Supplementary Material for Chemical Communications

Supplementary data

Experimental:

All reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI) and were used as received. Solvents for solid phase peptide synthesis were acquired from Applied Biosystems and were peptide synthesis grade. Other solvents were obtained from Fisher Scientific and were used as received unless stated otherwise. Ether was degassed with argon, passed through activated alumina columns and used immediately.

Peptides were synthesized using an Applied Biosystems 433A automated peptide synthesizer. NMR spectra were acquired on a Varian Inova 500 MHz spectrometer at room temperature. Electrospray mass spectra were collected on a Micromass Quattro II Triple Quadrupole HPLC/MS/MS Mass Spectrometer.

Basic minimization of structures was done using Chem 3D Ultra 6.0, with energy minimization run under the MM2 force field with and RMS value of 0.100. The dielectric constant chosen was 1.8 for water.

All NMR spectra were collected on an INOVA 500 Varian instrument. NOE spectra were acquired at a mixing time of 1 second with samples at a concentration of 20 mmol. HPLC traces were obtained on a Rainin Instruments HPLC on commercially available reverse phase column (C_{18}). Eluents (A): 0.1% Formic acid in water and (B): 80% acetonitrile/water containing 0.08% formic acid were used in a gradient of 100/0 to 0/100

A/B over 22 minutes at a flow rate of 1.5mL/min. The HPLC traces were tracked by UV absorption at 260 nm. Fluorescence emission and excitation spectra were conducted on an ISS PC1 photon counting steady-state fluorescence spectrometer equipped with a 300 W xenon arc lamp. Slit widths of 2 mm (16 nm band width) were used, and the xenon lamp power supply was set to 18 A. Excitation and emission wavelengths were set as stated in the text. Fluorescence lifetime measurements were obtained through phase modulation techniques on an ISS K2 fluorometer at the excitation wavelengths listed in the text. Reported lifetimes were measured versus LUDOX scattering solution.

Synthesis of **1.** 2-Formyl-3,5-dimethoxy-benzoic acid methyl ester (15.3 g, 68.2 mmol) was placed in a flask with lithium hydroxide (4.7 g, 205 mmol) in a 5:1 mixture of tetrahydrofuran and water. The reaction was stirred for 3 h at room temperature and monitored by TLC. When no more starting material was present, the reaction was quenched with hydrochloric acid. The THF was then evaporated under reduced pressure, and the product was filtered from the aqueous phase as a pale yellow solid (10g, 71% yield).

^{1}H NMR $CDCl_3$: 7.65 (m, 2H), 6.68 (s, 1H, CHOH), 4.51 (bs, 1H, OH), 3.8 (s, 6H, OMe). ^{13}C NMR CD_3OD: δ 189.5, 165.8, 163.2, 147.5, 104.4, 97.0, 55.4, 55.2. ESI, MS(CH_3OH): 210.

Synthesis of **2.** m-Bromo xylene (50 g, 270 mmol) was dissolved in carbon tetrachloride (300 mL). N-Bromosuccinamide (91 g, 513 mmol) and a catalytic amount of benzoyl peroxide was added and fit to a reflux condenser. The mixture was refluxed for 12 hh and then cooled. The solvent was evaporated, and the resulting product was dissolved in

a 1:1 mixture of methylene chloride and hexanes and run through silica. The fractions pure by TLC were collected and the solvent evaporated to afford a yellow solid (21 g, 23% yield).
^{1}H NMR CDCl3: 7.49 (s, 2H), 7.37 (s, 1H), 4.42 (s, 4H, CH_2Br). ^{13}C NMR CDCl3: 140.5,132.2, 128.6, 122.9, 31.8. ESI (CH_2Cl_2) = 343, 345.

Synthesis of **3**. Bromide (11 g, 32 mmol) was placed in a flask with triethyl phosphite (8.3 mL, 70.6 mmol) and the reaction flask was fitted with a distillation apparatus to isolate the ethyl bromide side product. The reaction was heated for 18 h at 130 ^{0} C. After cooling, the reaction mixture was diluted with ether and washed with water four times. The organic phase was collected, dried with magnesium sulfate and collected in vacuo as a yellow oil (10 g, 68% yield).

