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An Improved Solid-Phase Methodology for the Synthesis of Putative Hexa- and Heptapeptide Intermediates in Vancomycin Biosynthesis

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Experimental details of the synthesis of the amino acid building blocks are provided here.

Experimental

Abbreviations: Alloc, allyloxycarbonyl (= (prop-2-enyloxy)carbonyl); Boc, (*tert*-butoxy)carbonyl; Cht, ß-hydroxy-*m*-chlorotyrosine; Dhpg, D-3,5-dihydroxyphenylglycine; DMAP, 4-*N,N*-dimethylaminopyridine; DCC, dicyclohexyl-carbodiimide; DIC, 1,3-diisopropylcarbodiimide; DIEA, *N,N'*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; EDC, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide; Fmoc, [(9*H*-fluorenyl)-methoxy]carbonyl; HOBt, 1-hydroxybenzotriazole; Hpg, D-4-hydroxyphenylglycine; NMM, *N*-methylmorpholine; Pfp-OH, pentafluorophenol; TFA, trifluoroacetic acid. For solid-phase synthesis, DMF was redistilled under reduced pressure from ninhydrin, CH_2Cl_2 was redistilled under N_2 from CaH_2 and HPLC-grade MeOH was used. NMR spectra were recorded at 300 MHz (^{1}H) except where noted. Flash chromatography used normal flash silica gel.

Alloc-Hpg-OH (12)

To Hpg (1.67 g, 10 mmol) and $NaHCO_3$ (1.18 g, 14 mmol) in water/acetone (15 ml v/v 1:1) was added Alloc-OSu[28] (10 mmol). The resulting mixture was stirred at rt for 5 h and then 10% aq. HCl was added to pH 2-3. Volatiles were removed by rotary evaporation and the remaining aq. solution was extracted (3x) with EtOAc. The combined organic layers were dried over Na_2SO_4, and concentrated under reduced

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pressure to give an oil, which crystallized from methanol and ether to afford **12** as a white solid (2.47 g, 98% yield). m.p. 268-270 ^{o}C; $[\alpha]_D^{25}$ = -161.3 (*c* = 0.24 in MeOH); IR (KBr): ν 3387, 3190, 1737, 1648, 1518, 1253, 1221, 1178, 1060 cm^{-1}; ^{1}H NMR (d$_6$ DMSO) δ 9.47 (s, 1 H), 7.88 (d, *J* = 7.9 Hz, 1 H), 7.22 (d, *J* = 8.6 Hz, 2 H), 6.75 (d, *J* = 8.6 Hz, 2 H), 6.00-5.87 (m, 1 H), 5.36-5.18 (m, 2 H), 5.04 (d, *J* = 7.9 Hz 1 H), 4.52 (d, *J* = 5.3 Hz, 2 H); ^{13}C NMR (d$_6$ DMSO) δ 172.32, 156.98, 155.44, 133.36, 128.78, 127.06, 116.88, 114.96, 64.36, 57.34; MS(CI): *m/z* 252.0 (100) [M+H$^+$]; HRMS(CI) calc. for $C_{12}H_{14}NO_5$ 252.0872, found 252.0869.

Alloc-Asn-OH

To Asn.H_2O (2.97 g, 19.8 mmol) and $NaHCO_3$ (2.35 g, 28 mmol) in water/acetone (15ml v/v 1:1) was added fresh Alloc-OSu (20 mmol). The resulting mixture was stirred at rt for 5 h, then 10% aq. HCl was added to pH 2-3. Volatiles were removed by rotary evaporation and the remaining aq. solution was extracted (3x) with EtOAc. The combined organic layers were dried over Na_2SO_4, and concentrated under reduced pressure to give a solid, which was recrystallized from methanol and ether to afford a white solid (2.83 g, 66%): m.p. 288-290 ^{o}C; $[\alpha]_D^{25}$ = -3.8 (*c* = 0.32 in MeOH); IR (KBr): ν 3412, 3319, 1694, 1646, 1542, 1271, 1233, 1199, 1066 cm^{-1}; ^{1}H NMR (d$_6$ DMSO) δ 7.48 (s, 1 H), 7.45 (brs, 1 H), 7.01 (s, 1 H), 6.08-5.95 (m, 1 H), 5.44-5.37 (m, 1 H), 5.31-5.27 (m, 1 H), 4.58 (d, *J* = 5.3 Hz, 2 H), 4.47-4.40 (m, 1 H), 2.70-2.52 (m, 2 H); ^{13}C NMR (d$_6$ DMSO) δ 173.00, 171.07, 155.44, 133.33, 116.88, 64.30, 50.38, 36.57; MS(CI): *m/z* 217.1 (100) [M+H$^+$]; HRMS(CI) calcd for $C_8H_{13}N_2O_5$ 217.0824, found 217.0820.

