SUPPLEMENTAL METHODS

Materials and Methods

Fmoc amino acids were purchased from Chem-Impex and Novabiochem, coupling reagents were purchased from GL Biochem, and solvents and other reagents were purchased from Merck and used without further purification. Resins were purchased from Chem-Impex and sulfonated Cy5 carboxylic acid was purchased from Lumiprobe.

Preparative high-performance liquid chromatography (HPLC) was performed on an Agilent 1260 Prep HPLC system, employing a G1315D photodiode array detector and a Phenomenex Axia Luna C8 column (250 × 21.2 mm, 5 μm), with a flow rate of 10 mL/min. Analytical HPLC was performed on (i) a Waters Alliance 2690 fitted with a Waters 5996 PDA detector and a Phenomenex Luna C_8 column (150 × 4.60 mm, 5 μm), with a flow rate of 1 mL/min and a gradient from 5–100% MeCN in H_2O (with 0.1% TFA throughout) over 10 min (method A), or (ii) on an Agilent 1260 HPLC system equipped with a G1312B photodiode array detector and an Agilent Eclipse Plus C18 column (100 × 4.6 mm, 3.5 μm), with a flow rate of 1 mL/min and a gradient from 5–100% MeCN in H_2O (with 0.1% TFA throughout) over 10 min (method B). Liquid chromatography-mass spectrometry (LC-MS) was carried out using an Agilent 6100 Series Single Quad LC-MS coupled to an Agilent 1200 Series HPLC. High-resolution mass spectrometry (HRMS) was carried on a Waters LCT TOF LC-MS mass spectrometer coupled to a 2795 Alliance Separations module.

Synthesis of Cy5-PK-DPP

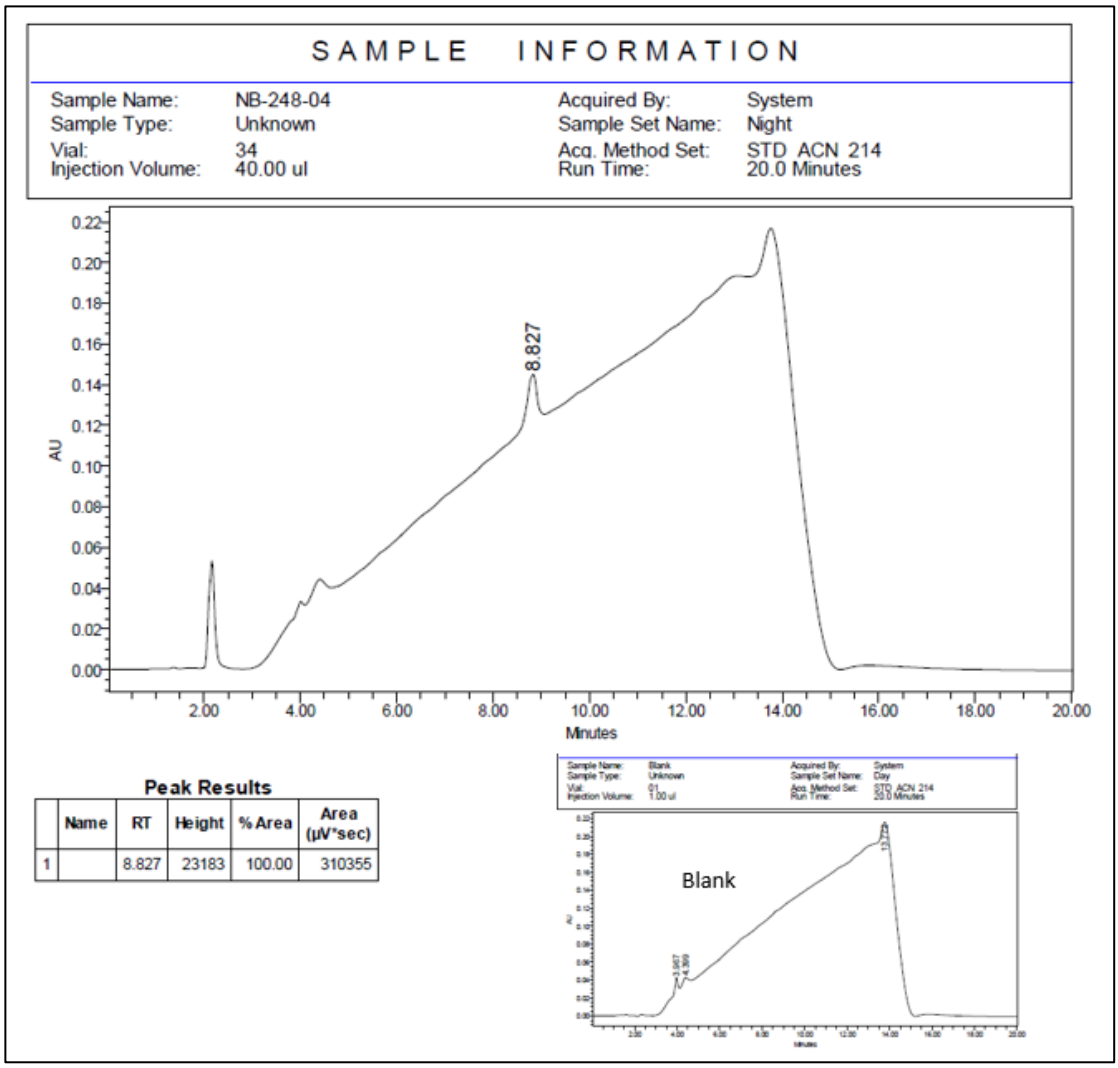
To a solution of diphenyl benzyloxycarbonylamino-(4-phthalimidobutyl)methanephosphonate (500 mg, 0.849 mmol) in MeOH (5 mL) was added 10% Pd/C (45 mg, 0.42 mmol) and the resulting suspension stirred for 2 h under an atmosphere of H_2. The suspension was filtered through celite and reduced *in vacuo.* The resulting solid was then dissolved in dry MeCN (10 mL), and added to a solution of Cbz-Pro-OH (708 mg, 2.84 mmol), HOBt (378 mg, 2.84 mmol), EDCI (538 mg, 2.84) and Et_3N (1.87 mL, 5.0 mmol) in dry MeCN (10 mL). After stirring for 15 h, the solvent was

