Supplementary Material

Synthesis of β-Hydroxy-β-(fluoronitrophenyl)alanines; Vital Components in the Assembly of Biologically Active Cyclic Peptides

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(*S*)-*N*-*tert*-Butyl-*N*$^{\alpha}$-phthaloyl-3-(4-fluoro-3-nitrophenyl)alaninamide (7a). A solution of (*S*)-4-fluoro-3-nitrophenylalanine hydrochloride **1a**[12] (326 mg, 1.23 mmol) and sodium carbonate (196 mg, 1.85 mmol) in water (5 mL) was treated with finely ground *N*-carbethoxyphthalimide (NCEP) (280 mg, 1.28 mmol). The suspension was stirred for 1 h, at which time most material had dissolved. The mixture was filtered, acidified to pH 3 with 10% aqueous HCl, and extracted with ethyl acetate (2 _ 5 mL). The combined extracts were washed with water (5 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude oil was dissolved in dry CH_2Cl_2 (5 mL) and treated with ethyl chloroformate (117 μL, 1.23 mmol) and triethylamine (170 μL, 1.23 mmol). The mixture was stirred 10 min, cooled to 0 °C, and treated with *tert*-butylamine (130 μL, 1.23 mmol). After stirring at 0 °C for 15 min the mixture was allowed to warm to room temperature and stirred for a further 30 min. The suspension was washed with 10% aqueous HCl (5 mL), water (5 mL), saturated aqueous $NaHCO_3$ (5 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Recrystallization from EtOAc–hexanes afforded **7a** (395 mg, 78%) as a white solid; mp 184–187 °C; $[\alpha]_D^{20}$ –77.0 (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.83 (dd, 1H, *J* = 2.5, 7.0 Hz), 7.80 (m, 2H), 7.74 (m, 2H), 7.47 (ddd, 1H, *J* = 2.5, 4.2, 8.5 Hz), 7.12 (dd, 1H, *J* = 8.5, 10.5 Hz), 5.94 (br s, 1H), 4.95 (dd, 1H, *J* = 6.6, 10.0 Hz), 3.60 (dd, 1H, *J* = 6.6, 14.4 Hz), 3.56 (dd, 1H, *J* = 10.0, 14.4 Hz), 1.32 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.8, 166.5, 154.4 (d, *J* = 265 Hz), 137.0 (d, *J* = 8 Hz), 136.0 (d, *J* = 8.4 Hz), 134.6, 134.3 (d, *J* = 4.6 Hz), 131.1, 126.3 (d, *J* = 2 Hz), 123.8, 118.6 (d, *J* = 20.6 Hz), 56.0, 51.9, 33.9, 28.6; *m/z* (EI) 414 ($M+H^+$, 18%), 413 ($M^{+\bullet}$, 21), 398 (4), 358 (7), 314 (91), 313 (87), 297 (100), 296 (25), 267 (23), 160 (41), 57 (37); HRMS *m/z* 413.1389 (M^+, $C_{21}H_{20}FN_3O_5$ requires 413.1387).

(*S*)-*N*-*tert*-Butyl-*N*$^{\alpha}$-phthaloyl-3-(3-fluoro-4-nitrophenyl)alaninamide (7b). (*S*)-3-fluoro-4-nitrophenylalanine hydrochloride **1b**[12] (64 mg, 0.24 mmol) was treated according to the preparation of **7a** from **1a**, to afford **7b** (74 mg, 74%) as a white solid; mp 209–211 °C; $[\alpha]_D^{22}$ –78.2 (c 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.90 (app t, 1H, *J* = 8.0 Hz), 7.82 (m, 2H), 7.75 (m, 2H), 7.11 (m, 2H), 5.82 (br s, 1H), 4.99 (dd, 1H, *J* = 7.3, 9.3 Hz), 3.62 (m, 2H), 1.33 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 166.3, 155.4 (d, *J* = 266 Hz), 146.7 (d, *J* = 8.4 Hz), 136.0 (d, *J* = 8 Hz), 134.7, 131.1, 126.3, 125.0 (d, *J* = 3.8 Hz), 123.9, 118.8 (d, *J* = 20.6 Hz), 55.5, 52.0, 34.6, 28.6; *m/z* (EI) 414 ($M+H^+$, 2%), 413 ($M^{+\bullet}$, 5), 398 (1), 358 (1), 314 (22), 313 (18), 297 (45), 296 (25), 267 (6), 160 (15), 91 (100), 57 (21); HRMS *m/z* 413.1392 ($M^{+\bullet}$, $C_{21}H_{20}FN_3O_5$ requires 413.1387).

