A Versatile Isocyanate-Mediated Strategy for Appending Chemical Tags onto Drug-like Small Molecules

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Chemical Methods

General Chemical Methods

Unless otherwise noted, commercially purchased chemical reagents were used without further purification. Reactions were monitored by thin layer chromatography (TLC) using glass plates pre-coated with silica gel (0.25 mm, Sigma). TLC plates were visualized using UV light at 254 nm. Column chromatography was performed on a Teledyne Isco CombiFlash Rf+ system using Teledyne RediSep Rf silica cartridges. Reversed phase high pressure liquid chromatography (HPLC) was conducted on an Interchim PuriFlash 4125, using an Uptisphere Strategy C18 5 µm 150x20mm prep-LC column. Preparative TLC was completed using glass plates precoated with amine functionalized silica gel (0.25 mm, Fujifilm Wako). Liquid chromatography mass spectrometry (LCMS) was performed on an Agilent 6125B mass spectrometer attached to an Agilent 1260 Infinity LC. LCMS samples were analyzed in methanol. High resolution mass spectrometry (HRMS) experiments were run on an Agilent Mass Spectrometer coupled to an Agilent 1260 Infinity II LC system in electrospray ionization mode (ESI+). Proton nuclear magnetic spectra (^{1}H NMR), carbon nuclear magnetic spectra (^{13}C NMR), fluoride nuclear magnetic spectra (^{19}F NMR) were performed on a Bruker Avance Neo 500 spectrometer (500MHz/125 MHz/470 MHz) running Bruker Topspin 4.0.5 with IconNMR. Fluorine chemical shifts are referenced to an external standard of 2,2,2-trifluoroethanol (-76 ppm). ^{1}H chemical shifts (δ) are reported in ppm relative the protonated solvent resonance employed as the internal standard ($CDCl_3$ δ = 7.26, CD_2Cl_2 δ = 5.32, $(CD_3)_2SO$ δ = 2.50, CD_3OD = 3.31 ppm). ^{13}C chemical shifts (δ) are expressed in ppm with the solvent resonance as the internal standard ($CDCl_3$ δ = 77.16, CD_2Cl_2 δ = 54, $(CD_3)_2SO$ δ = 39.52, CD_3OD = 49.00 ppm). Data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, p=pentet, s=sextet, h=heptet, m=multiplet, b=broad), coupling constants (Hz), and integration.

General Procedures for IMCT

General Procedure A: Sieber amide resin (Novabiochem, 0.75 mmol/g, 1 eq.) was swelled in amine free dimethylformamide (AF-DMF) (10 mL) in a reaction syringe (Supelco) for 30 minutes. AF-DMF was removed under reduced pressure. The resin was deprotected by stirring with 20% v/v piperidine/DMF (15 mL) for 10 minutes. This was repeated 4 times. For bead wash steps referenced in the remainder of the text, the following abbreviation will be used (15 mL × 4). The carboxylic acid (0.25 eq.) and hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) (0.25 eq.) were stirred in AF-DMF (2 mL) at room temperature for 30 minutes in a separate flask. The resin was then washed with AF-DMF (15 mL × 5), dichloromethane (DCM) (15 mL × 5), and methanol (15 mL × 5). The resin was reswelled in AF-DMF (10 mL) for 15 minutes. The generated activated ester was added to the resin, followed by *N,N*-diisopropylethylamine (DIPEA) (0.5 eq.). The reaction was stirred for 3 hours at room temperature. The resin was then washed five times with AF-DMF (15 mL × 5), DCM (15 mL × 5), and methanol (15 mL x 5). Unreacted sites on the resin were quenched by adding a solution of 5% acetic anhydride and 10% triethylamine (TEA) in AF-DMF (10 mL) and stirring for 30 min. The resin was then washed with AF-DMF (15 mL × 5), DCM (15 mL × 5), methanol (15 mL × 5), and dried under reduced pressure overnight. A test cleavage was performed, where a few beads of

resin were collected, resuspended in 5% TFA in DCM, placed on a shaker for 30 min, then filtered through cotton and analyzed by LCMS.

