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Supporting Information

Total Synthesis of the Ramoplanin A2 and Ramoplanose Aglycon

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Experimental Section

BOC-D-Orn(SES)-OH. Method A: A solution of BOC-D-Orn-OH (2.801 g, 12.1 mmol) in DMF (30 mL) was treated at 55 °C with trimethylsilyl chloride (TMSCl, 1.61 mL, 12.7 mmol) under Ar and the reaction mixture was stirred at 55 °C for 2 h. This reaction mixture was treated with Et_3N (3.52 mL, 25.3 mmol) and 2-trimethylsilylethanesulfonyl chloride (SES-Cl, 2.66 g, 13.3 mmol) at 25 °C. The resulting mixture was stirred for 18 h at 0 °C, then quenched with saturated aqueous $NaHCO_3$ (50 mL) at room temperature, and extracted with Et_2O (3 × 10 mL). The aqueous solution was acidified to pH 3 with 6 N aqueous HCl at 0 °C, and extracted with EtOAc (3 × 20 mL). The combined EtOAc extracts were dried ($MgSO_4$), and concentrated in vacuo to afford BOC-D-Orn(SES)-OH as a white solid (3.538 g, 4.782 g theoretical, 74%) which was employed directly in the next reaction without further purification: mp 65–68 °C; R_f = 0.10 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ –11 (*c* 0.50, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz) δ 4.09–4.02 (m, 1H), 3.05 (t, 2H, *J* = 6.5 Hz), 2.98–2.84 (m, 2H), 1.91–1.87 (m, 1H), 1.66–1.61 (m, 3H), 1.44 (s, 9H), 1.00–0.92 (m, 2H), 0.06 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.0, 158.1, 80.5, 61.5, 54.5, 43.4, 30.0, 28.7, 27.9, 11.4, –2.0; IR (neat) ν_{max} 3397, 2954, 1713, 1660, 1593, 1496, 1452, 1406, 1367, 1318, 1251, 1170, 1141, 1050 cm^{-1}; MALDI–FTMS (DHB) *m/z* 419.1645 ($M + Na^+$, $C_{15}H_{32}N_2O_6SSi$ requires 419.1642).

Method B: A solution of BOC-D-Orn-OH (780 mg, 3.36 mmol) in 50% THF–H_2O (16 mL) was treated at 0 °C with Na_2CO_3 (783 mg, 7.39 mmol) and 2-trimethylsilylethanesulfonyl chloride (SES-Cl, 809 mg, 4.0 mmol). The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h, then quenched with H_2O (5 mL). The aqueous solution was washed with Et_2O (20 mL), acidified to pH 3 with 10% aqueous HCl at 0 °C, and extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were washed with H_2O (20 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo to afford BOC-D-Orn(SES)-OH as a white solid (910 mg, 1.33 g theoretical, 68%).

BOC-D-Orn(SES)-OBn. A solution of BOC-D-Orn(SES)-OH (700 mg, 1.77 mmol) in DMF (5 mL) was treated at 0 °C with $NaHCO_3$ (148 mg, 1.77 mmol) and benzyl

bromide (0.25 mL, 2.12 mmol). The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 18 h, then quenched with H_2O (5 mL). The aqueous solution was extracted with EtOAc (3 × 10 mL), and the combined EtOAc extracts were washed with H_2O (10 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 3 × 18 cm, 20% EtOAc–hexanes) provided BOC-D-Orn(SES)-OBn as a white solid (790 mg, 859 mg theoretical, 92%): mp 48–52 °C; R_f = 0.42 (30% EtOAc–hexanes); $[\alpha]^{23}_D$ –1.1 (*c* 1.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38–7.24 (m, 5H), 5.0[illegible] (m, 2H), 4.50 (t, 1H, *J* = 9.7 Hz), 4.34–4.23 (m, 1H), 3.12–3.01 (m, 2H), 2.94–2.84 (m, 2H), 1.76–1.45 (m, 4H), 1.31 (s, 9H), 1.02–0.92 (m, 2H), 0.06 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.2, 155.4, 135.2, 128.6, 127.4, 127.3, 80.0, 67.1, 52.8, 48.6, 42.6, 29.9, 28.2, 26.2, 10.5, –2.0; IR (neat) ν_{max} 3321, 2954, 1713, 1508, 1453, 1366, 1321, 1253, 1168, 1084, 1021, 843 cm^{-1}; MALDI–FTMS (DHB) *m/z* 509.2126 (M + Na^+, $C_{22}H_{38}N_2O_6SSi$ requires 509.2117).

BOC-D-Hpg–D-Orn(SES)-OBn (4). A sample of BOC-D-Orn(SES)-OBn (170 mg, 0.35 mmol) was treated with 4 M HCl–EtOAc (2 mL) and the resulting mixture was stirred at room temperature for 1 h. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et_2O (3 mL) to the hydrochloride salt followed by its removal in vacuo. The white residue and BOC-D-Hpg-OH (93 mg, 0.35 mmol) were dissolved in DMF/CH_2Cl_2 (1:3, 2 mL). The mixture was treated sequentially at 0 °C with $NaHCO_3$ (29 mg, 0.35 mmol), HOAt (53 mg, 0.39 mmol), and EDCI (74 mg, 0.39 mmol). The reaction mixture was stirred at 0 °C for 6 h, then quenched with H_2O (3 mL). The aqueous layer was extracted with EtOAc (3 × 3 mL), and the combined EtOAc extracts were washed with H_2O (5 mL) and brine (5 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 3 × 17 cm, 33% EtOAc–hexanes) provided **4** as a white powder (210 mg, 223 mg theoretical, 94%): mp 155–158 °C; R_f = 0.42 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ –23 (*c* 0.13, $CHCl_3$); ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.36 (s, 1H), 7.68 (d, 1H, *J* = 12.5 Hz), 7.38–7.26 (m, 5H), 7.24 (d, 2H, *J* = 13.6 Hz), 6.75 (d, 2H, *J* = 13.6 Hz), 6.27 (d, 1H, *J* = 11.7 Hz), 5.93 (t, 1H, *J* = 10.0 Hz), 5.21 (d, 1H, *J* = 12.5 Hz), 5.07 (m, 2H), 4.51–4.48 (m, 1H), 3.06 (q, 2H, *J* = 10.3 Hz), 2.97–2.90 (m, 2H), 1.95–1.68 (m, 4H), 1.38 (s, 9H), 0.95 (m, 2H), 0.06 (s, 9H); ^{13}C NMR (CD_3OD, 150 MHz) δ 173.8, 173.0, 158.7, 157.5, 137.2, 130.0, 129.7, 129.5, 129.4,

129.3, 116.5, 80.9, 59.4, 53.7, 48.9, 43.4, 29.7, 28.9, 27.8, 15.3, 11.5, –1.9; IR (neat) ν_{max} 3319, 2954, 2917, 2848, 1739, 1666, 1514, 1454, 1367, 1317, 1251, 1169, 1139, 1051, 1021, 839, 697 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 768.1774 ($M + Cs^+$, $C_{30}H_{45}N_3O_8SSi$ requires 768.1751).

BOC-D-Hpg–D-Orn(SES)-OH (5). A solution of **4** (500 mg, 0.79 mmol) in CH_3OH (15 mL) was treated with 10% Pd–C (50 mg). The resulting black suspension was stirred under H_2 (1 atm) at room temperature for 2 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo to give **5** as a white solid (420 mg, 429 mg theoretical, 98%) which was employed directly in the next reaction without further purification: mp 74–76 °C; R_f = 0.10 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ –41 (*c* 1.1, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.23 (d, 2H, *J* = 12.5 Hz), 6.75 (d, 2H, *J* = 13.6 Hz), 5.07 (s, 2H), 4.46–4.36 (m, 1H), 3.05 (t, 2H, *J* = 10.3 Hz), 2.98–2.90 (m, 2H), 1.95–1.68 (m, 4H), 1.44 (s, 9H), 1.02–0.91 (m, 2H), 0.07 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.0, 173.5, 158.5, 157.2, 131.1, 129.9, 117.0, 116.4, 80.8, 59.4, 53.5, 48.8, 43.4, 30.1, 28.7, 27.6, 11.4, –2.0; IR (neat) ν_{max} 3354, 2954, 1653, 1616, 1516, 1456, 1368, 1315, 1251, 1168, 1139, 840, 757, 699 cm^{-1}; MALDI–FTMS (DHB) *m/z* 568.2138 ($M + Na^+$, $C_{23}H_{39}N_3O_8SSi$ requires 568.2125).

BOC-D-*a*Thr-OBn. A solution of D-*a*Thr-OH[21] (0.516 g, 4.33 mmol) in 50% THF–H_2O (15 mL) was treated with Na_2CO_3 (0.964 g, 9.10 mmol in 4 mL H_2O) and stirred for 10 min before the addition of Boc_2O (1.04 g, 4.76 mmol). The reaction mixture was stirred at 23 °C for 16 h. Water (5 mL) was added and the mixture was washed with ether (2 × 10 mL). The aqueous layer was acidified with 1 N aqueous HCl to pH 4 and extracted with EtOAc (5 × 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to give BOC-D-*a*Thr-OH as a white solid (0.826 g, 0.950 g theoretical, 87%): $[\alpha]^{23}_D$ +67 (*c* 0.021, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 4.94 (br s, 1H), 4.12 (m, 1H), 4.10–3.96 (m, 1H), 1.44 (s, 9H), 1.21 (d, 3H, *J* = 5.8 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.0, 158.0, 80.7, 68.9, 60.8, 28.7, 19.4; FABHRMS (DHB) *m/z* 242.1000 ($M + Na^+$, $C_9H_{17}NO_5$ requires 242.0999). This solid was dissolved in DMF (25 mL) and treated with BnBr (2.1 mL, 17.3 mmol) at 0 °C for 2 h. The reaction mixture was subsequentially stirred for 18 h at 23 °C. Water (50 mL) was added and the mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers was

washed with H_2O (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromotography (SiO_2, 10–33% EtOAc–hexanes) to provide BOC-D-*a*Thr-OBn as a wax-like solid (1.01 g, 1.34 g theoretical, 75%): R_f = 0.40 (40% EtOAc–hexanes); $[\alpha]^{23}_D$ +46 (*c* 0.026, EtOAc); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.35 (br s, 5H), 5.48 (br s, 1H), 5.20 (m, 2H), 4.42 (m, 1H), 4.15 (m, 1H), 1.44 (s, 9H), 1.13 (d, 3H, J = 5.9 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.3, 163.1, 156.2, 135.0, 128.6, 128.5, 128.3, 80.4, 69.0, 67.3, 59.1, 28.2, 18.6; FABHRMS (DHB) *m/z* 332.1478 (M + Na^+, $C_{16}H_{23}NO_5$ requires 332.1468).

BOC-*a*Thr-OH could be prepared from L-*a*Thr-OH in the same manner as BOC-D-*a*Thr-OH.

BOC-D-Hpg–D-Orn(SES)–D-*a*Thr-OBn (6). A solution of **5** (400 mg, 0.73 mmol) and D-*a*Thr-OBn hydrochloride (179 mg, 0.73 mmol) in 20 % DMF–CH_2Cl_2 (2 mL) was treated sequentially at 0 °C with $NaHCO_3$ (61.3 mg, 0.73 mmol), HOAt (105 mg, 0.77 mmol), and EDCI (148 mg, 0.77 mmol). The reaction mixture was stirred at 0 °C for 2 h and at 10 °C for 1 h, then quenched with H_2O (3 mL). The aqueous layer was extracted with EtOAc (3 × 5 mL), and the combined EtOAc extracts were washed with H_2O (10 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated in vacuo to afford **6** as a white solid (462 mg, 538 mg theoretical, 86%; typically 85–94%) which was employed directly in the next reaction without further purification: mp 120–122 °C; R_f = 0.15 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ –31 (*c* 0.11, $CHCl_3$); ^{1}H NMR (CD_3OD, 600 MHz) δ 7.37 (m, 5H), 7.20 (d, 2H, J = 13.0 Hz), 6.74 (d, 2H, J = 13.0 Hz), 5.17 (m, 2H), 5.09 (s, 1H), 4.48–4.44 (m, 1H), 4.40 (d, 1H, J = 5.7 Hz), 3.99 (t, 1H, J = 5.7 Hz); 3.00 (t, 2H, J = 10.3 Hz), 2.97–2.91 (m, 2H), 1.95–1.52 (m, 4H), 1.44 (s, 9H), 1.10 (d, 3H, J = 6.6 Hz), 1.01–0.95 (m, 2H), 0.07 (s, 9H); ^{13}C NMR (CD_3OD, 150 MHz) δ 173.8, 171.5, 158.8, 157.7, 137.3, 130.0, 129.7, 129.6, 129.5, 116.6, 81.0, 68.8, 68.1, 60.0, 53.9, 48.9, 43.4, 28.9, 23.9, 19.9, 14.6, 11.5, –1.8; IR (neat) ν_{max} 3301, 2958, 2928, 1735, 1701, 1654, 1513, 1457, 1367, 1318, 1261, 1169, 1140, 1024, 841, 754, 698 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 869.2258 (M + Cs^+, $C_{34}H_{52}N_4O_{10}SSi$ requires 869.2228).

BOC-D-Hpg–D-Orn(SES)–D-*a*Thr-OH (7). A solution of **6** (120 mg, 0.46 mmol) in MeOH (3 mL) was treated with 10% Pd–C (10 mg). The resulting black suspension was stirred under H_2 (1 atm) at room temperature for 2 h. The catalyst was removed by

filtration through Celite, and the filtrate was concentrated in vacuo to give **7** as a white solid (105 mg, 105 mg theoretical, quant) which was employed directly in the next reaction without further purification: mp 108–111 °C; R_f = 0.10 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ –28 (*c* 1.2, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.21 (d, 2H, *J* = 8.5 Hz), 6.74 (d, 2H, *J* = 8.5 Hz), 5.08 (s, 1H), 4.48 (dd, 1H, *J* = 5.2, 7.8 Hz), 4.36 (d, 1H, *J* = 5.1 Hz), 4.00–3.96 (m, 1H), 3.04 (t, 2H, *J* = 6.5 Hz), 2.96–2.91 (m, 2H), 1.94–1.88 (m, 1H), 1.76–1.69 (m, 4H), 1.60 (d, 3H, *J* = 6.4 Hz), 1.43 (s, 9H), 1.11 (d, 3H, *J* = 4.3 Hz), 0.99–0.94 (m, 2H), 0.06 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 177.0, 173.6, 173.1, 158.6, 157.5, 129.9, 129.4, 116.6, 116.6, 116.5, 116.5, 80.8, 68.7, 59.5, 58.4, 53.9, 49.2, 43.3, 30.2, 28.7, 27.5, 19.4, 11.4, –2.0; IR (neat) ν_{max} 3418, 2978, 1652, 1516, 1456, 1398, 1314, 1252, 1168, 1139, 841, 757 cm^{-1}; MALDI–FTMS (DHB) *m/z* 669.2632 (M + Na^+, $C_{27}H_{46}N_4O_{10}SSi$ requires 669.2602).

BOC-L-Hpg–D-Hpg-OBn (8). A solution of BOC-L-Hpg-OH (2.28 g, 8.53 mmol) and D-Hpg-OBn hydrochloride (2.50 g, 8.53 mmol) in 25% DMF–CH_2Cl_2 (45 mL) was treated sequentially at 0 °C with $NaHCO_3$ (716 mg, 8.53 mmol), HOAt (1.28 g, 9.38 mmol), and EDCI (1.80 g, 9.38 mmol). The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 6 h, then quenched with H_2O (30 mL). The aqueous solution was extracted with EtOAc (3 × 15 mL), and the combined EtOAc extracts were washed with H_2O (25 mL) and brine (30 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 10 × 30 cm, 50% EtOAc–hexanes) provided **8** as a white solid (4.01 g, 4.30 g theoretical, 93%): mp 147–149 °C; R_f = 0.3 (35% EtOAc–hexanes); $[\alpha]^{23}_D$ +5.4 (*c* 0.2, CH_3OH); ^{1}H NMR (CD_3OD, 600 MHz) δ 7.30–7.22 (m, 5H), 7.19 (d, 2H, *J* = 10.1 Hz), 7.08 (d, 2H, *J* = 10.1 Hz), 6.71 (d, 2H, *J* = 10.6 Hz), 6.69 (d, 2H, *J* = 10.6 Hz), 5.39 (s, 1H), 5.17 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.3, 172.0, 158.9, 158.5, 157.2, 137.1, 130.0, 129.9, 129.7, 129.5, 129.2, 129.0, 127.6, 116.5, 116.3, 80.8, 68.0, 59.3, 57.9, 28.6; IR (neat) ν_{max} 3477, 3411, 1723, 1654, 1613, 1514, 1452, 1367, 1217, 1172, 836, 697 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 639.1128 (M + Cs^+, $C_{28}H_{30}N_2O_7$ requires 639.1107).

