folded conformation of the LB30870 molecule.

Since Form Ib is not stable at ambient condition, Form Ibair-dried was similarly prepared by exposing Form Ib at ambient conditions for 12 h. Form Ib-air-dried was still isostructural to Form Ib (Figures 5–7 and S3–S4). The additional water molecules in the solvent channel in Form Ib-air-dried were reduced to ~2 water molecules, which led to a slight contraction of the solvent channel dimension (Figure 10) and to the eventual reduction of the unit cell volume (Table 2). Figure 11 shows the water molecules aligned along the onedimensional solvent channels of Form Ia-air-dried and Form Ibair-dried, respectively.

Simulated and Experimental PXRD Patterns of LB30870 Crystals. The simulated PXRD patterns of Form Ia, Form Ia-air-dried, and Form Ib are very similar to each other (Figure 12). On the other hand, the simulated PXRD pattern of Form Ib-air-dried is significantly different from the others. Such



Figure 10. Overlay of the two solvent channels of Form Ia-air-dried in red sticks and Form Ib-air-dried in blue sticks.



Figure 11. Water molecules in the solvent channels of (a) Form Ia-air-dried and (b) Form Ib-air-dried.



Figure 12. Simulated PXRD patterns derived from single crystal structures of Form Ia, Form Ib, Form Ia-air-dried, and Form Ib-air-dried.

a difference in the simulated PXRD patterns is due to slight contraction of the solvent channel in Form Ib-air-dried crystal compared to those of the other crystals.

In order to demonstrate the effect of dehydration on the crystal structures, experimental PXRD of single crystal samples were obtained after grinding in crystallization solution (wetground) or grinding in the dry state (dried-ground), and they were compared with simulated PXRD. The PXRD pattern of the wet-ground Form Ia is the same as the simulated PXRD pattern of Form Ia and is also very similar to but not the same as that of Form I (Figure 13a). On the other hand, while the



**Figure 13.** Comparison of simulated PXRD patterns derived from single crystal structures and experimental PXRD patterns of various samples. (a) From the top, simulated PXRD pattern of Form Ib-air-dried, experimental PXRD patterns of Form III and Form Ia-air-dried ground in dry state, simulated PXRD of Form Ia-air-dried, experimental PXRD patterns of Form I, and Form Ia-air-dried ground in the wet state, and simulated PXRD pattern of Form Ia. (b) From the top, simulated PXRD pattern of Form II. (b) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) From the top, simulated PXRD pattern of Form II. (c) Form I

simulated PXRD pattern of Form Ia-air-dried is the same as the PXRD pattern of Form I, the PXRD pattern of Form Ia-airdried ground at the dry state is the same as that of Form III. Form Ia-air-dried is, thus, assigned as Form I containing a nonstoichiometric amount of water molecules in the solvent channel, and it shows conversion to Form III when the sample is ground at dry state. Accelerated desolvation from its onedimensional solvent channel by dry-grinding Form Ia-air-dried would drive the conversion of Form I to Form III, which is consistent with the experimental results of hydrate conversion. Those characteristics of the PXRD patterns are probably due to variations of the solvent contents in the one-dimensional solvent channels in the samples for the PXRD measurements depending on the sample grinding condition, wet or dry. When the single Form Ib was exposed at ambient conditions for 12 h, only two water molecules remained at the solvent channel. Even when ground in the presence of the crystallization solution, it turned to Form III as shown in their PXRD pattern (Figure 13b). Grinding at dried state does not affect its PXRD pattern, which is still a pattern of Form III. These results support the assignment of Form Ia-air-dried and Form Ib-air-dried to hydrates Form I and III, respectively. Taken together, the conformation of LB30870 and the crystal packings are similar among Form Ia and Form Ib, wet or dried, and hydrates Form I and III with the only difference in the solvent channel structures which are responsible for interconversion among the hydrate forms without any structural disruption by hydration and dehydration.<sup>34</sup>

