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IMPROVED ENANTIOSELECTIVE SYNTHESIS OF PROTECTED (3*S*,4*S*)-4-AMINO-3,5-DIHYDROXYPENTANOIC ACID (ADPA)

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An improved enantioselective synthesis of protected (3S,4S)-4-amino-3,5-dihydroxypentanoic acid (ADPA) from L-serine has been developed. Enantioselectivity is improved by replacing the methyl ester with the* tert*-butyl ester and using neutral magnesium salt of esters to give β-keto ester.

Keywords: ADPA; γ-amino-β-hydroxy acids; enantioselective synthesis

INTRODUCTION

The stereoselective synthesis of γ-amino-β-hydroxy acids has received increasing attention because of their biological activity as peptide mimics.[1] Statine (**1**), (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (Fig. 1), as the γ-amino-β-hydroxy acid[2] first discovered, is an important component of pepstatin, which is a natural aspartate protease inhibitor. Its different γ-side chain analogs have been developed as potent inhibitors of pepsin, penicilloepsin, and rennin.

Other γ-amino-β-hydroxy acids are also found in natural products. For example, (3*S*,4*S*)-4-amino-3,5-dihydroxypentanoic acid (ADPA, **2**), an important member in this family, has been found in cyclic depsipeptide thalassosiramides A and B,[3] which are the first secondary metabolites isolated from marine α-proteobacterium *Thalassospira* sp. The thalassosiramides bearing three γ-amino acids have novel structures and show immunosuppressive activity in an interleukin-5 inhibition assay ($IC_{50} = 5\,\mu M$ for thalassospiramide B). As a part of the total synthesis of thalassosiramides, we herein report the efficient enantioselective synthesis of (3*S*,4*S*)-4-amino-3,5-dihydroxypentanoic acid (ADPA).

RESULTS AND DISCUSSION

One of the simplest conceptual approaches to the statine family is the $NaBH_4$ reduction of γ-(*N,N*-dibenzylamino)-β-keto esters (Scheme 1), which are available

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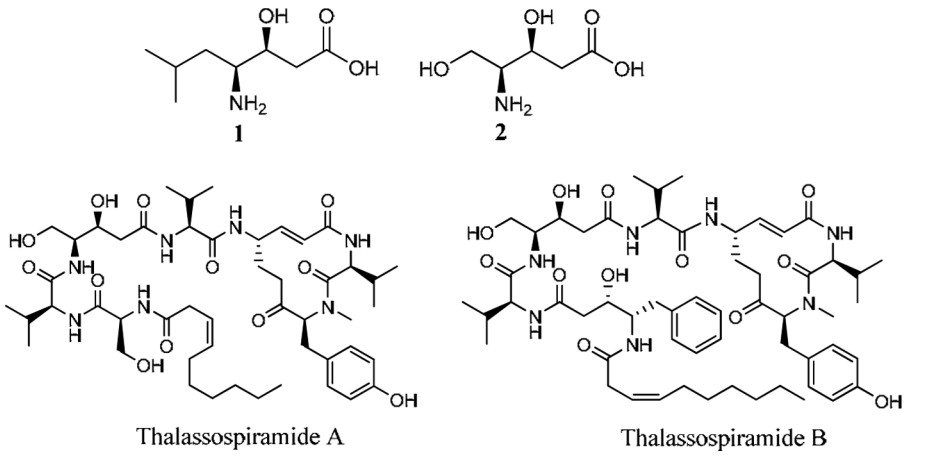
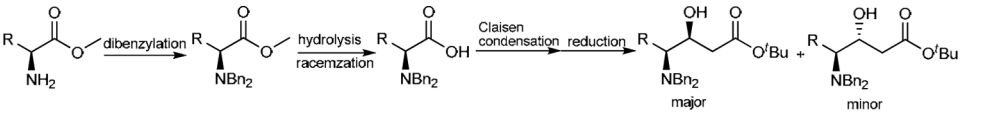
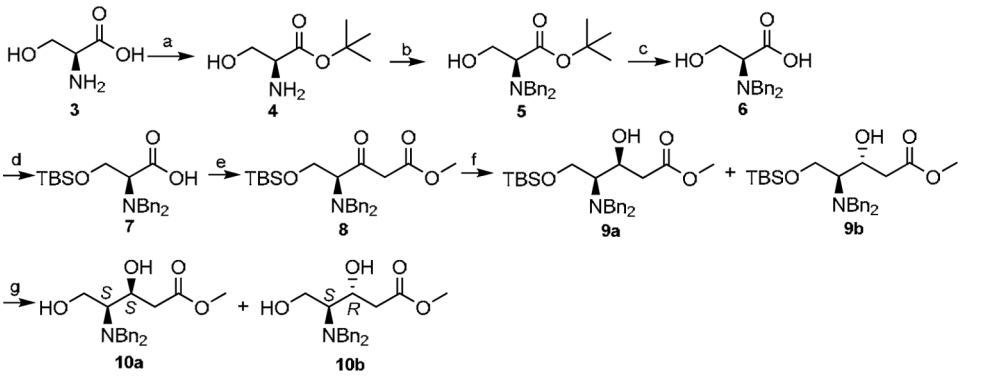


