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Article

Practical Synthesis of (+)-Biotin Key Intermediate by Calcium Borohydride Reduction and Temperature-Dependent Purity Upgrade during Crystallization

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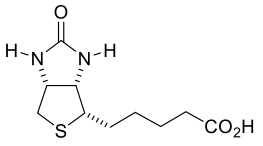
ABSTRACT: An expedient synthesis of a key intermediate for (+)-biotin has been accomplished through high-yielding reduction of chiral imide with calcium borohydride and efficient isolation of the desired isomer by crystallization at a specific temperature where only undesired isomer was converted to soluble anhydrate while the desired isomer kept unchanged as a less soluble monohydrate.

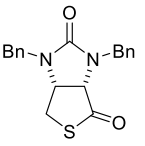
KEYWORDS: *(+)-biotin, calcium borohydride, preferential solvation, crystallization, solubility, van't Hoff equation*

INTRODUCTION

Over the past few decades, (+)-biotin (**1**) has received increasing interest due to the significance in human nutrition and animal health.[1] It has a highly functionalized heterobicyclic scaffold with properly oriented nitrogen, oxygen, and sulfur atoms in the molecule. It carries a 4-carboxybutyl side chain at 4 position along with three contiguous asymmetric centers, all being set up as *cis*-configuration. Unlike other vitamins, as much as 200 MTN of **1** is currently being produced using the total synthetic method due to lack of an efficient fermentation technology.[2] Among a number of synthetic approaches hitherto developed for **1**, synthesis using thiolactone (**2**) as a key intermediate has been considered the most efficient and viable method for large scale production of **1**.[3]

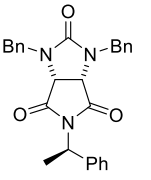


(+)-Biotin (**1**)



2

For the synthesis of **2**, considerable efforts have long been made to meet the ever growing demand of **1**.[3] Among them, a method based on optical resolution employing cinchonidine as the resolving agent has been considered one of the most practical approaches to obtain **2**.[4] However, lack of alkaloids from natural resources has inevitably forced the supplier to change the route of synthesis. In the meantime, as an alternative method, synthesis based on desymmetrization of *meso*-cyclic acid anhydride by (*S*)-1,1-diphenyl-1,2-propanediol has been reported.[5] It can produce required chiral centers with excellent selectivity and yield and has been considered the most reliable approach to obtain **2**.[6] Nonetheless, considering the price of the chiral alcohol, we thought that the method was not cheap enough and a more economical synthetic method that can cut manufacturing cost and reduce waste had to be explored. On the other hand, in 1975, synthesis of **2** based on sodium borohydride reduction of chiral imide **3** to amide alcohol **4a** was reported.[7] In sharp contrast to the above method, it employed very cheap (*R*)-1-methylbenzylamine (R-PEA, ca. $10/kg) as a chiral source to introduce chirality. However, selectivity and yield of the reduction of **3** to **4a** were not good enough to be applied for commercial production of **2**. Nonetheless, fascinated by the exceptionally low price and ready availability of R-PEA used as a chiral source, we decided to reconsider the possibility of the synthetic method as a practical approach to **2**. Herein, we report a much improved synthesis of **2** based on calcium borohydride reduction of chiral imide **3** and subsequent isolation of the desired isomer **4a** by controlled crystallization.



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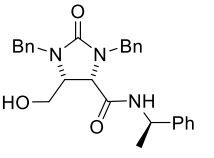

4a

RESULTS AND DISCUSSION

To begin with, the synthesis of chiral imide **3**,[7] a substrate for reduction, was investigated. The starting material cyclic carboxylic acid **5** was produced commercially from fumaric acid via dibromination, amination with benzylamine, and subsequent ureido formation.[3,14] For preparation of **3** from **5**, a sequential procedure was first tested that involved anhydride

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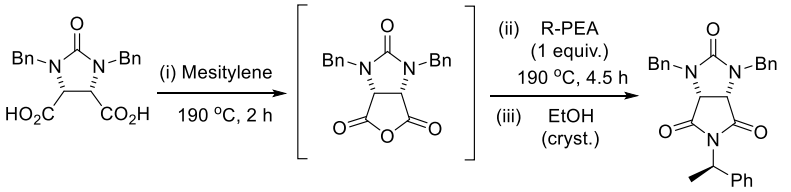
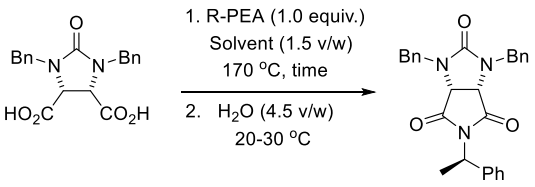
Scheme 1. Synthesis of Chiral Imide 3 Using a Sequential Procedure



formation, amidation with R-PEA, and final dehydration to **3** (Scheme 1). Owing to the poor solubility of the anhydride **6**, the reaction was required to be carried out at a high temperature (>190 °C) in mesitylene to avoid any crystallization during the reaction. Although the procedure provided **3** in 92.0% yield and 98.2% purity, it was difficult to scale up because very careful operation of the reaction was required. To overcome the drawback, the reactions in which **5** and R-PEA were blended in the beginning and heated in polar solvents were examined (Table 1).

Table 1. Screening of the Solvent for Imidation of 5 to 3[a]



entry	solvent	time (h)	yield[b] (%)	purity[c] (%)
1	NMP	2.5	92.4	98.85
2	DMSO	3.5	86.0	97.30
3	DMA	2	93.9	99.23

[a]The reaction was conducted using **5** (50 g, 0.141 mol) and R-PEA (17.1 g, 0.141 mol). [b]Isolated yield. [c]Area (%) of **3** in high-performance liquid chromatography (HPLC).

Use of 1-methyl-2-pyrrolidone (NMP, 1.5 v/w) as a solvent and heating at 170 °C for 2.5 h produced **3** in 92.4% yield and 98.85% purity (Table 1, entry 1). However, NMP is a potential hazard and needs to be avoided for scale up.[8] The use of DMSO (1.5 v/w) finished the reaction in 3.5 h, and it was accompanied by considerable sulfur odor and the yield was diminished (86.0%, Table 1, entry 2). Finally, to our delight, the reaction was found to go well using cheap *N*, *N*-dimethylacetamide (DMA, 1.5 v/w) to provide **3** in 93.9% yield and 99.23% purity. As expected, in all reactions employing polar solvents, heterogeneous nucleation was not observed during the reaction, which made the operation quite easy.

Then, we moved to the synthesis of amide alcohol **4a** by reduction of chiral imide **3**. In our initial study, reduction of **3** was tested using a previously reported procedure[7] employing $NaBH_4$ as a reductant (Scheme 2). When $NaBH_4$ reduction of **3** was run at 40 °C for 10 h, the reaction was completed, and upon simple addition of water, the desired isomer **4a** (monohydrate, vide infra, **4a**/**4b** = >99:1 after crystallization) was isolated as a white crystal. However, the yield (40.8%) was not high enough to be applied for commercial production.