BOC-L-Hpg–D-Hpg-OH (9). A solution of **8** (304 mg, 0.601 mmol) in MeOH (6 mL) was treated with 10% Pd–C (30 mg). The resulting black suspension was stirred under H_2 (1 atm) at room temperature for 2 h. The catalyst was removed by filtration

through Celite, and the filtrate was concentrated in vacuo to give **9** as a white solid (248 mg, 250 mg theoretical, 99%) which was employed directly in the next reaction without further purification: mp 191–195 °C; R_f = 0.15 (75% EtOAc–hexanes); $[\alpha]^{23}_D$ –21 (*c* 1.8, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.28 (d, 2H, *J* = 8.5 Hz), 7.10 (d, 2H, *J* = 8.5 Hz), 6.72 (d, 2H, *J* = 8.5 Hz), 6.70 (d, 2H, *J* = 8.5 Hz), 5.32 (s 1H), 5.16 (s, 1H), 1.41 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.9, 173.0, 158.7, 158.5, 157.3, 129.9, 129.8, 129.7, 128.5, 116.3, 80.8, 59.3, 57.6, 49.9, 28.6; IR (neat) ν_{max} 3336, 2977, 1734, 1684, 1654, 1516, 1456, 1394, 1368, 1257, 1163, 856, 754, 699 cm^{-1}; MALDI–FTMS (DHB) *m/z* 439.1478 (M + Na$^+$, $C_{21}H_{24}N_2O_7$ requires 439.1481).

BOC-L-*a*Thr–L-Phe-OBn (10). A solution of BOC-L-*a*Thr-OH[21] (250 mg, 1.15 mmol) and L-Phe-OBn hydrochloride (331 mg, 1.15 mmol) in DMF/CH_2Cl_2 (1:3, 6 mL) was treated sequentially at 0 °C with $NaHCO_3$ (95 mg, 1.15 mmol), HOAt (171 mg, 1.25 mmol), and EDCI (242 mg, 1.25 mmol). The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h, then quenched with H_2O (5 mL). The aqueous solution was extracted with EtOAc (3 × 10 mL), and the combined EtOAc extracts were washed with H_2O (15 mL) and brine (15 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 30% EtOAc–hexanes) provided **10** as a white solid (471 mg, 520 mg theoretical, 90%; typically 88–90%): mp 60–62 °C; R_f = 0.42 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ –20 (*c* 1.1, CH_3OH); ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.73 (s, 1H), 8.36 (s, 1H), 7.51 (d, 1H, *J* = 12.5 Hz), 7.31–7.08 (m, 10H), 5.11 (m, 2H), 4.78–4.68 (m, 1H), 4.04 (s, 1H), 3.98–3.88 (m, 1H), 3.19–2.96 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.9, 172.5, 152.2, 137.8, 136.9, 130.4, 130.0, 129.5, 129.4, 127.9, 122.1, 80.8, 68.8, 68.1, 61.6, 61.1, 55.3, 38.5, 28.7, 19.4; IR (neat) ν_{max} 3412, 3333, 3272, 2972, 2923, 1746, 1692, 1650, 1528, 1391, 1365, 1293, 1174, 1016, 697 cm^{-1}; MALDI–FTMS (DHB) *m/z* 479.2176 (M + Na$^+$, $C_{25}H_{32}N_2O_6$ requires 479.2158).

BOC-L-Hpg–D-Hpg–L-*a*Thr–L-Phe-OBn (12). A sample of **10** (589 mg, 1.29 mmol) was treated with 4 M HCl–EtOAc (5 mL) and the resulting mixture was stirred at room temperature for 50 min. The volatiles were removed in vacuo. The residue was dissolved in EtOAc (50 mL), and washed with saturated $NaHCO_3$ (2 × 50 mL). The organic layer was dried (Na_2SO_4), and concentrated in vacuo to give **11** as a white solid (405 mg). The residue **11** and **9** (474 mg, 1.14 mmol) were dissolved in THF (25 mL).

The mixture was treated sequentially with $NaHCO_3$ (192 mg, 2.29 mmol) and DEPBT (673 mg, 2.25 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temperature for 18 h, then quenched with H_2O (40 mL). The aqueous solution was extracted with EtOAc (3 × 30 mL), and the combined EtOAc extracts were washed with H_2O (30 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 5 × 20 cm, 50% EtOAc–hexanes) provided **12** as a white solid (684 mg, 866 mg theoretical, 79%; typically 77–83%): mp 189–194 °C; R_f = 0.40 (75% EtOAc–hexanes); $[\alpha]^{23}_D$ –21 (*c* 0.26, CH_3OH); ^1H NMR (acetone-d_6, 400 MHz) δ 8.39 (s, 1H), 8.36 (s, 1H), 7.95 (d, 1H, *J* = 4.6 Hz), 7.88 (d, 1H, *J* = 8.2 Hz), 7.67 (d, 1H, *J* = 8.2 Hz), 7.35–7.18 (m, 14H), 6.74 (d, 2H, *J* = 8.5 Hz), 6.71 (d, 2H, *J* = 8.0 Hz), 6.44 (d, 1H, *J* = 8.0 Hz), 5.40 (d, 1H, *J* = 6.5 Hz), 5.31 (d, 1H, *J* = 6.6 Hz), 5.13 (m, 2H), 4.75 (td, 1H, *J* = 7.8, 6.2 Hz), 4.33 (dd, 1H, *J* = 9.6, 6.8 Hz), 3.87 (q, 1H, *J* = 5.1 Hz), 3.15 (dd, 1H, *J* = 6.2, 13.8 Hz), 3.08 (dd, 1H, *J* = 7.8, 13.8 Hz), 1.37 (s, 9H), 0.88 (d, 3H, *J* = 6.3 Hz); ^{13}C NMR (acetone-d_6, 150 MHz) δ 178.3, 178.1, 171.8, 171.0, 158.0, 151.6, 151.4, 136.9, 130.3, 130.2, 130.0, 129.8, 129.4, 129.3, 129.3, 129.3, 129.1, 129.0, 128.8, 127.6, 116.1, 79.7, 68.7, 67.4, 59.0, 58.0, 54.8, 38.1, 30.7, 28.6, 19.7; IR (neat) ν_{max} 3115, 1636, 1504, 1490, 1400, 1210 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 887.2236 (M + Cs^+, $C_{41}H_{46}N_4O_{10}$ requires 887.2268).

In early studies enlisting other coupling reagents (see text), *epi*–**12** (BOC-L-Hpg–L-Hpg–L-*a*Thr–L-Phe-OBn was observed as a major byproduct, but was not detected in the reaction conducted with DEPBT: t_R (*epi*–**12**) = 22 min, t_R (**12**) = 31 min by HPLC on a Waters analytical Nova–Pak® C_{18} 3.9 × 300 mm column, 1 mL/min, 65% MeOH–CH_2Cl_2).

BOC-D-Hpg–D-Orn(SES)–D-*a*Thr–L-Hpg–D-Hpg–L-*a*Thr–L-Phe-OBn (14). A sample of **12** (291 mg, 0.39 mmol) was treated with 4 M HCl–EtOAc (2.5 mL) and the resulting mixture was stirred at room temperature for 90 min. The volatiles were removed in vacuo. The residue was dissolved in EtOAc (150 mL), and washed with saturated aqueous $NaHCO_3$ (2 × 50 mL). The organic layer was dried (Na_2SO_4), and concentrated in vacuo to give **13** as a white solid (219 mg, 255 mg theoretical, 86%). The residue **13**, **7** (220 mg, 0.34 mmol), HOAt (139 mg, 1.02 mmol), and EDCI (196 mg, 1.02 mmol) were dissolved in DMF (1 mL). The reaction mixture was stirred at room

temperature for 14 h. The DMF was evaporated and the crude material was triturated with EtOH. Successive washing with EtOH afforded **14** as a white solid (331 mg, 436 mg theoretical, 76%): mp 185–188 °C; $[\alpha]^{23}_{D}$ –20 (*c* 0.64, DMSO); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.36 (s, 1H), 9.33 (s, 1H), 9.32 (s, 1H), 8.78 (d, 1H, *J* = 8.1 Hz), 8.47 (d, 1H, *J* = 7.3 Hz), 8.25 (d, 1H, *J* = 8.4 Hz), 8.16 (d, 1H, *J* = 8.1 Hz), 8.10 (d, 1H, *J* = 8.1 Hz), 7.98 (d, 1H, *J* = 8.9 Hz), 7.31–7.36 (m, 3H), 7.17–7.25 (m, 9H), 7.14 (d, 2H, *J* = 8.6 Hz), 7.09–7.05 (m, 3H), 6.90 (t, 1H, *J* = 6.1 Hz), 6.65 (d, 2H, *J* = 8.6 Hz), 6.61 (d, 2H, *J* = 8.6 Hz), 6.57 (d, 2H, *J* = 8.6 Hz), 5.57 (d, 1H, *J* = 7.8 Hz), 5.46 (d, 1H, *J* = 8.1 Hz), 5.00–5.08 (m, 3H), 4.87 (d, 1H, *J* = 5.7 Hz), 4.67 (d, 1H, *J* = 5.1 Hz), 4.55 (q, 1H, *J* = 7.3 Hz), 4.24–4.34 (m, 3H), 3.67–3.74 (m, 2H), 3.05–2.96 (m, 2H), 2.88–2.80 (m, 4H), 1.58–1.68 (m, 1H), 1.39–1.52 (m, 3H), 1.36 (s, 9H), 0.91 (d, 3H, *J* = 6.2 Hz), 0.82–0.87 (m, 2H), 0.76 (d, 3H, *J* = 6.5 Hz), 0.00 (s, 9H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 171.2, 171.1, 170.3, 170.2, 170.1, 169.8, 169.0, 162.3, 158.4, 156.7, 154.7, 149.7, 139.5, 136.8, 135.7, 134.6, 129.2, 128.7, 128.4, 128.3, 128.1, 128.0, 127.9, 126.6, 120.0, 114.9, 114.7, 78.3, 67.0, 66.1, 57.8, 55.3, 54.6, 53.9, 46.8, 42.1, 36.8, 36.2, 35.8, 35.7, 34.2, 30.8, 28.2, 25.2, 20.0, 19.3, 15.7, 10.0, –1.9; IR (neat) ν_{max} 3287, 1631, 1458, 1430, 1410 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 1415.4404 (M + Cs$^+$, $C_{63}H_{82}N_8O_{17}SSi$ requires 1415.4342).

Fmoc-L-*threo*-HAsn(Trt)-OBn (16). Since our disclosure of the preparation of **16** which relied on a key Sharpless asymmetric aminohydroxylation (AA) reaction,[24] we have improved its preparation in several subtle ways. Benzyl carbamate (15.70 g, 104 mmol, freshly recrystallized) was dissolved in 122 mL of *n*-PrOH. A freshly prepared solution of NaOH (4.25 g, 106 mmol) in 191 mL of H_2O was added to this stirred solution, followed by a freshly prepared solution of *tert*-butyl hypochlorite (11.51 g, 106 mmol) and $(DHQD)_2PHAL$ (1.853 g, 2.260 mmol) in 70 mL of *n*-PrOH. The reaction vessel was immersed in a room-temperature water bath and stirred for a few minutes. Methyl 4-methoxycinnamate (8.69 g, 45.2 mmol) was added, followed by $K_2OsO_2(OH)_4$ (667 mg, 1.81 mmol). The reaction mixture was stirred for 1 h at 0 °C, and the reaction mixture was homogeneous at this point. The homogeneous mixture was stirred at 0 °C for an additional 1 h by which it transformed into a pale yellow slurry. The crystalline precipitate was isolated by filtration. One wash with ice-cold EtOH–H_2O (1:1, 15 mL) yielded methyl (2*S*,3*R*)-3-[(benzyloxycarbonyl)amino]-2-hydroxy-3-(4-methoxyphenyl)

propionate as a white solid (11.40 g, 71%, >99% ee). Less $K_2OsO_2(OH)_4$/$(DHQD)_2PHAL$ can result lower ee's and lower yields. A solution of this free alcohol in CH_2Cl_2 (0.25 M) at 0 °C was treated with 2,6-lutidine (3 equiv) followed by TBDMSOTf (1.2 equiv). The reaction mixture was stirred at 0 °C for 2.5 h. The reaction mixture was diluted with EtOAc and washed sequentially with 10% aqueous HCl, H_2O, and saturated aqueous NaCl. The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 25% EtOAc–hexane) provided methyl (2*S*,3*R*)-3-[(benzyloxycarbonyl)amino]-2-[(*tert*-butyldimethylsilyl)oxy]-3-(4-methoxyphenyl)propionate as a clear oil (98%) as previously described.[24] The Cbz protecting group was converted to Boc group in a 97% yield by treatment with Boc_2O, H_2 and 10% Pd–C in MeOH as previously described.[24] The yield of the amidation reaction utilized for the conversion of the methyl ester to the primary amide was improved simply by increasing the reaction time (sat. NH_3–MeOH, 25 °C, 7–10 d, 62–77%[24] vs 14 d, 99%). The latent carboxylic acid was unmasked followed an improved procedure. A solution of $NaIO_4$ (0.688 g, 3.22 mmol) in H_2O (16 mL) was treated with a solution of the substrate (0.201 g, 0.46 mmol) in EtOAc–CH_3CN (1:1, 4 mL) followed by $RuCl_3 \cdot 3H_2O$ (3.8 mg, 0.018 mmol) and $NaHCO_3$ (58 mg). The reaction mixture was stirred vigorously (mechanical stirring) at 25 °C for 27 h. The reaction mixture was extracted into saturated aqueous $NaHCO_3$ (30 mL) and washed with CH_2Cl_2 (20 mL). The aqueous layer was acidified with the addition of 10% aqueous HCl to pH 2–3 in an ice-bath and extracted with EtOAc (5 × 40 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo to provide the carboxylic acid as a white solid (112 mg, 65%). A solution of the L-*threo*-BOCNH-*β*-OTBDMS-Asn-OH in DMF (5.5 mL) at 0 °C was treated with $NaHCO_3$ and benzyl bromide as previously described.[24] The deprotection of TBS and Boc groups of L-*threo*-BOCNH-*β*-OTBDMS-Asn-OBn (0.612 g, 1.35 mmol) was accomplished by treating with 4 M HCl–EtOAc (10.1 mL, 40.6 mmol) at 25 °C for 2 h. The removal of excess HCl and EtOAc with N_2 provided a white solid. This white solid was dissolved in 50% dioxane–H_2O (20 mL) and treated with FmocCl (0.541 g, 2.03 mmol) and $NaHCO_3$ (227 mg, 2.70 mmol) for 12 h at 25 °C. The reaction mixture was partitioned between saturated aqueous $NaHCO_3$ (50 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (2 × 50 mL), and the combined organic layers was dried (Na_2SO_4),

filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 65% EtOAc–hexanes) provided L-*threo*-FmocNH-HAsn-OBn as a white solid (0.583 g, 0.623 g theoretical, 92%). As described,[24] L-*threo*-FmocNH-HAsn-OBn and trityl alcohol (10 equiv) in HOAc at 50 °C was treated successively with concentrated sulfuric acid (0.6 equiv) and acetic anhydride (2.5 equiv) to provide Fmoc-L-*threo*-HAsn(Trt)-Obn (**16**) in a 71% yield.

