Supporting Information for:

An Efficient Synthesis of a Probe for Protein Function: 2,3-Diaminopropionic Acid with Orthogonal Protecting Groups

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General Methods. Proton nuclear magnetic resonances (**^{1}H NMR**) and ^{13}C nuclear magnetic resonances (**^{13}C NMR**) were recorded in deuterated solvents on an iNOVA (500 MHz) or a Mercury (400 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (δ 0.00). **^{1}H NMR** splitting patterns are designated as singlet (s), doublet (d), triplet (t), doublet of doublets (dd). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). Coupling constants are reported in Hertz (Hz). Electrospray mass spectra (**ESI-MS**) were obtained using a Micromass Quattro II Triple Quadrapole HPLC/MS/MS Mass Spectrometer. Optical rotation was determined on an Optical Activity AA-100 Automatic Digital Polarimeter at wavelength 589 nm. All samples were done in MeOH at 24 ^{O}C, with 5 cm long path length. Acetonitrile and DMF were passed through activated alumina.[1] Unless otherwise noted, all other commercially available reagents and solvents were purchased from Aldrich and used without further purification. CuCl grade was 98+%. Benzyl alcohol was 99.8% anhydrous grade. Boc-Asp(OBn)-OH and Boc-Dap(Fmoc)-OH were purchased from Advanced ChemTech. Unless otherwise indicated, all reactions were performed under an inert atmosphere of N_2. Glassware was dried in an oven at 180 °C for 2 hours prior to use.

Abbreviations: DMF, *N,N*-dimethylformamide; DMAP, *N,N*-dimethyl-4-aminopyridine; THF, tetrahydrofuran; EtOAc, ethyl acetate; DCM, dichloromethane; TFA, trifluoroacetic acid; Boc_2O, di-*tert*-butyl dicarbonate

Boc-Asp(OBn)-OMe (**5**). Boc-Asp(OBn)-OH (4.8 g, 14.9 mmol) was dissolved in dry DMF (35 mL) in a 100 mL RBF. Finely ground K_2CO_3 (3.0 g, 22 mmol) was added to the solution to form a suspension. The mixture was cooled to 0 ^{O}C in an ice bath over five minutes. Methyl iodide (2.0 mL, 30 mmol) was then added to the RBF over 30 seconds. The resulting mixture was stirred at 0 ^{O}C for 3 hours. A yellow color developed within 20-30 minutes. The ice bath was removed, and H_2O (50 mL) was added, producing a grayish white precipitate. The mixture was extracted with EtOAc (3 x 50 mL). The organic layers were combined and washed with saturated $NaHCO_3$ (1 x 50 mL) and saturated NaCl (4 x 50 mL). The organic layer (which was yellow) was dried over Na_2SO_4, and passed through a plug of silica, eluting with ethyl acetate. Evaporation under reduced pressure afforded 2.52 g of a white powder (98 %). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34 (m, 5H, phenyl-C$\underline{H}$), 5.48 (d, *J* = 7.9 Hz, 1 H, N$\underline{H}$), 5.13 (dd, J_1 = 12.3 Hz, J_2 = 17.2 Hz, 2H, C$\underline{H}_2$), 4.59 (t, *J* = 3.8 Hz, 1 H, NH-C$\underline{H}$), 3.70 (s, 3 H, C$\underline{H}_3$), 3.05 (dd, J_1 = 4.0 Hz, J_2 = 16.8 Hz, 1 H, HC-$\underline{H}$), 2.86 (dd, J_1 = 4.5 Hz, J_2 = 13.8 Hz, 1 H, HC-$\underline{H}$), 1.44 (s, 9 H, tert-butly-C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 171.73, 171.00, 135.62, 128.83, 128.65, 128.54, 80.36, 67.02, 52.89, 50.18, 37.11, 28.51.

