An Operationally Simple and Efficient Synthesis of Orthogonally Protected L-*threo*-β-Hydroxyasparagine

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Abstract: A synthesis of orthogonally protected L-*threo*-β-hydroxyasparagine from L-aspartic acid is reported. Iodocyclization of 3-benzoylaminoaspartic acid provided an intermediate oxazoline dicarboxylate that was efficiently hydrolyzed to L-*threo*-β-hydroxyaspartic acid. The synthetic route for conversion of the free β-hydroxy-α-amino acid into the target compound is highly efficient and amenable to the preparation of various orthogonally protected asparagine derivatives, on a multigram scale.

Key words: asymmetric synthesis, amino acids, amides, peptides, protecting groups

Lysobactin (katanosin B) is a cyclic depsipeptide antibiotic that was isolated by the Shionogi Research Laboratories from a producing organism related to the genus *Cytophaga*.[1,2] Lysobactin was also isolated from a species of *Lysobacter* by workers at the Squibb Institute.[3–5] In a recent evaluation, lysobactin displayed very good antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), as well as vancomycin-resistant enterococci, with minimum inhibitory concentrations (MIC's) ranging from 0.39–0.78 mg/mL.[6] Given this promising antibacterial activity, lysobactin has emerged as an attractive target for chemical synthesis.

During the course of our ongoing investigation into the synthesis of lysobactin (**1**), and structurally related depsipeptide antibiotics, we required a dependable and efficient synthetic route to the nonproteinogenic amino acid L-*threo*-β-OH-Asn (Figure 1). The isomers of β-OH-Asn were originally isolated from human urine or synthesized as a racemate that was separated via resolution.[7,8] Several synthetic procedures have been published for the enantioselective synthesis of the isomers of β-hydroxyaspartic acid[9–14] and *erythro*-β-hydroxyasparagine.[15] In contrast, there are few descriptions of an asymmetric synthesis of *threo*-β-hydroxyasparagine.[16,17] Boger et al. reported a procedure that relies upon the Sharpless asymmetric aminohydroxylation reaction of (*E*)-4-methoxycinnamate, where the methoxy-substituted aromatic ring serves as masking group for the side chain carboxyl group.[16] Lectka also reported a procedure involving ring-opening of appropriately derivatized β-lactams deriving from a cinchona alkaloid catalyzed asymmetric [2+2] cycloaddition reaction of ketenes and imines.[17] We sought to develop a synthetic route to *threo*-hydroxyasparagine derivatives that would take advantage of readily available members of the chiral pool. Herein, we describe an efficient alternative asymmetric synthesis of orthogonally protected L-*threo*-FmocNH-β-OH-Asn(Tr)-OH (**2**) for use in our synthesis of lysobactin.

Our synthetic route (Scheme 1) relied upon the highly stereoselective conversion of L-aspartic acid to L-*threo*-β-hydroxyaspartic acid (**3**), as reported by Cardillo.[9] A two-step sequence was used to convert **3** into Boc-protected