Alloc-Asn-OPfp (13)

To Alloc-Asn-OH (2.55 g, 11.8 mmol) and Pfp-OH (7.3 g, 40 mmol) at 0 ^{o}C in dry dioxane was added DCC (3.1 g, 15 mmol). The resulting mixture was stirred at 0 ^{o}C for 2 h, and then at rt for another 2 h. After filtration, the filtrate was concentrated under reduced pressure to give an oil which crystallized from EtOAc and hexane to afford **13** as a white solid (3.13 g, 70%): m.p. 234-236 ^{o}C; $[\alpha]_D^{25}$ = -6.5 (*c* = 0.37 in DCM); IR (KBr): ν 3329, 1744, 1695, 1664, 1520, 1280 cm^{-1}; ^{1}H NMR (d$_6$

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DMSO) δ 7.31 (s, 1 H), 7.28 (brs, 1 H), 6.85 (s, 1 H), 5.92-5.79 (m, 1 H), 5.27-5.11 (m, 2 H), 4.48-4.42 (m, 2 H), 4.31-4.25 (m, 1 H), 2.99-2.76 (m, 1 H), 2.55-2.37 (m, 1 H); MS(CI): *m/z* 383.1 (33) [M+H$^+$]; HRMS(CI): calc. for $C_{14}H_{12}F_5N_2O_5$ [M+H$^+$]: 383.0666; found 383.0658.

Alloc-D-Tyr(Alloc)-OMe (14)

To D-tyrosine methyl ester hydrochloride[29] (2.8 g, 12.1 mmol) in dioxane/water (20 ml v/v 1:1) was added $NaHCO_3$ (10.2 g, 122 mmol) and Alloc-Cl (13 mL, 121 mmol). After stirring at rt for 9 h, 32% aq. HCl was added to pH 2-3. Volatiles were removed by rotary evaporation and the remaining aq. solution was extracted (3x) with EtOAc. The organic layers were dried over Na_2SO_4, and concentrated under reduced pressure to give an oil, which was purified by flash chromatography (hexane : EtOAc = 2 : 1) to afford **14** as a white solid (3.44 g, 78%): m.p. 116-118 ^oC; $[\alpha]_D^{25}$ = -45.0 (*c* = 0.20 in CH_2Cl_2); IR (KBr): ν 3321, 1754, 1741, 1691, 1543, 1298, 1263, 1216 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.18-7.11 (m, 4 H), 6.08-5.86 (m, 2 H), 5.48-5.33 (m, 2 H), 5.27-5.21 (m, 2 H), 4.76-4.73 (m, 2 H), 4.67-4.63 (m, 1 H), 4.57 (d, *J* = 5.5 Hz, 2 H), 3.73 (s, 3 H), 3.14-3.11 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 171.69, 155.36, 153.26, 150.16, 133.53, 132.42, 131.00, 130.18, 121.02, 119.41, 117.76, 69.03, 65.75, 54.57, 52.25, 37.51; MS(CI): *m/z* 364.2 (100) [M+H$^+$]; HRMS(CI): calc. for $C_{18}H_{22}NO_7$ [M+H$^+$]: 364.1396; found 364.1405.