Boc_2-Asp(OBn)-OMe (**6**). Di-*tert*-butyl dicarbonate (10.63 g, 49 mmol) and DMAP (3.97 g, 32.5 mmol) were placed into a dry 100 mL RBF. Product **5** (10.96 g, 32.5 mmol) was added and the solids were dissolved in dry acetonitrile (30 mL). The resulting solution was stirred for 24 hours, during which time the reaction color went from light to dark yellow. Water (50 mL) was added, and the solution became cloudy white. The resulting mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with 1 M HCl (1 x 50 mL) and saturated NaCl (4 x 50 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to afford 13.71 g of a viscous red oil (96 %). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34 (m, 5H, phenyl-C$\underline{H}$), 5.48 (dd, J_1 = 6.9 Hz, J_2 = 6.8 Hz, 1 H, N-C$\underline{H}$), 5.13 (dd, J_1 = 12.3 Hz, J_2 = 18.1 Hz, 2H, C$\underline{H}_2$), 3.70 (s, 3 H, C$\underline{H}_3$), 3.30 (dd, J_1 = 7.3 Hz, J_2 = 16.7 Hz, 1 H, HC-$\underline{H}$), 2.77 (dd, J_1 = 6.4 Hz, J_2 = 16.5 Hz, 1 H, HC-$\underline{H}$), 1.48 (s, 18 H, tert-butyl-C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.75, 170.49, 151.80, 135.96, 128.76, 128.47, 83.79, 66.86, 55.10, 52.73, 36.11, 28.17.

Boc_2-Asp-OMe (**7**). Product **6** (12.81 g, 29.3 mmol) was dissolved in MeOH (70 mL), and the solution was placed into a dry Parr reaction vessel. Palladium on activated carbon (2.5g, 10% by weight) was added to the solution. The solution was shaken on a Parr apparatus for 2 hours under an H_2 atmosphere of 50 psig. The Pd/C was filtered off through Celite 545, eluting with MeOH. The MeOH was evaporated under reduced pressure to give 9.02 g of a white powder (89 %). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.42 (dd, J_1 = 6.6 Hz, J_2 = 6.8 Hz, 1 H, N-C$\underline{H}$), 3.74 (s, 3 H, C$\underline{H}_3$), 3.30 (dd, J_1 = 7.0 Hz, J_2 = 17.0 Hz, 1 H, HC-$\underline{H}$), 2.81 (dd, J_1 = 6.4 Hz, J_2 = 17.0 Hz, 1 H, HC-$\underline{H}$), 1.49 (s, 18 H, *tert*-butyl-C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 176.96, 170.39, 151.76, 83.89, 54.81, 52.81, 35.85, 28.14.

Isocyanate 8. Product **7** (3.00 g, 8.6 mmol) was dissolved in dry THF (6 mL) in a 50 mL RBF, cooled to –10 ^{O}C via an ice bath of saturated NaCl solution, and stirred for 10 minutes. Ethylchloroformate (1.24 mL, 13 mmol) was added via syringe to the solution. Triethylamine (4 mL, 28.7 mmol) was added over 30 seconds and stirred for 30 minutes. A solution of NaN_3 (2.8 g, 43 mmol) in H_2O (15 mL) was added to the solution. The solution was allowed to stir for 1.5 hours as the temperature was maintained at –10 ^{O}C. The solution was then extracted with EtOAc (4 x 50 mL). The combined organic layers were washed with H_2O (1 x 50 mL) and saturated NaCl solution (3 x 50 mL), and then dried over Na_2SO_4 and evaporated under reduced pressure to give clear oil. NOTE! This solution was not evaporated to dryness. In some cases, acyl azides can explode when concentrated to dryness.[2] After concentration, toluene (100 mL) was added, and the resulting solution was refluxed for 2 hours. The toluene was then removed under reduced pressure to afford 2.52 g of a light red oil (85 %). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.13 (dd, J_1 = 5.5 Hz, J_2 = 9.1 Hz, 1 H, N-C$\underline{H}$), 3.94 (dd, J_1 = 4.9 Hz, J_2 = 13.4 Hz, 1 H, HC-$\underline{H}$), 3.83 (dd, J_1 = 9.2 Hz, J_2 = 13.4 Hz, 1 H, HC-$\underline{H}$), 3.75 (s, 3 H, C$\underline{H}_3$), 1.52 (s, 18 H, *tert*-butyl-C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 169.06, 152.09, 128.46, 84.23, 58.23, 52.74, 43.00, 28.16. IR: 2266 cm^{-1} (N=C=O), 1749 cm^{-1} (C=O), 1701 cm^{-1} (C=O).

