

Synthesis of All Nineteen Appropriately Protected Chiral α -Hydroxy Acid Equivalents of the α -Amino Acids for Boc Solid Phase Depsi-Peptide Synthesis

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Supporting Information

Materials and Methods. All common organic and inorganic chemicals were purchased from Aldrich Chemical or Fisher Scientific. Side-chain protected (L)- α -amino acids were purchased from Novabiochem or Advanced Chemtech. Methyl-(2*S*)-Glycidate and L- β -imidazolelactic acid were purchased from Aldrich. Anhydrous solvents were purchased from Fisher Scientific and were either used as received or further dried using a Dri-Solv solvent still. Column Chromatography was performed with 70-230 mesh silica gel (Fisher Scientific). Amberlite[®] IR-120 (plus) ion-exchange resin produced by Rohm and Haas Co. was purchased from Aldrich. Chiral HPLC (on a Chiracel OD-H column using hexane and isopropanol as eluents) was performed to determine optical purity of the products. The flow rate was 1 mL/min. A photodiode array detector (Water 996) was utilized. NMR spectra were acquired on a Bruker DMX 600 MHz or Bruker DMX 500 MHz spectrometer.

Diazotization Method A. Side chain-protected α -amino acids (1 mmol) were dissolved in 8:2 deionized water: acetic acid (v/v, 10 mL). Two mL (2 mmol) of an aqueous solution of sodium nitrite (2M) was added to a solution of amino acid slowly using a syringe pump over 15 min at 0 °C. The reaction was warmed to ambient temperature and stirred for 3 h. Once the

reaction was finished as shown by thin-layer chromatography (TLC) using ninhydrin as a stain, the reaction was quenched with 2 M methylamine solution in tetrahydrofuran (1 mL). For the more hydrophobic α -hydroxy acids, the tetrahydrofuran was removed under reduced pressure, the aqueous residue was acidified to pH 2.0, and then extracted with ethyl acetate (3 \times 50 mL). Alternatively, water soluble α -hydroxy acids were produced by acidification with 1N HCl and de-salted by ion exchange column chromatography on Amberlite[®] IR-120(plus) using deionized water as the eluent. The solvent (either ethyl acetate for the hydrophobic α -hydroxy acids or deionized water for the water soluble α -hydroxy acids) was evaporated and the residue was further purified (if necessary) by flash column chromatography on silica gel using 90:9:1 chloroform: methanol: acetic acid.

Diazotization Method B (used only for Arg(Tos) herein). Side chain-protected α -amino acids (1 mmol) were dissolved in glacial acetic acid (10 mL). Sodium nitrite (2 mmol, 138 mg) was added in five portions over 15 min at 0°C. The reaction was warmed to ambient temperature and stirred for 3 h. The reaction was quenched with 2 M methylamine solution in tetrahydrofuran (1 mL). The solvent was removed *in vacuo*. The residue was purified by flash column chromatography on silica gel using 90:9:1 chloroform: methanol: acetic acid.

(S)-2-Hydroxy-succinic acid 4-cyclohexyl ester or HO-Asp(OcHx)-OH (8). Compound **8** was synthesized from H-Asp(OcHx)-OH (**1**) using diazotization procedure A. HRMS (MALDI-FTMS): calcd for MNa⁺ 239.0890 found 239.0892. ¹H NMR (600 MHz, Acetone-d₆) δ 1.39-1.61 (m, 6H), δ 1.73-1.78 (m, 2H), δ 1.83-1.87 (m, 2H), δ 2.66 (dd, *J* = 7.6, 15.8 Hz, 1H), δ 2.80 (dd, *J* = 4.4, 15.8 Hz, 1H), δ 4.46 (dd, *J* = 4.4, 7.6 Hz, 1H), δ 4.75-4.81 (m, 1H) ¹³C NMR (600 MHz, Acetone-d₆) δ 24.7, 26.5, 32.5, 40.7, 68.9, 74.4, 162.3, 172.0 Chiral

HPLC of **8**-methyl ester on Chiracel OD-H using isocratic 10% isopropanol in hexane shows >99% ee (t_R = 8.78 min).

(S)-2-Hydroxy-pentanedioic acid 5-cyclohexyl ester or HO-Glu(OcHx)-OH (9).