To assess resin loading, ten milligrams of dry resin were weighed out and set aside for quantification. Resin was either stored under argon at 4 ˚C or used immediately in subsequent reactions. Bead loading was quantified using standard Fmoc cleavage protocols.[1]

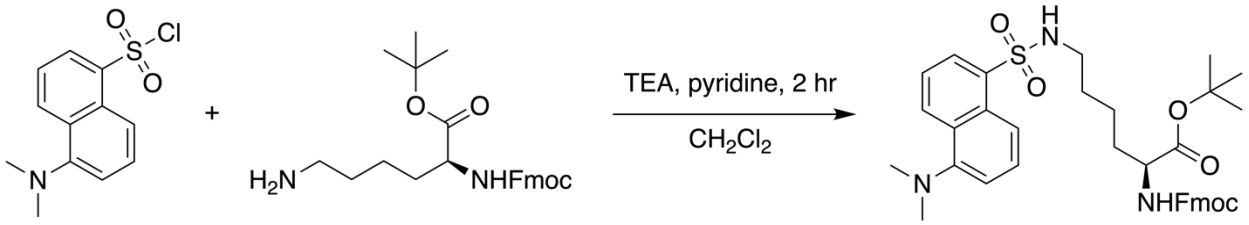
General Procedure B: Resin (~100-300 mg, 0.014 mmol – 0.031 mmol, 1 eq.) prepared using **General Procedure A** was weighed out in a new reaction syringe (Supelco) and swelled in AF-DMF (5 mL) for 30 minutes. Resin was deprotected by adding 20% v/v piperidine/AF-DMF for 10 minutes (5 mL × 4). The resin was then washed with AF-DMF (5 mL × 5), DCM (5 mL × 5), methanol (5 mL × 5), and dried under reduced pressure. Resin was reswelled in AF-DMF (5 mL) for 15 minutes. Diisocyanate (10 eq.) and TEA (5 eq.) were added to the stirred slurry. The reaction was rotated for 3 hours at room temperature. The resin was then washed with AF-DMF (5 mL x 5), DCM (5 mL × 5), tetrahydrofuran (THF) (5 mL × 5), and dried under reduced pressure. Dry resin was transferred to a glass round bottom (10 mL) containing molecular sieves. The flask was purged of air and kept under argon. Resin was swelled in anhydrous THF (4 mL) for 15 minutes. Nucleophile (1.5-5 eq.) was added to the slurry and allowed to stir 15 minutes. The slurry was then cooled to 0 ˚C in an ice bath and 1 M lithium bis(trimethylsilyl)amide in toluene (LiHMDS, 1 eq.) was added dropwise to the stirred slurry. The reaction was stirred overnight. Methanol was added to quench the reaction. Resin was transferred to a new reaction syringe and then washed with AF-DMF (5 mL × 5), DCM (5 mL × 5), and methanol (5 mL × 5). The resin was cleaved by stirring with 5% TFA in DCM for 5-18 hours. The cleavage solution was collected, along with the filtrate collected from DCM (5 mL × 5) and methanol (5 mL × 5) washes of the resin. The combined filtrates were filtered through cotton, concentrated under reduced pressure, dried azeotropically with toluene, resuspended in DMSO (500 µL), and purified with HPLC Method A or Method B. Method A: 10 to 50% acetonitrile (MeCN) containing 0.1% TFA in water containing 0.1%TFA over 7.5 column volumes (CV). Method B: 30 to 90% MeCN containing 0.1% TFA in water containing 0.1% TFA over 10 CV.

Reaction yields were calculated using two methods. The first method calculated the yield based on the weight of the product obtained via HPLC and the TFA salt content determined by ^{1}H NMR using trifluoroethanol as an internal standard. For the second method, a standard curve of the purified product was used to determine the amount of product in the crude cleavage sample. Dansyl amide was used as an internal standard for all samples to normalize for any variations in LCMS injection volumes. Extracted ion chromatogram (EIC) values were used to quantify the amount of substance in each sample.

General Procedure C: The acid (1 eq.) and HATU (1.2 eq.) were dissolved in AF-DMF (5 mL) under argon. N-Boc-ethylenediamine (2 eq.) and DIPEA (2 eq.) were added to the solution and stirred overnight at room temperature. The solution was diluted with water and extracted with ethyl acetate (3x). The organic layer was washed with aqueous saturated NaCl, dried over sodium sulfate, and the solvent was removed under reduced pressure. The crude product was then purified with HPLC Method C: 10 to 90% MeCN containing 0.1% trifluoroacetic acid (TFA) in water containing 0.1%TFA over 10 CV.