(2*S*,3*RS*)-3-Bromo-*N*-*tert*-butyl-*N*$^{\alpha}$-phthaloyl-3-(4-fluoro-3-nitrophenyl)alaninamide (8a). A solution of **7a** (100 mg, 0.24 mmol) in CCl_4 (4 mL) and CH_2Cl_2 (1 mL) was treated with NBS (85 mg, 0.48 mmol) and the mixture was heated at reflux while irradiated with a 300W tungsten lamp for 4 h. The mixture was washed with water (2 _ 5 mL), dried ($MgSO_4$), and concentrated *in vacuo* to afford a 1:1 mixture of the diastereomers of the bromide **8a** (118 mg, 99%) as a white foam; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.26 (dd, 0.5H, *J* = 2.5, 6.9 Hz), 8.08 (dd, 0.5H, *J* = 2.5, 6.9 Hz), 7.96 (m, 1H), 7.92 (ddd, 0.5H, *J* = 2.5, 3.9, 8.8 Hz), 7.84 (m, 1H), 7.75 (m, 1H), 7.72-7.66 (m, 1.5H), 7.35 (dd, 0.5H, *J* = 8.8, 10.2 Hz), 7.18 (dd, 0.5H, *J* = 8.8, 10.2 Hz), 6.46 (br s, 0.5H), 6.21 (br s, 0.5H), 6.16 (d, *J* = 11.4 Hz, 0.5H), 6.14 (d, *J* = 11.7 Hz, 0.5H), 5.24 (d, *J* = 11.4 Hz, 0.5H), 5.13 (d, *J* = 11.7 Hz, 0.5H), 1.38 (s, 4.5H), 1.13 (s, 4.5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 167.0, 164.2, 163.5, 155.3 (d, *J* = 268 Hz), 155.0 (d, *J* = 268 Hz), 137.1 (d, *J* = 8 Hz), 137.0 (d, *J* = 8 Hz), 135.7 (d, *J* = 8.5 Hz), 135.3 (d, *J* = 4 Hz), 135.2 (d, *J* = 4 Hz), 134.9 (d, *J* = 8.6 Hz), 134.8, 134.6, 131.1, 130.7, 126.1 (d, *J* = 2.0 Hz), 125.6 (d, *J* = 2.1 Hz), 124.0, 123.8, 119.1 (d, *J* = 21.2 Hz), 119.0 (d, *J* = 21.1 Hz), 62.4, 60.3, 52.3, 51.9, 48.1, 45.3, 28.5, 28.2; *m/z* (EI) 492/494 ($M+H^+$, 8%), 491/493 ($M^{+\bullet}$, 1), 436/438 (3), 391/393 (8), 313 (100), 312 (90), 266 (6), 249 (6), 104 (15), 57 (41); HRMS *m/z* 491.0487 ($M^{+\bullet}$, $C_{21}H_{19}{}^{79}BrFN_3O_5$ requires 491.0492).

(2*S*,3*RS*)-3-Bromo-*N*-*tert*-butyl-*N*$^{\alpha}$-phthaloyl-3-(3-fluoro-4-nitrophenyl)alaninamide (8b). A solution of **7b** (60 mg, 0.15 mmol) in CCl_4 (2.4 mL) and CH_2Cl_2 (0.6 mL) was treated with NBS (78 mg, 0.44 mmol) and the mixture was heated at reflux while irradiated with a 300W