As a solution to resolve the problem, we came up with an idea to employ $Ca(BH_4)_2$[10] as a reductant because Ca^{2+} cations are much more Lewis acidic and sterically demanding than Na^+.[9] Considering these favorable features of Ca^{2+} cations, use of $Ca(BH_4)_2$ might induce stronger and more selective coordination to one of the diastereomeric imide carbonyl groups of **3** to enhance selectivity of the reaction. To our delight, use of $Ca(BH_4)_2$ gave a higher selectivity (**4a**/**4b** = 70:30) than with $NaBH_4$ (**4a**/**4b** = 61:39, Table 2, entry 1 vs entry 2). Other borohydrides carrying different metal cations (K, Mg, and Al) were tested (Table 2, entries 3−5). However, they gave much lower selectivities (**4a**/**4b** = 59:41 to 66:34) and yields (0−20%). Screening of the solvent was then conducted (Table 2, entries 6−11). Although use of 1-PrOH gave a similar result (92% conversion, **4a**/**4b** = 71:29, Table 2, entry 6), considerably decreased conversions were observed for other alcoholic solvents (Table 2, entries 7−10). Furthermore, use of a well-employed ether-type solvent (diglyme) was found to be much less effective than EtOH (31% conversion, **4a**/**4b** = 61:39, Table 2, entry 11). Consequently, the reduction of **3** was found to proceed well with $Ca(BH_4)_2$, especially in EtOH.

A possible mechanism of the reduction of **3** by $Ca(BH_4)_2$ is shown in Figure 1. A phenyl group of **3** attached to the chiral

Scheme 2. Reduction of 3 with $NaBH_4$

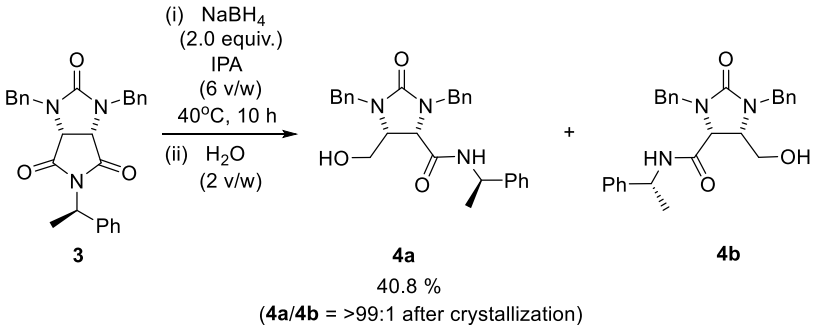


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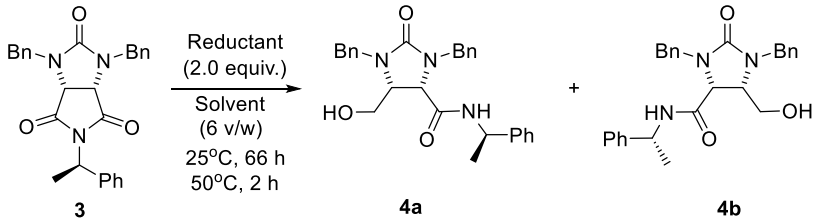
Table 2. Screening of the Reductant and Solvent for Reduction of 3 to 4a/4b[a]



entry	reductant	solvent	conversion[b] (%)	4a/4b[c] (%)
1	$Ca(BH_4)_2$	EtOH	100	70:30
2	$NaBH_4$	EtOH	100	61:39
3	KBH_4	EtOH	20	59:41
4	$Mg(BH_4)_2$	EtOH	6	66:34
5	$Al(BH_4)_3$	EtOH	0	65:35
6	$Ca(BH_4)_2$	1-PrOH	92	71:29
7	$Ca(BH_4)_2$	IPA	2	68:32
8	$Ca(BH_4)_2$	1-BuOH	8	69:31
9	$Ca(BH_4)_2$	2-BuOH	8	65:35
10	$Ca(BH_4)_2$	*t*-BuOH	0	-
11	$Ca(BH_4)_2$	diglyme	31	61:39

[a]The reaction was conducted using **3** (2.0 g, 4.6 mmol) and reductant (4.6 × 2 mmol based on $[BH_4]_n$ count). [b]Based on the ratio of area (%) of **3** consumed in HPLC. [c]Based on the ratio of area (%) of **4a** and **4b** in HPLC.

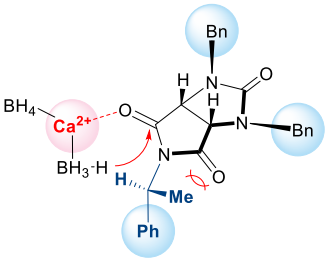


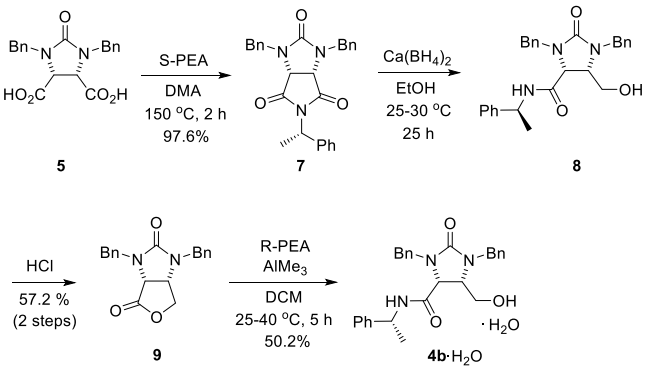
Figure 1. Possible mechanism of $Ca(BH_4)_2$ reduction of **3**.

center was assumed to orient to the concave side of the bicyclic ring due to a steric repulsion by two ureide benzyl groups. $Ca(BH_4)_2$ would preferentially attack to the left-hand side of the imide carbonyl group to avoid steric repulsion by the neighboring methyl group. Both higher coordination ability and steric bulkiness of Ca^{2+} are presumably the reasons for the better selectivity. It is worth noting that $Ca(BH_4)_2$ has never been used for reduction of imide to amide alcohol and represents the first example.

Although a better protocol for reduction of **3** was obtained using $Ca(BH_4)_2$, the regioselectivity was moderate (**4a**/**4b** = 70:30). To compensate this, we explored effective purification of **4a**. It was quite difficult to obtain enough amount of undesired isomer **4b** of high quality by means of recrystallization and/or chromatographic purification. Hence, an alternative synthesis of **4b** was devised (Scheme 3). Starting from cyclic carboxylic acid **5**, coupling with (*S*)-1-methylbenzylamine (S-PEA) gave chiral imide **7** with *S*-configuration. Then, **7** was subjected to $Ca(BH_4)_2$ reduction to provide amide alcohol **8**, which upon treatment with HCl gave lactone **9**. Finally, ring opening of **9** with R-PEA and Me_3Al followed by recrystallization from aqueous EtOH afforded desired **4b** as a monohydrate (vide infra).