Synthesis of Dansyl Precursor

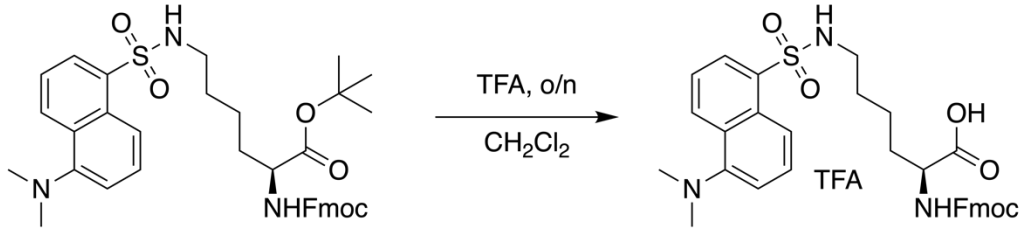
tert-butyl N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^6-((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-*L*-lysinate (P1)



P1

5-(dimethylamino)naphthalene-1-sulfonyl chloride (702.3 mg, 2.60 mmol, 2 eq.) and *tert*-butyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)-*L*-lysinate hydrochloride (600 mg, 1.30 mmol, 1 eq.) were dissolved in anhydrous DCM (10 mL). TEA (532 µL, 3.81 mmol, 3 eq.) and pyridine (523 µL, 6.50 mmol, 5 eq.) were added dropwise to the stirred solution. The reaction was stirred for 2 hours and then solvent was removed under reduced pressure. The crude product was purified by column chromatography (2:3 hexane/ethyl acetate) to afford a pale yellow solid (752.4 mg, 1.14 mmol, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (d, *J* = 8.5 Hz, 1H), 8.38 (d, *J* = 8.6 Hz, 1H), 8.19 (d, *J* = 6.8 Hz, 1H), 7.69 (d, *J* = 7.3 Hz, 2H), 7.53 (t, *J* = 8.0 Hz, 3H), 7.42 (t, *J* = 8.1 Hz, 1H), 7.36 – 7.29 (m, 2H), 7.29 – 7.21 (m, 3H), 5.15 (d, *J* = 8.1 Hz, 1H), 5.00 (s, 1H), 4.43 – 4.30 (m, 2H), 4.15 (t, *J* = 6.8 Hz, 1H), 4.11 – 4.02 (m, 1H), 2.98 (s, 6H), 2.88 – 2.77 (m, 2H), 1.66 – 1.55 (m, 1H), 1.36 (s, 12H), 1.28 – 1.06 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.4, 156.1, 144.0, 143.9, 143.8, 142.6, 141.3, 135.4, 129.9, 129.6, 129.4, 128.7, 127.9, 127.8, 127.1, 126.5, 125.2, 125.1, 124.6, 121.8, 120.0, 120.0, 116.3, 82.3, 66.9, 53.8, 47.2, 46.0, 42.9, 32.3, 28.8, 28.0, 21.9. HRMS (ESI) m/z: calcd. for $C_{37}H_{44}N_3O_6S$ $[M+H]^+$ 658.2945, found 658.2957; m/z: calcd. for $C_{37}H_{43}N_3O_6NaS$ $[M+Na]^+$ 680.2770, found 680.2772.

N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^6-((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-*L*-lysine trifluoracetic acid salt (1)