Fmoc-L-Asn(Trt)–L-*threo*-HAsn(Trt)-OBn (18). A solution **16** (966 mg, 1.38 mmol) in CH_2Cl_2 (18 mL) was treated with piperidine (0.9 mL, 9.09 mmol) and the reaction mixture was stirred at room temperature for 1.5 h. The solvent was evaporated in vacuo. Flash chromatography (SiO_2, 3 × 20 cm, 33–80% EtOAc–hexanes) afforded **17** a clear oil (658 mg, 660 mg theoretical, quant): R_f = 0.50 (66% EtOAc–hexanes); $[\alpha]^{23}_D$ –11 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.17 (s, 1H), 9.33 (s, 1H), 7.37–7.17 (m, 20H), 5.13–5.24 (m, 2H), 4.47 (d, 1H, *J* = 1.8 Hz), 4.29 (d, 1H, *J* = 1.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.9, 170.8, 144.6, 135.4, 128.9, 128.6, 128.6, 128.7, 128.4, 128.3, 128.2, 127.4, 73.1, 70.5, 67.7, 55.5; IR (neat) ν_{max} 3384, 3058, 3031, 2962, 2925, 1738, 1675, 1503, 1446, 1260, 1083, 1034, 799, 754, 698 cm^{-1}; MALDI–FTMS (DHB) *m/z* 503.1947 (M + Na^+, $C_{30}H_{28}N_2O_4$ requires 503.1941). The residue **17** and Fmoc-L-Asn(Trt)-OH (845 mg, 1.42 mmol) were dissolved in 17% DMF–CH_2Cl_2 (8.4 mL), and the mixture was treated at 0 °C with HOAt (241 mg, 1.77 mmol) and EDCI (339 mg, 1.77 mmol). The reaction mixture was stirred for 2.5 h and allowed to warm to 25 °C. The reaction mixture was diluted with EtOAc (100 mL) and the organic layer was washed with saturated aqueous $NaHCO_3$ (80 mL), and brine (80 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography (SiO_2, 3 × 25 cm, 50–75% EtOAc–hexane, crude product adsorbed on silica) provided **18** as a white solid (1.18 g, 1.46 g theoretical, 81%; typically 80–90%): mp 221 °C; R_f = 0.48 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ +7.8 (*c* 0.32, $CHCl_3$); ^{1}H NMR (10% CD_3OD–acetone-d_6, 400 MHz) δ 8.28 (s, 1H), 8.05 (s, 1H), 7.82 (d, 2H, *J* = 7.6 Hz), 7.64 (d, 1H, *J* = 7.3 Hz), 7.53 (d, 1H, *J* = 7.6 Hz), 7.10–7.36 (m, 39H), 5.12 (s, 2H), 5.05 (br s, 1H), 4.75 (t, 1H, *J* = 6.5 Hz), 4.64 (d, 1H, *J* = 2.2 Hz), 4.16 (m, 3H), 2.76 (d, 2H, *J* = 6.5 Hz); ^{13}C NMR (50% $CDCl_3$–CD_3OD, 125 MHz) δ 172.9, 170.7, 170.3, 156.7, 144.7, 144.6, 144.3, 143.9, 141.6, 141.5, 135.5, 129.1, 128.9, 128.8, 128.6, 128.5, 128.2, 128.0, 127.9, 127.4, 127.3,

127.1, 125.5, 125.4, 120.1, 71.8, 71.1, 70.6, 67.9, 67.6, 55.8, 52.3, 47.2, 39.8; IR (neat) ν_{max} 3411, 2923, 1732, 1667, 1494, 1447, 1219, 1035, 771, 699 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 1191.3360 ($M + Cs^+$, $C_{68}H_{58}N_4O_8$ requires 1191.3309).

BOC-L-Chp-OBn. A solution of BOC-L-Chp-OH[25] (1.28 g, 4.25 mmol) in DMF (20 mL) was treated with $NaHCO_3$ (357 mg, 4.25 mmol) and benzyl bromide (0.56 mL, 4.68 mmol). The reaction mixture was stirred at 0 °C for 2 h and at room temperature for another 10 h. Water (20 mL) was added at 0 °C and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with H_2O (15 mL) and brine (15 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 5 × 25 cm, 10–50% EtOAc–hexanes) provided BOC-L-Chp-OBn as a white foam (1.45 g, 1.67 g theoretical, 87%): R_f = 0.40 (30% EtOAc–hexanes); $[\alpha]^{23}_D$ +46 (*c* 2.6, $CHCl_3$); ^1H NMR (CD_3OD, 500 MHz) δ 7.27 (m, 5H), 7.20 (s, 1H), 7.09 (dd, 1H, *J* = 2.2, 8.1 Hz), 6.84 (d, 1H, *J* = 8.4 Hz), 5.13 (m, 3H), 1.42 (s, 9H); ^{13}C NMR (CD_3OD, 125 MHz) δ 172.4, 157.5, 154.5, 137.0, 130.2, 129.7, 129.4, 129.2, 129.0, 128.4, 117.6, 80.9, 68.0, 58.5, 28.7; IR (neat) ν_{max} 3364, 2978, 1738, 1683, 1608, 1499, 1423, 1368, 1338, 1257, 1214, 1161, 1076, 820, 752 cm^{-1}; FABHRMS (NBA–NaI) *m/z* 414.1074 ($M + Na^+$, $C_{20}H_{22}ClNO_5$ requires 414.1084).

BOC-L-Chp(OTBS)-OBn. A solution of BOC-L-Chp-OBn (500 mg, 1.69 mmol) in THF (3 mL) was treated with *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (2 mL, 8.5 mmol). The reaction mixture was stirred at 40 °C for 3 h, then quenched with saturated aqueous NH_4Cl (50 mL). EtOAc (80 mL) was added and the organic layer was further washed with saturated aqueous NH_4Cl (2 × 50 mL) and brine (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 5 × 25 cm, 10% EtOAc–hexanes) provided BOC-L-Chp(OTBS)-OBn as a clear oil (848 mg, 848 mg theoretical, quant): R_f = 0.50 (10% EtOAc–hexanes); $[\alpha]^{23}_D$ +56 (*c* 2.1, CH_2Cl_2); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.29 (m, 4H), 7.20 (m, 2H), 7.08 (dd, 1H, *J* = 1.8, 8.4 Hz), 6.80 (d, 1H, *J* = 8.4 Hz), 5.51 (br s, 1H), 5.26 (br s, 1H), 5.16 (m, 2H), 1.42 (s, 9H), 1.01 (s, 9H), 0.21 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.7, 154.7, 151.7, 135.1, 130.6, 128.9, 128.5, 128.3, 127.9, 126.5, 120.8, 80.3, 67.4, 56.7, 28.3, 25.6, 18.3, –4.4; IR (neat) ν_{max} 2933, 2861, 1738, 1713, 1492, 1364, 1292, 1251, 1164, 1056, 923, 841, 779

cm^{-1}; MALDI–FTMS (DHB) *m/z* 528.1949 (M + Na^+, $C_{26}H_{36}ClNO_5Si$ requires 528.1943).

BOC-L-Chp(OTBS)-OH (19). A solution of BOC-L-Chp(OTBS)-OBn (694 mg, 1.37 mmol) in EtOH (27 mL) was treated with 10% Pd–C (145 mg). The resulting black suspension was stirred under H_2 (1 atm) at room temperature for 1.5 h. The catalyst was removed by filtration through Celite and washed with EtOAc (100 mL). The filtrate was concentrated in vacuo to give **19** as a white foam (570 mg, 570 mg theoretical, quantitative) which was employed directly in the next reaction without further purification: $[\alpha]^{23}_D$ +112 (*c* 2.1, CH_2Cl_2); ^1H NMR (acetone-d_6, 400 MHz) δ 7.51 (s, 1H), 7.32 (d, 1H, *J* = 10.5 Hz), 7.02 (d, 1H, *J* = 10.5 Hz), 6.63 (m, 1H), 5.24 (d, 1H, *J* = 9.8 Hz), 1.40 (s, 9H), 1.04 (s, 9H), 0.26 (s, 6H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 172.4, 155.9, 152.1, 133.0, 130.2, 128.3, 125.8, 121.6, 79.6, 57.6, 28.6, 26.0, 18.9, –4.2; IR (neat) ν_{max} 3292, 2933, 2861, 2553, 1728, 1661, 1600, 1492, 1395, 1364, 1287, 1251, 1159, 1051, 923, 841, 779 cm^{-1}; MALDI–FTMS (DHB) *m/z* 438.1467 (M + Na^+, $C_{19}H_{30}ClNO_5Si$ requires 438.1474).

Fmoc-L-Asn(Trt)–L-*threo*-*O*-[BOC-L-Chp(OTBS)]-HAsn(Trt)-OBn (20). A solution of **18** (318 mg, 0.30 mmol), **19** (250 mg, 0.60 mmol) and DMAP (11 mg, 0.09 mmol) in CH_2Cl_2 (2.5 mL) was treated at 0 °C with EDCI (173 mg, 0.90 mmol). The reaction mixture was stirred at 0 °C for 1 h, then EtOAc (60 mL) was added. The organic layer was washed with saturated aqueous $NaHCO_3$ (50 mL), 1 N aqueous HCl (50 mL), and brine (50 mL), then dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography (SiO_2, 5 × 25 cm, 25% EtOAc–hexanes) provided **20** and a minor isomer. The major isomer constitutes the desired product **20** (380 mg, 437 mg theoretical, 87%) and the minor isomer (36 mg, 8%) constitutes the Chp α-CH epimerized product.

For the major diastereomer (**20**): white solid; mp 124–125 °C; R_f = 0.30 (30% EtOAc–hexanes); $[\alpha]^{23}_D$ +7.2 (*c* 0.29, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.24 (d, 1H, *J* = 8.1 Hz), 7.73 (m, 2H), 7.54 (d, 1H, *J* = 6.7 Hz), 7.38–7.04 (m, 34H), 6.97 (d, 1H, *J* = 8.6 Hz), 6.91 (m, 8H), 6.70 (d, 1H, *J* = 8.1 Hz), 6.32 (m, 1H), 6.24 (d, 1H, *J* = 7.8 Hz), 5.65 (s, 1H), 5.26 (m, 1H), 5.18 (dd, 1H, *J* = 3.2, 8.9 Hz), 5.11 (s, 2H), 4.95 (br s, 1H), 4.48 (br s, 1H), 4.21 (m, 2H), 4.08 (dd, 1H, *J* = 7.0, 14.0 Hz), 2.94 (m, 1H), 2.80 (m,

1H), 1.42 (s, 9H), 0.99 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1, 170.5, 169.4, 168.4, 164.6, 155.9, 154.5, 151.9, 144.2, 144.0, 143.6, 143.5, 141.2, 141.1, 134.9, 128.7, 128.5, 128.4, 128.2, 128.0, 127.9, 127.6, 127.1, 127.0 (2C), 126.9, 126.4, 125.3, 125.2, 120.9, 119.8, 80.5, 73.5, 71.0, 70.3, 67.9, 67.2, 56.7, 53.2, 50.8, 46.9, 39.2, 28.2, 25.5, 18.2, –4.3, –4.4; IR (neat) ν_{max} 3337, 3060, 2980, 1701, 1493, 1289, 1180, 1056, 752 cm^{-1}; MALDI–FTMS (DHB) *m/z* 1478.5590 (M + Na$^+$, $C_{87}H_{86}ClN_5O_{12}Si$ requires 1478.5628).

For the minor diastereomer (*epi*–**20**): white solid; R_f = 0.33 (30% EtOAc–hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.48 (br s, 1H), 7.84 (m, 3H), 7.65 (d, 1H, *J* = 7.3 Hz), 7.60 (br s, 1H), 7.48 (m, 4H), 7.41–7.18 (m, 34H), 7.04 (m, 2H), 7.00 (dd, 1H, *J* = 1.8, 8.4 Hz), 6.71 (d, 1H, *J* = 8.4 Hz), 6.43 (br s, 1H), 5.77 (br s, 1H), 5.43 (br s, 1H), 5.17 (m, 2H), 5.02 (d, 1H, *J* = 5.1 Hz), 4.85 (d, 1H, *J* = 12.5 Hz), 4.72 (d, 1H, *J* = 12.5 Hz), 4.46 (m, 1H), 4.40 (t, 1H, *J* = 7.3 Hz), 4.33 (t, 1H, *J* = 7.3 Hz), 4.21 (t, 1H, *J* = 7.3 Hz), 2.87 (br s, 2H), 1.42 (s, 9H), 1.10 (s, 9H), 0.26 (s, 3H), 0.25 (s, 3H); MALDI–FTMS (DHB) *m/z* 1478.5576 (M + Na$^+$, $C_{87}H_{86}ClN_5O_{12}Si$ requires 1478.5628).

BOC-L-Leu–D-Ala-OH (23). A solution of BOC-L-Leu-D-Ala-OMe (**22**, 1.01 g, 3.13 mmol) in THF/CH_3OH/H_2O (3:1:1, 22 mL) was treated with lithium hydroxide monohydrate (377 mg, 9.0 mmol) at room temperature and the reaction mixture was stirred for 3 h. The reaction mixture was acidified to pH 3 with 10% aqueous HCl at 0 °C and extracted with EtOAc (3 × 15 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo to give **23** as a white solid (900 mg, 947 mg, 95%) which was employed directly in the next reaction without further purification: mp 161–162 °C; $[\alpha]^{23}_D$ –23 (*c* 0.96, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz) δ 4.36 (q, 1H, *J* = 7.4 Hz), 4.10 (m, 1H), 1.67 (m, 1H), 1.51 (m, 2H), 1.44 (s, 9H), 1.38 (d, 3H, *J* = 7.4 Hz), 0.94 (d, 3H, *J* = 6.6 Hz), 0.92 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 175.6, 175.4, 157.8, 80.6, 54.5, 42.2, 28.7, 25.9, 23.4, 21.9, 17.8; IR (neat) ν_{max} 3305, 2959, 1698, 1652, 1520, 1455, 1393, 1367, 1250, 1165, 1048, 875, 756 cm^{-1}; MALDI–FTMS (DHB) *m/z* 325.1732 (M + Na$^+$, $C_{14}H_{26}N_2O_5$ requires 325.1739).

Fmoc-L-Asn(Trt)–L-*threo*-*O*-[BOC-L-Leu–D-Ala–L-Chp(OTBS)]-HAsn(Trt)-OBn (24). A sample of **20** (380 mg, 0.26 mmol) was treated with a 0.2 M solution of *B*-bromocatecholborane in CH_2Cl_2 (2.6 mL, 0.52 mmol) at 0 °C. The reaction mixture was

stirred at 0 °C for 2 h, quenched with water (50 mL) and diluted with EtOAc (50 mL). The organic layer was washed with 10% aqueous Na_2CO_3 (3 × 40 mL) and brine (40 mL), then dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue **21** and **23** (83 mg, 0.27 mmol) were dissolved in CH_2Cl_2/DMF (4:1, 2.5 mL), and the mixture was treated with HOAt (71 mg, 0.52 mmol) and EDCI (100 mg, 0.56 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h and diluted with EtOAc (60 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (40 mL), 1 N aqueous HCl (40 mL) and brine (40 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Column chromatography (SiO_2, 3 × 25 cm, 25–33% EtOAc–hexanes) provided **24** as a white solid (350 mg, 432 mg theoretical, 81%): mp 123–124 °C; R_f = 0.6 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ +10 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.05 (d, 1H, *J* = 7.3 Hz), 7.82 (m, 2H), 7.63 (d, 1H, *J* = 6.9 Hz), 7.50 (d, 1H, *J* = 6.6 Hz), 7.44 (t, 2H, *J* = 7.7 Hz), 7.40–7.20 (m, 32H), 7.13 (br s, 1H), 7.06 (d, 9H, *J* = 5.8 Hz), 6.91 (m, 1H), 6.52 (m, 2H), 5.69 (br s, 1H), 5.28–5.10 (m, 4H), 5.04 (br s, 1H), 4.64 (m, 1H), 4.50 (m, 1H), 4.30 (d, 2H, *J* = 6.2 Hz), 4.18 (m, 2H), 2.94 (br s, 2H), 1.67 (m, 1H), 1.52 (m, 2H), 1.42 (s, 9H), 1.29 (d, 3H, *J* = 5.8 Hz), 1.09 (s, 9H), 0.94 (s, 6H), 0.23 (s, 3H), 0.22 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4, 171.9, 171.2, 170.1, 169.0, 168.3, 164.5, 156.2, 152.1, 144.3, 143.7, 141.2, 134.9, 128.7, 128.4, 128.3, 128.0, 127.9, 127.7, 127.1, 127.0, 125.3, 125.2, 120.7, 119.9, 80.2, 73.7, 70.9, 70.5, 67.8, 67.4, 56.1, 53.5, 53.3, 51.3, 46.9, 40.8, 39.2, 28.2, 25.5, 24.6, 22.9, 21.7, 18.2, 16.8, –4.3, –4.4; IR (neat) ν_{max} 3315, 2927, 2910, 1724, 1598, 1264 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 1774.3641 (M + Cs^+, $C_{96}H_{102}ClN_7O_{14}Si$ requires 1774.3547).