Compound **9** was synthesized from H-Glu(OcHx)-OH (**2**) using diazotization procedure A. HRMS (MALDI-FTMS): calcd for MNa^+ 253.1046 found 253.1046. 1H NMR (600 MHz, Acetone- d_6) δ 1.29-1.48 (m, 6H), δ 1.79-1.83 (m, 2H), δ 1.86-1.92 (m, 2H), δ 1.93-2.06 (m, 1H), δ 2.11-2.23 (m, 1H), δ 2.45-2.52 (m, 2H), δ 4.21 (dd, J = 5.3, 9.7 Hz, 1H), δ 4.78-4.82 (m, 1H) ^{13}C NMR (600 MHz, Acetone- d_6) δ 24.7, 26.5, 30.6, 31.2, 32.6, 70.5, 74.0, 174.4, 177.4 Chiral HPLC of **9**-methyl ester on Chiracel OD-H using isocratic 10% isopropanol in hexane shows >99 % ee (t_R = 8.32 min).

(S)-6-(2-Chloro-benzyloxycarbonylamino)-2-hydroxy-hexanoic acid or HO-Lys(2-Cl-Z)-OH (10). Compound **10** was synthesized from H-Lys(2-Cl-Z)-OH (**3**) using diazotization procedure A. HRMS (MALDI-FTMS): calcd for MNa^+ 338.0766 found 338.0766. 1H NMR (600 MHz, Acetone- d_6) δ 1.47-1.67 (m, 5H), δ 1.79-1.81 (m, 1H), δ 3.17 (d, J = 6.1 Hz, 2H), δ 4.15 (dd, J = 4.4, 12.0 Hz, 1H), δ 5.14 (s, 2H), δ 6.48 (br. s, 1H), δ 7.34-7.49 (m, 4H) ^{13}C NMR (600 MHz, Acetone- d_6) δ 23.1, 34.7, 63.7, 70.8, 128.0, 130.2, 136.1, 139.0, 156.9, 159.2, 176.2, 211.4 Chiral HPLC of **10**-methyl ester on Chiracel OD-H using isocratic 10% isopropanol in hexane shows >99% ee (t_R = 27.43 min). Note that the shoulder in the chromatogram that appears after the peak for **10**-methyl ester is not due to the enantiomer because its UV spectrum does not match that of the product.

(S)-N^G-*p*-toluenesulfonyl-5-Guanidino-2-hydroxy-pentanoic acid or HO-Arg(Tos)-OH (11). Compound **11** was synthesized from H-Arg(Tos)-OH (**4**) using diazotization procedure B. After column chromatography, the acetyl group can be removed by base

hydrolysis using 1 M LiOH (2 equiv.) in 3:1:1 tetrahydrofuran: methanol: water. HRMS (MALDI-FTMS): calcd for MH⁺ 330.1205 found 330.1205. ¹H NMR (600 MHz, Methanol-d₄) δ 1.53-1.73 (m, 2H), δ 1.83-2.02 (m, 2H), δ 2.41 (s, 3H), δ 3.10-3.33 (m, 2H), δ 4.10 (t, J = 7.0 Hz, 1H), δ 7.31 (d, J = 8.3, 2H), δ 7.74 (d, J = 8.3, 2H) ¹³C NMR (600 MHz, Methanol-d₄) δ 21.4, 24.8, 30.1, 68.3, 71.0, 127.1, 130.5, 142.1, 144.1, 156.0, 177.6 Chiral HPLC of O-acetyl-**11**-methyl ester on Chiracel OD-H using isocratic 100% isopropanol shows 99% ee (t_R = 9.12 min).

(S)-3-Benzoyloxy-2-hydroxy-propionic acid or HO-Ser(Bzl)-OH (12). Compound **12** was synthesized from H-Ser(Bzl)-OH (**5**) using diazotization procedure A. HRMS (MALDI-FTMS): calcd for MNa⁺ 219.0628 found 219.0629. ¹H NMR (600 MHz, CDCl₃) δ 3.80 (dd, J = 3.3, 9.9 Hz, 1H), δ 3.83 (dd, J = 4.1, 9.9 Hz, 1H), δ 4.39 (dd, J = 3.3, 4.1 Hz, 1H), δ 5.14 (pair of d, J = 12.1 Hz, 2H), δ 7.31-7.39 (m, 5H) ¹³C NMR (600 MHz, Acetone-d₆) δ 70.8, 72.3, 73.1, 127.6, 127.7, 128.4, 138.8, 173.4 Chiral HPLC of **12**-methyl ester on Chiracel OD-H using isocratic 10% isopropanol in hexane shows >99% ee (t_R = 11.66 min).