Alloc-D-Tyr(Alloc)-OPfp (15)

To **14** (3.0 g, 8.3 mmol) in THF/water (20 ml v/v 1:1) at 0 ^oC, was added $LiOH.H_2O$ (420 mg, 10 mmol). After stirring for 1 h at 0 ^oC, 32% aq. HCl was added to pH 2-3. Volatiles were removed by rotary evaporation and saturated aq. $NaHCO_3$ solution was added to pH 7-8. After extraction (2x) with cold ether, 32% aq. HCl was added to pH 2-3, and the aq. layer was extracted (3x) with EtOAc. The organic layers were dried over Na_2SO_4, and concentrated under reduced pressure to give a colorless oil, which was used without further purification.

To Alloc-D-Tyr(OAlloc)-OH (1.87 g, 5.4 mmol) and pentafluorophenol (Pfp-OH) (1.0 g, 5.4 mmol) in dry dioxane at 0 ^oC was added DCC (1.2 g, 5.4 mmol). The

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mixture was stirred at 0 °C for 2 h, and then at rt for 2 h. After filtration and concentration under reduced pressure, the resulting oil was crystallized from EtOAc and cyclohexane to afford **15** as a white solid (2.0 g, 53% over two steps): m.p. 240 °C; $[\alpha]_D^{25}$ = +9.4 (*c* = 0.31 in CH_2Cl_2); IR (KBr): ν 3335, 1754, 1698, 1523, 1269, 996 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.21 (dd, *J* = 24.3, 8.6 Hz, 4 H), 6.07-5.83 (m, 2 H), 5.46-5.21 (m, 4 H), 5.16-4.98 (m, 1 H), 4.75-4.72 (m, 2 H), 4.58 (d, *J* = 5.7 Hz, 2 H), 3.37-3.20 (m, 2 H); MS(CI): *m/z* 516.2 (44) [M+H^+]; HRMS(CI): calc. for $C_{23}H_{19}F_5NO_7$ [M+H^+]: 516.1082; found 516.1083.

Alloc-D-Leu-OH (16)

To Boc-D-Leu-OH.H_2O (2.0 g, 8 mmol) was added TFA/CH_2Cl_2/triisopropylsilane (47.5/47.5/5) and stirred for 1 h. After removal of solvent, the residue was dissolved in dioxane/water (1:1). Alloc-Cl (4.3 mL, 40 mmol) and $NaHCO_3$ (3.4 g, 40 mmol) were added and the resulting solution was stirred at rt overnight. Volatiles were removed by rotary evaporation and saturated aq. $NaHCO_3$ solution was added to pH 7-8. After extraction (2x) with cold ether, 32% aq. HCl was added to pH 2-3 and the aq. layer was extracted (3x) with ether. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give **16** as a colorless oil (1.57 g, 84% over two steps), which was used without further purification: ^1H NMR ($CDCl_3$) δ 7.23 (brs, 1 H), 5.91-5.78 (m, 1 H), 5.27-5.14 (m, 2 H), 5.06 (brd, *J* = 8.2 Hz, 1 H), 4.52 (d, *J* = 5.1 Hz, 2 H), 4.36-4.31 (m, 1 H), 3.66 (s, 2 H), 1.71-1.46 (m, 3 H), 0.90 (d, *J* = 6.3 Hz, 6 H).

Alloc-*N*(Me)-D-Leu-OH (17)

Boc-*N*(Me)-D-Leu-OH·H_2O (392 mg, 1.6 mmol) was treated as above to give **17** as a colorless oil (348 mg, 95% over two steps), which was used without further purification: ^1H NMR ($CDCl_3$) δ 8.67 (brs, 1 H), 5.92-5.79 (m, 1 H), 5.27-5.14 (m, 2 H), 4.86-4.69 (m, 1 H), 4.57-4.55 (m, 2 H), 2.81 (s, 3 H), 1.71-1.60 (m, 2 H), 1.57-1.45 (m, 1 H), 0.89 (2d, 2 conformers evident, 6 H).