tungsten lamp for 8 h. The mixture was diluted with CH_2Cl_2 (5 mL), washed with water (2 _ 5 mL), dried ($MgSO_4$), and concentrated *in vacuo* to afford a 1:1 mixture of the diastereomers of the bromide **8b** (70 mg, 98%) as a clear film; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.09 (app t, 0.5H, *J* = 7.9 Hz), 7.96 (m, 1H), 7.92 (app t, 0.5H, *J* = 7.9 Hz), 7.82 (m, 1H), 7.74 (m, 1H), 7.70 (m, 1H), 7.53 (m, 1H), 7.32 (m, 1H), 6.28 (br s, 0.5H), 6.19 (br s, 0.5H), 6.15 (d, *J* = 11.4 Hz, 0.5H), 6.12 (d, *J* = 11.7 Hz, 0.5H), 5.22 (d, *J* = 11.4 Hz, 0.5H), 5.13 (d, *J* = 11.7 Hz, 0.5H), 1.37 (s, 4.5H), 1.12 (s, 4.5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 167.0, 164.1, 163.4, 155.3 (d, *J* = 266 Hz), 155.1 (d, *J* = 266 Hz), 146.6 (d, *J* = 7.6 Hz), 146.5 (d, *J* = 7.7 Hz), 137.1 (d, *J* = 8 Hz), 137.0 (d, *J* = 8 Hz), 134.9, 134.8, 131.1, 130.7, 126.7, 126.6, 124.6 (d, *J* = 4 Hz), 124.2, 124.1 (d, *J* = 4 Hz), 124.0, 118.6 (d, *J* = 22.1 Hz), 118.1 (d, *J* = 22.2 Hz), 61.5, 60.0, 52.4, 52.0, 47.9, 45.0, 28.5, 28.3; *m/z* (EI) 492/494 ($M+H^+$, 1%), 491/493 ($M^{+\bullet}$, 1), 436/438 (1), 391/393 (7), 313 (100), 312 (77), 266 (6), 249 (6), 104 (13), 57 (39); HRMS *m/z* 493.0486 ($M^{+\bullet}$, $C_{21}H_{19}{}^{81}BrFN_3O_5$ requires 493.0472).

(2*S*,3*R*)-3-Hydroxy-*N*-*tert*-butyl-*N*$^{\alpha}$-phthaloyl-3-(4-fluoro-3-nitrophenyl)alaninamide (9a). A solution of the bromide **8a** (110 mg, 0.22 mmol) in acetone (3 mL) was treated with a solution of silver sulfate (70 mg, 0.22 mmol) in water (2 mL). The mixture was heated at reflux (oil bath 65–70 °C) in the dark for 3 days, filtered through celite, acetone was removed *in vacuo* and the concentrate was diluted with water (5 mL). The mixture was extracted with CH_2Cl_2 (2 _ 5 mL), the combined extracts were washed with water (5 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Recrystallization from CH_2Cl_2–hexanes afforded the alcohol **9a** (81 mg, 85%) as a colorless crystalline solid; mp 201–202 °C; $[\alpha]_D^{18}$ –55.7 (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.08 (dd, 1H, *J* = 2.3, 7.0 Hz), 7.79 (m, 2H), 7.73 (m, 2H), 7.64 (ddd, 1H, *J* = 2.3, 3.9, 8.6 Hz), 7.20 (dd, 1H, *J* = 8.6, 10.4 Hz), 6.08 (br s, 1H), 5.66 (dd, 1H, *J* = 5.1, 7.3 Hz), 5.07 (d, 1H, *J* = 5.1 Hz), 4.74 (br d, 1H, *J* = 7.3 Hz), 1.33 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 165.1, 154.7 (d, *J* = 265 Hz), 137.5 (d, *J* = 4 Hz), 137.2 (d, *J* = 8 Hz), 134.6, 132.9 (d, *J* = 8.6 Hz), 131.2, 123.9, 123.7, 118.4 (d, *J* = 21.2 Hz), 70.8, 60.1, 52.3, 28.5; *m/z* (EI) 430 ($M+H^+$, 1%), 429 ($M^{+\bullet}$, 1), 329 (9), 312 (66), 282 (13), 260 (100), 226 (11), 204 (36), 187 (89), 160 (23), 132 (32), 104 (35), 84 (52), 57 (36); HRMS *m/z* 429.1338 ($M^{+\bullet}$, $C_{21}H_{20}FN_3O_6$ requires 429.1336).