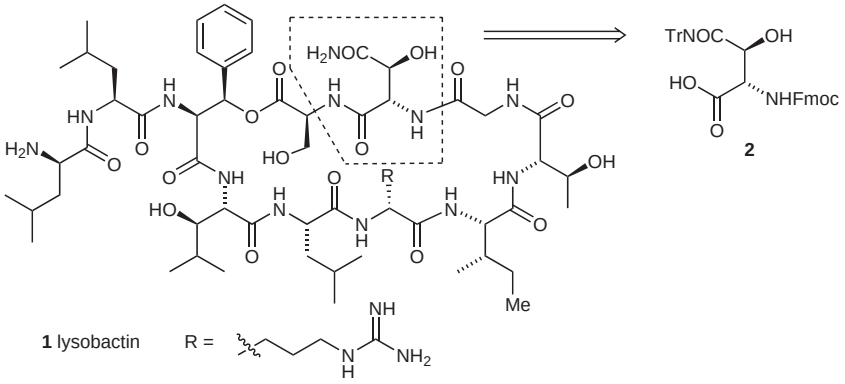

Figure 1 Structure of lysobactin

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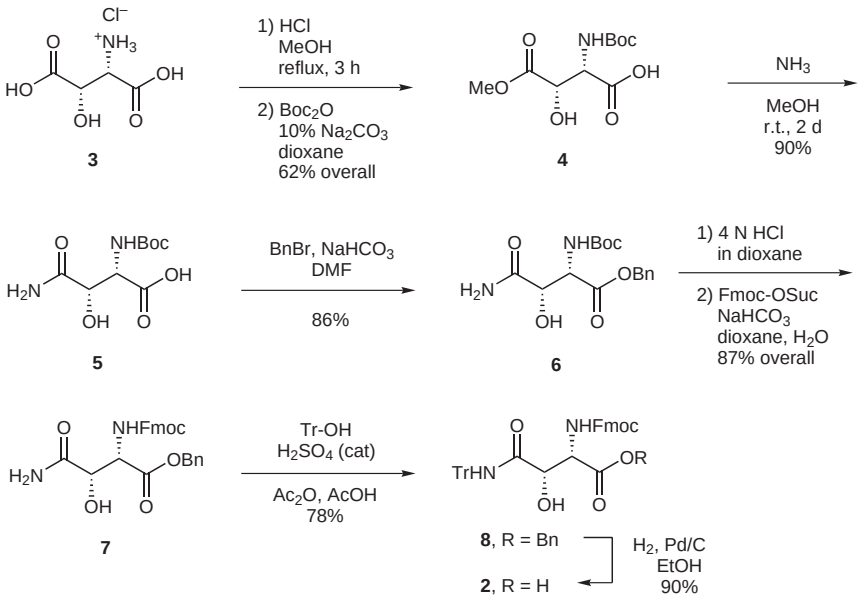

Scheme 1 Synthesis of orthogonally protected β-hydroxyasparagine

monoester **4**. Exposure to acidic methanol at reflux, to provide the side chain monoester,[18,19] followed by Boc-protection of the free amino group provided **4** in 62% overall yield. Direct aminolysis of methyl ester **4** (NH_3, MeOH, r.t., 48 h, 90%) provided carboxamide **5**. The free carboxyl group was efficiently protected as its benzyl ester (BnBr, $NaHCO_3$, DMF) and provided **6** in 86% yield. At this juncture, the protecting group scheme we envisaged for our total synthesis of lysobactin required exchange of the amine protective groups. Thus, removal of the Boc group (4 N HCl, dioxane) and reprotection of the free amino group (Fmoc-OSu, $NaHCO_3$, dioxane–H_2O) as its Fmoc carbamate provided the Fmoc-protected benzyl ester **7** in 87% overall yield.

Unprotected carboxamide groups of asparagine residues are susceptible to various side reactions during peptide coupling, for example, dehydration to nitriles and intramolecular cyclization onto activated carboxyl groups to provide succinimide by-products. In an effort to avoid these unwanted side reactions during the course of our synthetic effort toward lysobactin, a trityl protecting group was introduced onto the side chain carboxamide. The trityl group was readily installed (TrOH, cat. H_2SO_4, Ac_2O, AcOH, 78%) using the Boger modification[16] of the protocol initially reported by Sieber and co-workers.[20] Finally, careful hydrogenolysis of benzyl ester **8** (H_2, Pd/C, EtOH, 90%) provided the target compound L-*threo*-Fmoc-β-OH-Asn(Tr)-OH.[21]

In summary, we have accomplished an asymmetric synthesis of an orthogonally protected version of L-*threo*-β-hydroxyasparagine. The synthetic route utilizes a precursor that is readily available from the chiral pool. The synthetic sequence is sufficiently flexible such that other protecting group schemes could be utilized. The reaction sequence is also quite efficient and provides the possibility for the synthesis of these valuable building blocks on a multigram scale. The protecting group scheme employed for the synthesis of **2** was selected in order to maximize its utility in our synthetic efforts directed toward lysobactin. Efforts toward the synthesis of lysobactin, and related depsipeptide antibiotics, are in progress and will be presented in due course.

Acknowledgment

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(21) **L-*threo*-BocNH-β-OH-Asp(OMe)-OH (4)**: L-*threo*-β-Hydroxyaspartic acid (1.00 g, 5.41 mmol) was dissolved in a solution of concentrated HCl (0.89 mL, 10.8 mmol) and MeOH (18.0 mL) at 0 °C. The reaction was heated at reflux for 3 h, then cooled to r.t., and concentrated in vacuo. The crude L-*threo*-β-OH-Asp(OMe)-OH was triturated with Et_2O, collected by filtration, and used without further purification in the following reaction. An aqueous solution of 10% Na_2CO_3 (18.0 mL) was added to L-*threo*-β-OH-Asp(OMe)-OH and the resulting mixture was cooled in an iced bath. A solution of Boc_2O (3.65 g, 16.2 mmol) in dioxane (18.0 mL) was added dropwise to the reaction mixture and the resulting solution was stirred overnight at r.t. After concentration by rotary evaporation, the residue was dissolved in EtOAc (150 mL) and washed with 1 N HCl solution (3 × 100 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 35% EtOAc–hexanes followed by 10–25% MeOH–$CHCl_3$) provided **4** (0.88 g, 62%) as a foamy white solid; mp 109–110 °C; $[\alpha]^{23}_D$ +22 (*c* = 1.00, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ = 4.79 (s, 1 H), 4.50 (s, 1 H), 3.77 (s, 3 H), 1.46 (s, 9 H). ^{13}C NMR (125 MHz, CD_3OD): δ = 174.2, 170.2, 155.1, 80.4, 73.0, 59.0, 52.8, 28.7. IR (film): 3388, 2981, 2934, 2858, 1739, 1699, 1608, 1512, 1393, 1367, 1251, 1167, 1107, 1061 cm^{-1}. HR–EI–MS: *m*/*z* [M$^+$] calcd for $C_{10}H_{17}O_7N$: 263.1000; found: 263.1001.

