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A concise synthesis of (2*S*,3*S*)-BocAHPBA and (*R*)-BocDMTA, chiral building blocks for peptide-mimetic HIV protease inhibitors

Masaya Ikunaka,* Jun Matsumoto and Yukifumi Nishimoto

Research and Development Center, Nagase & Co., Ltd., 2-2-3, Murotani, Nishi-ku, Kobe 651-2241, Japan

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Abstract—Scalable syntheses of (2*S*,3*S*)-3-*N*-*tert*-butoxycarbonylamino-2-hydroxy-4-phenylbutanoic acid (BocAHPBA) **1** and (*R*)-3-*tert*-butoxycarbonyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (BocDMTA) **2** have been developed. Both **1** and **2** can serve as chiral building blocks in assembling JE-2147 (KNI-764) **3**, a potent HIV protease inhibitor. The synthesis of (2*S*,3*S*)-BocAHPBA **1** is achieved in 41% overall yield from (*S*)-2-*N*,*N*-dibenzylamino-3-phenylpropanal **4** in five steps where Tamao's reagent [Me_2(*i*-PrO)$SiCH_2MgCl$] is employed for a one-carbon homologation, and Zhao's oxidation protocol (TEMPO, $NaClO_2$, NaClO) is applied to convert a 1,2-glycol moiety into an α-hydroxy acid motif. (*R*)-BocDMTA **2** is synthesized with 99.4% ee in 24% yield via enantioselective hydrolysis of methyl (±)-5,5-dimethyl-1,3-thiazolidine-4-carboxylate **8b** by a *Klebsiella oxytoca* hydrolase; the unreacted (*S*)-ester **8b** can be recovered and racemized with NaOMe to afford (±)-**8b** in 46% yield for another round of the enzymatic processing. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

(2*S*,3*S*)-3-*N*-*tert*-Butoxycarbonylamino-2-hydroxy-4-phenylbutanoic acid (Boc AHPBA) **1** and (*R*)-3-*tert*-butoxycarbonyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (BocDMTA) **2** are chiral building blocks which are to be used for assembling potent HIV protease inhibitors, such as JE-2147 (KNI-764) **3** (Fig. 1);[1] these protease inhibitors have evolved from intensive structure–activity relationship studies on truncated peptide analogs that mimic the transition-state for the protease cleaving the *gag* and *gag*/*pol* gene products. In the present communication we will discuss chiral pool synthesis of (2*S*,3*S*)-BocAHPBA **1** commencing from (*S*)-2-*N*,*N*-dibenzylamino-3-phenylpropanal **4** (Scheme 1) and chemoenzymatic synthesis of (*R*)-BocDMTA **2** featuring kinetic resolution of methyl (±)-5,5-dimethyl-1,3-thiazolidine-4-carboxylate **8b** catalyzed by a thermally stable hydrolase of the *Klebsiella oxytoca* origin (Scheme 2).

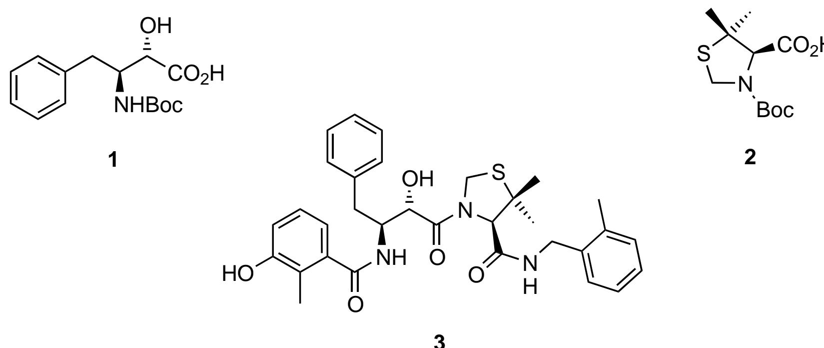

Figure 1. Structures of (2*S*,3*S*)-BocAHPBA **1**, (*R*)-BocDMTA **2**, and JE-2147 **3**.

* Corresponding author. Tel.: +81 78 992 3163; fax: +81 78 992 1050; e-mail: masaya.ikunaka@nagase.co.jp