^{1}H NMR $CDCl_3$: 7.36 (s, 2H), 7.18 (s, 1H), 4.05 (t, 4H, OCH_2CH_3, J = 7.3), 3.09 (d, 4H, CH_2P, J = 22 Hz), 1.28 (t, 6H, OCH_2CH_3, J = 6.8 Hz)**.** ^{13}C NMR $CDCl_3$: 134.3 (CCH_2P), 131.5 (CH), 130.2 (CBr), 62.6 (OCH_2CH_3), 34.1 (CCH_2P), 33.0 (CCH_2P), 16.7 (OCH_2CH3)**.** APCI, CH_2Cl_2: 457.1**.**

Synthesis of **4.** The aldehyde (4.5 g, 21.2 mmol) was placed in a dry flask. **3** (5.4 g, 11.8 mmol) was dissolved in dry ether and cannulated into the flask with the aldehyde. The reaction was cooled to 0 ºC, and potassium tert-butoxide (100 mL, 100 mmol) was then cannulated in dropwise. After the base was added, the reaction was allowed to warm to room temperature, stirring overnight under nitrogen. HCl was then added to quench the reaction, and the ether evaporated under reduced pressure. The product was then filtered from the aqueous solution to produce a a yellow solid in 52% yield.

^{1}H NMR d_6DMSO: δ 7.53 (s, 2H), 7.49 (s, 1H), 7.40 (d, J = 16.5 Hz, 2H), 7.03 (d, J = 16.5 Hz, 2H), 6.80 (s, 2H), 6.77 (s, 2H), 3.88 (s, 6H), 3.83 (s, 6H). $CDCl_3$: δ 169.6, 159.7, 159.3, 140.7, 133.2, 131.5, 127.9, 124.3, 119.6, 105.5, 102.0, 56.1, 55.8. ESI MS: 570.4 (MH^+).

Synthesis of **5**. Potassium hydroxide (622.8mg, 11.1 mmol) was placed in a dry flask with minimal DMSO. The mixture was stirred for 30 minutes under nitrogen. The acid (449.3 g, 2.3 mmol) in minimal DMSO was then added to the flask, and the reaction was cooled to -78 ºC and stirred for 15 minutes or until a viscous solid formed. Methyl iodide (1.13mL, 18.1 mmol) was added, and the ice bath was removed immediately after to allow for the reaction to occur. The reaction stirred under nitrogen for 3 h and was then quenched with ice water, and the product filtered to afford a yellow solid in 87% yield.

^{1}H NMR $CDCl_3$: δ 7.55 (s, 1H), 7.50 (s, 2 H), 7.42 (d, 2H, J = 19 Hz), 6.92 (d, 2H, J = 17 Hz), 6.85 (s, 2H), 6.62 (s, 2H), 3.89 (s, 12H OMe), 3.87 (s, 6H, COOMe), ^{13}C NMR $CDCl_3$: δ 166.9, 159.7, 159.3, 140.7, 133.2, 131.5, 127.9, 124.3, 119.6, 105.5, 102.0, 56.1, 55.8, 52.7. ESI MS: 598.10 (MH^+)

Synthesis of **6**. The bromide (2.2 g, 4.6 mmol) was placed in a dry flask with 4-carboxy phenyl boronic acid (836 mg, 5.1 mmol) and potassium carbonate (953 mg, 6.9 mmol). A mixture of DME:H_2O (3:1) was added, and the mixture was degassed with nitrogen for 30 minutes. The solution was then purged and the catalyst (286 mg, 0.23 mmol) was added. The reaction was heated at 80 ºC for 12 h. The reaction was then cooled and acidified with HCl. The acidified mixture was extracted with chloroform three times. The organic layer was collected, dried with magnesium sulfate and evaporated *in vacuo.*

The product was then purified by column chromatography and eluted with 5% methanol in methylene chloride to afford a deep yellow solid (1g, 71% yield).