Alloc-D-Leu-OPfp (18) and Alloc-*N*(Me)-D-Leu-OPfp (19)

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To **16** (1.57 g, 6.74 mmol) and Pfp-OH (1.24 g, 6.74 mmol) in dry dioxane at 0 ^{o}C was added DCC (1.39 g, 6.74 mmol). After stirring at 0 ^{o}C for 2 h, and at rt for 2 h, the solution was filtered, and the filtrate was concentrated under reduced pressure to afford **18** as an oil, which was used for peptide assembly without further purification. **19** was made in the same way starting with **17**.

4-Allyloxy-3-chloro-benzaldehyde (27)

4-Hydroxybenzaldhyde (8.54 g, 70 mmol) and N-chlorosuccinimide (14.1 g, 105 mmol) in $CHCl_3$ were refluxed for 3 days. After removal of solvent, the residue was partitioned between brine and ether. The ether layer was washed (4x) with brine dried over Na_2SO_4, and concentrated under reduced pressure to give 3-chloro-4-hydroxybenzaldehyde as a light yellow solid, which was used without further purification.

3-Chloro-4-hydroxybenzaldehyde (4.5 g, 29 mmol) in acetone was treated with allyl bromide (3.7 mL, 43.5 mmol) and K_2CO_3 (8.0 g, 58 mmol) and refluxed for 4 h. After removal of solvent, the residue was partitioned between ether and 10% aq. HCl. The aqueous layer was extracted (2x) with ether. The combined organic layer was dried over Na_2SO_4, and concentrated under reduced pressure to give an oil, which was purified by flash chromatography (hexane:EtOAc = 8:1) and vacuum distillation to afford **27** as a colorless oil (3.7 g, 65% over two steps): b.p. 110-114 ^{0}C/3 mmHg; IR (neat): ν 2840, 2731, 1693, 1595, 1496, 1272, 1194 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 10.04 (s, 1 H), 8.09 (t, J = 2.1 Hz, 1 H), 7.95-7.91 (m, 1 H), 7.24-7.21 (m, 1 H), 6.33-6.20 (m, 1 H), 5.73-5.66 (m, 1 H), 5.58-5.53 (m, 1 H), 4.92-4.89 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 189.47, 158.70, 131.51, 131.05, 130.23, 128.20, 123.87, 118.38, 112.81, 69.68; MS(CI): *m/z* 197.0 (100) [M+H$^+$]; HRMS(CI): calc. for $C_{10}H_{10}ClO_2$ [M+H$^+$]:197.0369; found 197.0364.

Methyl ester (28)

To a suspension of $Sn(OTf)_2$ (5.0 g, 12 mmol) and anhydrous *N*-ethylpiperidine (2 mL, 14.3 mmol) in anhydrous THF under N_2 at -78 ^{o}C was added isothiocyanate **26** [26] (3.0 g, 11 mmol) in THF. The resulting solution was stirred at -78 ^{o}C for 1.5 h, then a

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solution of aldehyde **27** (2.6 g, 13.2 mmol) in THF was added dropwise via syringe. The reaction mixture was stirred at -78 ^{o}C for 3 h and then quenched by the addition of aqueous pH 7 buffer. The resultant slurry was filtered through Celite. The filtrate was diluted with 1 N $NaHSO_4$, and extracted (3x) with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure to give an oil, which was purified by flash chromatography (hexane:EtOAc = 1:1) to afford product as a white foam (4.2 g, 81%): m.p. 159-161 ^{o}C; $[\alpha]_D^{25}$ = +157.9 (*c* = 0.38 in CH_2Cl_2); IR (KBr): ν 3394, 1780, 1703, 1502, 1393, 1258, 1173 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.86 (s, 1 H), 7.62-7.36 (m, 7 H), 7.12 (d, *J* = 8.6 Hz, 1 H), 6.51 (d, *J* = 5.2 Hz, 1 H), 6.30-6.17 (m, 1 H), 5.68-5.61 (m, 1 H), 5.54-5.49 (m, 1 H), 5.19-5.17 (m, 1 H), 5.00-4.92 (m, 1 H), 4.83-4.78 (m, 2 H), 4.60-4.50 (m, 2 H), 3.45-3.39 (m, 1 H), 3.19-3.12 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 188.34, 165.62, 154.88, 153.62, 133.84, 132.06, 129.55, 129.32, 129.12, 128.08, 127.77, 125.55, 123.63, 118.10, 113.81, 83.06, 69.67, 67.62, 65.07, 55.13, 37.46; MS(CI): *m/z* 473.1 (34) [M+H$^+$]; HRMS(CI): calc. for $C_{23}H_{22}ClN_2O_5S$ [M+H$^+$]: 473.0938; found 473.0924.