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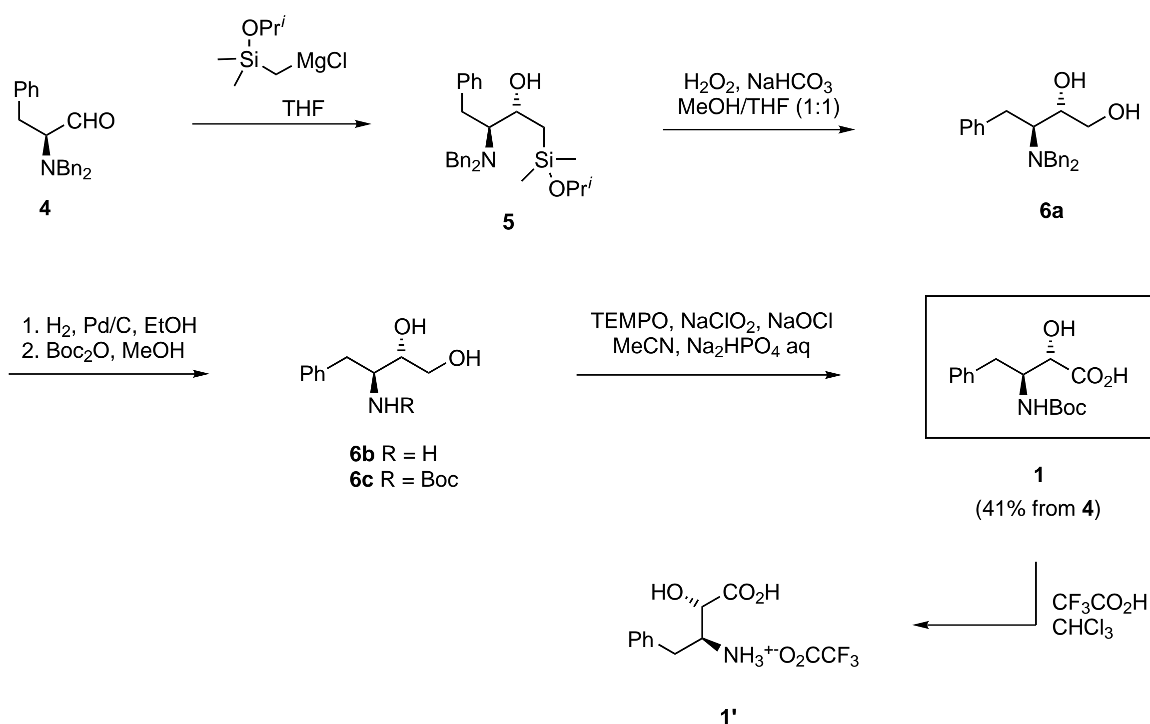
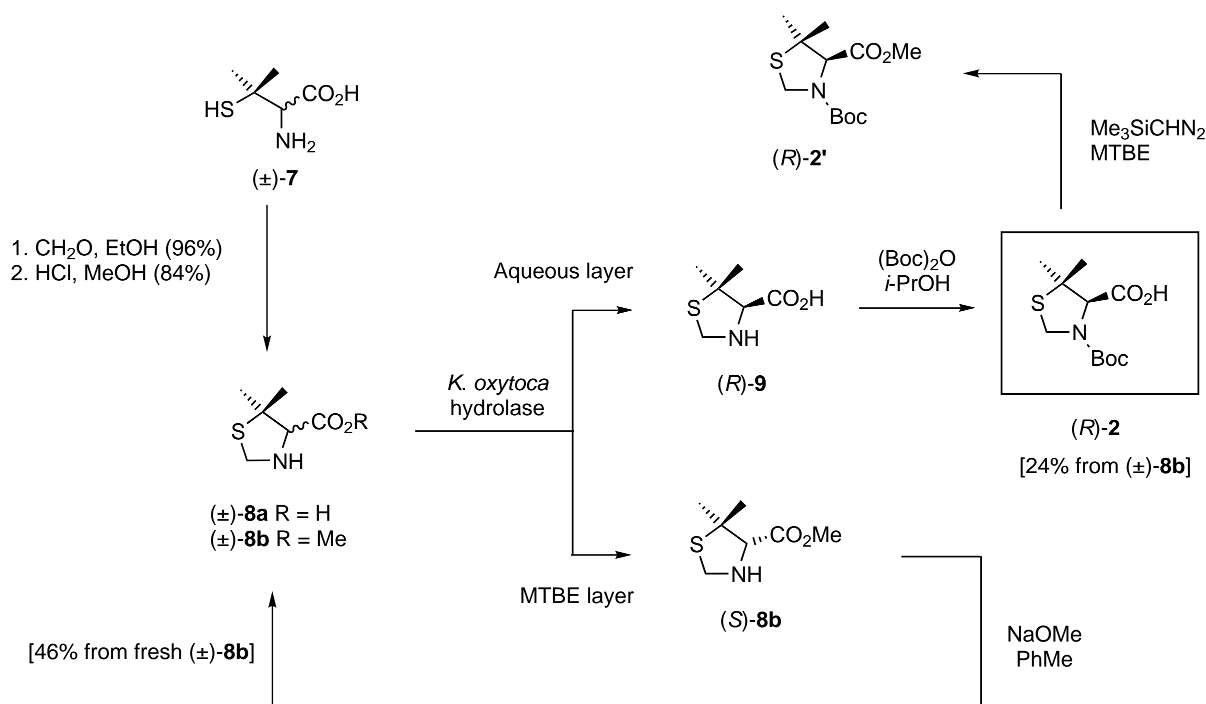


Scheme 1. Synthesis of (2*S*,3*S*)-BocAHPBA **1**.



Scheme 2. Synthesis of (*R*)-BocDMTA **2**.

2. Results and discussion

2.1. Chiral pool synthesis of (2*S*,3*S*)-BocAHPBA 1

2.1.1. Synthetic plan. Its β-amino-α-hydroxy acid motif being disposed as *erythro* configuration, synthesis of (2*S*,3*S*)-BocAHPBA **1** has presented an interesting challenge to the community of synthetic chemists, a few of whom have succeeded in developing enantioselective approaches: For example, using his unique hetero-bimetallic asymmetric catalyst, La-Li-(*R*)-Binol, Shibasaki achieved excellent diastereoselectivity in adding nitromethane to (*S*)-2-*N*-phthaloylamino-3-phenypropanal.[2] Without resorting to any external chi-

ral controller, Terashima also achieved relatively high diastereoselectivity in transferring a cyanide group from acetone cyanohydrin [$Me_2C(OH)CN$] to (*S*)-2-*N*,*N*-dibenzylamino-3-phenylpropanal **4**.[3]

However, each method has drawbacks from a practical perspective: (1) The use of the employed nucleophiles is undesirable, with nitromethane[2] and acetone cyanohydrin[3] being highly flammable and toxic, respectively; (2) each reaction takes as long as 72 h to go to completion;[2,3] (3) cryogenic conditions are needed for both Shibasaki's nitroaldol reaction[2] (−40°C) and Terashima's cyanohydrin formation[3] (−15°C); (4) Terashima's cyano group transfer reaction requires a halogenated solvent;[3] and (5) Shibasaki's and Terashima's recipes both end up requiring harsh, strongly acidic conditions to build a carboxylic acid functionality from the nitro and cyano groups.