Elemental analysis of **4a** and **4b** conducted under usual drying conditions (50 °C for 17 h in a tray dryer) revealed that both **4a** and **4b** existed as an monohydrate rather than as an anhydrate. This was further confirmed by Karl Fischer titration

Scheme 3. Preparation of Undesired Isomer 4b Monohydrate



of these compounds, which showed the water content of **4a** and **4b** to be 4.18 and 4.19%, respectively (calculated value for monohydrate: 3.90%). Monohydrates of **4a** and **4b** lost water on drying under a reduced pressure (1 mmHg) at 90 °C for 60 min and at 60 °C for 30 min, respectively, to produce corresponding anhydrates of **4a** and **4b**.[11] The X-ray diffraction data on hydrates and anhydrates of **4a** and **4b** showed different patterns, which substantiated that the water included was not adhering water but crystalline water (Figures 2 and 3).

Literature precedent showed that monohydrates are generally less soluble than anhydrates.[12] We have imagined, under certain conditions, that preferential crystallization of **4a** should be achieved if selective monohydration with **4a** rather than with **4b** is possible.

In the meantime, thermogravimetry−differential thermal analysis (TG−DTA) of monohydrates of **4a** and **4b** gave information on their structural changes by temperature. For **4a** monohydrate, 3.87% decrease of weight, close to the calculated value of one water loss (3.90%), was observed by heating at 72.1−85.0 °C (Figure 4), while a similar trend of weight loss (3.13%) was found for **4b** monohydrate, though at a much lower temperature (42.5−56.4 °C) (Figure 5). Consequently,

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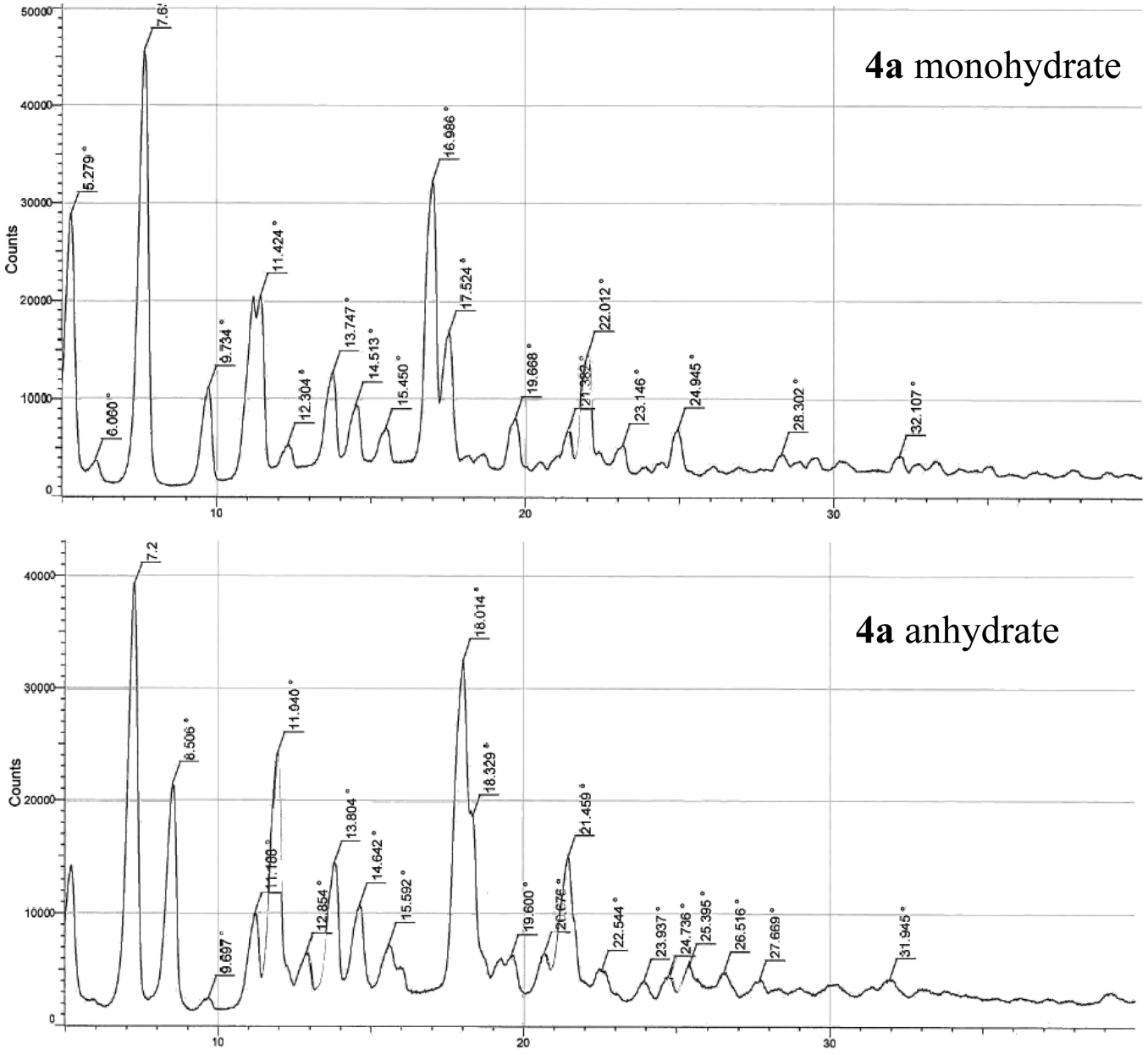

Figure 2. PXRD patterns of monohydrate and anhydrate of **4a**.

selective crystallization of **4a** over **4b** might be possible by crystallization within the temperature range of 42.5 and 72.1 °C.

To demonstrate the feasibility of our prediction, solubilities of monohydrates of **4a** and **4b** have been determined at different temperatures. As shown in Table 3 and Figure 6, solubility of **4b** monohydrate was found to increase dramatically at 303−313 K (30−40 °C), while that of **4a** monohydrate did not change significantly at the same temperature.

For dissolution of molecules, the van't Hoff equation shown in eq 1 is considered.[13] If linearity of the van't Hoff plot is observed, it can be concluded that there is no change in the crystal structure within the temperature.

$$\mathrm{Ln}\,\chi = -\Delta H/RT + \mathrm{const} \quad (1)$$

χ = mole fraction of molecule; ΔH = enthalpy; and R = ideal gas constant.

To gain a further insight into the crystal structures of **4a** and **4b** during the heating process, the van't Hoff plot on the basis of the solubility data shown in Table 3 and Figure 6 was plotted (Figure 7). For **4a** monohydrate, one linear regression line (R^2 = 0.975) was observed at 283−343 K (10−70 °C), while **4b** monohydrate showed two linear regression lines (R^2 = 0.9541 and 0.9521) whose transition was detected at 303−313 K (30−40 °C). This might suggest that **4a** and **4b** behave differently toward temperature: the crystal structure of **4b** altered from a monohydrate to an anhydrate at 303−313 K (30−40 °C) while that of **4a** kept unchanged as a monohydrate over the same temperature range.