The foregoing product (3.6 g, 7.6 mmol) in MeOH and dry CH_2Cl_2 (v/v 1:1) was added at 0 ^{o}C dropwise to a suspension formed by adding MeMgBr (3.0 M in ether, 2.8 mL, 8.4 mmol) to absolute MeOH. The resulting mixture was stirred for 3 min. and then quenched by the addition of 1 N $NaHSO_4$. The aqueous layer was extracted (3x) with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure to give an oil, which was purified by flash chromatography (hexane:EtOAc = 2:1) to afford **28** as an oil (2.16 g, 87%): $[\alpha]_D^{25}$ = +44.4 (*c* = 0.25 in CH_2Cl_2); IR (KBr): ν 3413, 1749, 1501, 1235, 1174 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.26 (s, 1 H), 7.65 (d, *J* = 2.2 Hz, 1 H), 7.49-7.46 (m, 1 H), 7.17 (d, *J* = 8.6 Hz, 1 H), 6.33-6.20 (m, 1 H), 6.09 (d, *J* = 6.1 Hz, 1 H), 5.72-5.64 (m, 1 H), 5.57-5.52 (m, 1 H), 4.87-4.84 (m, 2 H), 4.72 (d, *J* = 6.1 Hz, 1 H), 4.09 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 188.65, 168.09, 154.96, 132.03, 129.59, 127.77, 125.23, 123.80, 118.12, 113.81, 84.69, 69.70, 64.13, 53.45; MS(CI): *m/z* 328.0 (42) [M+H$^+$]; HRMS(CI): calc. for $C_{14}H_{15}ClNO_4S$ [M+H$^+$]: 328.0410; found 328.0394.

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Alloc-Cht(Allyl)-OH (29)

To a solution of **28** (1.84 g, 5.66 mmol) at rt in dry CH_2Cl_2 was added Alloc-Cl (670 μL, 6.22 mmol), DMAP (760 mg, 6.22 mmol) and Et_3N (870 μL, 6.22 mmol). After stirring at rt for 1.5 h and then cooling to 0 ^{0}C, 95% formic acid (1 mL) and 35% H_2O_2 (1 mL) were added. This mixture was stirred for 30 min and then poured into 1 N $NaHSO_4$. The aqueous layer was extracted (3x) with CH_2Cl_2. The organic layer was washed with 1 M $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under reduced pressure to give an oil, which was purified by flash chromatography (hexane:EtOAc = 2:1) to afford **product** as an oil (1.7 g, 76%): $[\alpha]_D^{25}$ = +66.7 (*c* = 0.21 in CH_2Cl_2); IR (KBr): ν 3431, 1831, 1738, 1504, 1378, 1320, 1221 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.69 (d, *J* = 2.1 Hz, 1 H), 7.53-7.49 (m, 1 H), 7.26 (d, *J* = 8.6 Hz, 1 H), 6.41-6.17 (m, 2 H), 5.80-5.58 (m, 5 H), 5.09-5.02 (m, 2 H), 5.00 (d, *J* = 4.1 Hz, 1 H), 4.95-4.93 (m, 2 H), 4.18 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 168.34, 154.93, 149.99,149.86, 132.00, 130.50, 129.65, 127.16, 124.46, 123.94, 119.46, 118.14, 113.86, 75.53, 69.70, 68.09, 63.18, 53.38; MS(CI): *m/z* 396.1 (17) [M+H$^+$]; HRMS(CI): calc. for $C_{18}H_{19}ClNO_7$ [M+H$^+$]: 396.0850; found 396.0831.