To address these issues, we explored another combination of nitrogen protection on (*S*)-2-amino-3-phenylpropanal and a functionalized C(1) nucleophile. Experimental investigation along with a literature search has led us to devise a new pragmatic methodology capitalizing on Tamao's silicon reagent, isopropoxydimethylsilylmethylmagnesium chloride,[4] in combination with Reetz's nitrogen protection tactics featuring an *N*,*N*-dibenzyl group[5] (Scheme 1).

2.1.2. Diastereoselective installation of a hydroxymethyl group. When (*S*)-2-*N*,*N*-dibenzylamino-3-phenylpropanal **4**, prepared in quantity according to Ng's procedures,[6] was treated with isopropoxydimethylsilylmethylmagnesium chloride in THF at 0°C, nucleophilic addition proceeded smoothly providing β-hydroxysilane **5**, which, without purification, was subjected to oxidative cleavage of the Si–C bond by basic hydrogen peroxide[4b] to give 3-amino-1,2-diol **6a** as a single stereoisomer. Its ^1H NMR spectrum was the same as that reported by Izawa et al.[7a] demonstrating that Tamao's reagent, under the influence of the *N*,*N*-dibenzyl group (nonchelation control based on the Felkin–Anh model[5a]), successfully established the desired *erythro* configuration.

It is noteworthy that complete *erythro*-selectivity was seen in the nucleophilic addition of Tamao's reagent to **4** at temperatures as high as 0°C. Indeed, **4** was shown to undergo nucleophilic addition of Grignard reagents under nonchelation control with high diastereoselectivity; however, the reported diastereoselectivity was less than complete, ranging from 92:8 (MeMgI) to 97:3 (PhMgBr) each in favor of the *erythro*-isomers.[5] Thus, in the present case, it seems probable that the silyl group [$Si(OPr^i)Me_2$] in Tamao's reagent exerted its steric bulk to augment the intrinsic propensity for **4** to undergo nonchelation-controlled addition. In addition, its branching being situated β to the nucleophilic center, Tamao's reagent could still complex with the nitrogen atom of **4** via the magnesium center to increase the passive volume of the amine functionality such that nonchelation control is further augmented.[5a]

Having served its role in controlling the stereochemistry of the reaction with Tamao's reagent, the *N*,*N*-dibenzyl group of **6a** was supposed to be exchanged for an *N*-Boc group, such that product isolation should be effected more easily in the later stage(s) of the synthesis. Catalytic hydrogenolysis (H_2, Pd/C) of the crude **6a** proceeded uneventfully to give the completely deprotected amino diol **6b**, which, because of its increased polarity and high crystallinity, could be isolated free from silicon-derived side products without recourse to chromatography; **6b** was obtained in 56% overall yield from **4** in three steps, its *erythro*-(2*S*,3*S*) configuration being corroborated by the ^1H NMR spectrum that was superimposed on that recorded by Moyano et al.[7b] The purified **6b** was then treated with $(Boc)_2O$ in methanol to protect its primary amine function as the carbamate **6c**.

2.1.3. Oxidation of 1,2-glycol to α-hydroxy acid. To oxidize the 1,2-glycol functionality of **6c** regioselectively to the α-hydroxy carboxylic acid in a single operation, we opted to apply Zhao's procedures[8] with reference to Wovkulich's precedent.[9] When crude diol **6c** was treated with NaOCl and $NaClO_2$ in the presence of catalytic 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO), regioselective oxidation took place and, albeit sluggishly, reached completion. Finally, single recrystallization furnished (2*S*,3*S*)-BocAHPBA **1** in a two-step overall yield of 74% from **6b** and a five-step overall yield of 41% from **4**. Its stereochemical integrity was confirmed unequivocally by comparing the physicochemical data with those recorded in the technical report[10] and by converting it to the trifluoroacetic acid salt of (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoic acid **1′**, ^1H NMR analysis of which showed the same spectrum as that recorded by Wong et al. to demonstrate the absence of the *threo* isomer.[11]

2.2. Chemoenzymatic synthesis of (*R*)-BocDMTA 2

2.2.1. Synthetic plan. Naive retrosynthetic analysis of (*R*)-BocDMTA **2** would result in (*R*)-penicillamine **7** as a deceptive starting material, because, being an unnatural form of penicillamine, (*R*)-**7** is available less abundantly; additionally, (*R*)-penicillamine **7** is notorious as a causative agent for optic atrophy leading to blindness.[12] In contrast, besides having played a key role in Sheehan's first total synthesis of penicillin V,[13] (*S*)-penicillamine **7** has been used as a chelating agent to remove heavy metals, such as lead, copper, and mercury, from the body.[12] Hence, to meet such medicinal need, scalable synthesis of (*S*)-**7** had been established where (±)-3-formyl-2,2,5,5-tetramethylthiazolidine-4-carboxylic acid, which, in turn, was assembled from (±)-**7**, was resolved via diastereomeric salt formation into its (*S*)-isomer and the latter was hydrolyzed to (*S*)-**7** itself. For the chiral separation process the following resolving agents were employed: brucine (a natural alkaloid),[13] (+)-(1*S*,2*S*)-pseudonorephedrine (an isomer of phenypropanolamine, used to alleviate cold symptoms),[14a] (1*S*,2*S*)-*threo*-2-amino-1-(4-nitrophenyl)-1,3-propanediol (an industrial precursor to chloramphenicol, an antibiotic produced by *Strepto-*

mycesvenezuelae),[14b] and (*S*)-lysine (a natural amino acid).[14c] However, because each antipode of the resolving agents is not easily obtainable commercially, neither approach for the resolution seemed applicable for a scalable synthesis of (*R*)-penicillamine **7**,[14b] and hence (*R*)-**2**.[15]

Thus, as part of our program to develop scalable chemoenzymatic processes, we opted to explore the kinetic resolution of methyl (±)-5,5-dimethyl-1,3-thiazolidine-4-carboxylate **8b** with a hydrolytic enzyme (Scheme 2), taking into account that the synthesis should commence with the least expensive commercially available (±)-**7**[13,16,17] and the fact that to the best of our knowledge, no chemoenzymatic approach had been used to access either enantiomerically enriched (*R*)-**8a** or (*R*)-**2**.