L-*threo*-BocNH-β-OH-Asn (5): A sample of L-*threo*-BocNH-β-OH-Asp(OMe)-OH (**4**; 1.30 g, 4.94 mmol) was dissolved in MeOH (16.5 mL) and treated with NH_3 gas until the solution became saturated. The resulting mixture was stirred at r.t. for 3 d. The reaction was concentrated by rotary evaporation and the crude material was purified by flash chromatography (SiO_2, 25% → 50% MeOH–$CHCl_3$), which provided **5** (1.10 g, 90%) as a crystalline solid; mp 165–166 °C; $[\alpha]^{23}_D$ –11 (*c* = 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 4.63 (s, 1 H), 4.48 (s, 1 H), 1.40 (s, 9 H). ^{13}C NMR (125 MHz, CD_3OD): δ = 177.8, 177.7, 157.6, 80.4, 73.6, 59.4, 28.8. IR (KBr): 3369, 2980, 2933, 1694, 1602, 1510, 1395, 1251, 1168, 1109, 1102, 1059, 1029 cm^{-1}. HR–EI–MS: *m*/*z* [M$^+$] calcd for $C_9H_{16}O_6N_2$: 249.1003; found: 249.1002.

L-*threo*-BocNH-β-OH-Asn-OBn (6): A solution of L-*threo*-BocNH-β-OH-Asn (**5**; 0.910 g, 3.67 mmol) in DMF (18.3 mL) at 0 °C was treated with $NaHCO_3$ (0.616 g, 7.33 mmol) and benzyl bromide (1.74 mL, 14.3 mmol). The resulting solution was stirred at 0 °C for 2 h and then for 24 h at r.t. The solution was cooled to 0 °C and H_2O (100 mL) was slowly added. The resulting mixture was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with H_2O (100 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography (SiO_2, 0% → 5% MeOH–$CHCl_3$), which provided **6** (1.06 g, 86%) as a crystalline solid; mp 135–136 °C; $[\alpha]^{23}_D$ –17 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.30–7.38 (m, 5 H), 5.19 (s, 2 H), 4.70 (d, *J* = 2.0 Hz, 1 H), 4.59 (d, *J* = 2.5 Hz, 1 H), 1.40 (s, 9 H). ^{13}C NMR (125 MHz, CD_3OD): δ = 176.2, 171.7, 157.8, 136.9, 129.3, 129.2, 129.0, 128.9, 80.8, 72.7, 68.2, 58.1, 28.7. IR (film): 3358, 2979, 1685, 1653, 1559 cm^{-1}. HR–EI–MS: *m*/*z* [M$^+$] calcd for $C_{16}H_{22}O_6N_2$: 338.1472; found: 338.1471.

L-*threo*-FmocNH-β-OH-Asn-OBn (7): L-*threo*-BocNH-β-OH-Asn-OBn (**6**; 0.740 g, 2.19 mmol) was dissolved in 4 N HCl–dioxane (16.4 mL) and stirred at r.t. for 2 h. The solution was concentrated by rotary evaporation and dried under high vacuum. The residue obtained was dissolved in 50% dioxane–H_2O (32.4 mL, 1:1) and the resulting solution was cooled to 0 °C. Fmoc-OSuc (1.14 g, 3.28 mmol) and $NaHCO_3$ (0.368 g, 4.37 mmol) were added to the solution and the resulting mixture was stirred at r.t. for 12 h. The reaction solution was diluted with EtOAc (100 mL) and sat. aq $NaHCO_3$ (150 mL). The aqueous layer was separated and extracted with EtOAc (2 × 75 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated by rotary evaporation. The crude product was dissolved in the minimal amount of 20% MeOH–$CHCl_3$ solution. Precipitation with hexanes and collection of the solid by filtration provided **7** as a white solid; mp 173–174 °C; $[\alpha]^{23}_D$ –18 (*c* = 0.80, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.78 (d, *J* = 7.2 Hz, 2 H), 7.64 (d, *J* = 6.0 Hz, 2 H), 7.26–7.38 (m, 9 H), 5.21 (d, *J* = 3.2 Hz, 2 H), 4.64 (d, *J* = 2.4 Hz, 1 H), 4.35 (dd, *J* = 3.6, 6.0 Hz, 1 H), 4.22 (m, 2 H). ^{13}C NMR (125 MHz, CD_3OD): δ = 176.4, 171.7, 158.8, 145.2, 145.1, 142.5, 142.4, 137.0, 129.5, 129.2, 129.1, 128.8, 128.7, 128.2, 128.1, 126.3, 126.2, 120.1, 72.8, 68.4, 68.3, 58.6, 48.2. IR (film): 3328, 1724, 1695, 1655, 1539, 1451, 1299, 1255, 1109 cm^{-1}. HR–EI–MS: *m*/*z* [M + H$^+$] calcd for $C_{26}H_{24}O_6N_2$: 460.1629; found: 460.1638.