On the basis of the thermodynamic properties of **4a** and **4b** mentioned above, selective isolation of **4a** monohydrate might

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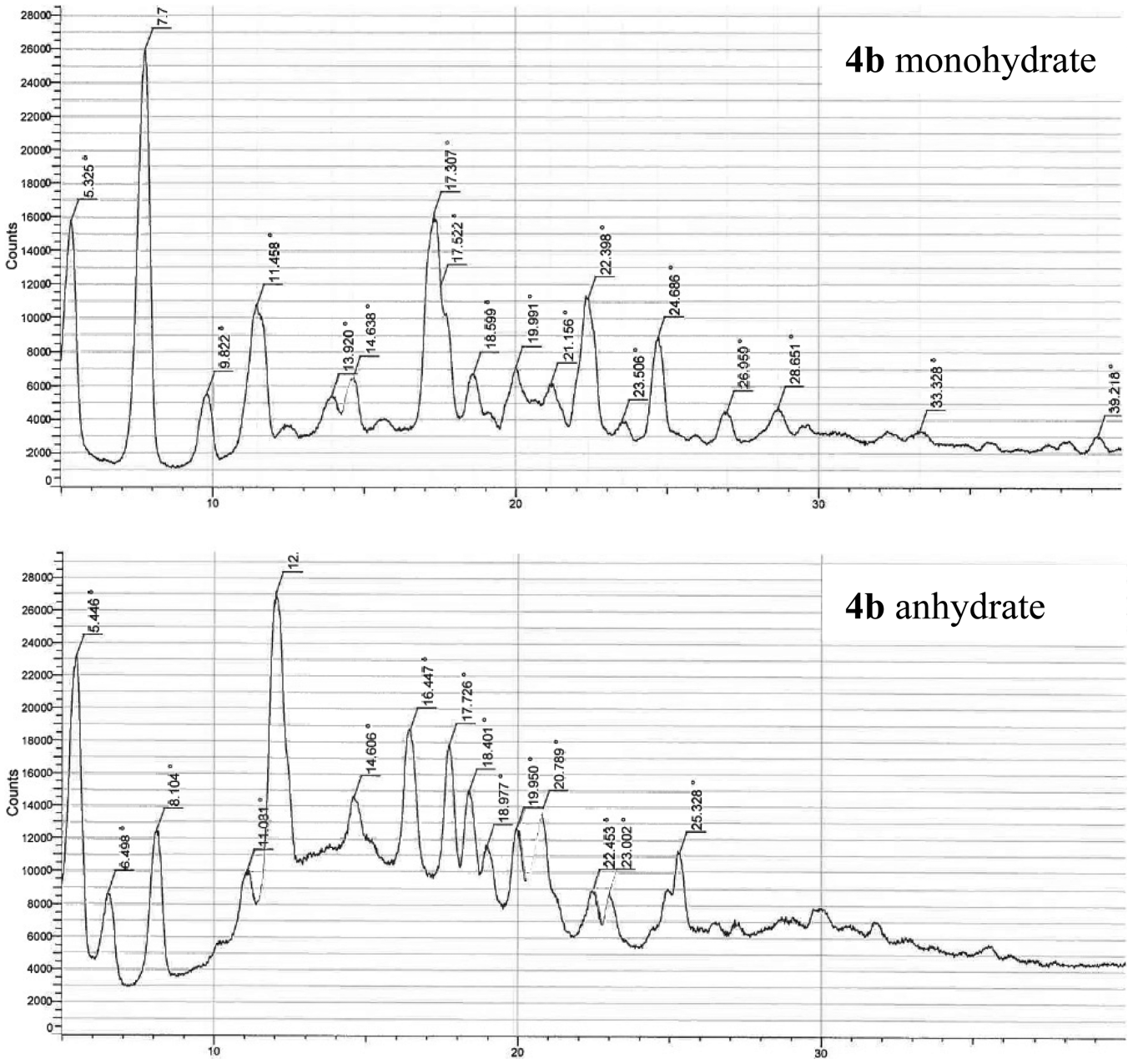

Figure 3. PXRD patterns of monohydrate and anhydrate of **4b**.

be possible by crystallization within the range of 42.5 and 72.1 °C where increase of solubility is possible only for **4b** by a structural change from monohydrate to anhydrate. Isolation of **4a** monohydrate from a mixture of monohydrates of **4a** and **4b** by crystallization was thus tested by changing the crystallization temperature (Table 4). When a mixture of monohydrates of **4a** and **4b** (**4a**/**4b** = 73:27) was crystallized from toluene (50 v/w) at 25−29 °C, incomplete isolation of **4a** resulted to afford an 80.1:19.9 mixture of monohydrates of **4a** and **4b** (Table 4, entry 1). In contrast, when it was carried out at 52−55 °C, complete separation of isomers was achieved to provide virtually pure **4a** monohydrate (**4a**/**4b** = 99.2:0.8, Table 4, entry 2). When a mixture of EtOH and H_2O (7:3) was employed as the solvent, a similar temperature dependence of the crystallization was noticed (**4a**/**4b** = 98.5:1.5 at 52−55 °C vs 95.8:4.2 at 25−29 °C, Table 4, entry 4 vs entry 3). It was thus concluded that our assumption on selective crystallization of **4a** monohydrate actually worked well to enable efficient isolation of **4a** monohydrate by simple adjustment of the crystallization temperature.

Taking account of the operational advantage as well as the fundamental insights on crystallization mentioned above into consideration, we conducted direct isolation of **4a** monohydrate from the reaction mixture by adding concentrated HCl (c-HCl) and water and concomitant crystallization at a specific temperature (40−60 °C, Scheme 4). Expectedly, to our delight, by implementing the elaborated procedure, diastereomerically pure **4a** was isolated in a good yield (63% based on **3**). As a consequence, the enabling process met the objective of increasing the yield of **4a** by more than 50% of the initial protocol based on the literature precedent (40.8%, Scheme 2).[7] Conceptually, recovery of **4b** would be possible by oxidation of **4b** and cyclization to **3**. However, due to the possibly high recovery cost, it was not considered.

