

Synthesis of Protected L-4-[Sulfonyl(difluoromethyl)]phenylalanine and its Incorporation into Peptides.

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Supporting Information

General. All starting materials and reagents were obtained from Aldrich Chemical Company (Oakville, ON, Canada) except for amino acids and resin used for peptide synthesis. Amino acids and Rink amide resin used for peptide synthesis were purchased from Novabiochem Corp. (San Diego, CA, USA). Solvents were purchased from Caledon Laboratories (Georgetown, Ontario, Canada) or BDH Canada (Toronto, Canada). Tetrahydrofuran (THF) was distilled from sodium metal in the presence of benzophenone under argon. CH_2Cl_2 was distilled from calcium hydride under argon. DMF and N,N-dimethylacetamide (DMAC) were dried and distilled over calcium hydride under vacuum and stored over 4 angstrom sieves under nitrogen. Silica gel chromatography was performed using silica gel 60A (230-400 mesh) obtained from Silicycle, (Laval, Quebec, Canada). ^1H , ^{19}F , ^{31}P , and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer. Chemical shifts (δ) for ^1H NMR spectra run in CDCl_3 are reported in ppm relative to the internal standard tetramethylsilane (TMS). Chemical shifts (δ) for ^1H NMR spectra run in CD_3OD are reported in ppm relative to residual solvent protons (δ 3.30). Chemical shifts (δ) for ^1H NMR spectra run in D_2O are reported in ppm relative to residual solvent protons (δ 4.79). For ^{13}C NMR spectra run in CDCl_3 , chemical shifts are reported in ppm relative to the CDCl_3 residual carbons (δ 77.0 for central peak). For ^{13}C NMR spectra run in CD_3OD , chemical shifts are reported in ppm relative to the CD_3OD residual carbons (δ 49.0 for central peak). For ^{13}C NMR spectra run in D_2O , chemical shifts are reported in ppm relative to CH_3OH in D_2O (external). For ^{19}F NMR spectra, chemical shifts are reported in ppm relative to trifluoroacetic acid (external). Low-resolution and high-resolution electron impact mass spectra (EIMS) were obtained on a Micromass 70-S-250 mass spectrometer. Electrospray ionization mass spectra (ESIMS) were obtained on a Micromass Quattro II mass spectrometer. Melting points were determined on Fisher-Johns melting point apparatus and are uncorrected.

Sodium (4-iodophenyl)methanesulfonate (5). To a stirred solution of *p*-iodobenzylbromide (30.0 g, 101.0 mmol) in acetone (200 mL) was added a solution of sodium sulfite (12.73 g, 101.0 mmol) in water (200 mL). A white precipitate formed. The reaction mixture was refluxed for 20 h. The mixture was concentrated to approximately one-half of the original volume and then filtered. After washing of the filter cake with acetone **5** was obtained as a white solid (30.0 g, 93%). Mp >300 °C; ^1H NMR (D_2O): δ 7.77 (2H, d, J = 7.8 Hz), 7.17 (2H, d, J = 7.8 Hz), 4.11 (2H, s); ^{13}C NMR (D_2O): δ 137.67, 132.32, 131.53, 93.30, 56.42; negative ion ESIMS m/z 297 (100, $\text{M}^+ - \text{Na}$).

(4-iodophenyl)methanesulfonyl chloride (6). To a suspension of **5** (25.0g, 78.1 mmol) in sulfolane:CH₃CN (1:1, 104 mL) was added freshly distilled POCl₃ (43.7ml mL, 468.6 mmol). After stirring at 60 °C for 2.5 h, the reaction was cooled to room temperature and then poured slowly into ice cold water (300 mL) which resulted in the formation of a white precipitate. The suspension was filtered, the solid was dissolved in CH₂Cl₂ (600 mL) then washed with water (3 x 150 mL) and brine (2 x 150ml), then dried (MgSO₄), filtered and concentrated to give **6** as a white solid (21.9 g, 89%). Mp 138-139 °C; ¹H NMR: δ 7.81 (2H, d, J = 7.8 Hz), 7.21 (2H, d, J = 7.8 Hz), 4.80 (2H, s); ¹³C NMR: δ 138.44, 132.90, 125.69, 96.92, 70.16; EIMS m/z 316 (9.6, M⁺), 217 (100, M⁺ - SO₂Cl), 90 (66); HREIMS calcd for C₇H₆SO₂ClII 315.8822, found 315.8818.