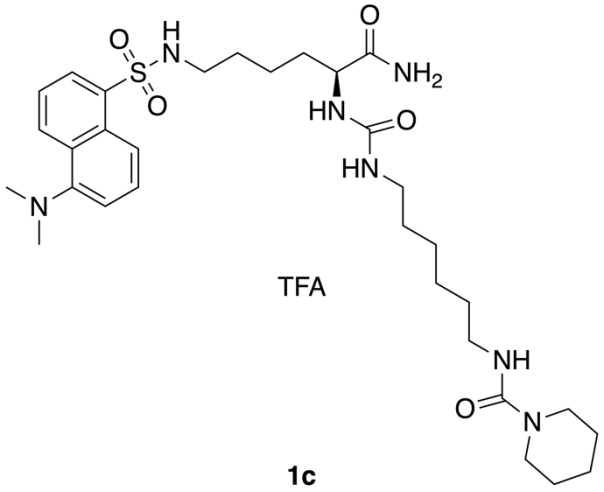
P1 **1**

P1 (752.4 mg, 1.14 mmol) was dissolved in DCM (3 mL). Trifluoracetic acid (2 mL) was added dropwise and the solution was stirred overnight at room temperature. The solvent was removed under reduced pressure and dried azeotropically with toluene yielding a pale yellow solid. **1** was used in subsequent reactions without further purification. ^{1}H NMR (500 MHz, CD_3OD) δ 8.74 – 8.67 (m, 1H), 8.48 (d, *J* = 8.6 Hz, 1H), 8.31 (d, *J* = 7.2 Hz, 1H), 7.79 (dd, *J* = 7.1, 5.2 Hz, 2H), 7.75 – 7.61 (m, 5H), 7.43 – 7.33 (m, 2H), 7.33 – 7.23 (m, 2H), 4.37 (d, *J* = 6.9 Hz, 2H), 4.21 (t, *J* = 6.8 Hz, 1H), 3.82 (dd, *J* = 9.3, 4.6 Hz, 1H), 3.22 (s, *J* = 12.8 Hz, 6H), 2.95 – 2.84 (m, 2H), 1.61 – 1.51 (m, 1H), 1.47 – 1.39 (m, 1H), 1.38 – 1.26 (m, 3H), 1.26 – 1.05 (m, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 174.3, 157.2, 144.0, 143.8, 141.2, 136.9, 129.4, 129.4, 127.4, 126.9, 126.8, 126.8,

125.0, 124.9, 124.8, 123.8, 119.6, 119.5, 117.3, 66.4, 53.6, 47.0, 45.5, 42.2, 30.6, 28.5, 22.4. HRMS (ESI) m/z: calcd. for $C_{33}H_{36}N_3O_6S$ $[M+H]^+$ 602.2319, found 602.2324; m/z: calcd. for $C_{33}H_{35}N_3NaO_6S$ $[M+Na]^+$ 624.2144, found 624.2144; calcd. for $C_{33}H_{39}N_4O_6S$ $[M+NH_4]^+$ 619.2590, found 619.2526.

Synthesis of Dansyl Derivatives using IMCT

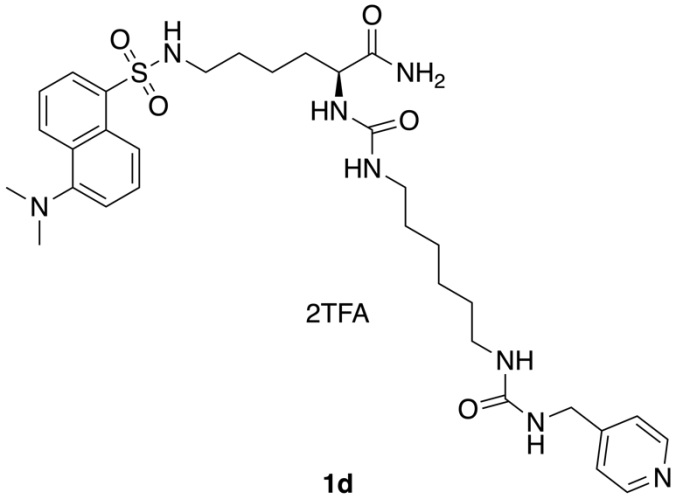
(*S*)-*N*-(6-(3-(1-amino-6-((5-(dimethylamino)naphthalene)-1-sulfonamido)-1-oxohexan-2-yl)ureido)hexyl)piperidine-1-carboxamide trifluoroacetic acid (1c)