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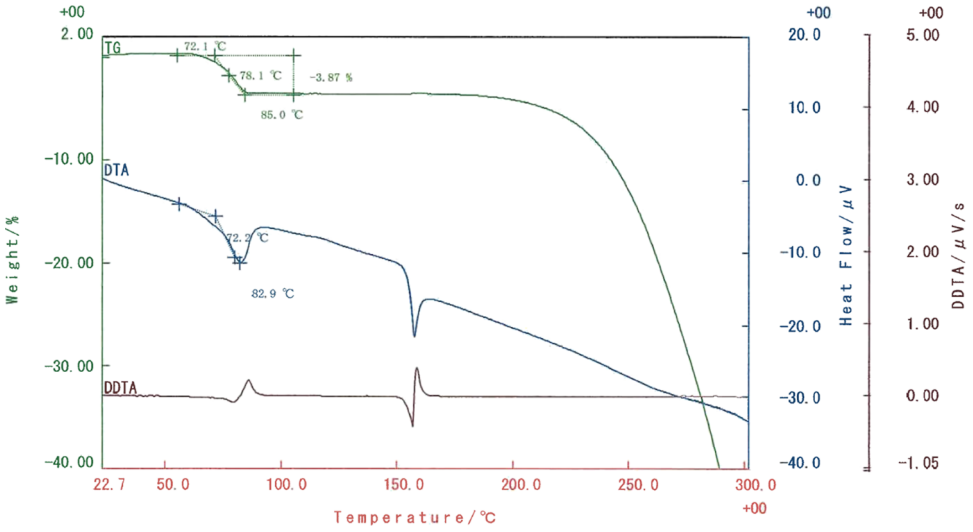

Figure 4. TG−DTA of **4a** monohydrate.

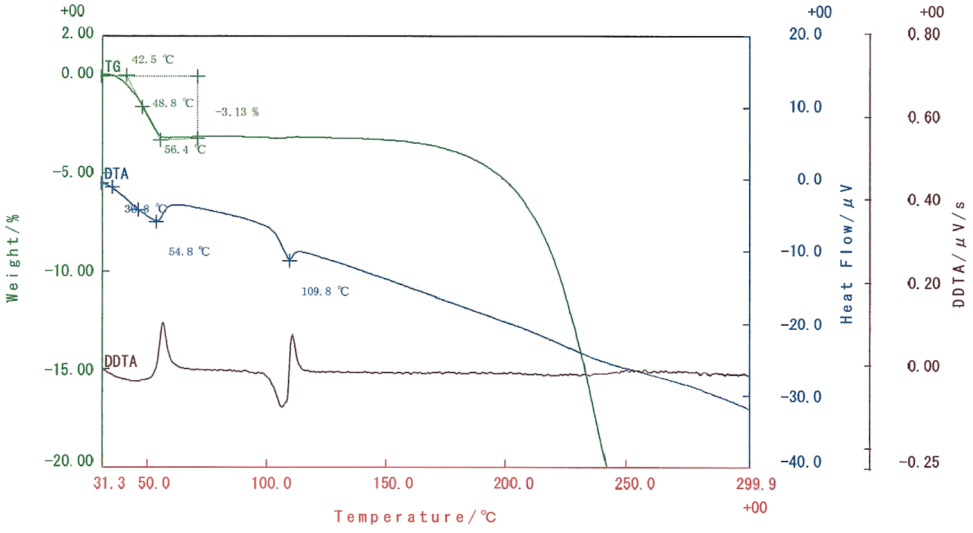

Figure 5. TG−DTA of **4b** monohydrate.

Table 3. Measured Solubility Expressed in Mole Fraction (χ) of Monohydrates of 4a And 4b in a Mixture of EtOH and H_2O (6:4 (v/v))

T (K)	**4a**·H_2O/χ	**4b**·H_2O/χ
283	0.00001921	0.00019901
293	0.00003202	0.00025701
303	0.00004484	0.00043806
313	0.00014754	0.00178659
323	0.00025701	0.00196904
333	0.00036682	0.00205044
343	0.00053545	0.00235050

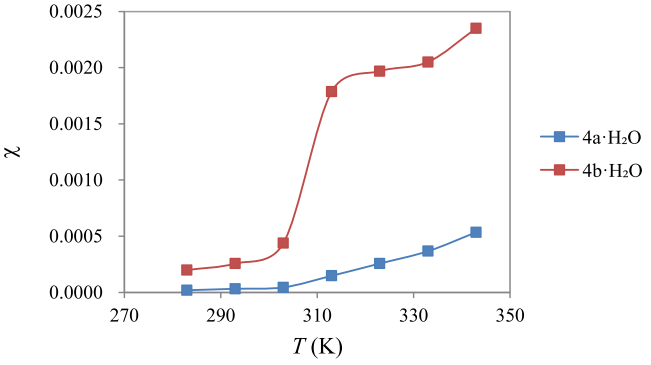

Figure 6. Solubility of monohydrates of **4a** and **4b** at various temperatures.

Amide alcohol **4a** thus obtained underwent further conversion to **2** as illustrated in Scheme 5. Treatment of **4a** with c-HCl in 1-methoxy-2-propanol at 100 °C for 15 min followed by crystallization by simple addition of water gave lactone **10** in 98.0% yield. Finally, **10** was converted to **2** in 86.9% yield using the reported procedure.[14] Thiolactone **2** was converted to (+)-biotin (**1**) using a well-established method involving Fukuyama coupling with a side-chain zinc reagent, hydrogenation, and subsequent removal of benzyl protecting groups.[3,15]

CONCLUSIONS

Practical synthesis of a key intermediate **2** for (+)-biotin (**1**) has been accomplished through high-yielding reduction of

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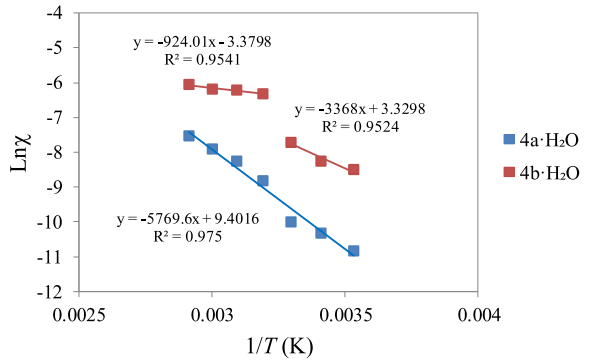
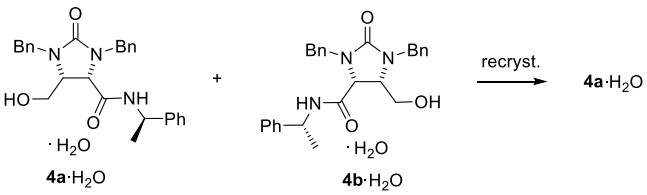


Figure 7. van't Hoff plot of the solubility of monohydrates of **4a** and **4b** at various temperatures.