Figure 1. Structures of statine, ADPA, and thalassospiramides A and B.



Scheme 1. General synthetic route of γ-amino-β-hydroxy acid.

from α-amino acids, to yield *syn* diastereoisomers in good diastereomeric ratios.[4] The excellent diastereoselectivity is attributed to the Fekin–Ahn transition state for the bulky *N,N*-dibenzyl-protected compounds. The carboxylate group is generally masked with methyl ester first to avoid benzylating before the amino group is dibenzylated. After hydrolysis of methyl ester, the amino acid is converted to a more reactive acylating agent to give β-keto ester via Claisen condensation. However,



Scheme 2. (a) AcO^{t}Bu, $HClO_4$, rt, 65%; (b) BnBr, $NaHCO_3$, THF, DMSO, reflux, 92%; (c) 50% TFA/DCM, rt, quant.; (d) TBSCl, imidazole, DMAP, THF, rt, 84%; (e) CDI, THF, and then $MgCl_2$, $KO_2CCH_2CO_2Me$, THF, 60%; (f) $NaBH_4$, MeOH, −40 °C, 89%, *de* 81%; and (g) TBAF, THF, 0 °C, 75%. DMAP, 4-dimethylamino pyridine; TBAF, tetrabutyl ammonium fluoride; and CDI, carbonyldiimidazole.

different degrees of racemization may occur when cleaving the methyl ester.[4] For the amino acid ADPA, it was reported that the saponification of methyl ester caused significant racemization for Bn_2-Ser(TBDPS)-OMe.[5] What is worse, the *O*-benzyl derivative was racemized completely in this step.[5] We turned to another method reported by Hoffman[4] to cleave the methyl ester. We treated the methyl ester with LiI/NaCN in refluxed pyridine, however, the amino acid was also racemized completely. It is necessary to develop a racemation-free route for (3*S*,4*S*)-4-amino-3, 5-dihydroxypentanoic acid.

Our improved synthetic route is displayed as Scheme 2. Treatment of L-serine with *tert*-butyl acetate with the catalysis of 0.2 eq of $HClO_4$ gave its *tert*-butyl ester **4** in 65% yield. The traditional methyl ester was replaced by *tert*-butyl ester to avoid the strong basic conditions of hydrolysis, and the *tert*-butyl ester could be removed by 50% trifluoroacetic acid/dichloromethane (TFA/DCM) that may not cause racemization. Notably, the quantity of catalyst must be controlled, or the hydroxyl group and the *tert*-butyl ether will be protected simultaneously. The free amine in Ser-O^{t}Bu was dibenzylated with BnBr and $NaHCO_3$ in tetrahydrofuran/dimethylsulfoxide (THF/DMSO) under reflux in 92% yield. The *tert*-butyl ester of **5** was removed by 50% TFA/DCM quantitatively. As expected, no significant racemization was detected by chiral high-performance liquid chromatography (HPLC) in this step (>98% *ee*). Compound **6** was converted to serine by hydrogenolysis in MeOH, and its enantiomeric excess was detected by chiral HPLC.