Boc_2-Dap(Cbz)-OMe (**9**). In a dry 50 mL RBF, CuCl (2.60 g, 26.3 mmol) and benzyl alcohol (2.72 mL, 26.3 mmol) were suspended in DMF (15 mL) to afford a yellowish green mixture. A solution of **8** (8.82 g, 25.6 mmol) in dry DMF (7 mL) was added. The resulting suspension turned brownish red, and was stirred for 1 hour. Water (100 mL) was added to the reaction, and the resulting mixture was extracted with EtOAc (4 x 100 mL). The combined organic layers were then washed with 1 M HCl (100 mL) and saturated NaCl (4 x 100 mL), dried over Na_2SO_4 and evaporated under reduced pressure to afford a brownish red oil. The oil was redissolved in EtOAc (100 mL), and eluted through a silica plug to afford 11.01 g of a light yellow oil (95 %). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34 (m, 5H, phenyl-C$\underline{H}$), 5.25 (t, 1 H, $\underline{H}$N-CH_2), 5.11 (m, 2H, C$\underline{H}_2$), 5.05 (t, 1 H, N-C$\underline{H}$), 3.85 (m, 1 H, HC-$\underline{H}$), 3.75 (s, 3 H, C$\underline{H}_3$), 3.69 (m, 1 H, HC-$\underline{H}$), 1.50 (s, 18 H, *tert*-butyl-C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 170.30, 151.96, 128.79, 128.35, 127.21, 83.91, 67.00, 57.87, 52.51, 41.35, 28.15.

Boc_2-Dap(Cbz)-OH (**10**). A solution of **9** (533 mg, 1.18 mmol in 10 mL of MeOH) was added to a 100 mL RBF. To the stirring solution, 2 M NaOH (5 mL) was added slowly. The color changed from a light yellow to gold. Precipitate briefly formed when all the NaOH solution had been added. After three minutes, the precipitate had dissipated. The reaction was left for two hours. The solution was concentrated by evaporation under reduced pressure, diluted with water (20 mL) and washed with ethyl ether (3 x 10 mL). The organic layer was washed with 2 M NaOH (2 x 25 mL). The combined aqueous layers were made acidic using 1 M HCl (pH ~3), which resulted in formation of a cloudy white solution. The aqueous mixture was extracted with ethyl acetate (3 x 50 mL). The combined organic layers (yellow) were washed with 1 M HCl (2 x 20 mL), and saturated NaCl (2 x 25 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give a sticky yellow solid. Addition of hexanes (~50 mL), followed by vigorous stirring, resulted in precipitation of a slightly yellow solid (350 mg, 68 %). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.36 (m, 5H, phenyl-C$\underline{H}$), 5.41 (m, 1 H, N-C$\underline{H}$), 5.25 (s, 1 H, $\underline{H}$N-CH_2), 5.11 (m, 2H, C$\underline{H}_2$), 3.85 (m, 1 H, HC-$\underline{H}$), 3.68 (m, 1 H. HC-$\underline{H}$), 1.50 (s, 18 H, *tert*-butyl-C$\underline{H}_3$).

Note! Due to some monodeprotection, there are small amounts of Boc-Dap(Cbz)-OH that are present along with **10**. There are clearly additional peaks present in the ^{1}H NMR when Boc-Dap(Cbz)-OH is present (i.e. additional Boc and methyl ester peaks). The amount of Boc-Dap(Cbz)-OH is most easily identified and quantified by examination of the α protons in the ^{1}H NMR spectrum. For Boc-Dap(Cbz)-OH, this proton appears at δ 4.4 ppm, and for Boc$_2$-Dap(Cbz)-OH the same proton appears at δ 5.4 ppm. Comparing the integrations of these two peaks gives the relative amounts of the two products. Using this method, there is never more that 10% of Boc-Dap(Cbz)-OH.