(2S,3R)-3-Benzoyloxy-2-hydroxy-butyric acid or HO-Thr(Bzl)-OH (13). Compound **13** was synthesized from H-Thr(Bzl)-OH (**6**) using diazotization procedure A. HRMS (MALDI-FTMS): calcd for MH⁺ 211.1052 found 211.1053. ¹H NMR (600 MHz, DMSO-d₆) δ 1.29 (d, J = 6.1 Hz, 2H), δ 3.98 (dd, J = 2.6, 6.1 Hz, 1H), δ 4.10 (d, J = 2.6 Hz, 1H), δ 4.62 (pair of d, J = 11.8 Hz, 2H), δ 7.31-7.37 (m, 5H) ¹³C NMR (600 MHz, Acetone-d₆) δ 15.7, 71.5, 74.5, 76.6, 128.0, 128.2, 128.8, 139.8, 174.3 Chiral HPLC of **13**-methyl ester on Chiracel OD-H using isocratic 10% isopropanol in hexane shows 99% ee (t_R = 8.74 min).

(S)-3-[4-(2,6-Dichloro-benzyloxy)-phenyl]-2-hydroxy-propionic acid or HO-Tyr(2,6-Cl₂-Bzl)-OH (14). Compound **14** was synthesized from H-Tyr(2,6-Cl₂-Bzl)-OH (**7**) using

diazotization procedure A. HRMS (MALDI-FTMS): calcd for MNa^+ 363.0161 found 363.0160.

^1H NMR (600 MHz, Acetone- d_6) δ 2.74 (dd, J = 9.7, 16.7 Hz, 1H), δ 2.91 (dd, J = 5.7, 16.7 Hz, 1H), δ 4.11 (dd, J = 5.7, 9.7 Hz, 1H), δ 5.19 (s, 2H), δ 6.95 (d, J = 10.1 Hz, 2H), δ 7.17 (d, J = 10.1 Hz, 2H), δ 7.47 (t, J = 5.3 Hz, 1H), δ 7.56 (d, J = 5.3 Hz, 2H) ^{13}C NMR (600 MHz, Acetone- d_6) δ 40.4, 65.8, 72.2, 115.2, 129.6, 131.4, 131.6, 132.0, 133.3, 137.5, 158.6, 175.4
Chiral HPLC of **14**-methyl ester on Chiracel OD-H using gradient 5-10% isopropanol in hexane over 60 min shows 99% ee (t_{R} = 12.96 min). Note that the shoulder in the chromatogram that appears after the peak for **14**-methyl ester is not due to the enantiomer because its UV spectrum does not match that of the product.

(S)-3-[1-(2,4-Dinitro-phenyl)-1H-imidazol-4-yl]-2-hydroxy-propionic acid or HO-His(Dnp)-OH (17) (Method C). L- β -imidazolelactic acid, **15** (360 mg, 2.3 mmol), 2,6-dinitrofluorobenzene, **16** (428 mg, 2.3 mmol), and triethylamine (0.64 mL 4.6 mmol) were dissolve in acetonitrile (20 mL). The mixture was allowed to stir at room temperature in the dark for 16 h. Upon completion, acetonitrile was evaporated. The residue was redissolve in water. Excess 2,6-dinitrofluorobenzene was removed by washing the aqueous layer with hexane. The aqueous layer was lyophilized to afford **17** as triethylamine salt. HRMS (MALDI-FTMS): calcd for MH^+ 323.0622 found 323.0617. ^1H NMR (600 MHz, Acetone- d_6) δ 1.25 (t, J = 5.5, 9H), δ 2.77 (dd, J = 7.9, 14.9 Hz, 1H), δ 3.10 ($-\text{CH}_2-$ of Et_3N J = 5.5 Hz, 6H and β -H, 1H), δ 4.16 (dd, J = 3.5, 7.9 Hz, 1H), δ 7.20 (s, 1H), δ 7.83 (s, 1H), δ 8.03 (d, J = 8.8, 1H), δ 8.69 (d, J = 8.8, 1H), δ 8.90 (t, 1H) ^{13}C NMR (600 MHz, Acetone- d_6) δ 34.8, 71.3, 117.7, 121.8, 122.0, 129.3, 130.4, 136.2, 142.8, 147.5, 178.6