^{1}H NMR Acetone: δ 7.64 (s, 2H), 7.57 (d, J = 16.5 Hz, 2H), 7.46 (d, J = 7.6 Hz, 2H), 7.37 (d, J = 7.6 Hz, 2H), 7.27 (m, 2H), 7.18 (d, J = 16.5 Hz, 2H), 6.93 (s, 1H), 6.76 (s, 2H), 3.91 (s, 9H), 3.85. ^{13}C NMR Acetone: δ 169.14, 1549.79, 141.56, 134.17, 130.74, 130.45, 139.94, 128.99, 125.19, 124.94, 122.59, 118.38, 106.25, 101.37, 55.7, 55.3. ESI MS: 639.2 (MH^+)

Synthesis of **7**. The acid (635 mg, 0.1 mmol) was placed in methylene chloride with HBTU (371 mg, 0.1 mmol) and DIEA (715 μL, 0.4 mmol). The mixture was stirred for 30 minutes and then N-acetyl ethylamine diamine (102 mg, 0.15 mmol) was added. The reaction was stirred for 12 h and the solvent evaporated off under reduced pressure. The crude mixture was then run on a column and eluted with 5% methanol in methylene chloride. The solvent was evaporated *in vacuo*, affording a yellow solid in 43% yield.

^{1}H NMR $CDCl_3$: δ 7.89 (d, 2H, J = 10 Hz), 7.70 (d, 2H, J = 9.5 Hz), 7.64 (m, 1H, NH), 7.55 (m, 3H), 7.43 (d, 2H, J = 20.5 Hz), 7.04 (d, 2H, J = 20.5 Hz), 6.82 (s, 2H), 6.78 (m, 1H, NH), 6.61 (s, 1H), 3.87 (s, 3H, OMe), 3.86 (s, 3H, OMe), 3.84 (s, 6H, COOMe), 3.57 (m, 2H, $NHCH_2$), 3.57 (m, 2H, CH_2NH), 2.00 (s, 3H, $COCH_3$). ^{13}C NMR CDCl3: δ 171.24, 169.65, 168.29, 159.58, 159.29, 139.38, 133.16, 132.63, 132.27, 130.93, 128.85, 127.61, 127.61, 125.77, 124.62, 123.59, 119.87, 105.40, 101.937, 56.14, 55.81, 53.68, 52.72, 23.46. ESI MS: 723.0 (MH^+).

Synthesis of **8**. The ester (280 mg, 0.347mmol) was placed in a 5:1 mixture of THF:water, and lithium hydroxide (142 mg, 3.47 mmol) was added. The reaction was allowed to stir at room temperature for 3 hours, and was then quenched with HCl. The

THF was evaporated *in vacuo*, and the product was extracted from HCl with chloroform. The crude was purified by column chromatography, and eluted with 10% methanol in methylene chloride. The eluent was evaporated *in vacuo* to obtain a yellow solid (86% yield).

^{1}H NMR d_8-THF: δ 8.12 (s, 1H), 7.97 (d, J = 8 Hz, 2H), 7.77 (d, J = 8.4 Hz, 2H), 7.58 (m, 5H), 7.15 (d, J = 16.4 Hz, 2H), 6.85 (s, 2H), 6.73 (s, 2H). ^{13}C NMR d_8-THF: δ 173.24, 170.4, 168.29, 159.8, 159.4, 140.7, 133.16, 132.54, 132.20, 130.93, 128.85, 127.61, 127.61, 125.77, 124.62, 123.59, 119.87, 105.40, 101.937, 56.14, 55.53, 51.42, 23.46. ESI MS: 695.4 (MH^+).

Synthesis of **6-FFKDEL** conjugate.

The peptide was synthesized using an Applied Biosystems 433A automated peptide synthesizer. The peptide was then removed from the resin into a manual shaker. **6** was activated in DMF with 1eq. HBTU and 12 equivalents of DIEA. After mixing for 10 minutes, the activated mixture was added to the shaker and allowed to react overnight. Coupling was repeated until a ninhyndrin test showed negative results. The conjugate was then cleaved from the resin with 3% TFA in methylene chloride for 15 minutes. The free peptide-conjugate was then collected, and the solvent was evaporated *in vacuo*. Cold ether was then added until the peptide precipitated out. The conjugate was then run on a preparative TLC in 10% methanol in methylene chloride. The product band was isolated and eluted with 10% methanol in methylene chloride. The peptide was collected and treated with anhydrous 4M HCl in dioxanes for 4 hours.