The primary hydroxyl group of **6** was protected as tert-butyldimethylsilyl (TBS) ether **7** in 84% yield. Next was the Claisen condensation. Lithium diisopropylamide (LDA), which is used to produce lithio *tert*-butyl acetate of Claisen condensation, is a strong base that may lead to racemization. We adopted a milder condition developed by Brooks et al.[6] to get γ-(*N,N*-dibenzylamino)-β-keto esters. $MgCl_2$ and $KO_2CCH_2CO_2Me$ were refluxed in THF for 5 h to be activated as K-Mg-enolate, and at the same time acid **7** was activated to the imidazolide. Then the activated amino acid was added dropwise to the K-Mg-enolate suspension at 0 °C, and the mixture was stirred for 16 h at rt, giving β-keto ester **8** in 60% yield. Stereoselective reduction of **8** with $NaBH_4$ in methanol at −40 °C gave **9a** and **9b** as a 9.5:1 diastereomeric mixture in 89% yield (81% *de*), but they were only separable by flash chromatography after desilylation with TBAF in THF to give **10a** and **10b** in the total yield of 75%. The physical data of pure **10a** was matched excellently to the literature. No detectable racemization was found in these steps.[7]

CONCLUSION

In conclusion, an improved enantioselective synthesis of protected ADPA derivativing from L-serine has been developed. Enantiospectivity is improved by replacing the methyl ester with the *tert*-butyl ester and using neutral magnesium salt of esters to give β-keto ester. No detectable racemization was found in these steps.

EXPERIMENTAL

Solvents were purified in a conventional manner. Thin-layer chromatography (TLC) was performed on precoated E. Merck silica-gel 60 F254 plates. Flash-column

chromatography was performed on silica gel (200–300 mesh, Qingdao, China). Optical rotations were determined with a Perkin-Elmer model 241 MC polarimeter. ^{1}H NMR and ^{13}C NMR spectra were taken on a Jeol JNM-ECP 600 spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are recorded in parts per million (ppm).

L-Serine *tert*-Butyl Ester (4)

$HClO_4$ (170 μL, 2.0 mmol) was added dropwise to a solution of L-serine (1.05 g, 10.0 mmol) in *tert*-butyl acetate (20 mL) at 0 °C. The reaction mixture was stirred at room temperature for 12 h and then washed with H_2O (2 × 10 mL) and 1 N HCl (2 × 10 mL). The resultant aqueous solution was adjusted to pH 9 by addition of 10% K_2CO_3 solution and then extracted with DCM (3 × 100 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated to give a yellow oil (1.04 g, 65.0%), which was directly used in the next step without further purification.

N,N-Dibenzylamino-L-serine *tert*-Butyl Ester (5)

Benzyl bromide (2.2 mL, 19.5 mmol) and $NaHCO_3$ (2.73 g, 32.5 mmol) were added to a solution of **4** (1.04 g, 6.5 mmol) in a mixture of THF (12 mL) and DMSO (3 mL). The reaction mixture was heated to reflux for 15 h, cooled to room temperature, and diluted with H_2O (100 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (eluent: heptane/EtOAc = 10/1 to 6/1) afforded **5** as a yellow oil (2.04 g, 92.0%). $[\alpha]_D^{20} = -5.8$ ($c = 0.2$, $CHCl_3$); IR (KBr): 3325, 1659, 1442, 1005, 745, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.21–7.41 (m, 5H, Ar-H), 3.96 (d, 2H, $J = 13.7$ Hz, NCH_2Ph), 3.72 (dd, 1H, $J = 8.8$, 7.1 Hz, CH_aH_bOH), 3.70 (d, 2H, $J = 13.7$ Hz, NCH_2Ph), 3.57 (dd, 1H, $J = 8.8$, 5.5 Hz, CH_aH_bOH), 3.44 (dd, 1H, $J = 7.1$, 6.0 Hz, $CHNBn_2$), 1.53 [s, 9H, $OC(CH_3)_3$]; HR ESI-MS calcd. $[M + H]^+$ 342.2003; found 342.1999.

N,N-Dibenzylamino-L-serine (6)