2.2.2. Enzyme-catalyzed enantioselective hydrolysis. According to the reported procedures,[18] (±)-**7**[13,16,17] was converted to (±)-1,3-thiazolidine-4-carboxylic acid **8a** in 96% yield, which, in turn, was esterified under acidic conditions to give (±)-**8b** uneventfully in 84% yield (Scheme 2). We then screened 43 different hydrolase preparations, such as lipases, proteases, and carboxyesterases, looking to identify hydrolytic enzymes able to digest the (*R*)-ester **8b** selectively. This led to identification of three hydrolases that could discriminate between the enantiomers of (±)-**8b**. As can be seen from Table 1, it was the thermally stable hydrolase from *K. oxytoca*[15] that showed superior enantioselectivity in the hydrolysis of the (±)-ester **8b**.

With the promising hydrolytic enzyme from *K. oxytoca* in hand, we examined parameters affecting the enzymatic hydrolysis, such as substrate concentrations, enzyme usage, reaction time, and temperatures, to maximize the space-time yield (Table 2). The *K. oxytoca* hydrolase being able to function at temperatures as high as 70°C, we took advantage of its thermal stability to increase the volume efficiency with (±)-**8b**. In the event, the substrate concentration {[(±)-**8b**]} could be increased to 3.0 M (526 g/L) without compromising the resolution efficiency ($E^{20}=145$), when the enzymatic hydrolysis was run at 60°C in the presence of the *K. oxytoca* hydrolase at a concentration of 0.3%; the (*R*)-acid **8a** was generated with 98% ee after 24 h when 35% conversion was reached.

2.2.3. Telescoping the enzymatic hydrolysis to incorporate carbamate formation. Being an amphoteric α-amino acid, the hydrolyzed (*R*)-acid **8a** was too difficult to isolate from the aqueous medium. Thus, it should be more tactically beneficial to postpone product isolation until the basic nitrogen function has been blocked as a carbamate. The telescoped process that was eventually established is as follows: At a 34% conversion of the enzymatic hydrolysis {[(±)-**8b**] = 3.0 M; [*K. oxytoca* hydrolase] = 0.3%; 60°C; 26 h} where (*R*)-acid **8a** was generated with 97.3% ee, the reaction mixture was basified with aqueous NaOH to pH 11.5, and the unreacted (*S*)-ester **8b**[21] of 50.3% ee was extracted into methyl *tert*-butyl ether (MTBE). The aqueous layer containing the (*R*)-acid **8a** was treated with *i*-PrOH and then with $(Boc)_2O$ under ice-cooling. On complete carbamate formation, the volatile components were evaporated in vacuo, and the aqueous residue was acidified with aqueous HCl to pH 3.0. Extraction with AcOEt followed by a single recrystallization [*n*-heptane–AcOEt (4:1)] provided (*R*)-BocDMTA **2**[22] of 99.4% ee in 24%

Table 1. Hydrolases able to catalyze the enantioselective hydrolysis of (±)-**8b**[a]

Enzyme	Microbial source	[Enzyme] (%)	Conversion (%)[d]	Ee (%) for (*S*)-**8b**[e]	Ee (%) for (*R*)-**9**[e]	E[f]
Protease[b] (XP-488)	*Aspergillus melleus*	0.5	13.3	14.0	91.0	24
Protease[b] (Bioprase)	*Bacillus subtillis*	5	37.5	41.4	69.2	8
Hydrolase[b,c]	*Klebsiella oxytoca*	0.1	41.6	71.1	99.7	>500

[a] The enzymatic reaction was run at 25°C in an unbuffered aqueous medium with (±)-**8b** at a concentration of 90 mM and with enzymes each at the indicated concentration for 24 h.
[b] Available from Nagase ChemteX Corporation.
[c] See Ref. 19.
[d] For the HPLC conditions used to monitor the progress of the reaction, see Section 4.3.3.
[e] For the HPLC conditions used to determine the enantiomeric purity, see Section 4.3.3.
[f] See Ref. 20.

Table 2. Enantioselective hydrolysis of (±)-**8b** with *K. oxytoca* hydrolase[a]

[(±)-**8b**] (M)	[Hydrolase] (%)	Time (h)	Temperature (°C)	Conversion (%)[b]	Ee (%) for (*R*)-**9**[c]	E[d]
0.5	0.1	24	40	43.7	99.1	>500
1.0	0.1	24	40	30.6	99.4	>500
1.0	0.1	24	60	41.8	96.5	118
3.0	0.3	24	60	34.7	97.7	145

[a] The enzymatic reaction was run in an unbuffered aqueous medium under the conditions detailed in Section 4.3.3.
[b] For the HPLC conditions used to monitor the progress of the reaction, see Section 4.3.3.
[c] For the HPLC conditions used to determine the enantiomeric purity, see Section 4.3.3.
[d] See Ref. 20.

overall yield from (±)-**8b**; the enantiomeric purity of (*R*)-**2** was determined unambiguously by converting it to the methyl ester (*R*)-**2′** with Me_3SiCHN_2[23] and analyzing the latter on a chiral HPLC column.

2.2.4. Racemization of the unwanted enantiomer (*S*)-8b. To increase the throughput (atom economy[24]) with the overall process of the kinetic resolution, the unreacted (*S*)-ester **8b**, recovered by extraction with MTBE, should be racemized to replenish the enzyme substrate (±)-**8b**. When a solution of (*S*)-**8b** (50.3% ee) in toluene was treated with catalytic NaOMe (28% in MeOH) at room temperature, racemization took place and the reaction went to completion in 4 h to regenerate (±)-**8b** in 46% overall yield from the fresh (±)-**8b**.