To the foregoing product (1.44 g, 3.6 mmol) in dioxane (70 mL) at rt was added $LiOH.H_2O$ (756 mg, 18 mmol) in water (7 mL). The resulting suspension was stirred at rt for 90 min. Then saturated $NaHSO_4$ was added until pH 2-3. Volatiles were removed by rotary evaporation. The residue was diluted with 1 N aq. $NaHSO_4$ and extracted (3x) with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure to give an oil, which was purified by HPLC (preparative C_{18} column, gradient from 10 - 100% MeCN/H_2O + 0.1% TFA) to afford **29** as a sirup (240 mg, 19%) and oxazolidinone **29a** as a white solid (620 mg, 58%).

29: $[\alpha]_D^{25}$ = -10.0 (*c* = 0.53 in MeOH); IR (KBr): ν 3342, 1750, 1696, 1503, 1408, 1260, 1059 cm^{-1}; ^{1}H NMR (d_6 DMSO) δ 7.54 (d, *J* = 1.9 Hz, 1 H), 7.39-7.35 (m, 1 H), 7.18 (d, *J* = 8.6 Hz, 1 H), 7.11 (d, *J* = 9.5 Hz, 1 H), 6.21-6.08 (m, 1 H), 5.97-5.82 (m, 1 H), 5.57-5.49 (m, 1 H), 5.40-5.17 (m, 4 H), 4.75-4.73 (m, 2 H), 4.49-4.47 (m, 2 H), 4.37-4.32 (m, 1 H); ^{13}C NMR (d_6 DMSO) δ 171.51, 155.86, 152.27, 135.42, 133.23, 133.10, 127.64, 125.77, 120.69, 117.37, 116.55, 113.35, 71.09, 68.82, 64.26,

60.07; MS(CI): *m/z* 338.0 (100) [M-H_2O+H^+]; HRMS(CI): calcd for $C_{16}H_{17}ClNO_5$ [M-H_2O+H^+]: 338.0795; found 338.0789.

29a: m.p. 303-305 ^oC (dec); $[\alpha]_D^{25}$ = +82.3 (*c* = 0.22 in MeOH); IR (KBr): ν 3328, 2930, 1751, 1689, 1510, 1246, 1013 cm^{-1}; ^1H NMR (d_6 DMSO) δ 8.44 (s, 1 H), 7.58 (d, *J* = 2.1 Hz, 1 H), 7.46-7.42 (m, 1 H), 7.31 (d, *J* = 8.6 Hz, 1 H), 6.21-6.09 (m, 1 H), 5.62 (d, *J* = 5.2 Hz, 1 H), 5.57-5.50 (m, 1 H), 5.41-5.36 (m, 1 H), 4.80-4.78 (m, 2 H), 4.39 (d, *J* = 5.4 Hz, 1 H); ^{13}C NMR (d_6 DMSO) δ 171.43, 157.31, 153.60, 132.85, 131.94, 127.62, 126.03, 121.44, 117.61, 114.16, 77.36, 68.97, 60.23; MS(CI): *m/z* 298.1 (86) [M+H^+]; HRMS(CI): calc. for $C_{13}H_{13}ClNO_5$ [M+H^+]: 298.0482; found 298.0469.

Alloc-Dhpg-OH (31)

Oxazolidinone **30**[24] (1.12 g, 1.85 mmol) in THF/H_2O (v/v 3:1) at 0 ^0C, was treated with LiOH.H_2O (100 mg, 2.4 mmol). After stirring at 0 ^0C for 90 min, 10% aq. HCl was added to pH 2-3. Volatiles were removed by rotary evaporation, and the remaining aq. solution was extracted (3x) with ether. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure to give Alloc-Dhpg$(Bn)_2$-OH as an oil, which was used without further purification.