(S)-2-Hydroxy-3-(4-methyl-benzylsulfanyl)-propionic acid or HO-Cys(4-MeBzl)-OH (19) (Method D). A flame-dried round bottom flask was charged with solution of methyl-

(2S)-glycidate, **18** (0.5 g, 4.9 mmol), 4-methylbenzenethiol (0.66 mL, 4.9 mmol), and triethylamine (0.69 mL, 4.9 mmol) in methanol (30 mL). The reaction was heated to reflux for 2 h. Once the reaction was completed (determined by TLC), the reaction mixture was cooled to room temperature and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (1:1 ethylacetate:hexane on silica gel) to afford the methyl ester of **19**. Ester hydrolysis was carried out in the presence of 1M LiOH (2 equiv.) in 3:1:1 tetrahydrofuran: methanol: water. After the reaction was completed (12 h), the mixture was acidified and extracted with ethylacetate (3x50 mL). The solvent was dried and evaporated to afford **19** as a colorless oil. HRMS (MALDI-FTMS): calcd for MH⁺ 227.0742 found 227.0743. ¹H NMR (600 MHz, Acetone-d₆) δ 2.29 (s, 3H), δ 2.70 (dd, *J* = 6.2, 13.6 Hz, 1H), δ 2.81 (dd, *J* = 4.7, 13.6 Hz, 1H), δ 3.79 (s, 2H), δ 4.10 (dd, *J* = 4.7, 6.2 Hz, 1H), δ 7.12 (d, *J* = 7.9 Hz, 2H), δ 7.23 (d, *J* = 7.9 Hz, 2H) ¹³C NMR (600 MHz, Acetone-d₆) δ 21.0, 35.5, 36.8, 72.0, 129.7, 129.8, 136.4, 137.1, 173.8. Chiral HPLC of **19**-methyl ester on Chiracel OD-H using isocratic 10% isopropanol in hexane shows 99% ee (*t*_R = 10.97 min).

(S)-2-Hydroxy-3-(1H-indol-3-yl)-propionic acid or HO-Trp-OH (20) (Method E). A flame-dried round bottom flask was charged with a solution of methyl-(2S)-glycidate, **18** (1.0 g, 9.8 mmol), Indole (1.5 g, 12.78 mmol) in 12 mL carbontetrachloride. After the reaction mixture was cooled down to 0°C, a 25°C solution of Tin(IV)chloride (1.26 mL, 10.74 mmol) in carbontetrachloride (8 mL) was added to the reaction mixture. The reaction was warmed to ambient temperature with continued stirring for 1 h. Once the reaction was complete, the reaction mixture was diluted with saturated sodium bicarbonate (20 mL). The mixture was extracted with chroloform (3x100 mL). The organic layers were combined and evaporated. The residue was purified by flash column chromatography (1:1 ethylacetate:hexane on silica gel) to

afford the methyl ester of **20**. Ester hydrolysis was carried out in the presence of 1M LiOH (2 equiv.) in 3:1:1 tetrahydrofuran: methanol: water. After the reaction was completed (12 h), the mixture was acidified and extracted with ethylacetate (3x50 mL). The solvent was dried and evaporated to afford **20** as colorless oil. HRMS (MALDI-FTMS): calcd for MH⁺ 206.0812 found 206.0813. ¹H NMR (500 MHz, Acetone-d₆) δ 3.26 (dd, J = 0.8, 5.3 Hz, 1H), δ 3.29 (dd, J = 0.8, 8.8 Hz, 1H), δ 3.79 (s, 2H), δ 4.46 (dd, J = 5.3, 8.8 Hz, 1H), δ 6.99 (dd, J = 1.3, 8.8 Hz, 1H), δ 7.01 (d, J = 8.8 Hz, 1H), δ 7.23 (s, 1H), δ 7.35 (dd, J = 1.3, 9.7 Hz, 1H), δ 7.62 (d, J = 9.7 Hz, 1H) ¹³C NMR (600 MHz, Acetone-d₆) δ 31.3, 71.9, 111.5, 112.2, 119.6, 119.8, 122.2, 124.8, 129.1, 137.7, 176.0. Chiral HPLC of **20**-methyl ester on Chiracel OD-H using isocratic 10% isopropanol in hexane shows 99% ee (t_R = 39.1 min).