Boc-Dap(Fmoc)-OMe (**13**). Boc-Dap(Fmoc)-OMe (0.098 g, 0.24 mmol, Advanced Chemtech) was combined with finely ground K_2CO_3 (0.075 g, .54 mmol) in dry DMF (1.0 mL). The resulting mixture was cooled to 0 ^{O}C in an ice bath. Methyl iodide (0.040 mL, 0.5 mmol) was added slowly via syringe, and the resulting solution was stirred for 3 hours. Water (10 mL) was added, and a cloudy white mixture was obtained. The mixture was extracted with EtOAc (1 x 25 mL), and the organic layer was washed with saturated $NaHCO_3$ solution (1 x 25 mL) and saturated NaCl solution (2 x 25 mL). Next, the organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to afford 105 mg of a fine white powder (>99 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76 (d, J = 7.49 Hz, 2H, Ar), 7.58 (d, J = 7.49 Hz, 2H, Ar), 7.36 (m, 4H, Ar), 5.44 (s, 1H, Boc-N$\underline{H}$), 5.19 (s, 1 H, Fmoc-N$\underline{H}$), 4.39 (m, 3 H, αC$\underline{H}$, Fmoc-CH-C$\underline{H}_2$O), 4.21 (t, J = 6.40 Hz, 1H, Fmoc-C$\underline{H}$-CH_2O), 3.76 (s, 3 H, CO_2C$\underline{H}_3$) 3.61 (m, 2 H, βC$\underline{H}_2$), 1.45 (s, 9 H, *tert*-butyl-C$\underline{H}_3$) $[\alpha]^{24}_D = -10^O$ (c 1.0, MeOH).

Boc-Dap(Fmoc)-OMe (**13**). A sample of **9** (1.10 g, 2.4 mmol) was dissolved in neat TFA (15 mL) in a 250 mL RBF. The reaction was allowed to stir for 3 hours. TFA was then evaporated under a stream of nitrogen to afford a thick, red oil. A crude ^{1}H NMR confirmed that the *tert*-butyl carbamate had been removed. A solution of saturated $NaHCO_3$ (20 mL) was added in order to quench any remaining TFA. Additional $NaHCO_3$ was added until the pH was ~ 8-9. Dioxane (20 mL) was added to the aqueous solution, followed by Boc$_2$O (0.795 g, 3.6 mmol in 10 mL of dioxane). The resulting solution was allowed to stir for 5 hours. The solution was then extracted with EtOAc (3 x 50 mL), the organic layers were washed with saturated $NaHCO_3$ solution (1 x 100 mL) and saturated NaCl solution (1 x 100 mL) and dried over Na_2SO_4. Evaporation under reduced pressure produced 0.720 g of yellow oil (85% crude). ^{1}H NMR confirmed the presence of single *tert*-butyl carbamate protecting group. The yellow oil was dissolved in MeOH (20 mL), then Pd/C (130 mg, 10% by weight) was added to the solution. The solution was put under an H_2 atmosphere (X psig) and agitated vigorously on a Parr apparatus for 3 hours. The solution was filtered through Celite 545, eluting with MeOH. Evaporation under reduced pressure afforded a yellow/green oil. A crude ^{1}H NMR showed complete loss of benzyl carbamate protecting group. The oil was dissolved in dry THF (10 mL). Triethylamine (0.250 mL, 1.75 mmol) was added to the solution, followed by Fmoc-OSu (0.590 g, 1.75 mmol). The resulting solution was stirred for 5 hours. The solution was then evaporated to dryness under reduced pressure to afford a grayish oil. The grayish oil was dissolved in EtOAc (50 mL), and the organic layer was washed with saturated $NaHCO_3$ (1 x 50 mL), 1 M HCl (1 x 50 mL) and saturated NaCl solution (2 x 50 mL). The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure to afford a white precipitate. This crude product was partially soluble in DCM. The white solid that did not dissolve in DCM was filtered off through Celite 545, eluting with DCM. The crude **13** that was obtained after filtration was further purified by flash column chromatography on silica gel eluting first with DCM (R_F ~ 0) followed by DCM/MeOH (95:5, R_F = 0.35). Purified **13** was obtained as a fine white powder (0.510 g, 65 %). The ^{1}H NMR was consistent with Boc-Dap(Fmoc)-OMe given above. $[\alpha]^{24}_D = -14^O$ (c 1.0, MeOH).

(1) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Trimmers, F. J. *Organometallics* **1996**, *15*, 1518.

(2) Ongeri, S.; Aitken, D. J.; Husson, H. P. *Synth*. *Commun*. **2000**, *30*, 2593.