Alloc-Dhpg$(Bn)_2$-OH was stirred in TFA/thioanisole (v/v 3:1) for 3.5 h at rt. After removal of solvent, the residue was partitioned between sat. aq. $NaHCO_3$ and EtOAc. The aq. layer was extracted (3x) with EtOAc and then 32% aq. HCl was added to pH 2-3, and extracted (4x) with ether. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure to give a brown oil, which was purified by HPLC (preparative C_{18} column, gradient from 10 - 40% MeCN/H_2O + 0.1% TFA) to afford **31** as a sirup (400 mg, 81% over two steps): $[\alpha]_D^{25}$ = +132.0 (*c* = 0.20 in MeOH); IR (KBr): ν 3410, 1696, 1607, 1520, 1158 cm^{-1}; ^1H NMR (d_6 DMSO) δ 9.32 (s, 2 H), 7.91 (d, *J* = 8.0 Hz, 1 H), 6.30 (d, *J* = 2.1 Hz, 2 H), 6.20 (t, *J* = 2.1 Hz, 1 H), 6.03-5.90 (m, 1 H), 5.40-5.22 (m, 2 H), 4.97 (d, *J* = 8.0 Hz, 1 H), 4.55-4.54 (m, 2 H); ^{13}C NMR (d_6 DMSO) δ 171.82, 158.18, 155.45, 138.55, 133.34, 116.86, 105.69, 101.92, 64.37, 57.84; MS(CI): *m/z* 268.1 (27) [M+H^+]; HRMS(CI): calc. for $C_{12}H_{14}NO_6$ [M+H^+]: 268.0821; found 268.0823.

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Alloc-Tyr-OMe (33)

L-Tyrosine methyl ester hydrochloride (4.47 g, 19.3 mmol) and $NaHCO_3$ (4.0 g, 48 mmol) in acetone/water (v/v 1:1) were treated with Alloc-OSu (20 mmol). After stirring at rt for 7 h, 10% aq. HCl was added to pH 2-3. Volatiles were removed by rotary evaporation. The remaining aq. solution was diluted with water and extracted (3x) with EtOAc. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure to give an oil, which was purified by flash chromatography (Si, hexane:EtOAc= 1:1) to afford **33** as a colorless oil (5.38 g, 100 %): $[\alpha]_D^{25}$ = +49.4 (*c* = 0.33 in CH_2Cl_2); IR (neat): ν 3358, 1697, 1516, 1222 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.20 (d, *J* = 8.5 Hz, 2 H), 6.97 (d, *J* = 8.3 Hz, 2 H), 6.16-6.07 (m, 1 H), 5.57-5.42 (m, 2 H), 4.88-4.78 (m, 3 H), 3.96 (s, 3 H), 3.33-3.24 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 172.18, 155.63, 155.01, 132.34, 130.26, 127.23, 117.84, 115.46, 65.86, 54.83, 52.27, 37.37; MS(CI): *m/z* 280.1 (34) [$M+H^+$]; HRMS(CI): calc. for $C_{14}H_{18}NO_5$ [$M+H^+$]: 280.1185; found 280.1184.

Alloc-Tyr-OH (34)

To **33** (4.6 g, 16.6 mmol) in THF/water (v/v 1:1) was added $LiOH.H_2O$ (1.68 g, 40 mmol) at 0 ^{0}C. After stirring for 1 h at 0 ^{o}C, 32% aq. HCl was added to pH 2-3. Volatiles were removed by rotary evaporation and saturated aq. $NaHCO_3$ was added to pH 7-8. After extraction with cold ether (2x), 32% aq. HCl was added to pH 2-3 and the aqueous layer was extracted with ether (3x). The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure to give **34** as a colourless oil (3.82 g, 87%). $[\alpha]_D^{25}$ = +10.0 (*c* = 0.26 in MeOH); IR (neat): ν = 3337, 1702, 1654, 1516, 1230 cm^{-1}; ^1H NMR (d_6 DMSO) δ 9.33 (brs, 1 H), 7.61 (d, *J* = 8.4 Hz, 1 H), 7.19 (d, *J* = 8.4 Hz, 2 H), 6.81 (d, *J* = 8.4 Hz, 2 H), 6.07-5.94 (m, 1 H), 5.41-5.27 (m, 2 H), 4.62-4.50 (m, 2 H), 4.26-4.18 (m, 2 H), 3.11-3.05 (m, 1 H), 2.91-2.83 (m, 1 H); ^{13}C NMR (d_6 DMSO) δ 173.32, 155.72, 155.66, 133.35, 129.83, 127.75, 116.76, 114.82, 64.19, 55.69, 35.59; MS(CI): *m/z* 266.1 (100) [$M+H^+$]; HRMS(CI): calc. for $C_{13}H_{16}NO_5$ [$M+H^+$]: 266.1028; found 266.1021.