Neopentyl(4-iodophenyl)methanesulfonate (7). To a solution of **6** (18.5 g, 58.4 mmol) in THF (200 mL) at 0 °C was added a solution of neopentyl alcohol (6.93ml, 64.3 mmol) and triethylamine (8.96 mL, 64.3 mmol) dropwise. The reaction mixture was stirred at room temperature for 20 h. The solvent was evaporated, the residue was dissolved in CH₂Cl₂ (500 mL), washed with water (3 x 150 mL) and brine (2 x 150ml), then dried (MgSO₄), filtered, and concentrated. Flash chromatography (hexane:ethyl acetate,1:5) of the crude product yielded pure **7** as a white solid (19.9 g, 93%). Mp 105-106 °C; ¹H NMR: δ 7.73 (2H, d, J = 8.3Hz), 7.15 (2H, d, J = 8.3 Hz), 4.28 (2H, s), 3.76 (2H, s), 0.92 (9H, s). ¹³C NMR: δ 138.09, 132.56, 127.80, 95.23, 79.68, 55.93, 31.90, 26.10; EIMS m/z 368 (15, M⁺), 217 (100, M⁺ - C₅H₁₁SO₃), 90 (47); HREIMS calcd for C₁₂H₁₇IO₃S 367.9943, found 367.9935.

2,2,2-Trichloroethyl(4-iodophenyl)methanesulfonate (8). Same procedure as described for **7** except 2,2,2-trichloroethanol was used (92% yield). Mp 137-138 °C; ¹H NMR: δ 7.77 (2H, d, J = 7.8 Hz), 7.19 (2H, d, J = 8.2 Hz), 4.54 (2H, s), 4.46 (2H, s), ¹³C NMR: δ 138.38, 132.64, 126.57, 95.90, 93.50, 77.72, 57.34; EIMS m/z 428 (5.2, M⁺), 430 (5.4, M⁺+2), 217 (100, M⁺ - C₂H₂SO₃Cl₃), 90 (44); HREIMS calcd for C₉H₈Cl₃IO₃S 427.8304, found 427.8302.

Neopentyldifluoro(4-iodophenyl)methanesulfonate (9). To a solution of **7** (5.0 g, 13.58 mmol) and N-fluorobenzenesulfonimide (NFSi, 10.28 g, 32.6 mmol) in THF (170 mL) at -78°C was added 1.0 M NaHMDS in THF (29.7 mL, 29.7 mmol) dropwise over 25 min. The reaction was stirred for 2 h at -78°C and then at room temperature for 2 h. The reaction was quenched with a saturated NH₄Cl solution (25 mL), and the resulting solution was extracted with ether (3 x 100 mL). The organic solution was washed with 5% NaHCO₃ (2 x 100 mL), brine (2 x 100 mL), then dried (MgSO₄), filtered and concentrated to give a yellow residue. Flash chromatography (hexane:ethyl acetate,1:5) of the crude product yielded pure **9** as a white solid (4.92 g, 90%). Mp 63-64 °C; ¹H NMR: δ 7.87 (2H, d, J = 8.9 Hz), 7.41 (2H, d, J = 8.3 Hz), 4.13 (2H, s), 1.01 (9H, s); ¹³C NMR: 138.02, 128.57 (t, J = 6.0 Hz), 127.54 (t, J = 22.8 Hz), 120.82 (t, J = 283.4 Hz), 99.80, 84.58, 32.10, 25.81; ¹⁹F NMR: -25.06; EIMS m/z 404 (2.0, M⁺), 253 (100, M⁺ - C₅H₁₁O₃S), 126 (28); HREIMS calcd for C₁₂H₁₅F₂IO₃S 403.9755, found 403.9756.