TFA (5.0 mL, 67.3 mmol) was added dropwise to a solution of **5** (2.00 g, 5.9 mmol) in DCM (5 mL) at 0 °C and stirred 2 h at 0 °C. The reaction was monitored by TLC, and after the starting material had disappeared, the reaction mixture was concentrated twice to give **6** as white crystals (2.04 g, 92.0%). $[\alpha]_D^{20} = -103.1$ ($c = 1.0$, CH_3OH), lit.[8] $[\alpha]_D^{20} = -79.0$ (2% CH_3OH); IR (KBr): 3421, 3196, 1617, 1355, 1050, 758, 700 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 7.19–7.36 (m, 10H, Ar-H), 4.18 (brs, 1H, OH), 3.80 (d, 2H, $J = 14.4$ Hz, NCH_2Ph), 3.77 (dd, 1H, $J = 11.0$, 7.7 Hz, CH_aH_bOTBS), 3.65 (d, 2H, $J = 14.3$ Hz, NCH_2Ph), 3.62 (dd, 1H, $J = 11.0$, 6.6 Hz, CH_aH_bOTBS), 3.24 (dd, $J = 7.7$, 6.6 Hz, $CHNBn_2$); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 140.4, 128.9, 128.7, 127.3, 63.3, 61.0, 55.2; HR ESI-MS calcd. $[M - H]^+$, 284.1366, found 284.1362.

(*S*)-2-(*N,N*-Dibenzylamino)-3-(*tert*-butyl-dimethylsiloxy)-propanoic Acid (7)

Imidazole (506.7 mg, 7.5 mmol) was added to a solution of **6** (1.65 g, 5.8 mmol) in THF (12 mL) under argon at 0 °C and stirred 0.5 h. Then *tert*-butyldimethylsilyl chloride (1.05 g, 7.0 mmol) was added. The reaction mixture was stirred at room temperature for 3 h (100 mL) and quenched by addition of H_2O (100 mL). The aqueous phase was extracted with EtOAc (3×100 mL). The combined organic phases were washed with 1 N HCl (2×20 mL) and brine (2×20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (eluent: chloroform/methanol = 100/1 to 40/1) afforded **7** as a yellow oil (1.95 g, 84.0%). $[\alpha]_D^{20} = -52.6$ ($c = 0.2$, CH_3OH), lit.[9] $[\alpha]_D^{20} = -50.85$ ($c = 0.35$, $CHCl_3$); IR (KBr): 3328, 3149, 1563, 1066, 834, 662 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ: 7.31–7.38 (m, 10H, Ar-H), 4.32 (dd, 1H, $J = 11.5$, 5.0 Hz, CH_aH_bOTBS), 3.75 (dd, 1H, $J = 11.5$, 8.7 Hz, CH_aH_bOTBS), 4.11 (d, 2H, $J = 11.0$ Hz, NCH_2Ph), 3.98 (d, 2H, $J = 11.0$ Hz, NCH_2Ph), 3.77 (dd, $J = 9.2$, 5.5 Hz, $CHNBn_2$), 0.96 [s, 9H, $(CH_3)_3CSi$], 0.14 (s, 3H, CH_3Si), 0.13 (s, 3H, CH_3Si); HR ESI-MS calcd. $[M - H]^+$; 398.2151; found 398.2148.

(*S*)-4-(*N,N*-Dibenzylamino)-3-oxo-5-(*tert*-butyl-dimethylsiloxy)-pentanoic Acid Methyl Ester (8)

$MgCl_2$ (730.8 mg, 7.7 mmol) was suspended in THF (40 mL), $KO_2CCH_2CO_2Me$ (1.39 g, 8.3 mmol) was added in one portion, and the resulting suspension heated to reflux for 5 h. The suspension was then cooled with an ice bath. In the meantime, **7** (1.90 g, 4.8 mmol) was dissolved in THF (10 mL). The solution was cooled in an ice bath, and CDI (843.4 mg, 5.2 mmol) was added in portions. The solution was stirred 1 h at 0 °C and 1 h at rt. This solution containing the activated amino acid was then added dropwise to the K-Mg-enolate suspension via addition funnel at 0 °C. The resulting suspension was stirred for 16 h at room temperature. The solvent was evaporated, the residue was taken up in EtOAc (50 mL), and 1 N HCl (50 mL) solution was added. The mixture was stirred for 10 min, resulting in a yellowish solution. The layers were separated, and the aqueous layer was extracted with EtOAc (3×100 mL). The combined organic layers were then washed with 1 N HCl (2×20 mL), 10% $NaHCO_3$ solution (2×20 mL), and brine (2×20 mL). The organic layer was dried over Na_2SO_4 and filtered, and the solvent was evaporated. Flash chromatography on silica gel (eluent: heptane/EtOAc = 80/1 to 20/1) afforded **8** as a yellow oil (1.95 g, 84.0%). $[\alpha]_D^{20} = -10.3$ ($c = 0.2$, $CHCl_3$); IR (KBr): 2932, 1742, 1455, 1140, 837, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ: 7.24–7.52 (m, 10H, Ar-H), 4.11 (dd, 1H, $J = 10.5$, 3.2 Hz, CH_aH_bOTBS), 4.03 (dd, 1H, $J = 10.5$, 5.1 Hz, CH_aH_bOTBS), 3.84 (d, 2H, $J = 13.5$ Hz, NCH_2Ph), 3.80 (d, 2H, $J = 13.5$ Hz, NCH_2Ph), 3.62 (s, 3H, OCH_3), 3.58 (dd, 1H, $J = 5.5$, 3.2 Hz, $CHNBn_2$), 3.51 (d, 1H, $J = 16.0$ Hz, CH_aH_bCO), 3.44 (d, 1H, $J = 16.0$ Hz, CH_aH_bCO), 0.92 [s, 9H, $(CH_3)_3CSi$], 0.10 (s, 3H, CH_3Si), 0.09 (s, 3H, CH_3Si); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 203.6, 166.7, 129.1, 128.4, 127.2, 81.5, 66.9, 60.3, 55.2, 48.2, 25.8, −3.4, −4.4; HR ESI-MS calcd. $[M + H]^+$, 456.2493; found 456.2491.