1c

Crude product **1c** synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (209 mg, 0.024 mmol, 1 eq.), hexamethylene diisocyanate (39 µL, 0.24 mmol, 10 eq.), TEA (16.7 µL, 0.12 mmol, 5 eq.), piperidine (11.8 µL, 0.12 mmol, 5 eq.), and LiHMDS (24 µL, 0.024 mmol, 1 eq.) had a calculated yield of 45% using a standard curve. To isolate pure product, additional **1c** was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (219.2 mg, 0.019 mmol, 1 eq.), hexamethylene diisocyanate (31 µL, 0.19 mmol, 10 eq.), TEA (13.2 µL, 0.095 mmol, 5 eq.), piperidine (9.6 µL, 0.095 mmol, 5 eq.), and LiHMDS (20 µL, 0.019 mmol, 1 eq.). Purification by HPLC Method A yielded a pale yellow oil (8.1 mg, 0.01 mmol, 56%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.56 (d, *J* = 8.6 Hz, 1H), 8.50 (d, *J* = 8.7 Hz, 1H), 8.24 (dd, *J* = 7.3, 1.0 Hz, 1H), 7.69 – 7.62 (m, 2H), 7.48 (d, J = 7.6 Hz, 1H), 4.04 (dd, J = 8.6, 5.1 Hz, 1H), 3.37 – 3.34 (m, 3H), 3.13 (ddd, J = 20.4, 10.3, 5.1 Hz, 4H), 3.04 (s, J = 6.4 Hz, 5H), 2.85 (t, *J* = 6.8 Hz, 2H), 1.66 – 1.59 (m, 2H), 1.56 – 1.45 (m, 8H), 1.38 – 1.33 (m, 5H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.9, 159.1, 158.8, 149.2, 136.1, 129.5, 129.0, 129.0, 128.7, 127.6, 123.7, 120.9, 115.9, 53.3, 52.7, 44.8, 44.5, 42.3, 41.4, 40.3, 39.4, 32.0, 29.9, 29.8, 28.9, 26.2, 26.2, 25.4, 24.2, 22.4. HRMS (ESI) m/z: calcd. for $C_{31}H_{50}N_7O_5S$ $[M+H]^+$ 632.3589, found 632.3597; m/z: calcd. for $C_{31}H_{49}N_7NaO_5S$ $[M+Na]^+$ 654.3414, found 654.3416; calcd. for $C_{31}H_{49}KN_7O_5S$ $[M+K]^+$ 670.3153, found 670.3170.

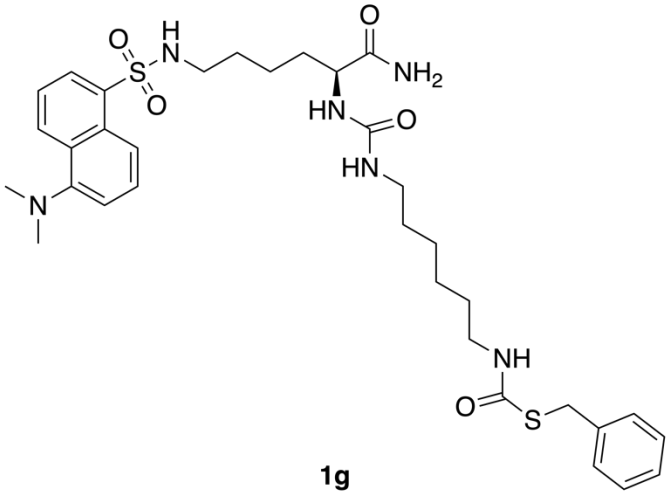
(*S*)-14-(4-((5-(dimethylamino)naphthalene)-1-sulfonamido)butyl)-3,12-dioxo-1-(pyridin-4-yl)-2,4,11,13-tetraazapentadecan-15-amide trifluoroacetic acid (1d)



1d

Crude product **1d** synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (204.5 mg, 0.023 mmol, 1 eq.), hexamethylene diisocyanate (37.7 µL, 0.23 mmol, 10 eq.), TEA (16.4 µL, 0.12 mmol, 5 eq.), 4-(aminomethyl)pyridine (8.8 µL, 0.12 mmol, 5 eq.), and LiHMDS (24 µL, 0.023 mmol, 1 eq.) had a calculated yield of 35% using a standard curve. To isolate pure product, additional **1d** was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (277.5 mg, 0.016 mmol, 1 eq.), hexamethylene diisocyanate (25.7 µL, 0.16 mmol, 10 eq.), TEA (11.1 µL, 0.08 mmol, 5 eq.), 4-(aminomethyl)pyridine (8.1 µL, 0.08 mmol, 5 eq.), and LiHMDS (16 µL, 0.016 mmol, 1 eq.). Purification by HPLC Method A yielded a pale yellow oil (3.8 mg, 0.004 mmol, 27%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.76 (s, 2H), 8.56 (d, *J* = 8.5 Hz, 1H), 8.48 (d, *J* = 8.7 Hz, 1H), 8.23 (dd, *J* = 7.2, 0.7 Hz, 1H), 7.98 (d, *J* = 4.8 Hz, 2H), 7.65 (ddd, *J* = 8.4, 7.7, 3.4 Hz, 2H), 7.46 (d, *J* = 7.6 Hz, 1H), 4.60 (s, 2H), 4.05 (dd, *J* = 8.6, 5.0 Hz, 1H), 3.19 – 3.10 (m, 4H), 3.02 (s, *J* = 6.8 Hz, 6H), 2.85 (t, *J* = 6.8 Hz, 2H), 1.55 – 1.32 (m, 14H). 13C NMR (126 MHz, CD3OD) δ 177.0, 163.3, 159.4, 159.1, 149.5, 141.0, 136.0, 129.5, 129.1, 128.9, 128.8, 127.6, 124.8, 123.6, 120.6, 115.8, 53.3, 44.8, 43.0, 42.3, 41.4, 39.6, 39.3, 32.0, 29.8, 29.7, 28.8, 26.0, 22.4, 8.6. HRMS (ESI) m/z: calcd. for $C_{32}H_{47}N_8O_5S$ $[M+H]^+$ 655.3385, found 655.3394; m/z: calcd. for $C_{32}H_{46}N_8NaO_5S$ $[M+Na]^+$ 677.3210, found 677.3215; calcd. for $C_{32}H_{46}KN_8O_5S$ $[M+K]^+$ 693.2949, found 693.2964.