Fmoc-L-Asn(Trt)–L-*threo*-*O*-[BOC-L-Leu–D-Ala–L-Chp]-HAsn(Trt)-OBn (25). A solution of **24** (350 mg, 0.213 mmol) in THF (2 mL) was treated at 0 °C with a 1 N buffered solution of Bu_4NF (0.64 mL, 0.64 mmol, 1 mL of 1 N Bu_4NF solution premixed with 0.06 mL of AcOH). The reaction mixture was stirred at 0 °C for 45 min and diluted with EtOAc (60 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (40 mL) and brine (40 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Column chromatography (SiO_2, 3 × 25 cm, 50% EtOAc–hexanes) provided **25** as a white solid (295 mg, 324 mg theoretical, 91%; typically 90–95%): mp 129–130 °C; R_f = 0.2 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ +16 (*c* 1.6, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.11 (br s,

1H), 7.85 (m, 2H), 7.67–7.45 (m, 5H), 7.40–7.18 (m, 32H), 7.08 (m, 8H), 6.92 (m, 2H), 6.55 (d, 2H, J = 7.8 Hz), 5.75 (br s, 1H), 5.30 (dd, 1H, J = 4.8, 8.8 Hz), 5.20 (br s, 4H), 4.71 (br s, 1H), 4.55 (br s, 1H), 4.32 (m, 2H), 4.20 (m, 1H), 4.15 (br s, 1H), 2.98 (br s, 2H), 1.67 (m, 1H), 1.52 (m, 2H), 1.43 (s, 9H), 1.33 (d, 3H, J = 6.2 Hz), 0.96 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.9, 172.5, 171.7, 170.5, 169.3, 168.7, 165.1, 156.6, 156.3, 152.8, 144.8, 144.4, 144.2, 144.1, 141.7, 135.4, 129.2, 128.9, 128.8, 128.5, 128.4, 128.1, 127.5, 127.4, 125.8, 125.7, 121.2, 120.4, 117.5, 80.6, 74.2, 71.4, 71.0, 68.3, 67.9, 56.7, 54.0, 53.9, 51.8, 47.4, 41.2, 39.7, 28.7, 25.1, 23.4, 22.2, 17.6; IR (neat) ν_{max} 3313, 3056, 2954, 1749, 1682, 1497, 1446, 1364, 1246, 1159, 1051, 908, 733, 697 cm^{-1}; MALDI–FTMS (DHB) *m/z* 1548.6042 (M + Na$^+$, $C_{90}H_{88}ClN_7O_{14}$ requires 1548.5975).

Fmoc-L-Asn(Trt)–L-*threo*-*O*-[BOC-L-Leu–D-Ala–L-Chp]-HAsn(Trt)-OH (26). A solution of **25** (255 mg, 0.167 mmol) in EtOH (1.5 mL) was treated with 10% Pd–C (178 mg). The resulting black suspension was stirred under H_2 (1 atm) at room temperature for 4.5 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo to give **26** as a white solid (226 mg, 240 mg theoretical, 94%) which was employed directly in the next reaction without further purification: mp 171–172 °C; R_f = 0.2 (10% EtOH–CH_2Cl_2); $[\alpha]^{23}_D$ +13 (*c* 0.15, CH_2Cl_2); ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.82 (br s, 1H), 8.26 (d, 1H, J = 8.2 Hz), 8.21 (s, 1H), 8.08 (br s, 1H), 7.84 (d, 2H, J = 7.6 Hz), 7.79 (s, 1H), 7.66 (d, 2H, J = 6.8 Hz), 7.54 (d, 1H, J = 7.3 Hz), 7.38 (t, 2H, J = 7.3 Hz), 7.34–7.06 (m, 32H), 7.01 (m, 3H), 6.65 (d, 1H, J = 8.4 Hz), 6.30 (d, 1H, J = 7.0 Hz), 5.66 (d, 1H, J = 2.0 Hz), 5.24 (br s, 2H), 4.79 (m, 1H), 4.46 (m, 1H), 4.15 (m, 3H), 4.04 (m, 1H), 2.95 (d, 2H, J = 6.2 Hz), 1.73–1.50 (m, 3H), 1.27 (m, 12H), 0.84 (m, 6H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 173.5, 172.9, 172.3, 170.2, 169.9, 169.5, 165.5, 156.3, 153.5, 145.4, 145.1, 144.9, 144.8, 144.7, 144.4, 141.6, 141.5, 129.4, 129.3 (2C), 129.2, 129.1, 128.3, 128.0, 127.5, 127.3, 127.1, 127.0, 125.9, 125.8, 120.8, 120.3, 117.3, 79.1, 74.5, 70.9, 70.7, 67.3, 56.5, 53.9, 53.3, 52.4, 47.4, 41.0, 39.9, 28.1, 24.9, 23.0, 21.6, 17.7; IR (neat) ν_{max} 3303, 3056, 2954, 1692, 1646, 1553, 1503, 1451, 1251, 1159, 1046, 749, 697 cm^{-1}; MALDI–FTMS (DHB) *m/z* 1458.5468 (M + Na$^+$, $C_{83}H_{82}ClN_7O_{14}$ requires 1458.5506).

BOC-L-Hpg–D-*a*Thr-OBn (27). A sample of BOC-D-*a*Thr-OBn (68 mg, 0.22 mmol) was treated with 4 M HCl–EtOAc (4 mL). The resulting mixture was stirred at

room temperature for 1 h, then the volatiles were removed under a stream of N_2. The residual HCl was further removed by adding Et_2O (1 mL) to the hydrochloride salt followed by its removal in vacuo to give a white solid. The residue and BOC-L-Hpg-OH (64 mg, 0.24 mmol) were dissolved in THF (1.5 mL). The mixture was treated with $NaHCO_3$ (39 mg, 0.46 mmol) and DEPBT (131 g, 0.44 mmol) at 0 °C. The reaction mixture was stirred for 18 h at room temperature, then poured into H_2O (3 mL), and extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were washed with saturated aqueous Na_2CO_3 (2 × 2 mL) and brine (2 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (SiO_2, 1 × 15 cm, 33–50% EtOAc–hexanes) afforded **27** as a white solid (83 mg, 101 mg theoretical; 82%): mp 44–46 °C; R_f = 0.20 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ +24 (*c* 1.7, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.38–7.32 (m, 5H), 7.20 (d, 2H, *J* = 8.5 Hz), 6.71 (d, 2H, *J* = 8.5 Hz), 5.17 (s, 2H), 5.10 (s, 1H), 4.40 (d, 1H, *J* = 6.8 Hz), 3.98 (t, 1H, *J* = 5.9 Hz), 1.43 (s, 9H), 1.07 (d, 3H, *J* = 5.9 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.7, 171.7, 158.6, 137.1, 130.1, 129.9, 129.6, 129.5, 129.3, 116.4, 116.4, 80.9, 68.8, 67.9, 60.0, 48.8, 28.7, 20.0; IR (neat) ν_{max} 3353, 1667, 1512, 1493, 1480, 1240, 1166 cm^{-1}; FABHRMS (NBA–NaI) *m*/z 481.1963 (M + Na$^+$, $C_{24}H_{30}N_2O_7$ requires 481.1951).

BOC-D-Orn(SES)–L-Hpg–D-*a*Thr-OBn (29). A sample of **27** (206 mg, 0.45 mmol) was treated with 4 M HCl–EtOAc (2 mL) and the resulting mixture was stirred at room temperature for 1 h. The volatiles were removed in vacuo. The residual HCl was further removed by adding Et_2O (3 mL) to the hydrochloride salt followed by its removal in vacuo. The residue **28** and Boc-D-Orn(SES)-OH (180 mg, 0.45 mmol) were dissolved in DMF/CH_2Cl_2 (1:4, 10 mL). The mixture was treated sequentially at 0 °C with $NaHCO_3$ (38 mg, 0.45 mmol), HOAt (64 mg, 0.47 mmol), and EDCI (90 mg, 0.47 mmol). The reaction mixture was stirred at 0 °C for 2 h and at 15 °C for 2 h, then quenched with H_2O (20 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL), and the combined EtOAc extracts were washed with H_2O (20 mL) and brine (30 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 4 × 17 cm, 75% EtOAc–hexanes) provided **29** as a white solid (300 mg, 334 mg theoretical, 90%): mp 55–58 °C; R_f = 0.52 (100% EtOAc); $[\alpha]^{23}_D$ +30 (*c* 1.0, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.45–7.24 (m, 5H), 7.21 (d, 2H, *J* = 8.8 Hz), 6.71 (d, 2H, *J* = 8.8 Hz), 5.36 (s, 1H), 5.18 (s,

1H), 4.41 (d, 1H, J = 6.5 Hz), 4.40–4.06 (m, 2H), 3.01 (t, 2H, J = 6.5 Hz), 2.95–2.90 (m, 2H), 1.78 (br, 1H), 1.63–1.54 (br, 3H), 1.42 (s, 9H), 1.08 (d, 2H, J = 6.5 Hz), 0.98–0.93 (m, 2H), 0.05 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.5, 172.8, 171.6, 158.7, 157.9, 137.2, 130.1, 130.0, 129.6, 129.3, 129.2, 116.5, 80.8, 68.8, 67.9, 60.0, 58.2, 55.7, 48.8, 43.3, 30.2, 28.7, 27.8, 20.1, 11.4, –2.0; IR (neat) ν_{max} 3373, 3275, 2954, 1734, 1694, 1636, 1540, 1509, 1473, 1456, 1367, 1317, 1251, 1171, 1140, 838, 756, 698 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 869.2262 (M + Cs$^+$, $C_{34}H_{52}N_4O_{10}SSi$ requires 869.2228).

BOC-D-Orn(SES)–L-Hpg–D-*a*Thr-OH (30). A solution of **29** (40 mg, 54 μmol) in CH_3OH (2 mL) was treated with 10% Pd–C (5 mg). The resulting black suspension was stirred under H_2 (1 atm) at room temperature for 2 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo to give **30** as a white solid (34 mg, 35 mg theoretical, 98%) which was employed directly in the next reaction without further purification: mp 124–128 °C; $[\alpha]^{23}_D$ +29 (*c* 0.94, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.25 (d, 2H, J = 8.5 Hz), 6.74 (d, 2H, J = 8.5 Hz), 5.39 (s, 1H), 4.39 (d, 1H, J = 6.5 Hz), 4.09–3.92 (m, 2H), 3.01 (t, 2H, J = 6.5 Hz), 2.95–2.90 (m, 2H), 1.79 (s, 1H), 1.59 (br, 3H), 1.43 (s, 9H), 1.11 (d, 2H, J = 6.5 Hz), 0.98–0.94 (m, 2H), 0.06 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.5, 173.3, 172.7, 158.7, 157.9, 130.1, 129.3, 116.4, 80.8, 68.9, 59.7, 58.2, 55.8, 48.8, 43.3, 30.2, 28.7, 27.9, 19.8, 11.4, –2.0; IR (neat) ν_{max} 3418, 2978, 1652, 1516, 1456, 1368, 1314, 1252, 1170, 1138, 839, 757 cm^{-1}; MALDI–FTMS (DHB) *m/z* 669.2620 (M + Na$^+$, $C_{27}H_{46}N_4O_{10}SSi$ requires 669.2602).

BOC-L-Hpg–Gly-OBn (31). A slurry of BOC-L-Hpg-OH (1.050 g, 3.93 mmol), the HCl salt of Gly-OBn (0.791 g, 3.92 mmol), and $NaHCO_3$ (0.661 g, 7.86 mmol) in THF (20 mL) maintained at 0 °C, was treated with DEPBT (2.38 g, 7.95 mmol). The reaction mixture was stirred for 1 h at 0 °C, then for 18 h at room temperature. Water (20 mL) was added, and the reaction mixture was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with water (50 mL), saturated aqueous $NaHCO_3$ (3 × 50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated. Recrystallization (CH_2Cl_2/hexanes) afforded **31** (1.41 g, 82%) as a white solid: mp 146.5–148 °C; R_f = 0.30 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ +84 (*c* 0.20, CH_3OH); ^{1}H NMR (CD_3OD, 500

MHz) δ 8.45 (brt, 1H, J = 4.4 Hz), 7.32–7.31 (m, 5H), 7.22 (d, 2H, J = 8.8 Hz), 6.72 (d, 2H, J = 8.8 Hz), 5.12 (s, 2H), overlapping with 5.12 (br, s, 1H), 3.98 (d, 2H, J = 4.4 Hz), 1.42 (s, 9H); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.4, 174.3, 171.0, 158.7, 157.5, 137.3, 130.2, 129.9, 129.7, 129.4, 116.5, 81.0, 68.0, 59.6, 42.5, 28.8; IR (neat) ν_{max} 3330, 2977, 1744, 1665, 1614, 1514, 1454, 1367, 1169, 1106, 1048 cm^{-1}; MALDI–FTMS (DHB) m/z 437.1680 (M + Na$^+$, $C_{22}H_{26}N_2O_6$ requires 437.1683).

BOC-D-Orn(SES)–L-Hpg–D-*a*Thr–L-Hpg–Gly-OBn (33). A sample of **31** (258 mg, 0.59 mmol) was treated with 4 M HCl–EtOAc (3 mL), and the reaction mixture was stirred at room temperature for 1 h. The volatiles were removed under a stream of N_2, and the residual HCl was further removed by addition of Et_2O (2 × 1 mL) to the hydrochloride salt **32**, followed by its removal in vacuo. The residue **32** and **30** (340 mg, 0.53 mmol) were dissolved in DMF/CH_2Cl_2 (1:1, 12 mL). The solution was cooled to 0 °C, and treated with $NaHCO_3$ (57 mg, 0.67 mmol), HOAt (80 mg, 0.59 mmol), and EDCI (114 mg, 0.59 mmol). The reaction mixture was stirred for 25 h and then triturated with EtOAc. The precipitate was collected and rinsed with EtOAc to afford **33** (448 mg, 498 mg theoretical, 90%) as a white solid: mp 210–212 °C (decomp); R_f = 0.30 (10% CH_3OH–CH_2Cl_2); $[\alpha]^{23}_D$ –78 (c 0.80, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.45–7.38 (m, 5H), 7.35 (d, 2H, J = 8.8 Hz), 7.31 (d, 2H, J = 8.8 Hz), 6.83 (d, 2H, J = 7.0 Hz), 6.81 (d, 2H, J = 7.0 Hz), 5.47 (1H), 5.38 (s, 1H), 5.23 (s, 2H), 4.32 (d, 1H, J = 7.0 Hz), 4.18–4.04 (m, 3H), 3.95 (m, 1H), 3.11–3.06 (m, 2H), 3.04–2.97 (m, 2H), 1.89–1.78 (m, 1H), 1.71–1.57 (m, 3H), 1.50 (s, 9H), 1.14 (d, 3H, J = 6.2 Hz), 1.08–1.02 (m, 2H), 0.14 (s, 9H); IR (neat) ν_{max} 3450, 1634, 1510, 1502 cm^{-1}; MALDI–FTMS (DHB) m/z 965.3792 (M + Na$^+$, $C_{44}H_{62}N_6O_{13}SSi$ requires 965.3757).

BOC-D-Orn(SES)–L-Hpg–D-*a*Thr–L-Hpg–Gly-OMe. A solution of BOC-Hpg-OH (66.8 mg 0.25 mmol) and the HCl salt of Gly-OMe (31.4 mg, 0.25 mmol) in 25% DMF–CH_2Cl_2 was treated sequentially with $NaHCO_3$ (21 mg, 0.25 mmol), HOAt (37.4 mg, 0.275 mmol), and EDCI (52.7 mg, 0.275 mmol). The reaction mixture was stirred at 0 °C for 2 h and at 25 °C for 10 h before 3 mL of H_2O was added. The aqueous layer was extracted with EtOAc (3 × 5 mL), and the combined EtOAc extracts were washed with H_2O (5 mL) and brine (5 mL), dried (Na_2SO_4), and concentrated in vacuo to afford BOC-Hpg–Gly-OMe (70 mg, 84.6 mg theoretical, 83%): $[\alpha]^{23}_D$ +79 (c 2.0, $CHCl_3$); ^{1}H NMR

($CDCl_3$, 400 MHz) δ 7.08 (d, 2H, J = 8.5 Hz), 6.71 (t, 1H, J = 5.3 Hz), 6.58 (d, 2H, J = 8.6 Hz), 5.76 (s, 1H), 5.09 (s, 1H), 4.04 (dd, 1H, J = 5.6, 18.2 Hz), 3.93 (dd, 1H, J = 5.3, 18.2 Hz), 3.69 (s, 3H), 1.04 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.3, 170.1, 156.5, 155.3, 128.7, 128.5, 115.9, 100.0, 80.5, 52.5, 41.3, 28.3; IR (neat) ν_{max} 3352, 2978,1794, 1669, 1615, 1515, 1456, 1368, 1219, 1164, 1040, 1024, 838, 756 cm^{-1}.