removed *in vacuo* and the residue dissolved in Et_2O (50 mL), washed successively with H_2O (2 × 50 mL), 0.5 M HCl (2 × 50 mL), saturated $NaHCO_3$ solution (2 × 50 mL), H_2O (50 mL) and brine (50 mL), and then dried over $MgSO_4$. The resulting crude was purified by silica gel chromatography (60% EtOAC in petroleum spirits) to provide diphenyl Cbz-Pro-(4-phthalimidobutyl)methanephosphonate (300 mg, 43%) as a while solid. LC-MS: *m/z* 696.4 $[M+H]^+$. To a solution of diphenyl Cbz-Pro-(4-phthalimidobutyl)methanephosphonate (30 mg, 43 μmol) in MeOH (2 mL) was added 10% Pd/C (5 mg, 5 μmol). This solution was stirred under an atmosphere of H_2 for 6 h. The solution was filtered through a syringe filter (0.22 μm), reduced *in vacuo* to provide diphenyl Pro-(4-phthalimidobutyl)methanephosphonate (10 mg, 41%) as a colorless oil. LC-MS: *m/z* 562.3 $[M+H]^+$. To a solution of diphenyl Pro-(4-phthalimidobutyl)methanephosphonate (3 mg, 5 μmol) in dry MeCN (2 mL) was added sulfonated Cy5 carboxylic acid (3 mg, 5 μmol), HCTU (4 mg, 10 μmol) and Et_3N (3 μL, 20 μmol). After stirring overnight, the crude reaction mixture was purified by preparative HPLC (0–60% MeCN in H_2O with 0.1% TFA throughout) and the eluate fractions containing diphenyl Cy5-Pro-(4-phthalimidobutyl)methanephosphonate combined. These combined fractions were made basic by dropwise addition of 30% hydrazine solution and stirred at room temperature for 15 h. The resulting solution was acidified to pH 3 with 1 M HCl and purified by preparative HPLC (0–42% MeCN in H_2O with 0.1% TFA throughout) to provide Cy5-PK-DPP (1.2 mg, 21%) as a blue solid after lyophilization. HRMS: *m/z* calc'd for $[M]^+$, M = $C_{55}H_{69}N_5O_{11}PS_2$: 1070.4167, found: 1070.4151. Analytical HPLC (method A): retention time 8.82 min, purity > 95%.