3. Conclusion

The tactical features that have benefited the synthesis of (2*S*,3*S*)-BocAHPBA **1** are twofold: In combination with *N*,*N*-dibenzyl protection of an α-amino aldehyde, Tamao's reagent enabled the facile construction of an *erythro*-amino alcohol structure at 0°C. Zhao's oxidation protocol proved to be applicable for regioselective oxidation of a 1,2-glycol to the α-hydroxy acid functionality.

In the synthesis of (*R*)-BocDMTA **2** the following measures have been taken for the kinetic resolution of (±)-**8b** with the *K. oxytoca* hydrolase to be industrially viable: (i) Because of the thermally stable nature of the hydrolase, the enzymatic hydrolysis was run at 60°C such that (±)-**8b** could be processed at concentrations as high as 3 M. (ii) The enzymatic process was telescoped into carbamate formation to facilitate the product isolation. (iii) The (*S*)-ester **8b** that survived the enzymatic hydrolysis and could be recovered easily from the spent reaction mixture was racemized by the catalytic action of NaOMe to augment the throughput of the kinetic resolution.

4. Experimental

4.1. General

Melting points were measured on an Electrothermal 1A8104 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz on a Varian UNITY-400 spectrometer in a $CDCl_3$ or DMSO-d_6 solution and in a D_2O solution with tetramethylsilane and 2-(trimethylsilyl)ethanesulfonic acid, sodium salt, as an internal standard, respectively. FT-IR spectra were recorded on a Nicolet Avatar 360 FT-IR spectrometer. Mass spectra were recorded on a Hitachi M-8000 mass spectrometer (ESI). Elemental analyses were performed on an Elementar vario EL analyzer. Optical rotations were measured on a Horiba SEPA-200 polarimeter. Thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 plates (0.25 mm thick, art 1.05714.)

4.2. Synthesis of (2*S*,3*S*)-BocAHPBA 1

4.2.1. (2*S*,3*S*)-3-*N*,*N*-Dibenzylamino-1-isopropoxydimethylsilyl-4-phenylbutan-2-ol 5. Under an atmosphere of N_2, a portion (5 mL) of a solution of (isopropoxydimethylsilyl)methyl chloride (available from Sigma-Aldrich; 4.6 g, 28 mmol) in dry THF (40 mL) was added to Mg turnings (0.67 g, 28 mg atom). To the stirred mixture was added 1,2-dibromoethane (50 μL) at room temperature and an exothermic reaction started in several minutes. The remainder of the THF solution of (isopropoxydimethylsilyl)methyl chloride was added dropwise over 30 min so that the reaction temperature did not exceed 40°C. After the addition was complete, the grey-silver mixture was stirred and heated at reflux for 30 min. The mixture was cooled to 0°C, and a solution of (*S*)-2-*N*,*N*-dibenzylamino-3-phenylpropanal **4** (prepared according to Ng's procedures;[6] 5.5 g, 15 mmol) in dry THF (30 mL) was added dropwise at the same temperature over 30 min. The mixture was stirred at 0°C for 30 min, and 10% aqueous NH_4Cl solution (80 mL) was added at the same temperature. The mixture was extracted with AcOEt (70 mL). The AcOEt extract was washed with saturated aqueous NaCl solution (50 mL), dried (Na_2SO_4), and concentrated in vacuo to give crude **5** as a pale yellow oil (7.6 g): TLC [*n*-hexane/EtOAc (4:1)] $R_f = 0.60$; IR ν_{max} (KBr) 3492, 2970, 2802, 1740, 1603, 1495, 1454, 1369, 1252, 1122, 1018, 841, 744 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) 7.25–7.05 (m, 15H), 4.12–4.05 (m, 1H), 3.98–3.90 (m, 1H), 3.70–3.50 (m, 4H), 3.00–2.70 (m, 4H), 1.08 (dd, $J = 9.6$ Hz, 6.4 Hz, 6H), 0.83–0.78 (m, 2H), 0.11–0.04 (m, 6H). This was employed in the next step without further purification.

4.2.2. (2*S*,3*S*)-3-*N*,*N*-Dibenzylamino-4-phenylbutane-1,2-diol 6a. To a stirred mixture of crude **5** (7.6 g, 15 mmol), $NaHCO_3$ (1.6 g, 19 mmol), MeOH (40 mL), and THF (40 mL) was added 30% aqueous H_2O_2 solution (12 mL) dropwise at room temperature. The mixture was stirred and heated at 70°C for 2.5 h, and 10% aqueous $Na_2S_2O_3$ solution (150 mL) was added dropwise carefully at temperatures between 10 and 25°C. Stirring was continued at the same temperature range for 30 min, and the mixture was extracted with AcOEt (150 mL). The AcOEt extract was washed with saturated aqueous NaCl solution (2×50 mL), dried (Na_2SO_4), and concentrated in vacuo to give crude **6a** (6.30 g) as a pale yellow oil: TLC [*n*-hexane/EtOAc (1:1)] $R_f = 0.40$ for **6a**, 0.80 for **5**; IR ν_{max} (KBr) 3396, 3061, 3026, 2928, 1950, 1877, 1809, 1738, 1602, 1495, 1454, 1373, 1244, 1028, 974, 906, 876, 744, 698, 611, 509 cm^{-1}; ^{1}H NMR δ ($CDCl_3$) 7.31–7.15 (m, 15H), 3.86–3.80 (m, 1H), 3.77 (d, $J = 13.6$ Hz, 2H), 3.68–3.58 (m, 2H), 3.54 (d, $J = 13.6$ Hz, 2H), 3.15 (dd, $J = 13.2$ Hz, 6.4 Hz, 1H), 3.06–2.92 (m, 2H). This was employed in the next step without further purification.