A sample of BOC-Hpg–Gly-OMe (117 mg, 0.35 mmol) was treated with 4 M HCl–EtOAc (2.0 mL). The resulting mixture was stirred at 25 °C for 1 h before the volatiles were removed under a stream of N_2. The residual HCl was further removed by adding Et_2O (1 mL) to the hydrochloride salt followd by its removal in vacuo to give a white solid (95 mg, 95 mg theoretical, >99%) which was employed directly in the next reaction without purification. A solution of this HCl salt (14.4 mg, 52.5 μmol) and **30** (34.0 mg, 52.5 μmol) in 50% DMF–CH_2Cl_2 (0.8 mL) was treated sequentially with $NaHCO_3$ (4.4 mg 52.5 μmol), HOAt (7.5 mg, 55 μmol), and EDCI (10.5 mg, 55.0 μmol). The reaction mixture was stirred at 0 °C for 2 h and at 25 °C for 6 h before 1 mL of H_2O was added. The aqueous solution was extracted with EtOAc (3 × 5 mL), and the combined EtOAc extracts were washed with H_2O (5 mL) and brine (5 mL), dried (Na_2SO_4), and concentrated in vacuo to give BOC-D-Orn(SES)–L-Hpg–D-*a*Thr–L-Hpg–Gly-OMe as a white solid (25.3 mg, 45.5 mg theoretical, 56%): R_f = 0.15 (75% EtOAc–hexanes); ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.85 (s, 1H), 8.81 (s, 1H), 7.28 (m, 4H), 6.76 (m, 4H), 6.40 (m, 1H), 6.28 (m, 1H), 5.47 (d, 1H, J = 6.0 Hz), 5.43 (m, 1H), 4.52 (m, 1H), 4.29 (m, 1H), 4.14 (m, 1H), 4.00 (d, 1H, J = 5.2 Hz), 3.97 (d, 1H, J = 4.8 Hz), 3.85 (m, 1H), 3.02 (m, 2H), 2.92 (m, 2H), 1.04 (s, 9H), 1.01 (d, 3H, J = 5.2 Hz), 0.96 (m, 2H), 0.05 (s, 9H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 174.5, 173.5, 171.6, 158.8, 158.7, 152.8, 130.6, 130.6, 130.6, 130.2, 128.9, 128.8, 116.5, 116.4, 68.4, 68.1, 60.3, 58.7, 58.6, 55.6, 52.6, 43.3, 42.0, 30.3, 28.7, 27.8, 20.2, 11.4, –2.0; FABHRMS (NAB–CsI) *m/z* 999.2571 (M + Cs^+, $C_{38}H_{58}N_6O_{13}SSi$ requires 999.2006).

BOC-D-Orn(SES)–L-Hpg–D-*a*Thr–L-Hpg–Gly-OH (34). A solution of **33** (41 mg, 0.044 mmol) in CH_3OH (5 mL) was treated with 10% Pd–C (32 mg). The resulting black suspension was stirred under H_2 (1 atm) at room temperature for 5 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo to give **34**

(34 mg, 92%) as a white solid: mp 213–215 °C (decomp); $[\alpha]^{23}_D$ +80 (*c* 0.17, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.28 (d, 2H, *J* = 8.5 Hz), 7.23 (d, 2H, *J* = 8.5 Hz), 6.75 (d, 2H, *J* = 8.5 Hz), 6.74 (d, 2H, *J* = 8.5 Hz), 5.39 (s, 1H), 5.30 (s, 1H), 4.26 (d, 1H, *J* = 7.0 Hz), 4.26–3.96 (m, 3H), 3.77 (m, 1H), 3.02 (t, 2H, *J* = 6.3 Hz), 2.95–2.91 (m, 2H), 1.75–1.62 (m, 1H), 1.61–1.52 (m, 3H), 1.42 (s, 9H), 1.05 (d, 3H, *J* = 6.2 Hz), 0.98–0.93 (m, 2H), 0.05 (s, 9H); ^{13}C NMR (CD_3OD, 150 MHz) δ 174.7, 173.5, 173.0, 158.9, 158.8, 158.0, 130.7, 130.3, 129.0, 129.0, 129.0, 116.7, 116.6, 81.0, 68.5, 58.9, 55.7, 43.5, 43.5, 42.1, 32.9, 30.5, 28.9, 27.9, 20.4, 11.5, –1.8; IR (neat) ν_{max} 3287, 1633, 1514, 1251 cm^{-1}; MALDI–FTMS (DHB) *m/z* 875.3291 ($M + Na^+$, $C_{37}H_{56}N_6O_{13}SSi$ requires 875.3288).

Fmoc-L-Asn(Trt)–L-*threo*-*O*-[BOC-L-Leu–D-Ala–L-Chp]-HAsn(Trt)–D-Hpg–D-Orn(SES)–D-*a*Thr–L-Hpg–D-Hpg–L-*a*Thr–L-Phe-OBn (36). A sample of **14** (36 mg, 28.0 μmol) in a reaction vessel was treated with 4 M HCl–dioxane (0.48 mL). The resulting suspension was stirred at room temperature for 30 min before the volatiles were removed with a stream of N_2. The residual HCl was removed by adding Et_2O (3 × 0.8 mL) to the hydrochloride salt **15** followed by its removal with a stream of N_2, and the resulting solid **15** was dried in vacuo for 2 h. A sample of **26** (40 mg, 28.0 μmol), DEPBT (25.4 mg, 42.0 μmol), and $NaHCO_3$ (7.2 mg, 42.0 μmol) were added to the residue **15** and the solid mixture was dissolved in DMF (0.20 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 20 h. The mixture was diluted with EtOAc (4 mL) and the resulting white solid was removed by filtration. The filtrate was concentrated in vacuo. Column chromatography (SiO_2, 1 × 5 cm, 2–10% EtOH–CH_2Cl_2) provided **36** as a pale yellow solid (42.3 mg, 72.9 mg theoretical, 58%; typically 50–68%): mp 174–176 °C; R_f = 0.52 (10% CH_3OH–CH_2Cl_2); $[\alpha]^{23}_D$ –12 (*c* 0.22, CH_2Cl_2); ^1H NMR (DMSO-d_6, 600 MHz) δ 10.04 (s, 1H), 9.32 (s, 1H), 9.31 (s, 1H), 9.30 (s, 1H), 8.78 (d, 1H, *J* = 8.3 Hz), 8.59 (m, 1H), 8.55 (s, 1H), 8.50 (m, 1H), 8.47 (d, 1H, *J* = 7.0 Hz), 8.33 (m, 1H), 8.24 (m, 2H), 8.12 (m, 1H), 8.03 (m, 1H), 7.90 (d, 3H, *J* = 7.4 Hz), 7.74 (d, 1H, *J* = 7.4 Hz), 7.70 (d, 1H, *J* = 7.5 Hz), 7.41 (m, 2H), 7.34–7.01 (m, 51H), 6.92 (m, 1H), 6.87 (d, 1H, *J* = 7.9 Hz), 6.83 (m, 1H), 6.63 (d, 2H, *J* = 8.8 Hz), 6.59 (m, 4H), 5.60 (d, 1H, *J* = 7.9 Hz), 5.56 (d, 1H, *J* = 7.9 Hz), 5.52 (s, 1H), 5.47 (d, 1H, *J* = 7.9 Hz), 5.33 (d, 1H, *J* = 6.5 Hz), 5.13 (m, 1H), 5.08 (d, 1H, *J* = 12.7 Hz), 5.03 (d, 1H, *J* = 12.7 Hz), 4.86 (d, 1H, *J* = 6.1 Hz),

4.68 (d, 1H, J = 4.9 Hz), 4.65 (m, 1H), 4.56 (dd, 1H, J = 7.4, 14.4 Hz), 4.43 (m, 1H), 4.34 (m, 2H), 4.27 (m, 2H), 4.16 (m, 2H), 3.95 (m, 1H), 3.75 (m, 1H), 3.69 (m, 1H), 3.01 (m, 2H), 2.84 (m, 5H), 2.62 (m, 1H), 1.64 (m, 1H), 1.60–1.35 (m, 7H), 1.31 (s, 9H), 1.16 (d, 3H, J = 6.5 Hz), 0.96 (d, 3H, J = 6.1 Hz), 0.85 (m, 8H), 0.78 (d, 3H, J = 6.1 Hz), 0.00 (s, 9H); IR (neat) ν_{max} 3298, 3067, 2923, 1638, 1512, 1446, 1250, 1174, 836 cm^{-1}; MALDI–FTMS (DHB) m/z 2623.0097 (M + Na^+, $C_{141}H_{154}ClN_{15}O_{28}SSi$ requires 2623.0158).

Fmoc-L-Asn(Trt)–L-*threo*-*O*-[BOC-D-Orn(SES)–L-Hpg–D-*a*Thr–L-Hpg–Gly–L-Leu–D-Ala–L-Chp]-HAsn(Trt)–D-Hpg–D-Orn(SES)–D-*a*Thr–L-Hpg–D-Hpg–L-*a*Thr–L-Phe-OBn (37). A sample of **36** (15.0 mg, 5.73 μmol) in CH_2Cl_2 (0.06 mL) was treated with a solution of *B*-bromocatecholborane (11.4 mg, 57.4 μmol) in CH_2Cl_2 (0.06 mL) at 0 °C for 30 min. The reaction mixture was quenched with H_2O (1 mL) and extracted with CH_2Cl_2 (1 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (1 mL), dried (Na_2SO_4), filtered, and concentrated. A solution of the residue, **34** (4.9 mg, 5.7 μmol) and HOAt (2.3 mg, 17 μmol) in DMF (0.06 mL) maintained at 0 °C was treated with EDCI (3.2 mg, 17 μmol). The reaction mixture was stirred for 20 h at 0 °C, and then quenched by the addition of EtOAc. The slurry was washed with 1 N aqueous HCl (5 mL), saturated aqueous $NaHCO_3$ (5 mL) and brine (5 mL), dried (Na_2SO_4), filtered, and concentrated to provide **37** (11.5 mg, 19.2 mg theoretical, 60%; typically 60–82%) as a white solid: mp 193–195 °C; R_f = 0.50 (10% EtOH–$CHCl_3$); $[\alpha]^{23}_D$ +35 (c 0.095, CH_3OH); ^1H NMR (DMSO-d_6, 500 MHz) δ 10.01 (s, 1H), 9.34 (s, 1H), 9.33 (s, 1H), 9.31 (s, 1H), 9.30 (s, 1H), 9.29 (s, 1H), 8.80–8.77 (m, 1H), 8.62–8.59 (m, 1H), 8.55–8.51 (m, 2H), 8.46 (d, 2H, J = 7.7 Hz), 8.36–8.34 (m, 1H), 8.32–8.30 (m, 1H), 8.25–8.21 (m, 2H), 8.16 (d, 2H, J = 7.7 Hz), 8.12–8.10 (m, 1H), 8.07–8.04 (m, 1H), 7.90 (d, 2H, J = 7.7 Hz), 7.84–7.82 (m, 1H), 7.74 (d, 1H, J = 7.3 Hz), 7.71–7.67 (m, 1H), 7.43–7.38 (m, 2H), 7.35–6.84 (m, 60H), 6.66 (d, 4H, J = 7.0 Hz), 6.62 (d, 2H, J = 8.4 Hz), 6.57 (d, 2H, J = 8.4 Hz), 6.54–6.51 (m, 2H), 5.60 (d, 1H, J = 7.7 Hz), 5.56–5.53 (m, 1H), 5.50–5.48 (m, 1H), 5.47 (d, 1H, J = 8.1 Hz), 5.41 (d, 1H, J = 7.4 Hz), 5.36 (d, 1H, J = 7.7 Hz), 5.31 (d, 1H, J = 6.2 Hz), 5.15–5.12 (m, 1H), 5.07 (d, 1H, J = 12.5 Hz), 5.02 (d, 1H, J = 12.5 Hz), 4.87–4.81 (m, 2H), 4.67 (d, 2H, J = 4.8 Hz), 4.57 (d, 1H, J = 7.4 Hz), 4.53 (d, 1H, J = 7.4 Hz), 4.45–4.40 (m, 1H), 4.37–4.23 (m, 9H), 4.17–4.15 (m, 2H),

4.00–3.94 (m, 1H), 3.77–3.62 (m, 2H), 3.02–2.98 (m, 6H), 2.86–2.82 (m, 6H), 1.64–1.38 (m, 11H), 1.37 (s, 9H), 1.13 (d, 3H, J = 6.6 Hz), 0.96 (d, 3H, J = 5.9 Hz), 0.87–0.76 (m, 16H), 0.01 (s, 9H), –0.01 (s, 9H); IR (neat) ν_{max} 3286, 1684, 1637, 1513, 1253, 1170, 1139 cm^{-1}; MALDI–FTMS (DHB) m/z 3391.3037 ($M + Na^+$, $C_{176}H_{198}ClN_{21}O_{38}S_2Si_2$ requires 3391.2767). In rare instances where necessary, column chromatography of impure **37** (SiO_2, 1 × 5 cm, 2–8% EtOH–CH_2Cl_2) provided pure **37**.

Cbz-D-Orn(SES)–L-Hpg–D-*a*Thr–L-Hpg–Gly-OH (39). A sample of **34** (37 mg, 0.043 mmol) in EtOAc (3 mL) was treated with 4 M HCl–EtOAc (0.5 mL, 2 mmol). The resulting mixture was stirred at 25 °C for 1 h, then the volatiles were removed under a stream of N_2. The residual HCl was removed by adding Et_2O (1 mL) to the hydrochloride salt followed by its removal in vacuo to give a white solid. The residue was dissolved in 50% dioxane–H_2O (4 mL). The resulting solution was treated with $NaHCO_3$ (18 mg, 0.21 mmol) and CbzCl (36 mg, 0.21 mmol) at 25 °C. The reaction mixture was stirred at 25 °C for 30 min, then saturated aqueous $NaHCO_3$ (5 mL) was added. The mixture was washed with Et_2O (1 mL), and the aqueous layer was acidified with 1 N aqueous HCl to pH 1–2. The reaction mixture was extracted with EtOAc (3 × 80 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to afford **39** (30 mg, 38 mg theoretical, 78%) as a solid which was employed directly in the next coupling reaction without further purification: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.40 (s, 1H), 9.38 (s, 1H), 8.62–8.55 (m, 1H), 8.40 (d, 2H, J = 8.1 Hz), 8.34 (d, 1H, J = 8.4 Hz), 7.47–7.32 (m, 5H), 7.30 (d, 2H, J = 8.8 Hz), 7.25 (d, 2H, J = 8.4 Hz), 6.96 (t, 1H, J = 5.9 Hz), 6.73 (d, 4H, J = 8.8 Hz), 5.48 (d, 2H, J = 8.1 Hz), 5.09 (s, 2H), 4.86 (d, 2H, J = 6.3 Hz), 4.32 (t, 1H, J = 8.4 Hz), 4.21–4.13 (m, 1H), 3.87–3.74 (m, 2H), 2.98–2.88 (m, 4H), 1.73–1.64 (m, 1H), 1.58–1.44 (m, 3H), 0.94–0.86 (m, 5H), 0.08 (s, 9H).

Fmoc-L-Asn(Trt)–L-*threo*-*O*-[Cbz-D-Orn(SES)–L-Hpg–D-*a*Thr–L-Hpg–Gly–L-Leu–D-Ala–L-Chp]-HAsn(Trt)–D-Hpg–D-Orn(SES)–D-*a*Thr–L-Hpg–D-Hpg–L-*a*Thr–L-Phe-OBn (40). A sample of **36** (5.0 mg, 1.9 μmol) in CH_2Cl_2 (0.06 mL) was treated with a 0.2 M solution of *B*-bromocatecholborane in CH_2Cl_2 (0.06 mL, 12 μmol) at 0 °C for 30 min with appearance of a precipitate. A few drops of 5% MeOH–CH_2Cl_2 were added to solubilize the reaction mixture for purification. Column chromatography (SiO_2, 0.7 × 7 cm, 5–8% MeOH–CH_2Cl_2) provided the amine as a white solid (3.0 mg). The

residue and **39** (1.3 mg, 1.4 μmol) were treated with a solution of EDCI (0.69 mg, 3.6 μmol) and HOAt (0.49 mg, 3.6 μmol) in 25% DMF–CH_2Cl_2 (0.04 mL) at 0 °C. The reaction mixture was stirred for 17 h at 0 °C, and then solvents were evaporated with a stream of N_2. PTLC (SiO_2, two 10 × 15 cm plates, 12% MeOH–CH_2Cl_2) provided **40** (3.3 mg, 4.0 mg theoretical, 82%) as a white solid: mp 193–195 °C (decomp); R_f = 0.70 (12% MeOH–$CHCl_3$); $[\alpha]^{23}_D$ +21 (*c* 0.14, CH_3OH); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.02 (s, 1H), 9.36 (s, 1H), 9.35 (s, 1H), 9.32 (s, 1H), 9.31 (s, 1H), 9.30 (s, 1H), 8.80–8.77 (m, 1H), 8.62–8.59 (m, 1H), 8.55–8.51 (m, 2H), 8.48 (d, 2H, *J* = 7.0 Hz), 8.39 (d, 2H, *J* = 7.0 Hz), 8.30 (m, 2H), 8.25 (m, 2H), 8.19 (m, 1H), 8.11 (m, 1H), 8.06 (m, 1H), 7.90 (d, 2H, *J* = 7.4 Hz), 7.84 (m, 1H), 7.75 (d, 1H, *J* = 7.0 Hz), 7.72 (m, 2H), 7.41 (m, 3H), 7.35–7.28 (m, 10H), 7.27–7.05 (m, 42H), 7.02 (m, 7H), 6.93 (m, 3H), 6.86 (m, 1H), 6.66 (dd, 2H, *J* = 3.1, 8.3 Hz), 6.63 (d, 2H, *J* = 8.8 Hz), 6.58 (d, 2H, *J* = 8.8 Hz), 6.53 (d, 2H, *J* = 8.8 Hz), 5.60 (d, 1H, *J* = 7.9 Hz), 5.56 (m, 1H), 5.50 (br s, 1H), 5.47 (d, 1H, *J* = 7.9 Hz), 5.42 (d, 1H, *J* = 7.0 Hz), 5.38 (d, 1H, *J* = 6.5 Hz), 5.31 (d, 1H, *J* = 6.6 Hz), 5.14 (m, 1H), 5.07 (d, 1H, *J* = 12.7 Hz), 5.02 (m, 3H), 4.84 (t, 2H, *J* = 7.4 Hz), 4.68 (d, 2H, *J* = 4.9 Hz), 4.57 (d, 1H, *J* = 7.4 Hz), 4.55 (d, 1H, *J* = 7.4 Hz), 4.43 (m, 1H), 4.39–4.24 (m, 6H), 4.17 (m, 2H), 4.14 (m, 1H), 3.74 (m, 2H), 3.68 (m, 3H), 3.00 (m, 6H), 2.85 (m, 6H), 1.63 (m, 2H), 1.56–1.36 (m, 9H), 1.13 (d, 3H, *J* = 7.0 Hz), 0.97 (d, 3H, *J* = 6.1 Hz), 0.84 (m, 13H), 0.78 (d, 3H, *J* = 6.2 Hz), 0.01 (s, 9H), –0.01 (s, 9H); IR (neat) ν_{max} 3405, 2923, 1631, 1508, 1451, 1256, 1067, 872 cm^{-1}; MALDI–FTMS (DHB) *m/z* 3391.3037 ($M + Na^+$, $C_{176}H_{198}ClN_{21}O_{38}S_2Si_2$ requires 3391.2767).