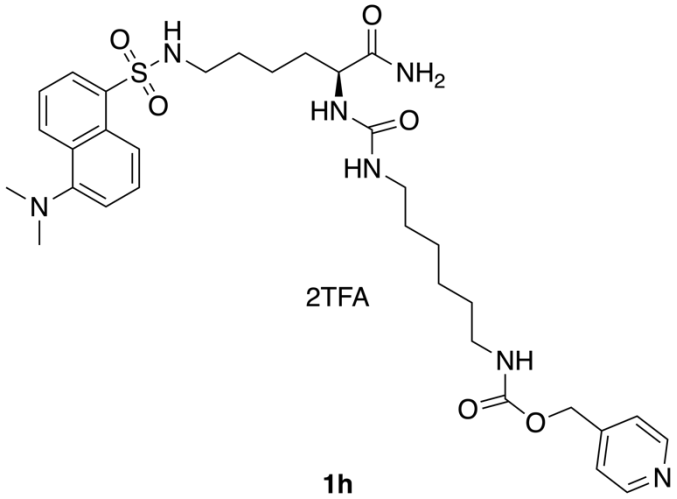
***S*-benzyl (*S*)-(6-(3-(1-amino-6-((5-(dimethylamino)naphthalene)-1-sulfonamido)-1-oxohexan-2-yl)ureido)hexyl)carbamothioate (1g)**



1g

Crude product **1g** synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (260.2 mg, 0.015 mmol, 1 eq.), hexamethylene diisocyanate (24.1 µL, 0.15 mmol, 10 eq.), TEA (10.5 µL, 0.075 mmol, 5 eq.), benzyl mercaptan (8.8 µL, 0.075 mmol, 5 eq.), and LiHMDS (15 µL, 0.015 mmol, 1 eq.) had a calculated yield of 35% using a standard curve. To isolate pure product, additional **1g** was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (309.7 mg, 0.033 mmol, 1 eq.), hexamethylene diisocyanate (52.3 µL, 0.33 mmol, 10 eq.), TEA (22.6 µL, 0.17 mmol, 5 eq.), benzyl mercaptan (19.1 µL, 0.17 mmol, 5 eq.), and LiHMDS (32 µL, 0.033 mmol, 1 eq.). Purification by HPLC Method A yielded an impure product, which was further purified using preparative TLC (8% methanol in ethyl acetate). Silica was extracted with methanol/ethyl acetate (1:9) solution (3 x1 mL) and solvent was removed under reduced pressure to yield a pale yellow oil (3.5 mg, 0.005 mmol, 16%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.57 (d, *J* = 8.5 Hz, 1H), 8.37 (d, *J* = 8.7 Hz, 1H), 8.20 (dd, *J* = 7.2, 0.9 Hz, 1H), 7.59 (dd, *J* = 16.1, 8.0 Hz, 2H), 7.35 – 7.18 (m, 5H), 4.15 – 4.05 (m, 3H), 3.22 (t, *J* = 7.0 Hz, 2H), 3.17 – 3.03 (m, 2H), 2.90 (s, 6H), 2.83 (t, *J* = 6.8 Hz, 2H), 1.53 – 1.33 (m, 13H). ^{13}C NMR (126 MHz, CD_3OD)) δ 176.9, 167.6, 159.1, 151.8, 138.9, 135.7, 129.8, 129.7, 129.6, 128.9, 128.3, 128.1, 127.7, 126.6, 122.9, 119.2, 53.2, 44.4, 42.3, 40.6, 39.4, 33.1, 32.1, 29.7, 29.1, 28.9, 26.1, 26.0, 22.3. HRMS (ESI) m/z: calcd. for $C_{33}H_{47}N_6O_5S_2$ $[M+H]^+$ 671.3044, found 671.3053; m/z: calcd. for $C_{33}H_{46}N_6NaO_5S_2$ $[M+Na]^+$ 693.2869, found 693.2873; calcd. for $C_{33}H_{46}KN_6O_5S_2$ $[M+K]^+$ 709.2608, found 709.2606.