Table 4. Recrystallization of a Mixture of Monohydrates of 4a and 4b at Different Temperatures



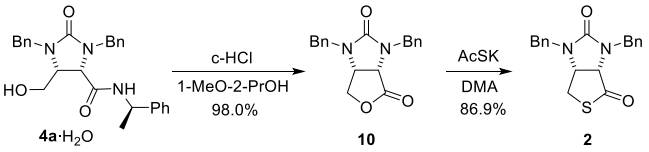
4a/4b = 73/27

entry[a]	solvent (v/w)	temp (°C)	time (h)	yield[b] (%)	4a/4b[c]
1	toluene (50)	25−29	2	76.5	80.1:19.9
2	toluene (20)	52−55	1	64.3	99.2:0.8
3	EtOH/H_2O = 7:3 (20)	25−29	2	65.3	95.8:4.2
4	EtOH/H_2O = 7:3 (10)	52−55	1	62.3	98.5:1.5

[a]The recrystallization was tested using crude **4a** (10 g, **4a**/**4b** = 73:27). [b]Isolated yield. [c]Based on the area (%) of **4a** and **4b** in HPLC.

chiral imide with $Ca(BH_4)_2$ and subsequent efficient separation of the desired isomer by crystallization. Although the selectivity of the reduction was moderate, the recovery rate of the desired isomer was excellent (90%) to afford the product in a very cost-effective way. Ready availability of raw materials, ease of operation, and a high overall yield of the current process would permit ready access to (+)-biotin (**1**), a compound of growing interest.

Scheme 5. Synthesis of 2 from 4a Monohydrate

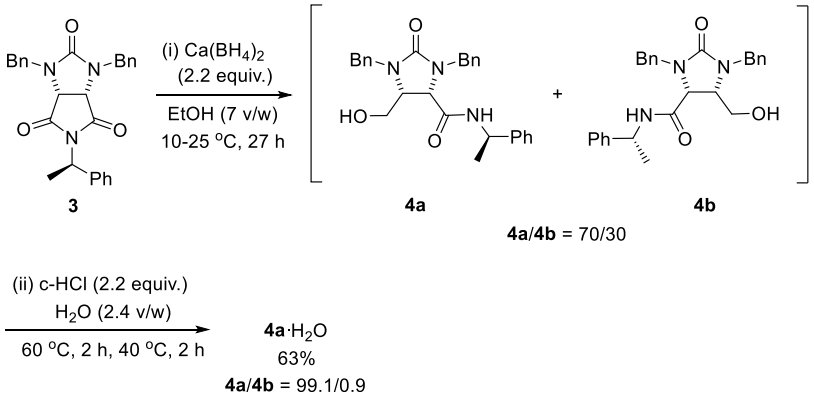


EXPERIMENTAL SECTION

General. Melting points were uncorrected. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra (JEOL Resonance, 400 and 100 MHz, respectively) were recorded with tetramethylsilane as an internal standard. TG−DTA was conducted using a Rigaku TG−DTA-8120. X-ray diffraction (XRD) patterns were recorded on a Rigaku SmartLab diffractometer equipped with a 5-axis goniometer and operated at 30 kV and 10 mA. The source of radiation was Cu Kα, and its wavelength was set to be 1.5060 Å. 2θ covered the range between 4.998° and 39.998° at a speed of 1°/min with a step size of 0.020°. The solubility measurement was performed using ChemiStation (EYELA, Japan). An excess quantity of sample was added to each tube of the instrument followed by the solvent. While the temperature of the slurry was controlled, the slurry was stirred for 2 h and then filtrated through a 0.45 μm membrane filter. The concentration of the filtrate was measured by HPLC to determine the solubility of the sample. HPLC analyses were performed using an Xbridge C18, 5 μm (4.6 mm × 150 mm) column at a flow rate of 1 mL/mL, elution solvent of 40−100% aq. CH_3CN (0−20 min), detection wavelength of 210 nm, and column temperature of 30 °C. All solvents and reagents were used as received.

[5(*R*)-*cis*]-Tetrahydro-5-(1-Phenylethyl)-1,3-Bis-(Phenylmethyl)Pyrrolo[3,4-*d*]Imidazole-2,4,6(5*H*)-Trione (3).[7] To a suspension of cyclic carboxylic acid **5** (200 g, 0.564 mol) in DMA (300 mL) was added R-PEA (68.4 g, 0.564 mol) and the mixture was stirred at 150 °C for 2 h. The mixture was cooled down to 90 °C and water (60 mL) was added gradually. After precipitation starts, the mixture was further stirred for 30 min. Then, water (840 mL) was added at 85 °C for over 1 h. The mixture was stirred at 25−30 °C for 2 h. The crystals formed were filtered and washed with water and dried at 60 °C for 25 h in a tray dryer to provide **3** as a white crystal (232 g, 93.9%). HPLC purity: 99.23 A%. Mp: 157−159 °C; ^1H NMR (400 MHz, $CDCl_3$) δ =7.28−7.35 (m, 15H), 5.33−5.40 (m, 1H), 5.02−5.09 (m, 2H), 4.21−4.29 (m, 2H), 3.88−3.95 (m, 2H), 1.79 (t, J = 7.2 Hz, 3H).

Scheme 4. Synthesis of 4a by $Ca(BH_4)_2$ Reduction of 3 and Subsequent Controlled Crystallization of 4a



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[5(*S*)-*cis*]-Tetrahydro-5-(1-Phenylethyl)-1,3-Bis-(Phenylmethyl)Pyrrolo[3,4-*d*]Imidazole-2,4,6(5*H*)-Trione (7). To a suspension of cyclic carboxylic acid **5** (20 g, 0.0564 mol) in DMA (30 mL) was added S-PEA (6.84 g, 0.0564 mol) and the mixture was stirred at 150 °C for 2 h. The mixture was cooled to 95 °C and water (6 mL) was added gradually. After precipitation starts, the mixture was further stirred for 30 min. Then, water (84 mL) was added at 85 °C for over 1 h. After completion of the addition, the mixture was stirred at 50 °C for 1 h. The crystals formed were filtered and washed with water and dried at 60 °C for 12 h in a tray dryer to provide **7** as a white crystal (24.2 g, 97.6%). HPLC purity: 99.40 A%. Mp: 159.1−159.4 °C; $[\alpha]_D^{25}$−76.3° (*c*, 0.5, THF); IR (neat): ν_{max} = 3033, 2940, 1713, 1685, 1449, 1358, 1233 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.26−7.34 (m, 15H), 5.34−5.40 (m, 1H), 5.03−5.06 (m, 2H), 4.24−4.30 (m, 2H), 3.89−3.95 (m, 2H), 1.80 (t, *J* = 7.2 Hz, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ = 172.9, 172.8, 157.6, 138.6, 135.8, 135.7, 128.9, 128.7, 128.3, 128.1, 127.5, 53.1, 53.0, 50.8, 46.43, 46.39, 16.4. HRMS (ESI-TOF): $[M + H]^+$ calcd for $C_{27}H_{25}N_3O_3$, 440.1929; found, 440.1947.