Fmoc-L-Asn(Trt)–L-*threo*-*O*-[BOC-L-Leu–D-Ala–L-Chp]-HAsn(Trt)–D-Hpg–D-Orn(SES)–D-*a*Thr–L-Hpg–D-Hpg–L-*a*Thr–L-Phe–D-Orn(SES)–L-Hpg–D-*a*Thr–L-Hpg–Gly-OBn (41). A sample of **34** (2.7 mg, 2.8 μmol) in a reaction vessel was treated with 4 M HCl–dioxane (0.4 mL). The resulting suspension was stirred at room temperature for 30 min before the volatiles were removed with a stream of N_2. The residual HCl was removed by adding Et_2O (3 × 0.4 mL) to the hydrochloride salt **35** followed by its removal with a stream of N_2, and the resulting solid **35** was dried in vacuo for 2 h. A solution of **36** (7.4 mg, 2.8 μmol) in C_2H_5OH (1.0 mL) was treated with 10% Pd–C (7 mg). The resulting black suspension was stirred under H_2 (1 atm) at room temperature for 4 h. The catalyst was removed by filtration through Celite, and the

filtrate was concentrated in vacuo to give the carboxylic acid as a white solid. To the residue was added $NaHCO_3$ (0.7 mg, 8.3 μmol), HOAt (1.4 mg, 10 μmol), and a solution of **35** (2.5 mg, 2.8 μmol) in DMF (100 μL). The mixture was treated at 0 °C with EDCI (1.5 mg, 7.8 μmol), and the reaction mixture was stirred for 20 h at 0 °C, and then quenched by the addition of EtOAc. The reaction mixture was washed with 5% aqueous HCl (3 mL), saturated aqueous $NaHCO_3$ (3 mL), and brine (3 mL), dried (Na_2SO_4), and concentrated. PTLC (10% EtOH–CH_2Cl_2) provided **41** (7.1 mg, 75%) as a white solid: mp 217 °C (decomp); R_f = 0.51 (10% EtOH–$CHCl_3$); $[\alpha]^{23}_D$ +40 (*c* 0.050, MeOH); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.04 (s, 1H), 9.36 (s, 1H), 9.34 (s, 1H), 9.31 (s, 1H), 9.30 (s, 1H), 9.28 (s, 1H), 8.73–8.67 (m, 2H), 8.62–8.58 (m, 1H), 8.57–8.54 (br s, 1H), 8.52–8.47 (m, 2H), 8.45–8.40 (m, 2H), 8.38 (d, 1H, *J* = 7.5 Hz), 8.33 (d, 1H, *J* = 7.0 Hz), 8.26–8.21 (m, 2H), 8.14 (s, 1H), 8.14 (m, 3H), 7.90 (d, 1H, *J* = 7.5 Hz), 7.93–7.89 (m, 1H), 7.74 (d, 1H, *J* = 7.5 Hz), 7.70 (d, 1H, *J* = 7.5 Hz), 7.43–7.39 (m, 2H), 7.37–7.08 (m, 46H), 7.05–6.99 (m, 8H), 6.94–6.89 (m, 2H), 6.89–6.86 (m, 2H), 6.85–6.81 (m, 1H), 6.68–6.53 (m, 11H), 5.62 (d, 1H, *J* = 6.6 Hz), 5.57 (d, 1H, *J* = 8.3 Hz), 5.54–5.48 (m, 2H), 5.44–5.40 (m, 2H), 5.33–5.30 (m, 1H), 5.16–5.12 (m, 1H), 5.11–5.08 (m, 2H), 4.88–4.84 (m, 1H), 4.81 (d, 1H, *J* = 6.1 Hz), 4.68–4.61 (m, 1H), 4.49–4.38 (m, 5H), 4.38–4.31 (m, 1H), 4.31–4.25 (m, 2H), 4.19–4.10 (m, 2H), 3.93–3.88 (m, 1H), 3.74–3.68 (m, 1H), 3.68–3.62 (m, 1H), 3.61–3.57 (m, 1H), 2.89–2.80 (m, 8H), 1.69–1.20 (m, 15H), 1.31 (s, 9H), 1.16 (d, 3H, *J* = 7.0 Hz), 0.96 (d, 3H, *J* = 5.7 Hz), 0.88–0.77 (m, 13H), 0.74 (d, 3H, *J* = 5.7 Hz), 0.02 (s, 9H), –0.01 (s, 9H); MALDI–FTMS (DHB) *m/z* 3357.3070 (M + Na^+, $C_{173}H_{200}ClN_{21}O_{38}S_2Si_2$ requires 3357.2923).

Cyclo-Fmoc-L-Asn(Trt)–[*O*-[D-Hpg–D-Orn(SES)–D-*a*Thr–L-Hpg–D-Hpg–L-*a*Thr–L-Phe–D-Orn(SES)–L-Hpg–D-*a*Thr–L-Hpg–Gly–L-Leu–D-Ala–L-Chp]–L-*threo*-HAsn(Trt)] (38). Method A (closure site at Phe^9–Orn^{10} from **37**): A suspension of **37** (3.3 mg, 1.0 μmol) in CH_3CN (0.1 mL) was treated with a solution of *B*-bromocatecholborane (2.0 mg, 10 μmol) in CH_3CN (100 μL) and the mixture was stirred at 0 °C for 3 h. Et_2O (1 mL) was added to the mixture, and the resulting white precipitate was collected by filtration to give the crude amine. A solution of the amine in EtOH (100 μL) was treated with 10% Pd–C (6.6 mg). The resulting black suspension was stirred under H_2 (1 atm) at 25 °C for 2 h. The catalyst was removed by filtration through Celite,

and the filtrate was concentrated in vacuo to give a white solid. The residue was treated at 0 °C with a solution of EDCI (5.0 μmol) and HOAt (5.0 μmol) in DMF/CH_2Cl_2 (1:2, 1.0 mL). The reaction mixture was stirred at 0 °C for 18 h, and then quenched by the addition of EtOAc. The reaction mixture was washed with 5% aqueous HCl (0.5 mL) and brine (0.5 mL), dried (Na_2SO_4), and concentrated. PTLC (SiO_2, 8 × 10 cm plate, 15% EtOH–CH_2Cl_2) provided **38** as a white solid (1.7 mg, 54%; typically 54–72%): R_f = 0.60 (15% EtOH–$CHCl_3$); $[\alpha]^{23}_D$ +90 (*c* 0.050, EtOH); ^{1}H NMR (50% D_2O–DMSO-d_6, 500 MHz) δ 7.85 (d, 2H, *J* = 6.6 Hz), 7.75–7.70 (m, 1H), 7.65–7.58 (m, 3H), 7.42–7.29 (m, 6H), 7.20–6.99 (m, 29H), 6.88–6.72 (m, 11H), 6.68 (d, 2H, *J* = 8.1 Hz), 6.50 (t, 4H, *J* = 8.5 Hz), 6.40 (d, 1H, *J* = 7.7 Hz), 6.35 (d, 2H, *J* = 8.5 Hz), 6.27 (d, 2H, *J* = 8.4 Hz), 6.18–6.10 (m, 3H), 5.95–5.90 (m, 1H), 5.78–5.74 (m, 1H), 5.59–5.55 (m, 1H), 5.38–5.35 (m, 1H), 4.91–4.88 (m, 1H), 4.81–4.75 (m, 1H), 4.62–4.55 (m, 1H), 4.20–4.10 (m, 3H), 4.08–4.01 (m, 2H), 3.88–3.85 (m, 2H), 3.82–3.76 (m, 2H), 3.72–3.67 (m, 1H), 3.65–3.62 (m, 1H), 3.52–3.44 (m, 1H), 3.15–3.07 (m, 1H), 2.94–2.74 (m, 6H), 2.27–2.20 (m, 1H), 2.18–2.10 (m, 1H), 1.93–1.87 (m, 1H), 1.55–1.32 (m, 4H), 1.30–1.10 (m, 10H), 1.08–1.00 (m, 2H), 0.92 (d, 3H, *J* = 5.5 Hz), 0.87–0.75 (m, 13H), 0.60–0.58 (m, 3H), 0.52–0.50 (m, 3H), 0.34–0.30 (m, 3H), –0.06 (s, 9H), –0.09 (s, 9H); IR (neat) ν_{max} 3295, 1637, 1513, 1249 cm^{-1}; MALDI–FTMS (DHB) *m/z* 3149.1625 (M + Na^+, $C_{161}H_{184}ClN_{21}O_{35}S_2Si_2$ requires 3149.1824).

Purification of the intermediate amino acid (2.5 mg, 0.79 μmol) and cyclization under the same conditions provided **38** (2.2 mg, 2.5 mg theoretical, 89%).

Method B (closure site at Phe^9–Orn^{10} from **40**): A solution of **40** (4.5 mg, 1.33 μmol) in 50% MeOH–DMF (0.6 mL) was treated with 10% Pd–C (4.2 mg). The resulting black suspension was stirred under H_2 (1 atm) at 25 °C for 4 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo to give a white solid (4.2 mg). The residue was treated at 0 °C with a solution of EDCI (6.65 μmol) and HOAt (6.65 μmol) in 25% DMF–CH_2Cl_2 (0.7 mL). The reaction mixture was stirred at 0 °C for 18 h, and solvents were evaporated with a stream of N_2. PTLC (SiO_2, 8 × 10 cm plate, 12% MeOH–CH_2Cl_2) provided **38** as a white solid (2.7 mg, 4.2 mg theoretical, 65%; typically 60–71%).

Method C (closure site at Gly^{14}–Leu^{15} from **41**): A suspension of **41** (7.1 mg, 2.1 μmol) in CH_3CN (0.2 mL) was treated with a solution of *B*-bromocatecholborane (4.0 mg, 21 μmol) in CH_3CN (200 μL) and the mixture was stirred at 0 °C for 3 h. Et_2O (2 mL) was added to the mixture, and the resulting white precipitate was collected by filtration to give the crude amine. A solution of the amine in EtOH (200 μL) was treated with 10% Pd–C (13.2 mg) and the resulting black suspension was stirred under H_2 (1 atm) at 25 °C for 2 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo to give a white solid. The residue was treated at 0 °C with a solution of EDCI (1.6 mg, 10.0 μmol) and HOAt (1.4 mg, 10.0 μmol) in 33% DMF–CH_2Cl_2 (2.0 mL). The reaction mixture was stirred at 0 °C for 18 h, and then quenched by the addition of EtOAc. The reaction mixture was washed with 5% aqueous HCl (0.5 mL) and brine (0.5 mL), dried (Na_2SO_4), and concentrated. PTLC (SiO_2, 8 × 10 cm plate, 15% EtOH–CH_2Cl_2) provided **38** as a white solid (3.3mg, 50%).

(2*Z*,4*E*)-7-Methyl-2,4-octadienoic Acid Anhydride (43). A solution of 18-crown-6 (1.47 g, 4.81 mmol, 1:1 complex with CH_3CN) and bis(2,2,2-trifluoroethyl) (methoxycarbonylmethyl)phosphonate (0.23 mL, 1.06 mmol) in THF (17 mL) at –78 °C was treated with a 0.5 M solution of KHMDS in THF (5.8 mL). The reaction mixture was stirred for 15 min before a solution of (2*E*)-5-methyl-2-hexenal[S1] (108 mg, 0.963 mmol) in THF (2 mL) was added at –78 °C. The reaction mixture was stirred for 30 min and quenched with saturated aqueous NH_4Cl (20 mL), and Et_2O (60 mL) was added. The ethereal layer was washed with saturated aqueous NH_4Cl (2 × 30 mL) and brine (30 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo [Product R_f = 0.50 (5% EtOAc–hexanes), >20:1 *Z*:*E*]. The concentrate was dissolved in a mixture of 25% H_2O–CH_3OH (10 mL) and lithium hydroxide monohydrate (115 mg, 4.80 mmol) was added. The reaction mixture was stirred at room temperature for 40 h. CH_3OH was removed in vacuo, water (50 mL) and Et_2O (50 mL) were added, and the organic layer was discarded. The aqueous layer was acidified to pH 1–2 with 2 N aqueous HCl, and extracted with Et_2O (3 × 50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to give (2*Z*,4*E*)-7-methyl-2,4-octadienoic acid **42** as a pale oil (126 mg, 151 mg theoretical, 86%): R_f = 0.50 (30% EtOAc–hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33 (dd, 1H, *J* = 11.3, 15.0 Hz), 6.66 (dd, 1H, *J* = 11.3, 11.3 Hz), 6.11 (td, 1H, *J* = 7.7, 15.0

Hz), 5.58 (d, 1H, J = 11.3 Hz), 2.11 (dd, 2H, J = 7.7, 7.7 Hz), 1.73 (m, 1H), 0.92 (d, 6H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.2, 147.7, 146.0, 128.0, 114.7, 42.3, 28.3, 22.4; IR (neat) ν_{max} 3046, 2957, 1688, 1633, 1600, 1443, 1247, 1231, 963 cm^{-1}. Anal. Calcd for $C_9H_{14}O_2$: C, 70.10; H, 9.15. Found: C, 69.94; H, 9.50.

A solution of (2*Z*,4*E*)-7-methyl-2,4-octadienoic acid (126 mg, 0.82 mmol) in CH_2Cl_2 (1 mL) was treated at 25 °C with EDCI (82 mg, 0.43 mmol) under Ar and the reaction mixture was stirred at 25 °C for 1 h. The reaction mixture was diluted with hexanes (5 mL) and the urea byproduct precipitated. The solution was decanted from the solid urea. The solid urea was washed with hexanes (2 × 5 mL) and the combined solution was concentrated in vacuo to afford a pale oil. Column chromatography (acetone deactivated SiO_2, 1 × 4 cm, 15% EtOAc–hexanes) provided (2*Z*,4*E*)-7-methyl-2,4-octadienoic acid anhydride (**43**) as a pale oil (94 mg, 119 mg theoretical, 83%): R_f = 0.50 (10% EtOAc–hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38 (dd, 1H, J = 11.2, 15.0 Hz), 6.74 (dd, 1H, J = 11.2, 11.2 Hz), 6.19 (td, 1H, J = 7.7, 15.0 Hz), 5.60 (d, 1H, J = 11.4 Hz), 2.14 (dd, 2H, J = 7.4, 7.4 Hz), 1.75 (m, 1H), 0.93 (d, 6H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.7, 149.5, 147.7, 128.0, 113.7, 42.3, 28.3, 22.4; MALDI–FTMS (DHB) *m/z* 313.1772 (M + Na$^+$, $C_{18}H_{26}O_3$ requires 313.1774).