2, 2, 2-Trichloroethyl difluoro(4-iodophenyl)methanesulfonate (10). To a solution of **8** (215 mg, 0.5 mmol) in THF (20 mL) at -78°C was added dropwise a 1.0 M NaHMDS in THF (0.5 mL, 0.5 mmol) over a period of 3 min, and the mixture was stirred for 15 min at -78 °C. A solution of NFSi (197 mg, 0.625 mmol) in THF (5 mL) was added dropwise over 4 min, and the mixture was stirred at -78 °C for 40 min. The above process was repeated (0.5 mL 1.0 M NaHMDS in THF, 197 mg NFSi). The reaction was quenched with saturated aq NH₄Cl solution (25 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The combined extracts were dried (MgSO₄) filtered and concentrated. Flash chromatography (CH₂Cl₂:hexane, 1:10) of the crude product yielded pure **10** as a white solid (166 mg, 71%). Mp 94-95 °C; ¹H NMR: δ 7.91 (2H, d, J = 8.9Hz), 7.43 (2H, d, J = 8.9 Hz), 4.85 (2H, s); ¹³C NMR: δ 138.29, 128.58 (t, J = 6.1Hz), 126.36 (t, J = 22.5 Hz), 121.01 (t, J = 285.5 Hz), 100.55 (t, J = 2.3 Hz), 92.58, 80.74, ¹⁹F NMR: δ -24.32; EIMS m/z 464 (1.0, M⁺), 253 (100, M⁺ - C₂H₂Cl₃SO₃), 126 (42); HREIMS calcd for C₉H₆Cl₃F₂IO₃S 463.8116, found 463.8126. In addition to the desired product **10**, the side product, 2,2-dichlorovinyldifluoro(4-iodophenyl)methanesulfonate (**11**), was also isolated (34 mg, 15.0%). Mp 49-50 °C; ¹H NMR: δ 7.92 (2H, d, J = 8.7Hz), 7.43 (2H, d, J = 8.7 Hz), 7.11 (1H, s), ¹³C NMR: δ 138.37, 133.79, 128.58 (t, J = 5.8 Hz), 125.91 (t, J = 22.2 Hz), 121.26 (t, J = 287.4 Hz), 117.52, 100.85 (t, J = 2.2 Hz), ¹⁹F NMR: δ -23.72; EIMS m/z 253 (100, M⁺ - C₂HCl₂SO₃), 126 (42).

Methyl 3-(4-{difluoro[(neopentyloxy)sulfonyl]methyl}phenyl)-2-[(9H-9-fluorenylmethoxy)carbonyl]amino}propanoate (13). To a solution of N-Fmoc-3-iodo-L-alanine methyl ester¹ (**12**, 902.6 mg, 2.0 mmol) in THF-DMAC (4 mL, 1:1) under N₂ was added acid washed zinc dust (130.7 mg, 2 mmol). The solution was warmed to 65–70 °C and stirred for 1h. In a separated flask a solution of **9** (404 mg, 1.0 mmol) in THF-DMAC (1.0 mL, 1:1) was added to a solution containing a catalyst that was prepared by reaction of a solution of Cl₂Pd(PPh₃)₂ (35 mg, 0.05 mmol) in THF-DMAC (1.0 mL, 1:1) with diisobutylaluminum hydride (1.0M in hexane, 0.1 mL, 0.1 mmol), and the mixture was stirred at room temperature for 10 min. This mixture was transferred via canula to the solution of zinc reagent. The mixture was stirred at 65–70 °C for 6h, then cooled to 0 °C, diluted with saturated NH₄Cl (10 mL) and then extracted with ethyl acetate (3 x 10 ml). The combined extracts were dried (MgSO₄), filtered, and concentrated to give a brown residue. The residue was placed under high vacuum for 16 h to remove residual DMAC. Flash chromatography (gradient elution with ethyl acetate in hexane, 10%, 12%, 14%, 16%, 18%, 20%) of the crude product yielded pure **13** as a white foam (271 mg, yield 45%). Mp 53-54 °C; ¹H NMR: δ 7.77 (2H, d, J = 8.0 Hz), 7.61 (2H, d, J = 8.1 Hz), 7.57 (2H, d, J = 7.4 Hz), 7.41 (2H, t, J = 7.4 Hz), 7.32 (2H, t, J = 7.4 Hz), 7.22 (2H, d, J = 7.7 Hz), 5.26 (1H, d, J = 8.0 Hz), 4.68 (1H, m), 4.36-4.50 (2H, m), 4.21 (1H, dd, J = 6.8 Hz, 6.2 Hz), 4.10 (2H, s), 3.73 (3H, s), 3.18 (2H, m), 1.01 (9H,s); ¹³C NMR: δ 171.38, 155.44, 143.70, 143.57, 141.30, 141.27, 140.83, 129.65, 127.72, 127.30 (t, J = 6.1 Hz), 127.03, 126.70 (t, J = 22.9 Hz), 124.99, 124.90, 120.93 (t, J = 283.4 Hz), 119.98, 119.95, 84.38, 66.85, 54.47, 52.47, 47.09, 37.98, 32.06, 25.79, ¹⁹F NMR: δ -24.30; positive ion ESIMS m/z 624 (100, M⁺ + Na), 602 (8, MH⁺), 554 (84, MH⁺ - C₅H₁₁ + Na).