Relative Stability of LB30870 Solid Forms. The single crystal X-ray structures of Form I, Form III, and their corresponding wet crystals, Form Ia and Form Ib, elucidated the one-dimensional solvent channel structure responsible for conversion between these solid forms. Such conversion observed with single crystals or their ground samples are consistent with the results from DVS or hydration/dehydration by the desiccator method. Notably, the weight decreases in the third step in TGA of Form I and the second one of Form III, correspond to the loss of the last structural water molecule (Figure 6a) with stronger binding energy than other water molecules in the solvent channel or in the crystal lattice. The two structural water molecules involved in the folded conformation of LB30870 are in slightly different environments in the crystal structures. While one structural water molecule (O1W) is exposed to the solvent channel, the other structural water molecule (O2W) is buried in a hydrophobic pocket (Figure 14). Since O1W, which is exposed to the solvent channel, is more susceptible to dehydration, O2W is likely to be responsible for the last dehydration steps observed during TGA or DSC of Form I or Form III. Form II is not stable solid form as it only exists at RH less than 5% and extensive vacuumdrying of Form II at 40 °C resulted in amorphous character as shown in Figure 4. Although a single crystal of Form II could



**Figure 14.** Space-filling model of the two structural water molecules involved in the folded conformation of LB30870 molecule around the solvent channel of Form III crystal. The oxygen atoms of O1W and O2W are represented in cyan and green spheres, respectively.

not be obtained, its TGA profile implies the presence of two structural water molecules with a slightly different environment (Figure 6a). The weight decrease of about 6% occurred with a slight inflection in the middle, its first derivative better representing such inflection. That is, dehydration of one water molecule buried in the hydrophobic pocket would start at a higher temperature than the other. Form III was stable down to 10% RH during desorption, which implies that the hydrogen bonding networks including the solvent channel structure are important in stabilizing the overall crystal structure of Form III.

All three hydrate forms undergo rapid conversion to Form I in the aqueous slurry and have a similar solubility of more than 20 mg/mL in the fasted state simulating gastric fluid (FaSSGF).<sup>37,38</sup> The human gastric transit time of typical solid dosage forms are known to range from 0.25 to 3 h.<sup>39</sup> Therefore, the different hydrate forms of LB30870 are unlikely to present any meaningful differences in terms of in vivo dissolution rate or oral bioavailability. However, it would be critical to identify a stable form of the hydrate and control the manufacturing process to ensure consistent production of the desired product quality because of the molecular weight differences among the hydrates. If the potential change in the hydrate form during manufacturing is not recognized early, one might assume the same molecular weight even after the hydrate form changes, which would then result in products out of specification. Selecting a stable hydrate and controlling the drug substance manufacturing for consistent hydrate formation would circumvent such deviation during further development.

#### CONCLUSIONS

The relationship of three different hydrates of LB30870 is summarized in Figure 15. Form I, Form II, and Form III were



Figure 15. Conversion diagram of LB30870 pseudopolymorphs.

identified as hexa-, di-, and tetrahydrates, respectively. Three hydrates undergo interconversion by water sorption/desorption or in the aqueous slurry. Four single crystal X-ray structures show a clear view of the solvent channel structures which are sensitive to water sorption/desorption, while the conformation of LB30870 and the crystal packings are similar. The rapid conversion of other forms to Form I in aqueous phase suggests that Form I is thermodynamically more stable in water. Nonetheless, Form III is recommended for further development considering its stable water content at a wide range of RH, and the stable hydration water molecules observed in the single structure.

# ASSOCIATED CONTENT

#### **Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.cgd.7b00927.

Details of single crystal analysis and data (PDF)

#### **Accession Codes**

CCDC 1543302–1543305 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data\_request/cif, or by emailing data\_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

# AUTHOR INFORMATION

#### **Corresponding Authors**

\*(M.S.L.) E-mail: mslah@unist.ac.kr.

\*(A.K.) Tel: +82 31 881 7166. Fax: +82 31 881 7219. E-mail: arkim@cha.ac.kr.

# ORCID <sup>0</sup>

Myoung Soo Lah: 0000-0001-9517-7519 Aeri Kim: 0000-0003-3597-2363

#### **Author Contributions**

<sup>#</sup>J.L. and D.K. are co-first authors that equally contributed to this work.

#### Notes

The authors declare no competing financial interest.

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