(2*Z*,4*E*)-7-Methyl-2,4-octadienoyl–L-Asn(Trt)–L-*threo*-*O*-[BOC-L-Chp]-HAsn-(Trt)-OBn (45). A sample of **20** (5.0 mg, 3.4 μmol) was treated with a 0.20 M solution of *i*-PrOH in DMF (34 μmol, 170 μL) at 25 °C for 5 min followed by a 0.04 M solution of Bu_4NF in DMF (7.0 μmol, 175 μL). The reaction solution was sonicated for 10 min at 25 °C. EtOAc (10 mL) was added to the mixture, and the solution was washed with brine (2 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo to provide the unstable free amine **44** as a white solid (3.9 mg, 4.2 mg theoretical, 91%) which was dissolved in DMF (170 μL) without purification. This free amine solution was treated with **43** (6.8 μmol, 2.0 μL) and the reaction mixture was stirred at 25 °C for 1 h. The solvents were removed in vacuo and the mixture was washed with hexanes (2 × 2 mL) to provided crude **45**. PTLC (SiO_2, 10 × 15 cm, 33% EtOAc–hexanes) provided **45** (3.5 mg, 3.9 mg theoretical, 90%) as a white solid: mp 123–124 °C; R_f = 0.6 (50% EtOAc–hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.30 (d, 1H, J = 6.7 Hz), 7.48 (s, 1H), 7.45 (d, 1H, J = 13.0 Hz),

7.42 (s, 1H), 7.36–6.99 (m, 36H), 6.89 (m, 4H), 6.84 (br s, 1H), 6.79 (d, 1H, J = 7.7 Hz), 6.38–6.31 (m, 2H), 5.99 (td, 1H, J = 7.3, 15.0 Hz), 5.73 (br s, 1H), 5.42 (s, 1H), 5.33–5.26 (m, 1H), 5.20 (dd, 1H, J = 3.0, 8.8 Hz), 5.13 (br s, 2H), 4.98 (m, 1H), 4.72 (m, 1H), 3.08–3.00 (m, 1H), 2.78–2.70 (m, 1H), 2.11 (dd, 2H, J = 7.3, 7.3 Hz), 1.77–1.67 (m, 3H), 1.41 (s, 9H), 0.92 (d, 3H, J = 6.6 Hz), 0.92 (d, 3H, J = 6.6 Hz); MALDI–FTMS (DHB) *m/z* 1278.4980 (M + Na^+, $C_{84}H_{90}ClN_7O_{13}$ requires 1278.4965).

(2*Z*,4*E*)-7-Methyl-2,4-octadienoyl–L-Asn(Trt)–L-*threo*-*O*-[BOC-L-Leu–D-Ala–L-Chp]-HAsn(Trt)-OBn (46). A sample of **24** (5.0 mg, 3.0 μmol) was treated with a 0.20 M solution of *i*-PrOH in DMF (30 μmol, 150 μL) at room temperature for 5 min followed by a 0.04 M solution of Bu_4NF in DMF (6.1 μmol, 151 μL). The reaction solution was sonicated for 10 min at 25 °C. EtOAc (10 mL) was added to the mixture, and the solution was washed with brine (2 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo to provide the unstable free amine as a white solid (4.0 mg) which was dissolved in DMF (150 μL) without purification. This free amine solution was treated with **43** (6.0 μmol, 1.8 μL) and the reaction mixture was stirred at 25 °C for 1 h. The solvents were removed in vacuo and the mixture was washed with hexanes (2 × 2 mL) to provided crude **46**. PTLC (SiO_2, 10 × 15 cm, 33% EtOAc–hexanes) provided **46** (3.9 mg, 4.4 mg theoretical, 90%) as a white solid: mp 195 °C (decomp); R_f = 0.6 (50% EtOAc–hexanes); $[\alpha]^{23}_D$ +15 (*c* 0.050, EtOH); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.21 (s, 1H), 8.74 (s, 1H), 8.49 (s, 1H), 8.47 (d, 1H, J = 9.7 Hz), 8.45 (d, 1H, J = 6.6 Hz), 8.33 (d, 1H, J = 8.3 Hz), 7.89 (d, 1H, J = 7.9 Hz), 7.56 (dd, 1H, J = 11.4, 15.1 Hz), 7.34–6.96 (m, 36H), 6.93 (dd, 1H, J = 1.8, 8.6 Hz), 6.83 (d, 1H, J = 7.9 Hz), 6.70 (d, 1H, J = 8.3 Hz), 6.50 (dd, 1H, J = 11.4, 11.4 Hz), 5.98 (td, 1H, J = 7.0, 15.1 Hz), 5.83 (d, 1H, J = 11.4 Hz), 5.72 (d, 1H, J = 2.8 Hz), 5.25 (d, 1H, J = 6.1 Hz), 5.22 (dd, 1H, J = 2.8, 9.0 Hz), 5.16 (d, 1H, J = 11.8 Hz), 5.05 (d, 1H, J = 11.8 Hz), 5.01 (m, 1H), 4.39 (m, 1H), 3.92 (m, 1H), 2.75–2.68 (m, 1H), 2.55–2.47 (m, 1H), 1.98 (dd, 2H, J = 7.0, 7.0 Hz), 1.68–1.60 (m, 1H), 1.57–1.49 (m, 1H), 1.35–1.28 (m, 2H), 1.29 (s, 9H), 1.16 (d, 3H, J = 7.0 Hz), 0.85 (d, 3H, J = 6.6 Hz), 0.84 (d, 3H, J = 7.0 Hz), 0.82 (d, 3H J = 6.6 Hz), 0.80 (d, 3H, J = 6.6 Hz); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 172.6, 172.1, 172.0, 169.2, 168.5, 168.2, 165.0, 164.4, 155.3, 153.1, 144.7, 143.9, 140.9, 140.6, 135.3, 128.6, 128.5, 128.4, 128.4, 128.2, 127.5, 127.4, 126.4,

126.2, 119.7, 119.2, 116.4, 78.0, 72.9, 69.5, 69.3, 67.1, 53.2, 53.0, 49.0, 47.7, 41.7, 40.6, 40.0, 28.1, 27.8, 24.2, 22.9, 22.2, 22.2, 21.5, 18.6; MALDI–FTMS (DHB) *m/z* 1462.6183 ($M + Na^+$, $C_{84}H_{90}ClN_7O_{13}$ requires 1462.6199).

N^1-Cyclo-[*O*-[D-Hpg–D-Orn(SES)–D-*a*Thr–L-Hpg–D-Hpg–L-*a*Thr–L-Phe–D-Orn(SES)–L-Hpg–D-*a*Thr–L-Hpg–Gly–L-Leu–D-Ala–L-Chp]–L-*threo*-HAsn(Trt)]–(2*S*)-[(2*Z*,4*E*)-7-Methyl-2,4-octadienoylamino]–N^4-Trityl-Succinamide (47). A sample of **38** (2.0 mg, 0.64 μmol) was treated with a 0.020 M solution of *i*-PrOH in DMF (6.4 μmol, 320 μL) at 25 for 5 min followed by a 0.016 M solution of Bu_4NF in DMF (5.1 μmol, 320 μL). The reaction solution was sonicated for 60 min at 25 °C. EtOAc (30 mL) was added to the mixture, and the resulting solution was washed by brine (2 × 20 mL), dried ($MgSO_4$), and concentrated in vacuo to provide the unstable free amine as a white solid (2.0 mg) which was employed directly in the next reaction without purification: t_R = 26.8 min (Waters analytical Nova–Pak® C_{18} 3.9 × 300 mm column, 1 mL/min, 30 min gradient of 30–100% CH_3CN–H_2O); MALDI–FTMS (DHB) *m/z* 2905.1339 ($M + H^+$, $C_{146}H_{174}ClN_{21}O_{33}S_2Si_2$ requires 2905.1324). The free amine (2.0 mg, 0.64 μmol) in DMF (320 μL) was treated with a 0.02 M solution of **43** in CH_2Cl_2 (1.2 μmol) at 25 °C for 14 h. The solvents were removed in vacuo and the crude mixture was washed with hexanes (4 × 2 mL) to provided crude **47** as a white solid. HPLC purification [Waters semipreparative LC 25 mm column, 10 mL/min, 30 min gradient of 30–100% CH_3CN–H_2O (t_R = 28.9 min)] provided pure **47** as a white solid (1.4 mg, 69% over two steps from **38**): t_R = 34.2 min (Waters analytical Nova–Pak® C_{18} 3.9 × 300 mm column, 1 mL/min, 30 min gradient of 30–100% CH_3CN–H_2O); $[\alpha]^{23}_D$ +53 (*c* 0.004, EtOH); ^{1}H NMR (33% D_2O–DMSO-d_6, 600 MHz, 70 °C) δ 7.38 (dd, 1H, *J* = 11.4, 14.5 Hz), 7.29 (s, 1H), 7.29 (s, 1H), 7.22–7.16 (m, 6H), 7.15–6.96 (m, 30H), 6.87–6.78 (m, 6H), 6.77 (s, 1H), 6.75 (s, 1H), 6.69–6.59 (m, 4H), 6.58–6.54 (m, 1H), 6.53–6.47 (m, 6H), 6.43 (d, 1H, *J* = 8.3 Hz), 6.39 (s, 1H), 6.37 (s, 1H), 6.33 (s, 1H), 6.32 (s, 1H), 6.24 (d, 1H, *J* = 8.3 Hz), 6.08 (s, 1H), 6.03 (td, 1H, *J* = 7.5, 14.5 Hz), 5.91 (s, 1H), 5.65 (s, 1H), 5.59 (s, 1H), 5.57 (d, 1H, *J* = 11.4 Hz), 5.36 (s, 1H), 5.31–5.25 (m, 2H), 5.03 (s, 1H), 4.77–4.74 (m, 1H), 4.65–4.60 (m, 1H), 4.54 (d, 1H, *J* = 8.8 Hz), 4.34–4.28 (m, 2H), 4.12–4.07 (m, 1H), 4.05–3.99 (m, 1H), 3.81–3.73 (m, 2H), 3.71–3.66 (m, 1H), 3.64 (d,

1H, J = 4.4 Hz), 3.53–3.47(m, 1H), 3.43–3.38 (m, 1H), 2.94–2.88 (m, 1H), 2.88–2.83 (m, 2H), 2.82–2.77 (m, 2H), 2.04 (dd, 1H, J = 7.5, 7.5 Hz), 1.94–1.89 (m, 2H), 1.82–1.74 (m, 1H), 1.61 (m, 1H), 1.47–1.40 (m, 4H), 1.21–1.15 (m, 16H), 1.13–1.02 (m, 4H), 0.97 (d, 3H, J = 7.0 Hz), 0.93 (d, 3H, J = 6.1 Hz), 0.87–0.73 (m, 12H), 0.62 (d, 3H, J = 6.6 Hz), 0.49 (d, 3H, J = 6.1 Hz), 0.43 (d, 3H, J = 6.1 Hz), –0.04 (s, 9H), –0.07 (s, 9H); MALDI–FTMS (DHB) *m/z* 3063.2037 ($M + Na^+$, $C_{155}H_{186}ClN_{21}O_{34}S_2Si_2$ requires 3063.2031).

Ramoplanin A2 Aglycon Dihydrochloride (48). From **47**: Anhydrous HF (4–5 mL) was condensed in a Kel-F vessel charged with **47** (1.2 mg, 0.39 μmol, crude) and anisole (80 μL) at –78 °C. The reaction mixture was warmed to 0 °C and stirred for an additional 90 min. The HF was removed at 0 °C under a stream of N_2 for 90 min. CH_3OH was added (1 mL), and the solvent was removed in vacuo. The residue was dissolved in 0.1 N aqueous HCl and lyophilized to provide crude **48**, HPLC >90%. HPLC purification [Waters semipreparative LC 25 mm column, 8 mL/min, 30 min gradient of 20–50% CH_3CN–$HCOONH_4$ (aq, 0.05 M, t_R = 32.9 min)] provided pure ramoplanin A2 aglycon as a mixture with $HCOONH_4$. The mixtures were lyophilized until they reached a constant weight, and then lyophilized with 0.1 N aqueous HCl (1 mL) to give **48** (0.3 mg, >30%) as a white solid identical in all respects with an authentic sample. When this reaction was conducted with EtOAc trituration of the crude reaction product, **48** (0.72 mg, 82%) was isolated as a white solid.

***N,N'*-Bis(2-trimethylsilylethanesulfonyl)-Ramoplanin A2 Aglycon (49).** From **47**: A sample of **47** (1.4 mg, 0.46 μmol, crude from **38**) was treated with a solution of 5% H_2O–TFA (1 mL) at 25 °C for 5 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (15 mL). The aqueous solution was extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated in vacuo to provide crude **49** as a solid: t_R = 16.6 min [Waters analytical Nova–Pak® C_{18} 3.9 × 300 mm column, 1 mL/min, 30 min gradient of 30–100% CH_3CN–$HCOONH_4$ (aq, 0.05 M)]. HPLC purification [Waters semipreparative LC 25 mm column, 10 mL/min, 50 min gradient of 30–70% CH_3CN–$HCOONH_4$ (aq, 0.05 M, t_R = 30.4 min)] provided pure **49** as a mixture with $HCOONH_4$. The mixtures were lyophilized until they reached a constant weight, and then lyophilized with 0.1 N aqueous

HCl (1 mL) to give **49** as a white solid (0.2 mg, 18% over three steps from **38**) identical in all respects with an authentic sample.

Ramoplanin A2 Aglycon Dihydrochloride (48). From **49**: Anhydrous HF (2–3 mL) was condensed in a Kel-F vessel charged with **49** (0.4 mg, 0.16 μmol) and anisole (20 μL) at –78 °C. The reaction mixture was warmed to 0 °C and stirred for an additional 90 min. The HF was removed at 0 °C under a stream of N_2 for 90 min. CH_3OH was added (1 mL), and the solvent was removed in vacuo. The residue was dissolved in 0.1 N aqueous HCl and lyophilized. HPLC purification [Waters semipreparative LC 25 mm column, 8 mL/min, 30 min gradient of 20–50% CH_3CN–$HCOONH_4$ (aq, 0.05 M, t_R = 32.9 min)] provided pure ramoplanin A2 aglycon as a mixture with $HCOONH_4$. The mixtures were lyophilized until they reached a constant weight, and then lyophilized with 0.1 N aqueous HCl (1 mL) to give **48** (0.3 mg, 83%) identical with an authentic sample.

Ramoplanin A2 Aglycon Dihydrochloride (48). Method A: From **1–3**: A solution of the ramoplanin complex (73 mg, 0.027 mmol) in DMF (0.88 mL) was treated with a 5% w/v solution of anhydrous HCl in BuOH (0.88 mL). The reaction mixture was stirred at 68 °C for 7 h, with addition of 0.1 mL of HCl–BuOH every 1.5 h. The reaction mixture was cooled to 0 °C and solid $NaHCO_3$ was added until pH 4–5. The mixture was filtered and the solid was washed with BuOH/DMF (1:1, 20 mL). The filtrate was evaporated and the resulting solid was washed with Et_2O (3 × 10 mL) and dried in vacuo. HPLC purification [Waters semi-preparative LC 25 mm column, 8 mL/min, 30 min gradient of 20–50% CH_3CN–$HCOONH_4$ (aq, 0.05 M, 19.2 min)] provided pure ramoplanin A2 aglycon as mixtures with $HCOONH_4$. The solid was dissolved in 5% MeOH–H_2O and the solution was passed through a short column of reverse phase C18 silica gel. The column was eluted with H_2O until the Nessler test became negative (presence of ammoniun ion), then with MeOH to collect the aglycon as a formate salt. The combined fractions were evaporated and the resulting solid was lyophilized with 0.1 N aqueous HCl (1 mL) to give **48** (13 mg, 20%; typically 20–32%) as a white solid.