L-*threo*-FmocNH-β-OH-Asn(Tr)-OBn (8): A solution of L-*threo*-FmocNH-β-OH-Asn-OBn (**7**; 0.863 g, 1.87 mmol) and Tr-OH (5.05 g, 18.7 mmol) in HOAc (6.52 mL) was heated to 50 °C and treated with concd H_2SO_4 (65 μL, 1.12 mmol) and Ac_2O (0.443 mL, 4.69 mmol). The resulting mixture was stirred at 50 °C for 2.5 h. After cooling to r.t., the reaction solution was diluted with EtOAc (100 mL) and sat. aq $NaHCO_3$ (150 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography (SiO_2, 15% → 30% EtOAc–hexanes) provided **8** (1.03g, 1.46 mmol, 78%) as a white solid; mp 74–75 °C; $[\alpha]^{23}_D$ –14 (*c* = 0.93, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.79 (d, *J* = 3.2 Hz, 1 H), 7.77 (d, *J* = 3.2 Hz, 1 H), 7.67 (d, *J* = 7.6 Hz, 1 H), 7.62 (d, *J* = 7.2 Hz, 1 H), 7.17–7.38 (m, 24 H), 5.20 (s, 2 H), 4.81 (d, *J* = 2.0 Hz, 1 H), 4.65 (d, *J* = 2..0 Hz, 1 H), 4.51 (dd, *J* = 4.0, 9.6 Hz, 1 H), 4.17 (m, 2 H), ^{13}C NMR (125 MHz, $CHCl_3$): δ = 172.2, 171.8, 158.9, 145.7, 145.3, 145.0, 142.5, 142.4, 137.1, 129.9, 129.7, 129.5, 129.2, 129.0, 128.9, 128.8, 128.7, 128.2, 128.1, 126.5, 126.2, 120.9, 73.4, 71.5, 68.6, 68.3, 58.6, 48.2. IR (film): 3347, 3061, 3022, 2915, 2522, 1723, 1709, 1670, 1493, 1451, 1333, 1218 cm^{-1}. HR–EI–MS: *m*/*z* [M$^+$] calcd for $C_{45}H_{38}O_6N_2$: 702.2724; found: 702.2721.

L-*threo*-FmocNH-β-OH-Asn(Tr)-OH (2): Pd/C (0.086 g) was cautiously added to a solution of L-*threo*-FmocNH-β-OH-Asn(Tr)-OBn (**8**; 0.860 g 1.22 mmol) in EtOH (12.2 mL). The solution was placed under an atmosphere of hydrogen gas for 1 h. The catalyst was removed by filtration through Celite and the filter cake was washed with EtOH.

The combined filtrates were concentrated in vacuo and purified by flash chromatography (SiO_2, 25% → 50% MeOH–$CHCl_3$), which provided **2** (0.674g, 90%) as a white solid; mp 189–190 °C (dec.); $[\alpha]^{23}_D$ –17 [*c* = 1.00, MeOH–$CHCl_3$ (1:1)]. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.29 (s, 1 H), 7.88 (d, *J* = 7.5 Hz, 2 H), 7.76 (d, *J* = 6.5 Hz, 1 H), 7.65 (d, *J* = 7.0 Hz, 1 H), 7.38 (q, *J* = 5.0, 7.0 Hz, 2 H), 7.08–7.27 (m, 18 H), 4.52 (d, *J* = 12 Hz, 1 H), 4.35 (m, 3 H), 4.17 (t, *J* = 6.5 Hz, 1 H). ^{13}C NMR (DMSO, 125 MHz): δ = 173.8, 171.0, 156.1, 144.7, 143.8, 143.7, 140.7, 128.4, 127.7, 127.1, 126.7, 125.3, 120.1, 72.8, 69.1, 65.8, 57.7, 46.7. IR (KBr): 3387, 3062, 2926, 2857, 1954, 1695, 1601, 1510, 1448, 1404, 1328, 1248, 1106, 1058 cm^{-1}. HR–EI–MS: *m*/*z* [M^+] calcd for $C_{38}H_{32}O_6N_2$: 612.2255; found: 612.2256.