4.2.3. (2*S*,3*S*)-3-Amino-4-phenylbutane-1,2-diol 6b. To a solution of crude **6a** (4.7 g) in AcOEt (50 mL) was added activated charcoal (Darco G-60, 0.5 g) at room temperature, and the mixture was stirred at the same temperature for 30 min. The activated charcoal was

filtered off, and the filtrate was concentrated in vacuo to give a slightly yellow oil (4.6 g), which was dissolved in EtOH (50 mL). 5% Pd/C (K type, N.E. Chemcat Corporation; 0.92 g; water content, 55.58%) was added, and the mixture was stirred and heated at 50°C under an atmosphere of H_2 (initial pressure, 4.0 kg/cm^2) for 6.5 h. (When hydrogen absorption is too sluggish, the mixture should be filtered and the Pd catalyst replenished.) When **6a** was consumed completely as confirmed by TLC *n*-hexane/EtOAc (1:1)], the Pd catalyst was filtered off, and washed with EtOH (20 mL). The combined filtrate and washing were concentrated in vacuo. To the residue was added EtOH (15 mL) followed by *n*-hexane (40 mL), and the precipitated solids were collected by filtration to give white crystals (1.5 g), which were suspended in MeOH (100 mL) and filtered. The filter cake was washed with MeOH (20 mL). The combined filtrate and washing were concentrated in vacuo to give **6b** as white crystals (1.14 g, 55.6% overall yield from **4**): TLC [*n*-hexane/EtOAc (1:1)] $R_f=0.05$; mp 110–113°C (lit.:[7b] mp 105–107°C); $[\alpha]_D^{25}=-35.2$ (*c* 0.95, MeOH) {lit.:[7b] $[\alpha]_D^{25}=-34.2$ (*c* 1.37, MeOH)}; IR ν_{max} (KBr) 3305, 3081, 2916, 2860, 2703, 1608, 1493, 1454, 1436, 1377, 1078, 1043, 958, 906, 878, 844, 750, 700, 649, 592, 511 cm^{-1}; ^{1}H NMR δ (CD_3OD) 7.30–7.18 (m, 5H), 3.75–3.60 (m, 2H), 3.58–3.52 (m, 1H), 3.08–2.98 (m, 2H), 2.58–2.42 (m, 1H); MS *m*/*z* 182 {[M+H]$^+$}.

4.2.4. (2*S*,3*S*)-3-*N*-*tert*-Butoxycarbonylamino-4-phenylbutane-1,2-diol 6c. To a stirred solution of **6b** (0.50 g, 2.76 mmol) in MeOH (15 mL) was added di-*tert*-butyl dicarbonate (Boc_2O, 0.63 g, 2.88 mmol) dropwise at room temperature. Stirring was continued at room temperature for 2 h, and the mixture was concentrated in vacuo. The residue was extracted with AcOEt (50 mL) and the AcOEt extract was washed with 1.0 M aqueous $KHSO_4$ solution (2×30 mL), and saturated aqueous NaCl solution (2×30 mL), dried ($MgSO_4$), and concentrated in vacuo to give crude **6c** as a white solid (0.90 g, quantitative yield): TLC [*n*-hexane/EtOAc (1:1)] $R_f=0.40$; IR ν_{max} (KBr) 3361, 2981, 2930, 1685, 1605, 1525, 1444, 1365, 1315, 1269, 1250, 1175, 1040, 1013, 890, 757, 702, 648 cm^{-1}; ^{1}H NMR δ (DMSO-d_6) 7.30–7.10 (m, 5H), 6.56 and 6.10 (each d, $J=9.2$ Hz, $J=9.2$ Hz, 1H), 4.80–4.70 (m, 1H), 4.50–4.40 (m, 1H), 3.62–3.56 (m, 1H), 3.50–3.30 (m, 3H), 2.95 (dd, $J=$ 13.6, 3.2 Hz, 1H), 2.58–2.52 (m, 1H), 1.35–1.20 (m, 9H). This was employed without further purification.

4.2.5. (2*S*,3*S*)-3-*N*-*tert*-Butoxycarbonylamino-2-hydroxy-4-phenylbutanoic acid 1. To a stirred mixture of crude **6c** (0.30 g, 0.92 mmol), phosphate buffer (Na_2HPO_4, pH 6.7, 5.0 mL), and MeCN (5.0 mL) was added 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO, 40 mg, 0.26 mmol) followed by sodium chlorite ($NaClO_2$, 80%, 0.21 g, 1.86 mmol) at room temperature. The mixture was warmed to 35°C, and 0.28% aqueous sodium hypochlorite (NaOCl) solution (0.75 mL, 2.8×10^{-2} mmol) was added at the same temperature. The mixture was stirred with warming at 35°C for 6.5 h, and TEMPO (20 mg, 0.13 mmol) and 0.28% aqueous NaOCl solution (0.75 mL, 0.94×10^{-2} mmol) were added at the same temperature. The mixture was stirred at 35°C for 24.5 h, and further TEMPO (20 mg, 0.13 mmol) and 0.28% aqueous NaOCl solution (0.50 mL, 1.88×10^{-2} mmol) were added at the same temperature. The mixture was stirred at 35°C for 23.5 h, then allowed to cool to room temperature. To the mixture was added H_2O (10 mL), and the pH of the mixture was adjusted to 8.0 with 2.0 M aqueous NaOH solution (2.0 mL). Six percent aqueous sodium sulfite (Na_2SO_3) solution (10 mL) was added with ice-cooling below 20°C, and pH of the mixture turned 9.0 after the addition was complete. The mixture was stirred at room temperature for 30 min and washed with MTBE (20 mL). To the aqueous layer was added AcOEt (50 mL). To the mixture was added 2.0 M aqueous HCl solution (3.0 mL) with stirring to adjust pH of the aqueous layer to 3. The layers were separated. The AcOEt layer was washed with H_2O (25 mL), and saturated aqueous NaCl solution (2×30 mL), dried ($MgSO_4$), and concentrated in vacuo to give crude **1** (0.35 g) as white solids, to which was added AcOEt (2.0 mL) followed by *n*-hexane (6.0 mL). The suspension was stirred under reflux for 15 min then cooled to 5°C, and kept at the same temperature for 30 min. The precipitated solids were collected by filtration, washed with *n*-hexane (10 mL), air-dried at an oven temperature of 50°C overnight to give purified **1** as white crystals (0.20 g, 74% overall yield from **6b**): TLC [BuOH/H_2O/AcOH (4:1:1)] $R_f=0.80$; mp 147–148°C (lit.:[10a] mp 147–148°C); $[\alpha]_D^{20}=+2.69$ (*c* 1.00, MeOH) {lit.:[10b] $[\alpha]_D^{20}=-2.10$ (*c* 1, MeOH) for the (2*R*,3*R*)-isomer}; IR ν_{max} (KBr) 3402, 3353, 2985, 2938, 1695, 1518, 1446, 1390, 1288, 1269, 1250, 1169, 1067, 1051, 1027, 933, 845, 760, 704, 656, 602, 515 cm^{-1}; ^{1}H NMR δ (DMSO-d_6) 12.80–12.40 (br, 1H), 7.30–7.10 (m, 5H), 6.72 and 6.20 (each d, $J=8.8$ Hz, $J=8.8$ Hz, total 1H), 5.80–5.20 (br, 1H), 4.00 (d, $J=4.8$ Hz, 1H), 4.00–3.80 (m, 1H), 2.80–2.60 (m, 2H), 1.35–1.20 (m, 9H); MS *m*/*z* 294 {[M−H]$^-$}. Anal. found: C, 60.8; H, 7.3; N, 4.7. Calcd for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74%.