(2*S*,3*S*)-3-Hydroxy-*N*-*tert*-butyl-N^{α}-phthaloyl-3-(3-fluoro-4-nitrophenyl)alaninamide (9b). A solution of the bromide **8b** (60 mg, 0.12 mmol) in acetone (1.5 mL) was treated with a solution of silver sulfate (38 mg, 0.12 mmol) in water (1 mL). The flask was sealed and heated at 85 °C in the dark for 4 days, filtered through celite, acetone was removed *in vacuo* and the concentrate was diluted with water (5 mL). The mixture was extracted with CH_2Cl_2 (2 _ 5 mL), the combined extracts were washed with water (5 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Elution through a silica column with hexanes–ethyl acetate afforded the bromide **8b** (7 mg, 12%), and the alcohol **9b** (31 mg, 59%, 67% based on recovered starting material) as a crystalline solid; mp 236–239 °C; $[\alpha]_D^{18}$ –82 (c 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.98 (app t, 1H, *J* = 7.9 Hz), 7.81 (m, 2H), 7.74 (m, 2H), 7.33 (m, 2H), 5.99 (br s, 1H), 5.63 (dd, 1H, *J* = 4.7, 7.9 Hz), 5.14 (d, 1H, *J* = 4.7 Hz), 4.99 (d, 1H, *J* = 7.9 Hz), 1.36 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.6, 164.7, 155.5 (d, *J* = 266 Hz), 149.3 (d, *J* = 7.6 Hz), 136.6 (d, *J* = 8 Hz), 134.8, 131.2, 126.2, 124.0, 121.6 (d, *J* = 4.1 Hz), 115.9 (d, *J* = 22.7 Hz), 71.2, 59.6, 52.5, 28.6; *m/z* (EI) ; HRMS *m/z* 429.1342 ($M^{+\bullet}$, $C_{21}H_{20}FN_3O_6$ requires 429.1336).

(2*S*,3*S*)-3-Hydroxy-3-(4-fluoro-3-nitrophenyl)alanine (10a). Alcohol **9a** (30 mg, 0.070 mmol) was dissolved in a mixture of 5N HCl and glacial acetic acid (2:1, 2 mL). The solution was heated at 100 °C for 5 h, then was cooled and evaporated to dryness *in vacuo*. The solid residue was taken up in water (1 mL) and the suspension was filtered to remove phthalic acid. The filtrate was evaporated to dryness *in vacuo*, and the residue dissolved in a minimal amount of 3N HCl. The solution was neutralised with aqueous ammonia solution and the precipitated solid isolated and dried to give the amino acid **10a** as an off-white powder (14 mg, 82%); ^{1}H NMR (D_2O/DCl, 300 MHz) δ 8.16 (dd, 1H, *J* = 1.8, 7.1 Hz), 7.75 (m, 1H), 7.47 (dd, 1H, *J* = 8.5, 10.9 Hz), 4.98 (d, 1H, *J* = 8.9 Hz), 3.88 (d, 1H, *J* = 8.9 Hz); *m/z* (FAB) 245 ($M+H^+$). Spectral details in accordance with reported values.[7]

(2*S*,3*S*)-3-Hydroxy-3-(3-fluoro-4-nitrophenyl)alanine (10b). The alcohol **9b** (16 mg, 0.037 mmol) was treated according to the preparation of **10a** from **9a** to give the amino acid **10b** as an off-white powder (7 mg, 78%); ^{1}H NMR (D_2O/DCl, 300 MHz) δ 8.16 (app t, 1H, *J* = 8.0 Hz), 7.40 (m, 2H), 4.99 (d, 1H, *J* = 8.2 Hz), 3.87 (d, 1H, *J* = 8.2 Hz); *m/z* (FAB) 245 ($M+H^+$).

(*S*)-*N*-Phthaloyl-3-(4-fluoro-3-nitrophenyl)alanine Methyl Ester (2). A solution of (*S*)-4-fluoro-3-nitrophenylalanine hydrochloride **1a**[12] (200 mg, 0.76 mmol) and sodium carbonate (125