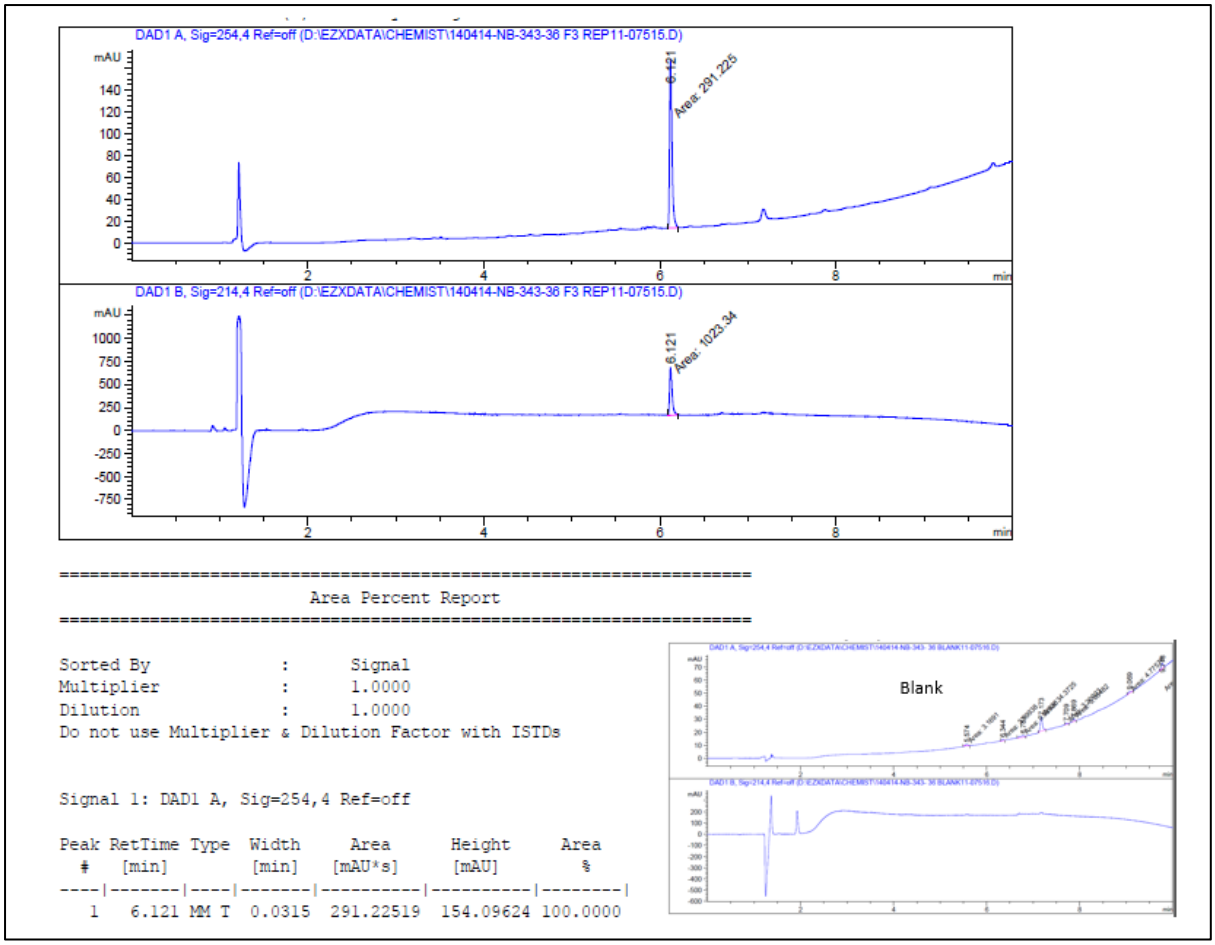
Synthesis of Cy5-V-DPP

A solution of isobutylaldehyde (200 mg, 2.78 mmol), benzylcarbamate (420 mg, 2.78 mmol) and triphenylphosphite (860 mg, 2.78 mmol) in glacial AcOH (10 mL) was heated at 100°C for 2 h. Solvent was removed *in vacuo*, the residue partitioned between H_2O (50 mL) and Et_2O (50 mL), made basic by the addition of a saturated aqueous solution of $NaHCO_3$ and then extracted with further washings of Et_2O (2 × 50 mL). The combined organic washings

were dried over $MgSO_4$ and purified by silica gel chromatography (0–20% EtOAc in petroleum spirits) to provide diphenyl benzyloxycarbonylaminoisopropylmethanephosphonate (796 mg, 65%). LC-MS: *m/z* 440.3 $[M+H]^+$. To a solution of diphenyl benzyloxycarbonylaminoisopropylmethanephosphonate (10 mg, 23 µmol) in MeOH (1 mL) was added 10% Pd/C (1 mg, 0.9 µmol) and the resulting suspension stirred for 2 h under an atmosphere of H_2. The mixture was filtered through a syringe filter (0.22 µm), reduced *in vacuo,* and redissolved in dry MeCN (1 mL). To this solution was added sulfonated Cy5 carboxylic acid (5 mg, 8 µmol), PyBOP (5 mg, 9 µmol) and Et_3N (50 uL, 71 µmol). After stirring for 5 h at room temperature, the reaction mixture was purified by preparative HPLC (0–70% MeCN in H_2O with 1% TFA throughout) to provide Cy5-V-DPP (2.1 mg, 30%) as a blue solid after lyophilization. LC-MS: *m/z* 942.6 $[M-2H]^-$. Analytical HPLC (method B): retention time 6.61 min, purity > 95%. HRMS: *m/z* calc'd for $[M]^+$, M = $C_{49}H_{59}N_3O_{10}PS_2$: 944.3374, found: 944.3385.



Supplemental Figure 1. Analytical HPLC of Cy5-PK-DPP.



Supplemental Figure 2. Analytical HPLC of Cy5-V-DPP.