(3a*R*,6a*S*)-Tetrahydro-1,3-Bis(Phenylmethyl)-1*H*-Furo[3,4-d]Imidazole-2,4-Dione (9).[16] Calcium chloride (4.77 g, 0.0430 mol) was added to ethanol (126 mL) and the mixture was stirred at 25 °C for 30 min. The mixture was cooled down to 10 °C and $NaBH_4$ (3.41 g, 0.0902 mol) was added, and the mixture was stirred at 10 °C for 5 min. To the suspension was added portionwise **7** (18 g, 0.0410 mol) below 10 °C for over 15 min and the mixture was stirred below 5−15 °C for 1 h and at 20−25 °C for 22 h. Then, c-HCl (9.12 g) was added for over 15 min, and the mixture was warmed up to 60 °C and H_2O (43 mL) was added at 25−35 °C for over 30 min. The mixture was stirred at 60 °C for 2 h followed by at 40 °C for 2 h. The solids formed were filtered and washed with a mixture of MeOH (14.4 mL) and water (20 mL) to give the **8** monohydrate (wet, 20.24 g). Into the monohydrate **8** (wet, 20.24 g) was added DMA (10.8 mL) and c-HCl (5.4 g) for over 10 min. The mixture was stirred at 100 °C for 2 h. After completion of the reaction, the mixture was cooled down to 30 °C and CH_2Cl_2 (43.2 mL) and 10% aq. NaCl (43 g) were added. The organic layer was washed with H_2O (43.2 mL × 3) and evaporated to give **9** as a white crystal (57.2% based on **7**). HPLC purity: 99.33 A%. Mp: 115.8−116.8 °C; $[\alpha]_D^{25}$−162.9° (*c*, 0.5, THF); IR (neat): ν_{max} = 3034, 2921, 1777, 1698, 1445, 1416, 1211 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.25−7.38 (m, 10H), 5.06 (d, *J* = 15.2 Hz, 1H), 4.64 (d, *J* = 15.2 Hz, 1H), 4.38−4.40 (m, 2H), 4.10−4.17 (m, 3H), 3.92 (d, *J* = 8.4 Hz, 1H).

(4*R*,5*S*)-5-(Hydroxymethyl)-2-Oxo-*N*-[(1*R*)-1-Phenylethyl]-1,3-Bis(Phenylmethyl)-4-Imidazolidinecarboxamide Monohydrate (4b Monohydrate). To a solution of R-PEA (0.66 g, 5.45 mmol) in CH_2Cl_2 (8 mL) was added Me_3Al (2.8 mL, 2.0 M in toluene) at −76 °C under a N_2 atmosphere and the mixture was stirred at 20−30 °C for 30 min. To the solution was added dropwise **9** (2.0 g, 6.21 mmol) in CH_2Cl_2 (8 mL) for over 4 min. After the mixture was stirred at 25 °C for 3 h and at 40 °C for 2 h, a mixture of c-HCl (0.59 g) and H_2O (6 mL) was carefully added for over 20 min. The organic phase was separated and washed with H_2O (6 mL × 3). To the mixture was added *n*-hexane (15 mL) and solids formed were filtered and washed with AcOEt (10 mL). The crude **4b** thus obtained was dissolved in EtOH (15 mL) at 60 °C and H_2O (15 mL) was added, and the mixture was stirred at 25 °C for 1 h. Solids formed were filtered, washed with a mixture of EtOH and H_2O (1/1, 5 mL), and dried at 50 °C for 3 h in a tray dryer to give a white crystal of **4b** monohydrate (1.44 g, 50.2%). Mp: 93.4−93.9 °C; $[\alpha]_D^{25}$ + 180.8 ° (*c*, 0.5, THF); IR (neat): ν_{max} = 3468, 3267, 2976, 1680, 1645, 1465, 1452, 1055 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.12−7.36 (m, 15H), 6.67−6.69 (m, 1H), 5.14−5.18 (m, 1H), 4.96 (d, *J* = 14.8 Hz, 1H), 4.75 (d, *J* = 15.2 Hz, 1H), 4.19 (d, *J* = 15.2 Hz, 1H), 3.94 (d, *J* = 9.6 Hz, 1H), 3.84 (d, *J* = 14.8 Hz, 1H), 3.63−3.69 (m, 2H), 3.47−3.53 (m, 1H), 3.05 (dd, *J* = 4.8, 9.6 Hz, 1H), 1.50 (d, *J* = 7.2 Hz, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ = 168.5, 161.1, 142.1, 136.5, 135.8, 129.1, 128.9, 128.8, 128.3, 128.1, 128.0, 127.8, 126.1, 60.1, 59.4, 57.3, 49.2, 47.7, 46.4, 21.3. Anal. calcd for $C_{27}H_{31}N_3O_4$; C, 70.26; H, 6.77; N, 9.10. Found: C, 69.70; H, 6.70; 8.90. PXRD (2θ in degrees): 5.325, 7.770, 9.822, 11.458, 13.920, 14.638, 17.307, 17.522, 18.599, 19.991, 21.156, 22.398, 23.506, 24.686, 26.959, 28.651, 33.328, 39.218.

(4*R*,5*S*)-5-(Hydroxymethyl)-2-Oxo-*N*-[(1*R*)-1-Phenylethyl]-1,3-Bis(Phenylmethyl)-4-Imidazolidinecarboxamide Anhydrate (4b Anhydrate). **4b** monohydrate obtained above was dried at 60 °C for 30 min under vacuum (1 mmHg) to give **4b** anhydrate as a white crystal. Mp: 93.1−93.7 °C; $[\alpha]_D^{25}$ + 181.3 ° (*c*, 0.5, THF); IR (neat): ν_{max} = 3398, 3324, 2933, 1672, 1651, 1471, 1451, 1060 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.12−7.36 (m, 15H), 6.64 (d, *J* = 8.4 Hz, 1H), 5.14−5.18 (m, 1H), 4.96 (d, *J* = 14.8 Hz, 1H), 4.75 (d, *J* = 15.2 Hz, 1H), 4.19 (d, *J* = 15.6 Hz, 1H), 3.94 (d, *J* = 10.0 Hz, 1H), 3.84 (d, *J* = 14.8 Hz, 1H), 3.63−3.69 (m, 2H), 3.47−3.53 (m, 1H), 3.05 (dd, *J* = 4.8 Hz, 9.6 Hz, 1H), 1.50 (d, *J* = 7.2 Hz, 3H). PXRD (2θ in degrees): 5.446, 6.498, 8.104, 11.081, 12.018, 14.606, 16.447, 17.726, 18.401, 19.950, 20.789, 22.453, 23.002, 25.328.