^{1}H NMR (d_6-DMSO): 7.87 (br s, 2H), 7.71 (br s, 4H), 7.56 (br s, 3H), 7.47 (br s, 2H), 7.42 (br s, 4H), 7.36 (br s, 2H), 7.27 (br s, 5H, Phe aromatic), 7.15 (br s, 6H, Phe aromatic), 4.16 – 4.46 (m, 6H, C_α), 3.98 (s, 6H, COOMe), 3.95 (m, 12H, $ArOCH_3$), 3.6 (m, 2H, Lys H_ε), 3.14 (m, 4H, Phe H_β), 2.67 (m, 2H, Asp H_β), 2.34 (m, 2H, Glu H_β), 2.15 (m, 1H, Leu H_β), 2.07 (s, 2H, Lys H_δ), 1.88 (m, 4H, Lys $H_\beta + H_\gamma$), 1.72 (m, 2H, Glu H_γ), 1.15 (br s, 1H, Leu H_γ), 0.94 (br s, 6H, Leu H_δ). ESI MS m/z = 710 ($MH^{+2}/2$). 95.6% pure by HPLC.

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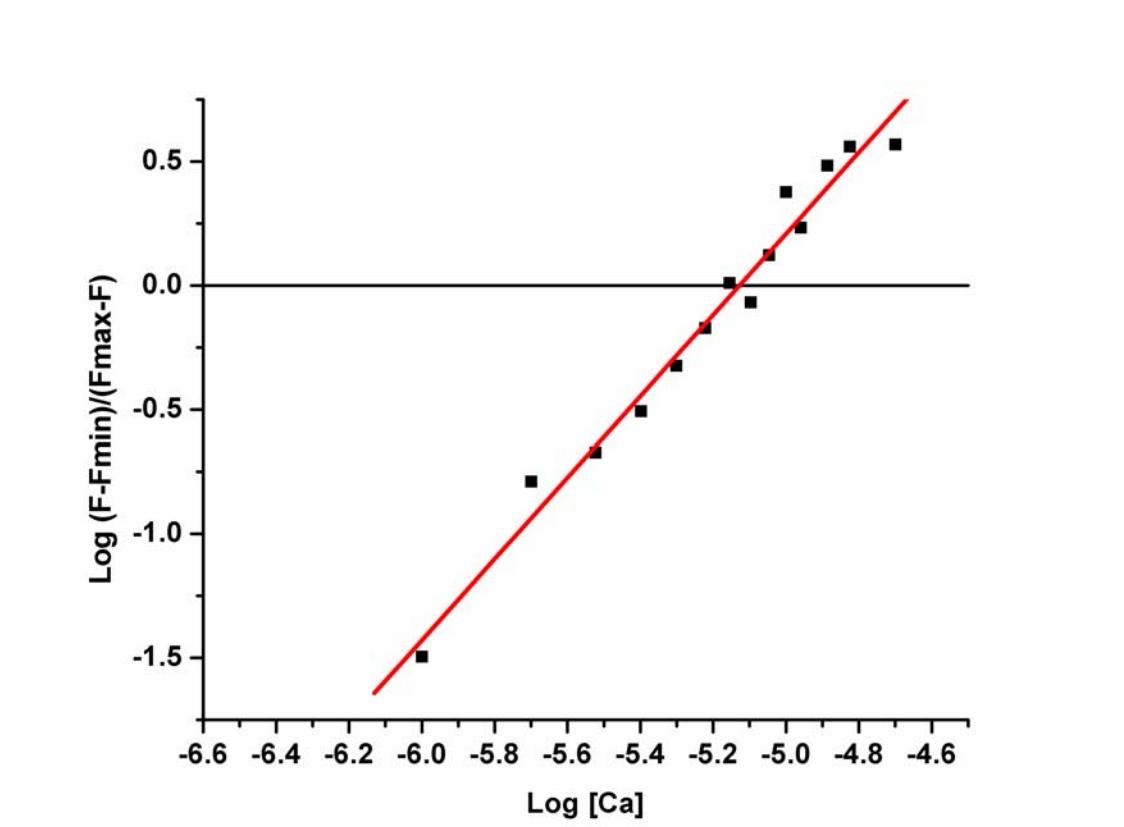
Determination of Kd from fluorescence titration (procedure from ref. 1)



Dissociation constant. The apparent Kd is at the X intercept, at a value of –5.12. The inverse log of this is 6.3μM. The equation is a double log plot that compares the log of calcium concentration to the log of ((F-Fmin)/(Fmax-F)).