(3*S*,4*S*)- and (3*R*,4*S*)-4-(*N*,*N*-Dibenzylamino)-3-hydroxy-5-(*tert*-butyl-dimethyl siloxy)-pentanoic Acid Methyl Ester (9a and 9b)

Compound **8** (1.30 g, 2.9 mmol) in methanol (50 mL) was cooled to −40 °C and treated with $NaBH_4$ (220.4 mg, 5.8 mmol). The reaction was monitored by TLC. After 3 h, the solution was quenched with H_2O (100 mL) at pH, 5–6 (adjusted by 1 N HCl solution), extracted with ethyl ether (3 × 100 mL), washed with brine (3 × 20 mL), dried over $MgSO_4$, and filtered, and the solvent was evaporated. Flash chromatography on silica gel (eluent: heptane/EtOAc = 80/1 to 15/1) afforded compounds **9a** and **9b** (1.18 g, 89.0%, 81% *de* based on ^{1}H NMR) as a yellow oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: **9a**: 7.22–7.31 (m, 10H, Ar-H), 4.13–4.16 (m, 1H, CHOH), 4.01 (d, 2H, J = 13.2 Hz, NCH_2Ph), 3.90 (dd, 1H, J = 10.5, 5.0 Hz, CH_aH_bOTBS), 3.88 (dd, 1H, J = 10.5, 5.5 Hz, CH_aH_bOTBS), 3.65 (s, 3H, OCH_3), 3.58 (d, 2H, J = 13.2 Hz, NCH_2Ph), 2.64–2.67 (m, 1H, Bn_2NCH), 2.50 (dd, 1H, J = 14.9, 2.8 Hz, CH_aH_bCO), 2.31 (dd, 1H, J = 14.9, 8.8 Hz, CH_aH_bCO), 0.94 [s, 9H, $SiC(CH_3)_3$], 0.12 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$); **9b**: 7.22–7.32 (m, 10H, Ar-H), 4.28–4.31 (m, 1H, CHOH), 4.10 (dd, 1H, J = 10.5, 5.0 Hz, CH_aH_bOTBS), 4.03 (dd, 1H, J = 10.5, 5.5 Hz, CH_aH_bOTBS), 3.88 (d, 2H, J = 13.7 Hz, NCH_2Ph), 3.66 (s, 3H, OCH_3), 3.62 (d, 2H, J = 13.2 Hz, NCH_2Ph), 3.31 (d, 1H, J = 4.4 Hz, CHOH), 2.95 (dd, 1H, J = 16.0, 2.8 Hz, CH_aH_bCO), 2.64–2.67 (m, 1H, Bn_2NCH), 2.26 (dd, 1H, J = 16.0, 9.4 Hz, CH_aH_bCO), 0.94 [s, 9H, $(CH_3)_3CSi$], 0.13 (s, 3H, CH_3Si), 0.12 (s, 3H, CH_3Si); HR ESI-MS calcd. $[M + H]^+$; 458.2650; found 458.2650.