4.2.6. (2*S*,3*S*)-3-Amino-2-hydroxy-4-phenylbutanoic acid trifluoroacetate 1′. To a mixture of **1** (50 mg, 0.17 mmol) in $CHCl_3$ (2.0 mL) was added trifluoroacetic acid (TFA, 1.0 mL) at room temperature. Stirring was continued at room temperature for 4 h, during which the progress of the reaction was monitored by TLC [*n*-BuOH/H_2O/AcOH (4:1:1); $R_f=0.4$ for **1′**]. The mixture was concentrated in vacuo. The residue was diluted with $CHCl_3$ (2.0 mL), and the mixture was concentrated in vacuo. This operation was repeated three times to remove the residual TFA completely; **1′** (60 mg) was obtained quantitatively: $[\alpha]_D^{24}=-3.2$ (*c* 0.74, N HCl) {lit.:[11] $[\alpha]_D^{24}=-2.2$ (*c* 0.67, 1M HCl)}; ^{1}H NMR δ (D_2O) 7.28–7.05 (m, 5H), 4.42 (d, $J=3.2$ Hz, 1H), 3.94–3.88 (m, 1H), 2.92–2.76 (m, 2H).

4.3. Synthesis of (*R*)-Boc DMTA 2

4.3.1. (±)-5,5-Dimethyl-1,3-thiazolidine-4-carboxylic acid 8a. To a stirred suspension of (±)-penicillamine **7** (available from Sigma-Aldrich; 50 g, 0.34 mol) in EtOH (250

mL) was added 37% aqueous solution of formaldehyde (50 mL) dropwise. The mixture was stirred at room temperature for 21 h, during which the progress of the reaction was monitored by TLC [*n*-BuOH/AcOH/H_2O (7:2:1); ninhydrin; R_f = 0.27 for (±)-**7** (yellow), 0.32 for (±)-**8a** (red)]. The precipitated solids were collected by filtration, washed with EtOH (25 mL), and air-dried at an oven temperature of 50°C overnight to give (±)-**8a** (52.1 g, 96.4%) as white crystals: mp 195.2–196.2°C (lit.:[18] mp 194–196°C); IR ν_{max} (KBr) 2979, 2511, 1642, 1578, 1459, 1382, 1338, 1269, 1192, 1125, 1003, 902, 785, 686 cm^{-1}; ^1H NMR δ (D_2O) 1.25 (s, 3H), 1.50 (s, 3H), 3.81 (s, 1H), 4.25 (d, J = 10.4 Hz, 1H), 4.32 (d, J = 10.4 Hz, 1H).

4.3.2. Methyl (±)-5,5-dimethyl-1,3-thiazolidine-4-carboxylate 8b. A stirred suspension of (±)-**8a** (28.6 g, 0.18 mol) in MeOH (500 mL) was heated to reflux, and HCl gas bubbled into the refluxing mixture over a period of 4 h. The resulting clear solution was concentrated in vacuo. To the residue was added H_2O (100 mL), and the pH of the mixture was adjusted to 11.5 with solid K_2CO_3 (19 g). The mixture was extracted with MTBE (2×100 mL). The combined MTBE extracts were dried ($MgSO_4$), and concentrated in vacuo to give (±)-**8b** as white crystals (26.0 g, 84%): TLC [*n*-BuOH/AcOH/H_2O (7:2:1); ninhydrin] R_f = 0.76 (yellow); mp 39.7–40.7°C [lit.:[21] mp 36°C for the (*S*)-isomer]; IR ν_{max} (KBr) 3309, 2962, 1736, 1448, 1345, 1289, 1236, 1200, 1179, 1146, 1124, 1011, 932, 856, 797 cm^{-1}; ^1H NMR δ ($CDCl_3$) 1.23 (s, 3H), 1.67 (s, 3H), 3.10 (s, 1H), 3.52 (s, 1H), 3.78 (s, 3H), 4.23 (d, J = 10.0 Hz, 1H), 4.35 (d, J = 10.0 Hz, 1H).

4.3.3. (*R*)-3-*tert*-Butoxycarbonyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid 2. A stirred suspension of (±)-**8b** (10.5 g, 59.9 mmol) in distilled H_2O (20 mL) was heated to 60°C, and *K. oxytoca* hydrolase (available from Nagase ChemteX Corporation;[19] 60 mg) was added. The mixture was stirred with heating at 60°C for 26 h, during which the progress of the reaction was monitored by HPLC using one of two methods.