Supplementary Material for Organic & Biomolecular Chemistry

Alloc-*N*(Me)-D-Leu-D-Tyr(Allyl)-OH (40)

To D-Tyr(Allyl)-OMe.TFA (733 mg, 2.1 mmol) in dry DMF was added at 0 ^{o}C DIEA (400 μL, 2.3 mmol) and then a solution of EDC (420 mg, 2.7 mmol), **17** (467 mg, 2.04 mmol) and HOBt (826 mg, 5.4 mmol) in dry DMF. The resulting mixture was allowed to warm to rt overnight. After removal of solvent, the residue was partitioned between 1 N aq. $NaHSO_4$ solution and CH_2Cl_2, the aqueous layer was extracted (3x) with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure to give an oil, which was purified by flash chromatography (hexane:EtOAc = 2:1) to afford Alloc-*N*(Me)-D-Leu-D-Tyr(Allyl)-OMe as a colourless oil (760 mg, 84%).

To the foregoing product (760 mg, 1.70 mmol) in THF and water (v/v 1:1) at 0 ^{o}C, was added $LiOH.H_2O$ (93 mg, 2.21 mmol). The resulting solution was stirred for 1 h at 0 ^{o}C. Then 32% aq. HCl was added to pH 2-3. Volatiles were removed by rotary evaporation and saturated aq. $NaHCO_3$ solution was added to pH 7-8. After extraction with cold ether(2x), 32% aq. HCl was added to pH 2-3 and the aq. layer was extracted (3x) with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure to give **40** as a colourless sirup (700 mg, 95%): $[\alpha]_D^{25}$ = +42.7 (*c* = 0.22 in MeOH); IR (KBr): ν 2958, 1682, 1512, 1400, 1316, 1243, 1177 cm^{-1}; ^{1}H NMR (d_6 DMSO) δ 8.20 (brs, 1 H), 7.30 (d, *J* = 8.6 Hz, 2 H), 7.01 (d, *J* = 8.6 Hz, 2 H), 6.28-6.11 (m, 2 H), 5.59-5.37 (m, 4 H), 4.80-4.69 (m, 5 H), 4.60-4.52 (m, 1 H), 3.23-3.17 (m, 1 H), 3.09-3.02 (m, 1 H), 2.75 (s, 3 H), 1.76-1.54 (m, 3 H), 1.07 (d, *J* = 6.6 Hz, 3 H), 1.04 (d, *J* = 6.5 Hz, 3 H); ^{13}C NMR (d_6 DMSO) δ 172.74, 170.33, 156.64, 155.71, 133.67, 133.22, 129.88, 129.60, 116.98, 116.60, 114.16, 67.94, 65.23, 55.84, 53.38, 36.72, 35.26, 29.15, 24.21, 22.91, 21.21; MS(CI): *m/z* 433.3 (100) [M+H^{+}]; HRMS(CI): calc. for $C_{23}H_{33}N_2O_6$ [M+H^{+}]: 433.2339; found 433.2339.

Alloc-*N*(Me)-D-Leu-D-Tyr(Allyl)-OPfp (41)

To **40** (226 mg, 0.52 mmol) and Pfp-OH (96 mg, 0.52 mmol) in dry dioxane at 0 ^{o}C, was added DCC (107 mg, 0.52 mmol). The resulting mixture was stirred at 0 ^{o}C for 2 h, and then at rt for 2 h. After filtration, the filtrate was concentrated under reduced

pressure to give **41** as an oil, which was used for peptide assembly without further purification.

References

28 J. Blaakmeer, T. Tijsse-Klasen and G. I. Tesser, *Int. J. Peptide Protein Res.*, 1991, **37,**, 556.

29 A. N. Hulme and E. M. Rosser, *Org. Lett.*, 2002, **4**, 265-267.