Methyl 3-(4-{difluoro[(2, 2, 2-trichloroethoxy)sulfonyl]methyl}phenyl)-2-[(9H-9-fluorenylmethoxy)carbonyl]amino}propanoate (14). Prepared using the same procedure

described above for **13** except **10** (232 mg, 0.5 mmol) was used as starting material (145 mg, 44%). Mp 52-54 °C; ¹H NMR: δ 7.78 (2H, d, J = 7.4 Hz), 7.63 (2H, d, J = 7.8 Hz), 7.57 (2H, d, J = 7.4 Hz), 7.41 (2H, t, J = 7.4 Hz), 7.20-7.35 (4H, m), 5.26 (1H, d, J = 8.0 Hz), 4.80 (2H, s), 4.62-4.75 (1H, m), 4.36-4.52 (2H, m), 4.21, (1H, dd, J = 6.9 Hz, 6.2 Hz), 3.73 (3H, s), 3.10-3.28 (2H, m); ¹³C NMR: δ 171.32, 155.41, 143.66, 143.56, 141.56, 141.30, 141.27, 129.90, 127.73, 127.36 (t, J = 6.0 Hz), 127.03, 125.50 (t, J = 22.0 Hz), 124.96, 124.87, 121.12 (t, J = 285.2 Hz), 119.99, 119.96, 92.67, 80.64, 66.81, 54.44, 52.50, 47.08, 38.04; ¹⁹F NMR: δ -23.6; positive ion ESIMS m/z 684 (100, $M^+ + Na$), 686 (99, $M^+ + 2 + Na$).

3-(4-{Difluoro[(neopentyloxy)sulfonyl]methyl}phenyl)-2-[(9H-9-fluorenylmethoxy)carbonyl]amino}propanoic acid (15). To a stirred solution of compound **13** (902 mg, 1.5 mmol) in THF (15 mL) at 0 °C was added 0.2 N LiOH (15 mL, 3.0 mmol) dropwise, and the mixture was stirred at 0 °C for 20 min. The reaction mixture was acidified with 0.2 N HCl to pH 3 and extracted with ethyl acetate (3 x 30 ml). The combined organic layer was dried over anhydrous MgSO₄, filtered and concentrated to afford a colorless oil. Flash chromatography of the crude material (MeOH:CHCl₃, 1:9) gave pure **15** as a white foamy solid (802 mg, 91%). Mp 81-82 °C; ¹H NMR (MeOH): δ 7.73 (2H, d, J = 7.4 Hz), 7.51 (4H, d, J = 7.8 Hz), 7.57 (2H, d, J = 7.4 Hz), 7.10-7.38 (6H, m), 4.38 (1H, dd, J = 8.8 Hz, 4.5 Hz), 4.16-4.29 (2H, m), 4.09 (1H, dd, J = 7.1 Hz, 6.8 Hz), 3.98 (2H, s), 3.19-3.31 (1H, m), 2.99 (1H, dd, J = 13.7 Hz, 9.3 Hz), 0.89 (9H, s); ¹³C NMR (MeOH): δ 175.83, 158.23, 145.18, 145.10, 144.34, 142.51, 130.95, 128.75, 128.17 (t, J = 6.1 Hz), 127.19, 127.48 (t, J = 22.8 Hz), 126.22, 126.17, 122.34 (t, J = 282.1 Hz), 120.91, 85.50, 67.86, 56.78, 48.24, 38.45, 32.86, 26.10; ¹⁹F NMR (MeOH): δ -25.44; negative ion ESIMS m/z 586 (47, $M^- - H$), 390 (100, $M^- - 1 - C_{14}H_{12}O$), 364 (65, $M^- - Fmoc$).