(4*S*,5*R*)-5-(Hydroxymethyl)-2-Oxo-*N*-[(1*R*)-1-Phenylethyl]-1,3-Bis(Phenylmethyl)-4-Imidazolidinecarboxamide Monohydrate (4a Monohydrate).[7] Calcium chloride (46.4 g, 0.418 mol) was added to ethanol (1225 mL) and the mixture was stirred at 25−30 °C. After dissolution was confirmed, the mixture was cooled down to 10 °C and $NaBH_4$ (33.1 g, 0.876 mol) was added and the mixture was stirred below 10 °C for 5 min. To the suspension was added portionwise **3** (175 g, 0.398 mol) below 10 °C for over 15 min and the mixture was stirred below 5−15 °C for 1 h and at 20−25 °C for 26 h. Then, dil. aq. HCl (c-HCl (88.7 g) + H_2O (420 mL)) was added at 25−35 °C for over 1 h. The mixture was stirred at 60 °C for 2 h followed by at 40 °C for 2 h, and the solids formed were filtered and washed with a mixture of MeOH (140 mL) and water (210 mL) and dried at 70 °C for 18 h in a tray dryer to provide **4a** monohydrate as a white crystal (113.2 g, 63%). **4a**/**4b** = 99.1:0.9. HPLC purity: 98.90 A%. Mp: 155.5−156.1 °C; $[\alpha]_D^{25}$ + 93.0 ° (*c*, 0.5, THF); IR (neat): ν_{max} = 3429, 3271, 1682, 1652, 1453, 1240, 1064 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.24−7.32 (m, 15H), 6.55 (d, *J* = 8.0 Hz, 1H), 5.07−5.11 (m, 1H), 4.81 (d, *J* = 15.2 Hz, 1H), 4.64 (d, *J* = 15.6 Hz, 1H), 4.10−4.21 (m, 2H), 3.99 (d, *J* = 9.6 Hz, 1H), 3.60−3.70 (m, 1H), 3.45−3.55 (m, 1H), 3.20−3.30 (m, 1H), 2.91−2.95 (m, 1H), 1.34 (d, *J* = 7.2 Hz, 3H). $^{13}C\{^1H\}$ NMR δ = 168.6, 161.3, 142.3, 136.4, 129.1, 128.9, 128.8, 128.5, 128.2, 127.9, 127.8, 127.8, 126.2, 60.1, 59.9, 57.8, 49.1, 48.3, 46.6, 21.3. Anal. Calcd for $C_{27}H_{31}N_3O_4$; C, 70.26; H, 6.77; N, 9.10. Found: C, 70.10; H, 6.60; 8.90. PXRD (2θ in degrees): 5.279, 6.060, 7.658, 9.734, 11.424, 12.304, 14.513,

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15.450, 16.986, 17.524, 19.668, 21.382, 22.012, 23.146, 24.945, 28.302, 32.107.

(4*S*,5*R*)-5-(Hydroxymethyl)-2-Oxo-*N*-[(1*R*)-1-Phenylethyl]-1,3-Bis(Phenylmethyl)-4-Imidazolidinecarboxamide Anhydrate (4a Anhydrate). **4a** monohydrate obtained above was dried at 90 °C for 60 min under vacuum (1 mmHg) to give **4a** anhydrate as a white crystal. Mp: 155.4−155.9 °C; $[\alpha]_D^{25}$ + 94.0° (*c*, 0.5, THF); IR (neat): ν_{max} = 3480, 3276, 1685, 1651, 1453, 1237, 1041 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.23−7.33 (m, 15H), 6.53 (d, *J* = 8.0 Hz, 1H), 5.07−5.10 (m, 1H), 4.81 (d, *J* = 15.2 Hz, 1H), 4.64 (d, *J* = 15.6 Hz, 1H), 4.12−4.21 (m, 2H), 4.00 (d, *J* = 9.6 Hz, 1H), 3.63−3.70 (m, 1H), 3.45−3.54 (m, 1H), 3.20−3.27 (m, 1H), 2.87 (dd, *J* = 4.4 Hz, 9.6 Hz, 1H), 1.33 (d, *J* = 6.8 Hz, 3H). PXRD (2θ in degrees): 7.252, 8.506, 9.697, 11.188, 11.940, 12.854, 13.804, 14.642, 15.592, 18.329, 19.600, 20.676, 21.459, 22.544, 23.937, 24.736, 25.395, 26.516, 27.669, 31.945.

(3a*S*,6a*R*)-Tetrahydro-1,3-Bis(Phenylmethyl)-1*H*-Furo[3,4-d]Imidazole-2,4-Dione (10).[7,14] **4a** monohydrate (4.15 g, 9.0 mmol) was added to a mixture of 1-methoxy-2-propanol (8.3 mL) and c-HCl (2.1 g) at 25−30 °C and the mixture was stirred at 100 °C for 15 min. After completion of the reaction, the mixture was cooled down to 25−30 °C and water (83 mL) was added. The mixture was stirred at 25−30 °C for 2 h, and solids formed were filtered and washed with water and dried for 16 h in a tray dryer to provide **10** as a white crystal (2.84 g, 98.0%). HPLC purity: 98.36 A%. ^1H NMR (400 MHz, $CDCl_3$) δ = 7.26−7.38 (m, 10H), 5.06 (d, *J* = 15.2 Hz, 1H), 4.64 (d, *J* = 15.2 Hz, 1H), 4.34−4.40 (m, 2H), 4.10−4.17 (m, 3H), 3.92 (d, *J* = 8.4 Hz, 1H); HRMS (ESI-TOF): $[M + H]^+$ calcd for $C_{19}H_{18}N_2O_3$, 323.1351; found, 323.1398.

(3a*S*,6a*R*)-Tetrahydro-1,3-Bis(Phenylmethyl)-1*H*-Thieno[3,4-d]Imidazole-2,4-Dione (2).[7,14] A solution of **10** (28.0 g, 86.8 mmol) in degassed DMA (42 mL) was added to S-potassium thioacetate (AcSK) (14.88 g, 130.3 mmol) at 125 °C under a N_2 atmosphere and the mixture was stirred at 125 °C for 1.5 h. After completion of the reaction, the reaction mixture was cooled down to 100 °C and water (140 mL) was added, and the slurry was stirred at 25 °C for 2 h. The solids formed were filtered to give crude **2** (wet, 40.4 g), which was dissolved in 2-butanol (196 mL) at 70 °C and treated with activated carbon (1.4 g) at 70 °C and filtered. The filtrate was evaporated to 112 g and stirred at −10 to −5 °C for 3 h. The solids formed were filtered and dried at 60 °C for 12 h in a tray dryer to give **2** (25.5 g, 86.9%) as a white crystal. HPLC purity: 99.92 A%. Mp: 125−126 °C; $[\alpha]_D^{25}$ + 80.0° (*c*, 1.0, DMF); ^1H NMR (400 MHz, $CDCl_3$) δ = 7.25−7.36 (m, 10H), 5.02 (d, *J* = 14.8 Hz, 1H), 4.68 (d, *J* = 15.6 Hz, 1H), 4.33−4.38 (m, 2H), 4.09−4.16 (m, 1H); 3.80 (d, *J* = 8.0 Hz, 1H), 3.34−3.39 (m, 1H), 3.29−3.294 (m. 1H); HRMS (ESI-TOF): $[M + H]^+$ calcd for $C_{19}H_{18}N_2O_2S$, 339.1123; found, 339.1181.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.1c00196.

^1H- and ^{13}C-NMR spectra of the products (PDF)

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Notes

The authors declare no competing financial interest.

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