Method B: From **1–3**: Anhydrous HF (4–5 mL) was condensed in a Kel-F vessel charged with ramoplanin complex (97 mg, 0.036 mmol) and anisole (0.5 mL) at –78 °C. The reaction mixture was warmed to 0 °C and stirred for an additional 90 min. The HF was removed at 0 °C under a stream of N_2 over 90 min. EtOH was added (1 mL), and the

solvent was removed in vacuo. The residue was dissolved in 0.1 N aqueous HCl and lyophilized. HPLC purification [Waters semi-preparative LC 25 mm column, 8 mL/min, 30 min gradient of 20–50% CH_3CN–$HCOONH_4$ (aq, 0.05 M; t_R = 17.4 min (A1); 19.2 min (A2); 21.3 min (A3))] provided pure ramoplanin A2 aglycon, and mixtures of ramoplanin A1/A2, and A2/A3 aglycons, as mixtures with $HCOONH_4$. HPLC purification (same conditions as above) of the A1, A2, and A3 aglycon mixtures provided pure ramoplanin A1, A2, and A3 aglycons, as mixtures with $HCOONH_4$. The mixtures were lyophilized until they reached a constant weight, and then lyophilized with 0.1 N aqueous HCl (1 mL) to give A1 aglycon (2.5 mg, 3%), A2 aglycon (**48**) (40 mg, 46%), and A3 aglycon (2.3 mg, 3%) as white solids. For **48**: mp > 212 °C (decomp); R_f = 0.38 ($BuOH/H_2O/HOAc$: 4/1/1); $[\alpha]^{23}_D$ +48 (*c* 0.050, MeOH); ^1H NMR (80% D_2O–DMSO-d_6, 600 MHz) δ 7.37 (d, 2H, *J* = 8.8 Hz), 7.14–7.06 (m, 6H), 6.96–6.90 (m, 5H), 6.83 (d, 2H, *J* = 8.0 Hz), 6.76 (d, 1H, *J* = 8.3 Hz), 6.73 (s, 1H), 6.68 (s, 1H), 6.61–6.58 (m, 5H), 6.50 (d, 2H, *J* = 8.8 Hz), 6.47–6.45 (m, 1H), 6.37–6.34 (m, 3H), 6.27–6.25 (m, 3H), 6.09 (s, 1H), 5.99–5.93 (m, 1H), 5.91 (s, 1H), 5.58 (s, 1H), 5.40 (d, 1H, *J* = 11.4 Hz), 5.34 (s, 1H), 5.31 (s, 1H), 4.84–4.81 (m, 1H), 4.75 (s, 1H), 4.19–4.16 (m, 2H), 4.14–4.09 (m, 2H), 4.00–3.98 (m, 1H), 3.89–3.88 (m, 1H), 3.82–3.76 (m, 2H), 3.60–3.57(m, 2H), 2.94–2.77 (m, 3H), 2.29–2.24 (m, 1H), 2.03–1.98 (m, 2H), 1.95–1.80 (m, 5H), 1.75–1.72 (m, 1H), 1.59–1.45 (m, 6H), 1.28 (m, 5H), 0.94 (d, 3H, *J* = 6.5 Hz), 0.83 (d, 3H, *J* = 11.4 Hz), 0.73–0.70 (m, 6H), 0.65–0.64 (m, 3H), 0.61–0.59 (m, 6H); IR (neat) ν_{max} 3260, 2917, 1737, 1632, 1514, 1237, 1179 cm^{-1}; MALDI–FTMS (DHB) *m/z* 2228.9405 ($M + H^+$, $C_{107}H_{134}ClN_{21}O_{30}$ requires 2228.9366).

Method C: From **2**: Anhydrous HF (4–5 mL) was condensed in a Kel-F vessel charged with pure ramoplanin A2 (**2**) (4.6 mg, 1.7 μmol) and anisole (0.1 mL) at –78 °C. The mixture was warmed to 0 °C and stirred for 90 min. The HF was removed at 0 °C under a stream of N_2 over 90 min. The residue was dissolved in EtOH (1 mL), transferred, and the solvent was removed in vacuo, dissolved in 0.1 N aqueous HCl (1 mL) and lyophilized to give a light brown solid. The solid was triturated with EtOAc (3 × 0.5 mL) to give HPLC pure **48** (3.8 mg, 4.1 mg theoretical, 92%) as a white solid.

***N,N'*-Bis(2-trimethylsilylethanesulfonyl)-Ramoplanin A2 Aglycon (49).** From **48**: A solution of ramoplanin A2 aglycon dihydrochloride (**48**) (5.2 mg, 2.3 μmol) in DMF

(100 μL) maintained at –20 °C was successively treated with a solution of Et_3N in DMF (2.67 M, 10 μL) and a solution of SES-Cl in DMF (0.97 M, 12 μL). The reaction mixture was stirred for 2 h at –20 °C and then quenched by the addition of EtOH (200 μL). The solvent was evaporated with a stream of N_2, and the resulting solid was washed with Et_2O (3 × 1 mL). HPLC purification [Waters semipreparative LC 25 mm column, 10 mL/min, 50 min gradient of 30–70% CH_3CN–$HCOONH_4$ (aq, 0.05 M)] afforded pure **49**, N^{10}-(2-trimethylsilylethanesulfonyl)-ramoplanin A2 aglycon, and recovered ramoplanin A2 aglycon as mixtures with $HCOONH_4$. The mixtures were lyophilized until they reached a constant weight to give **49** (1.2 mg, 20%, t_R = 30.4 min), N^{10}-(SES)-ramoplanin A2 aglycon (0.9 mg, 17%, t_R = 25.3 min), and recovered ramoplanin A2 aglycon diformate (0.6 mg, 12%, t_R = 15.7 min) as white solids.

For **49**: $[\alpha]^{23}_D$ +20 (*c* 0.015, EtOH); ^{1}H NMR (50% D_2O–DMSO-d_6, 600 MHz) δ 8.27 (br s, 4H), 7.33 (d, 2H, *J* = 8.3 Hz), 7.15 (d, 2H, *J* = 8.3 Hz), 7.10 (t, 3H, *J* = 7.0 Hz), 7.05 (t, 1H, *J* = 7.9 Hz), 6.95 (d, 2H, *J* = 8.3 Hz), 6.87 (d, 2H, *J* = 7.9 Hz), 6.78 (m, 3H), 6.72 (s, 1H), 6.66 (d, 2H, *J* = 8.3 Hz), 6.62 (d, 1H, *J* = 8.3 Hz), 6.59 (d, 2H, *J* = 8.3 Hz), 6.50 (d, 4H, *J* = 6.6 Hz), 6.35 (m, 4H), 6.25 (d, 2H, *J* = 8.8 Hz), 6.17 (br s, 1H), 6.08 (br s, 1H), 5.95 (m, 2H), 5.47 (s, 1H), 5.44 (d, 1H, *J* = 11.4 Hz), 5.31 (d, 2H, *J* = 5.3 Hz), 4.82 (s, 1H), 4.75 (m, 1H), 4.67 (m, 1H), 4.54 (m, 1H), 4.19 (d, 1H, *J* = 4.9 Hz), 4.16 (m, 1H), 4.11 (q, 1H, *J* = 7.4 Hz), 4.03 (m, 1H), 3.97 (m, 1H), 3.79–3.74 (m, 5H), 3.60 (m, 1H), 3.50 (m, 1H), 2.91–2.81 (m, 7H), 2.15 (m, 1H), 1.93 (m, 2H), 1.84 (m, 3H), 1.73 (m, 1H), 1.55 (m, 1H), 1.45–1.34 (m, 6H), 1.30 (d, 3H, *J* = 7.4 Hz), 1.27 (m, 1H), 1.12 (m, 7H), 0.97 (m, 1H), 0.90 (d, 3H, *J* = 6.1 Hz), 0.84 (m, 1H), 0.80 (d, 3H, *J* = 5.7 Hz), 0.75 (d, 2H, *J* = 6.6 Hz), 0.73 (d, 2H, *J* = 6.6 Hz), 0.64 (d, 3H, *J* = 5.3 Hz), 0.62 (d, 3H, *J* = 5.3 Hz), 0.54 (d, 3H, *J* = 6.6 Hz), –0.07 (s, 9H), –0.13 (s, 9H); IR (neat) ν_{max} 3284, 2955, 1631, 1596, 1508, 1255, 1091, 1026 cm^{-1}; MALDI–FTMS (DHB) *m/z* 2578.9560 (M + Na^+, $C_{117}H_{158}ClN_{21}O_{34}S_2Si_2$ requires 2578.984).

For N^{10}-(2-trimethylsilylethanesulfonyl)-ramoplanin A2 aglycon (**50**): ^{1}H NMR (50% D_2O–DMSO-d_6, 600 MHz) δ 7.33 (d, 2H, *J* = 8.8 Hz), 7.18 (d, 2H, *J* = 8.3 Hz), 7.10 (m, 3H), 7.07 (t, 1H, *J* = 7.9 Hz), 6.98 (d, 2H, *J* = 8.3 Hz), 6.88 (d, 2H, *J* = 8.3 Hz), 6.79 (d, 2H, *J* = 7.5 Hz), 6.76 (s, 1H), 6.72 (s, 1H), 6.65 (d, 2H, *J* = 8.3 Hz), 6.62 (d, 1H, *J* = 8.3 Hz), 6.58 (d, 2H, *J* = 8.7 Hz), 6.55 (d, 2H, *J* = 8.3 Hz), 6.51 (d, 2H, *J* = 8.8 Hz), 6.41–

6.32 (m, 2H), 6.34 (d, 2H, J = 8.3 Hz), 6.25 (d, 2H, J = 8.3 Hz), 6.22 (s, 1H), 6.01 (s, 1H), 6.00–5.93 (m, 1H), 5.91 (s, 1H), 5.48 (s, 1H), 5.46 (d, 1H, J = 11.4 Hz), 5.32 (s, 1H), 5.30 (s, 1H), 4.88 (s, 1H), 4.80–4.75 (m, 1H), 4.66–4.61 (m, 1H), 4.58–4.53 (m, 1H), 4.21 (d, 1H, J = 3.6 Hz), 4.18 (m, 1H), 4.13 (q, 1H, J = 8.0 Hz), 4.10–4.05 (m, 2H), 3.86 (m, 1H), 3.79–3.73 (m, 2H), 3.63 (m, 1H), 3.52 (m, 1H), 3.30–2.97 (m, 1H), 2.95–2.86 (m, 6H), 2.42 (m, 1H), 2.30 (m, 1H), 2.20 (m, 1H), 2.10 (m, 1H), 1.93 (m, 1H), 1.88–1.76 (m, 3H), 1.60–1.52 (m, 2H), 1.49–1.33 (m, 6H), 1.32 (d, 3H, J = 7.0 Hz), 1.15 (m, 1H), 0.97 (m, 2H), 0.92 (d, 3H, J = 6.1 Hz), 0.81 (d, 3H, J = 6.1 Hz), 0.80–0.75 (m, 2H), 0.76 (d, 3H, J = 6.5 Hz), 0.74 (d, 3H, J = 6.6 Hz), 0.66 (d, 3H, J = 5.3 Hz), 0.63 (d, 3H, J = 5.3 Hz), 0.57 (d, 3H, J = 6.6 Hz), –0.11 (s, 9H); MALDI–FTMS (DHB) *m/z* 2392.9634 ($M + Na^+$, $C_{112}H_{146}ClN_{21}O_{32}SSi$ requires 2392.9694).

Structure determination for N^{10}-(2-trimethylsilylethanesulfonyl)-ramoplanin A2 aglycon: Comparative NMR studies on the ramoplanin A2 aglycon (**48**), the mono-SES ramoplanin A2 aglycon **50** and the bis-SES derivative **49** were conducted under the identical conditions, same 600 MHz instrument, parameters, and concentration of 1 mg material in 0.7 mL 50% D_2O–DMSO-d_6. The 1D and 2D ^1H NMR spectra of the mono-SES ramoplanin A2 aglycon versus the ramoplanin A2 aglycon indicated that: a) the meta-H chemical shifts of Hpg^{11} and Hpg^{13} change while the meta-H chemical shift of Hpg^3 is unaltered; b) the δ-H_2 chemical shift of Orn^{10} exhibited a 0.1 ppm shift, the Phe^9 α-H exhibited a 0.07 ppm change and the Thr^{12} α-H exhibited a change of 0.03 ppm; while c) the chemical shifts of $HAsn^2$–Hpg^3–Orn^4–Thr^5 exhibited no change. These observations establish that the SES protecting group is on the free amine of Orn^{10} and not on Orn^4.

HPLC analysis [Nova-Pak® C18 column, 3.9 × 300 mm, 1 mL/min, 30 min gradient 30–100% CH_3CN–$HCOONH_4$ (aq, 0.05 M)]: t_R (**48**) = 12.4 min, t_R (mono–SES product, **50**) = 15.2 min, t_R (di SES product, **49**) = 16.6 min.

Hydrolysis of Ramoplanin A2 (2).[18] A sample of ramoplanin A2 (**2**, 2.10 mg, 0.823 μmol) was treated with 0.5 mL of 1% v/v Et_3N–H_2O for 1 h at 25 °C and lyophilized to give a white solid. The solid was triturated with EtOAc (2 × 1 mL) and CH_2Cl_2 (2 × 1 mL), dissolved in 1 mL of 0.1 N aqueous HCl and lyophilized to give a pure acyclic ramoplanin A2 (2.04 mg, 97%): t_R = 21.3 min [Waters analytical Nova–Pak® C_{18} 3.9 ×

M)]; ^{1}H NMR (50% D_2O–DMSO-d_6, 600 MHz) δ 7.21–7.15 (m, 3H), 7.15–7.08 (m, 8H), 7.08–7.01 (m, 2H), 7.01–6.97 (m, 2H), 6.96–6.90 (m, 2H), 6.89–6.83 (m, 2H,), 6.80 (d, 2H, J = 7.9 Hz), 6.79–6.74 (m, 2H), 6.71–6.65 (m,1H), 6.68 (d, 2H, J = 7.5 Hz), 6.63 (d, 2H, J = 7.5 Hz), 6.50–6.42 (m, 3H), 6.42–6.36 (m, 1H), 6.00–5.93 (m, 1H), 5.67 (s, 1H), 5.52 (d, 1H, J = 11.0 Hz), 5.39 (s, 1H), 5.31 (s, 1H), 5.26 (s, 1H), 4.88 (s, 1H), 4.85 (s, 2H), 4.68–4.60 (m, 3H), 4.43–4.33 (m, 6H), 4.20–4.10 (m, 2H), 3.88 (s, 1H), 3.87–3.81 (m, 2H), 3.81–3.73 (m, 1H), 3.72–3.66 (m, 2H), 3.66–3.60 (m, 4H), 3.59–3.54 (m, 3H), 3.53–3.44 (m, 4H), 3.41–3.35 (m, 3H), 3.13–3.09 (m, 1H), 2.93–2.84 (m, 4H), 2.84–2.79 (m, 1H), 2.76–2.65 (m, 2H), 2.63–2.53 (m, 2H), 2.45–2.37 (m, 2H), 1.93–1.87 (m, 1H), 1.67–1.59 (m, 1H), 1.59–1.50 (m, 2H), 1.50–1.30 (m, 6H), 1.23 (d, 3H, J = 7.5 Hz), 1.21 (d, 3H, J = 7.0 Hz), 1.03– 0.98 (m, 1H), 0.85–0.63 (m, 15H).

Hydrolysis of Ramoplanin A2 Aglycon (48). A 0.5 mL solution of 1% v/v Et_3N–H_2O was added into a vial charged with ramoplanin A2 aglycon (**48**, 1.46 mg, 0.655 μmol). The mixture was stirred for 40 min and lyophilized to give a white solid. The solid was triturated with EtOAc (2 × 1 mL) and CH_2Cl_2 (2 × 1 mL), dissolved in 1 mL of 0.1 N aqueous HCl and lyophilized to give a pure acyclic ramoplanin A2 aglycon (1.40 mg, 96%): t_R = 22.3 min [Waters analytical Nova–Pak® C_{18} 3.9 × 300 mm column, 0.8 mL/min, 30 min gradient of 20–50% CH_3CN–$HCOONH_4$ (aq, 0.05 M)]; ^{1}H NMR (50% D_2O–DMSO-d_6, 600 MHz) δ 7.26–7.20 (m, 3H), 7.20–7.16 (m, 1H), 7.15–7.08 (m, 6H), 7.05 (d, 2H, J = 7.4 Hz), 6.98 (d, 2H, J = 7.0 Hz), 6.94 (d, 2H, J = 7.4 Hz), 6.91–6.83 (m, 2H,), 6.82–6.75 (m, 1H), 6.79 (d, 2H, J = 8.8 Hz), 6.68 (d, 2H, J = 7.9 Hz), 6.65 (d, 2H, J = 8.3 Hz), 6.58 (d, 2H, J = 7.5 Hz), 6.51–6.42 (m, 3H), 6.43–6.36 (m, 1H), 6.00–5.93 (m, 1H), 5.87–5.78 (m, 1H), 5.52 (d, 1H, J = 11.4 Hz), 5.39–5.34 (m, 1H), 5.30–5.24 (m, 1H), 5.23 (s, 1H), 5.10 (s, 1H), 4.68–4.61 (m, 2H), 4.58–4.30 (m, 2H), 4.20–4.15 (m, 1H), 4.14–4.10 (m, 1H), 3.86–3.81 (m, 1H), 3.81–3.73 (m, 3H), 3.73–3.66 (m, 3H), 3.66–3.53 (m, 4H), 3.48–3.41 (m, 1H), 3.41–3.35 (m, 1H), 2.90–2.65 (m, 3H), 2.47–2.40 (m, 1H), 1.98–1.85 (m, 3H), 1.65–1.30 (m, 8H), 0.80 (d, 3H, J = 5.3 Hz), 0.76 (d, 3H, J = 6.1 Hz), 0.75 (d, 3H, J = 6.6 Hz), 0.73 (d, 3H, J = 7.0 Hz), 0.72 (d, 3H, J = 7.0 Hz), 0.70 (d, 3H, J = 8.3 Hz), 0.65 (br s, 3H).

(S1) Vig, O. P.; Bari, S. S.; Puri, S. K.; Dua, D. M. *Indian J. Chem.* **1981**, *20B*, 342