mg, 1.18 mmol) in water (5 mL) was treated with finely ground *N*-carbethoxyphthalimide (170 mg, 0.78 mmol). The mixture was stirred for 1 h, filtered, acidified to pH 3 with 10% aqueous HCl, and extracted with ethyl acetate (2 _ 5 mL). The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was dissolved in dry methanol which had been pre-treated with thionyl chloride (1 mL), and the solution was stirred under anhydrous conditions for 16 h. The solution was concentrated *in vacuo* and the residue dissolved in CH_2Cl_2, washed with 10% aqueous Na_2CO_3, dried ($MgSO_4$) and concentrated *in vacuo* to afford a colorless oil which solidified on standing. Recrystallization from EtOAc–hexanes afforded **2** (238 mg, 85%) as a white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.86 (dd, 1H, *J* = 7.0, 2.4 Hz), 7.79 (m, 2H), 7.72 (m, 2H), 7.47 (ddd, 1H, *J* = 8.6, 3.9, 2.4 Hz), 7.13 (dd, 1H, *J* = 10.6, 8.6 Hz), 5.12 (dd, 1H, *J* = 10.7, 5.5 Hz), 3.76 (s, 3H), 3.64 (dd, 1H, *J* = 14.4, 5.5 Hz), 3.56 (dd, 1H, *J* = 14.4, 10.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 167.2, 154.3 (d, *J* = 265 Hz), 137.0, 135.9 (d, *J* = 8.0 Hz), 134.4, 134.0, 131.2, 126.3, 123.6, 118.5 (d, *J* = 21 Hz), 53.0, 52.4, 33.7.

(2*S*,3*RS*)-*N*-Phthaloyl-3-bromo-3-(4-fluoro-3-nitrophenyl)alanine Methyl Ester (3). A solution of **2** (100 mg, 0.27 mmol) in CCl_4 (10 mL) was treated with NBS (72 mg, 0.40 mmol) and the mixture was heated at reflux while irradiated with a 300W tungsten lamp for 6 h. The mixture was washed with water (2 _ 10 mL), dried ($MgSO_4$), and concentrated *in vacuo* to afford a 1:1 mixture of the diastereomers of the bromide **3** (121 mg, 100%) as a pale yellow foam; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.29 (dd, 0.5H, *J* = 2.5, 6.9 Hz), 8.06 (dd, 0.5H, *J* = 2.5, 6.9 Hz), 7.94 (m, 1H), 7.88 (ddd, 0.5H, *J* = 2.5, 3.9, 8.8 Hz), 7.82 (m, 1H), 7.76-7.66 (m, 2.5H), 7.34 (dd, 0.5H, *J* = 8.8, 10.2 Hz), 7.16 (dd, 0.5H, *J* = 8.8, 10.3 Hz), 5.97 (d, *J* = 11.2 Hz, 0.5H), 5.92 (d, *J* = 9.9 Hz, 0.5H), 5.53 (d, *J* = 9.9 Hz, 0.5H), 5.41 (d, *J* = 11.2 Hz, 0.5H), 3.80 (s, 1.5H), 3.59 (s, 1.5H).

(2*S*,3*R*)-*N*-Phthaloyl-3-hydroxy-3-(4-fluoro-3-nitrophenyl)alanine Methyl Ester (4). A solution of the bromide **3** (60 mg, 0.13 mmol) in acetone (2 mL) was treated with a solution of silver nitrate (35 mg, 0.21 mmol) in water (2 mL). The mixture was heated at 50 °C in the dark for 4 days, filtered through celite, acetone was removed *in vacuo* and the concentrate was diluted with water (5 mL). The mixture was extracted with CH_2Cl_2 (2 _ 5 mL), the combined extracts were washed with water (5 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Analysis of the crude product by ^{1}H NMR spectroscopy indicated the production of a 95:5 mixture of the alcohol **4** and the corresponding *erythro*-diastereomer, as determined by integration of the peaks at δ 5.44

and 4.96 (corresponding to the α-protons of the *threo*- and *erythro*-isomers, respectively). Elution of the mixture through a silica column with hexanes–ethyl acetate afforded the alkene **5** (24 mg, 50%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.13 (dd, 1H, *J* = 2.2, 7.1 Hz), 8.04 (s, 1H), 7.92 (m, 2H), 7.83 (m, 2H), 7.66 (ddd, 1H, *J* = 2.2, 4.2, 8.7 Hz), 7.25 (dd, 1H, *J* = 8.7, 10.2 Hz), 3.86 (s, 3H). Further elution afforded the alcohol **4** (18 mg, 35%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.10 (dd, 1H, *J* = 2.5, 7.0 Hz), 7.82 (m, 2H), 7.76 (m, 2H), 7.61 (ddd, 1H, *J* = 2.5, 3.9, 8.7 Hz), 7.21 (dd, 1H, *J* = 8.7, 10.4 Hz), 5.72 (br d, 1H, *J* = 4.4 Hz), 5.44 (d, 1H, *J* = 4.4 Hz), 5.16 (br s, 1H), 3.87 (s, 3H).