3-(4-{Difluoro[(2, 2, 2-trichloroethoxy)sulfonyl]methyl}phenyl)-2-[(9H-9-fluorenylmethoxy)carbonyl]amino}propanoic acid (16). Prepared using the same procedure described above for **15** except **14** was used as starting material (428 mg, 89%). Mp 80-81 °C; ¹H NMR (MeOH): δ 7.78 (2H, d, J = 7.7 Hz), 7.66 (2H, d, J = 8.2 Hz), 7.60 (2H, d, J = 7.8 Hz), 7.47 (2H, d, J = 7.8 Hz), 7.38 (2H, dd, J = 7.9 Hz, 7.4 Hz), 7.26-7.32 (2H, m), 4.93 (2H, s), 4.48 (1H, dd, J = 9.6 Hz, 4.5 Hz), 4.23-4.35 (2H, m), 4.15 (1H, dd, J = 7.1 Hz, 6.3 Hz), 3.26-3.38 (1H, m), 3.06 (1H, dd, J = 13.7 Hz, 9.7 Hz); ¹³C NMR (MeOH): δ 174.58, 158.36, 145.26, 145.23, 144.84, 142.62, 131.19, 128.80, 128.37 (t, J = 6.0 Hz), 128.17, 126.67 (t, J = 22.3 Hz), 126.27, 126.23, 122.60 (t, J = 283.5 Hz), 120.94, 94.42, 82.00, 67.95, 56.33, 48.37, 38.41; ¹⁹F NMR (MeOH): δ -24.85; negative ion ESIMS m/z 646 (19, $M^- - H$), 450 (96, $M^- - H - C_{14}H_{12}O$), 452 (100, $M^- - H - C_{14}H_{12}O + 2$), 424 (65, $M^- - Fmoc$), 426 (58, $M^- - Fmoc + 2$).

Synthesis of DADE-F₂Smp-LNH₂, (17)

Solid phase peptide synthesis was carried out manually using the Fmoc strategy using the Rink amide AM resin (0.69 mmol/g). The resin was preswollen in DMF for 1 h. Coupling of the first amino acid was accomplished by reacting the resin with pentafluorophenyl ester (4 equiv) of Fmoc-L-leucine and HOAt (4 equiv) in DMF for 30 minutes. The resin was washed with DMF (6 x 2min), CH₂Cl₂ (2 x 2min) and then deprotected with 20% piperidine in DMF for two times (10 + 10 minutes) followed by washing with DMF and CH₂Cl₂. All subsequent Fmoc

deprotections were carried out in an identical manner. All subsequent coupling were carried out using Fmoc-protected amino acids in the following manner. To a solution of the protected amino acid (4 equiv) in DMF was added HATU (4 equiv), HOAt (4 equiv) and DIEA (4 equiv) and left stirred at rt for 10 min. The mixture was added to the resin and shaken for 30 minutes. The resin was filtered and washed with DMF. The coupling was carried out twice for each residue with the exception of the protected L-F₂Smp which was done once. The side chains of asp and glu were protected as *t*-butyl esters. After completion of the peptide assembly, the resin was washed with CH₂Cl₂ and dried in vacuo overnight. A solution of reagent K (82.5% TFA, 2.5% EDT, 5% H₂O, 5% thioanisole and 5% phenol – prepared fresh, 1.5 mL per 100 mg peptide-resin) was added to a vial containing resin and stirred for 2 h. The solution was filtered and the filtrate was concentrated to a volume that just covers the bottom of the flask. This solution was pipetted into a 50 mL centrifuge tube about two-thirds filled with cold methyl *t*-butyl ether and then centrifuged at 3500 rpm at 0 °C for 10 min. The solution was decanted, cold methyl *t*-butylether was added to about two-thirds of the total centrifuge tube volume, shaken vigorously and centrifuged as above for 10 minutes. This washing process was repeated two more times. After the final wash, the pellet was allowed to air dry in the centrifuge tube for approximately 1 h. The pellet was dissolved in acetonitrile-water (1:1) containing 0.1% TFA and stirred for 4-5 days and then purified by preparative HPLC using a Vydac 218TP1022 C-18 column using a linear gradient of 95% water (0.025% TFA)/5% acetonitrile (0.025% TFA) to 20% water (0.025% TFA)/80% acetonitrile (0.025% TFA) over a period of 120 minutes. The purity of the peptide was assessed by analytical reverse phase HPLC (Vydac 218TP54 C18 column, linear gradient of 95% water (0.025% TFA)/5% acetonitrile (0.025% TFA) to 20% water (0.025% TFA)/80% acetonitrile (0.025% TFA) over a period of 30 minutes) and ESIMS. Analytical HPLC showed only a single peak (retention time = 12.4 minutes). Negative ion ESIMS *m/z* calculated for C₃₂H₄₅F₂N₇O₁₅S 837.27. Found 836.37 [100, (M – H)⁻], 837.33 (40, M⁻).

References

1. Smyth, M.S. and Burke, T.R. *Tetrahedron Lett.* **1994**, 35, 551-554.