(3*S*,4*S*)-4-(*N*,*N*-Dibenzylamino)-3,5-dihydroxypentanoic Acid Methyl Ester (10a)

A solution of tetrabutylammonium fluoride (576.8 mg, 2.2 mmol) in THF (10 mL) was slowly added to a solution of **9a** and **9b** (1.00 g, 2.2 mmol) in THF (5 mL) at 0 °C. The mixture was stirred for 2 h at 0 °C and quenched by addition of water (100 mL). The aqueous phase was extracted with ethyl ether (3 × 50 mL), and the combined organic extracts were washed with brine (3 × 20 mL), dried over $MgSO_4$, concentrated, and chromatographed on silica gel (eluent: heptane/EtOAc = 6/1 to 1/1) to afford compound **10a** (512.7 mg, 75%) as a colorless oil. $[\alpha]_D^{20} = -5.9$ (c = 1.0, $CHCl_3$); IR (KBr): 3265, 2983, 1684, 1368, 1125, 754, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.24–7.33 (m, 10H, Ar-H), 4.22–4.26 (m, 1H, CHOH), 4.12 (d, 2H, J = 13.2 Hz, NCH_2Ph), 3.80 (d, 2H, J = 13.2 Hz, NCH_2Ph), 3.72 (dd, 1H, J = 10.4, 4.8 Hz, CH_aH_bOH), 3.88 (dd, 1H, J = 10.4, 6.8 Hz, CH_aH_bOH), 3.62 (s, 3H, OCH_3), 2.71–2.76 (m, 1H, Bn_2NCH), 2.41 (dd, 1H, J = 15.4, 3.3 Hz, CH_aH_bCO), 2.36 (dd, 1H, J = 15.4, 8.8 Hz, CH_aH_bCO); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 172.3, 129.3, 128.5, 127.4, 67.7, 66.8, 62.9, 58.3, 54.7, 40.1; HR ESI-MS calcd. $[M + H]^+$, 344.1785; found 344.1784.

REFERENCES

1. Gante, J. Peptidomimetics-tailored enzyme inhibitors. *Angew Chem., Int. Ed. Engl.* **1994**, *33*, 1699–1720.
2. Umezawa, H.; Aoyagi, T.; Morishima, H.; Matauzaki, H.; Hamada, M.; Takeuchi, T. Pepstatin, a new pepsin inhibitors produced by actinomycetes. *J. Antibiot.* **1970**, *23*, 259–260.

3. Oh, D.; Strangman, W. K.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. Thalassosiramides A and B, immunosuppressive peptides from the marine bacterium. *Thalassospira* sp. *Org. Lett.* **2007**, *9*, 1525–1528.
4. Hoffman, R. V.; Tao, J. An improved enantiospecific synthesis of statine and statine analogs via 4-(*N,N*-dibenzylamino)-3-keto esters. *J. Org. Chem.* **1997**, *62*, 2292–2297.
5. Hulme, A. N.; Curley, K. S. Approaches to the synthesis of (2*R*,3*S*)-2-hydroxymethylpyrrolidin-3-ol (CYB-3) and its C(3) epimer: A cautionary tale. *J. Chem. Soc., Perkin Trans. 1* **2002**, *1*, 1083–1091.
6. Brooks, D. W.; Lu, D. L.; Masamune, S. *C*-Acylation under virtually neutral conditions. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 72–74.
7. The methyl ester of **10a** was converted to *tert*-butyl ester, $[\alpha]_D^{20} = -4.4$ (c = 1.0, $CDCl_3$), lit. $[\alpha]_D^{20} = -3.8$ (c = 1.0, $CDCl_3$). See Andres, J. M.; Pedrosa, R.; Pérez, A.; Pérez-Encabo, A. Diastereoselective synthesis of enantiopure γ-amino-β-hydroxy acids by Reformatsky reaction of chiral α-dibenzylamino aldehydes. *Tetrahedron* **2001**, *57*, 8521–8530.
8. Amiard, G.; Heymes, R.; Velluz, L. *N,N*-Dibenzylamino acids. Fr. Patent 66245 19560605, 1956.
9. Chandrasekhar, S.; Reddy, C. R.; Rao, R. J. Facile and selective cleavage of allyl ethers, amines, and esters using polymethylhydrosiloxane-$ZnCl_2$/$Pd(PPh_3)_4$. *Tetrahedron* **2001**, *57*, 3435–3438.