Method 1: [Sumichiral OA-5000 (Sumika), 25 cm×4.6 mm ϕ; 2 mM solution of $CuSO_4$ in H_2O/MeCN (85:15); 1.0 mL/min; 25°C; UV (254 nm); an aliquot (0.05–0.1 mL) of the reaction mixture was diluted with MeOH (0.1 mL) and distilled H_2O (0.8 mL), well agitated, and centrifuged to give a supernatant, 0.01 mL of which was injected; t_R = 12.1 min for (*R*)-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid **8a**, 20.7 min for (*S*)-**8a**[18]].

Method 2: [Chiralpak AD (Daicel), 25 cm×4.6 mm ϕ; *n*-hexane/*i*-PrOH/Et_2NH (75:25:0.3); 1.0 mL/min; 25°C; UV (254 nm); an aliquot (0.05–0.1 mL) of the reaction mixture was diluted with saturated aqueous K_2CO_3 solution (0.1 mL) and *n*-hexane (1.0 mL), agitated, and centrifuged to separate the *n*-hexane layer, 0.01 mL of which was injected; t_R = 6.0 min for methyl (*S*)-5,5-dimethyl-1,3-thiazolidine-4-carboxylate **8b**,[21] 7.0 min for (*R*)-**8b**.

When HPLC analysis showed that 34.1% of (±)-**8b** was consumed and that (*R*)-**8a** was produced with 97.3% ee, the mixture was basified to pH 11.5 with 48% aqueous NaOH solution (4.0 mL). The mixture was extracted with MTBE (3×20 mL). The aqueous layer was stirred with ice-cooling, and diluted with *i*-PrOH (20 mL). Boc_2O (5.0 g, 23 mmol) was added, and the mixture was stirred at room temperature for 4 h. The *i*-PrOH was evaporated off in vacuo, and the residue was ice-cooled and acidified to pH 3.0 with 2.0 M HCl aqueous solution (8.0 mL). The mixture was extracted with AcOEt (2×100 mL). The combined AcOEt extracts were washed with H_2O (2×10 mL), dried (Na_2SO_4), and concentrated in vacuo. The solid residue was recrystallized from *n*-heptane (16.0 mL) and AcOEt (4.0 mL). The precipitated solids were collected by filtration, washed with *n*-heptane (5.0 mL), and air-dried at an oven temperature of 50°C overnight to give **2** as white crystals (3.70 g, 23.6%): TLC [*n*-hexane/AcOEt/AcOH (5:5:0.5); ninhydrin] R_f = 0.40 (red); mp 109.4–110.4°C; $[\alpha]_D^{24} = -76.4$ (*c* 1.0, EtOH) {lit.:[22] $[\alpha]_D^{24} = -76.2$ (*c* 1.0, EtOH)}; IR ν_{max} (KBr) 3001, 2977, 1752, 1736, 1660, 1642, 1474, 1402, 1371, 1307, 1231, 1184, 1165, 1149, 1115, 887, 860, 773, 762, 662 cm^{-1}; ^1H NMR δ ($CDCl_3$) 1.43 (s, 3H), 1.52 (s, 9H), 1.61 (s, 3H), 4.21 and 4.35 (each s, total 1H), 4.62 (s, 1H), 4.69 (s, 1H), 8.11 (s, 1H); HRMS (70 eV; CI) m/z 261.1030 (M^+), calcd for $C_{11}H_{19}NO_4S$: 261.1035. Anal. found: C, 50.56; H, 7.09; N, 5.33; S, 12.21. Calcd for $C_{11}H_{19}NO_4S$: C, 50.56; H, 7.33; N, 5.36; S, 12.27%. The enantiomeric purity of (*R*)-**2** was determined to be 99.4% ee by the following method: To a stirred solution of (*R*)-**2** (30 mg) in MTBE (0.3 mL) was added a 10% solution of Me_3SiCHN_2 in *n*-hexane (available from Tokyo Kasei Kogyo Co.; 0.01 mL). The solution was agitated vigorously, and concentrated in vacuo to give (*R*)-**2′** as a white solid, which was dissolved in *i*-PrOH (0.2 mL). The solution was diluted with *n*-hexane (0.8 mL), and 0.02 mL of the solution was subjected to HPLC analysis [Chiralcel OD (Daicel), 25 cm×4.6 mm ϕ; *n*-hexane/*i*-PrOH (98:2); 1.0 mL/min; 25°C; UV (254 nm); t_R = 7.7 min for (*R*)-**2′** (99.7%), 13.3 min for the (*S*)-isomer (0.3%)].

4.3.4. Racemization of methyl (*S*)-5,5-dimethyl-1,3-thiazolidine-4-carboxylate 8b. The MTBE extracts from the spent reaction mixture that were obtained in Section 4.3.3 were washed with H_2O (10 mL), dried (Na_2SO_4), and concentrated in vacuo to give crude (*S*)-**8b** of 50.3% ee [Chiralpak AD (Daicel); *Method 2* described in Section 4.3.3], which was dissolved in PhMe (20 mL). To the solution was added 28% NaOMe solution in MeOH (0.7 mL) with stirring at room temperature. Stirring was continued at room temperature for 4 h, when the complete racemization was confirmed by the HPLC analysis [Chiralpak AD (Daicel); using *Method 2* described in Section 4.3.3]. The mixture was washed with H_2O (3×10 mL), and concentrated in vacuo. The solid residue was recrystallized from *n*-heptane (8.0 mL). The precipitated solids were collected by filtration, washed with ice-chilled *n*-heptane (2.0 mL), dried in vacuo at room temperature to give (±)-**8b** as white crystals [4.80 g, 46% overall yield from (±)-**8b**].

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