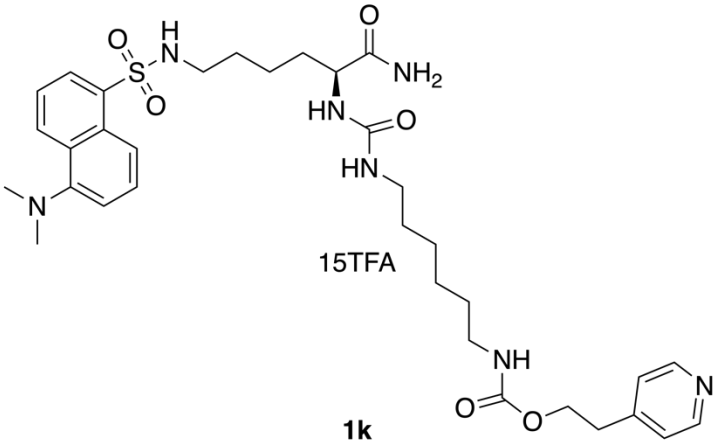
pyridin-4-ylmethyl (*S*)-(6-(3-(1-amino-6-((5-(dimethylamino)naphthalene)-1-sulfonamido)-1-oxohexan-2-yl)ureido)hexyl)carbamate trifluoroacetic acid (1h)



1h

Crude product **1h** synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (205.6 mg, 0.024 mmol, 1 eq.), hexamethylene diisocyanate (37.9 µL, 0.24 mmol, 10 eq.), TEA (16.4 µL, 0.12 mmol, 5 eq.), 4-pyridinemethanol (12.9 mg, 0.12 mmol, 5 eq.), and LiHMDS (24 µL, 0.024 mmol, 1 eq.) had a calculated yield of 46% using a standard curve. To isolate pure product, additional **1h** was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (152.2 mg, 0.016 mmol, 1 eq.), hexamethylene diisocyanate (25.7 µL, 0.16 mmol, 10 eq.), TEA (11.1 µL, 0.08 mmol, 5 eq.), 4-pyridinemethanol (8.7 mg, 0.08 mmol, 5 eq.), and LiHMDS (16 µL, 0.016 mmol, 1 eq.). Purification by HPLC Method A yielded a pale yellow oil (4.9 mg, 0.006 mmol, 35%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.81 (s, 2H), 8.56 (d, *J* = 8.5 Hz, 1H), 8.43 (d, *J* = 8.7 Hz, 1H), 8.22 (dd, *J* = 10.9, 4.2 Hz, 1H), 7.99 (s, 2H), 7.69 – 7.57 (m, 2H), 7.39 (d, *J* = 7.5 Hz, 1H), 5.38 (s, 2H), 4.07 (dd, *J* = 8.6, 5.0 Hz, 1H), 3.18 – 3.10 (m, 4H), 2.97 (s, *J* = 5.7 Hz, 5H), 2.84 (t, *J* = 6.7 Hz, 2H), 1.63 – 1.48 (m, 6H), 1.41 – 1.33 (m, 8H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.9, 159.1, 156.3, 150.4, 142.0, 135.9, 129.5, 129.4, 129.3, 129.2, 128.8, 127.6, 123.9, 123.3, 120.0, 115.5, 63.4, 53.3, 44.6, 42.3, 40.4, 39.3, 32.1, 29.8, 29.3, 28.9, 26.0, 26.0, 22.4. HRMS (ESI) m/z: calcd. for $C_{32}H_{46}N_7O_6S$ $[M+H]^+$ 656.3225, found 656.3235; m/z: calcd. for $C_{32}H_{45}N_7NaO_6S$ $[M+Na]^+$ 678.3050, found 678.3055; calcd. for $C_{32}H_{45}KN_7O_6S$ $[M+K]^+$ 694.2789, found 694.2804.

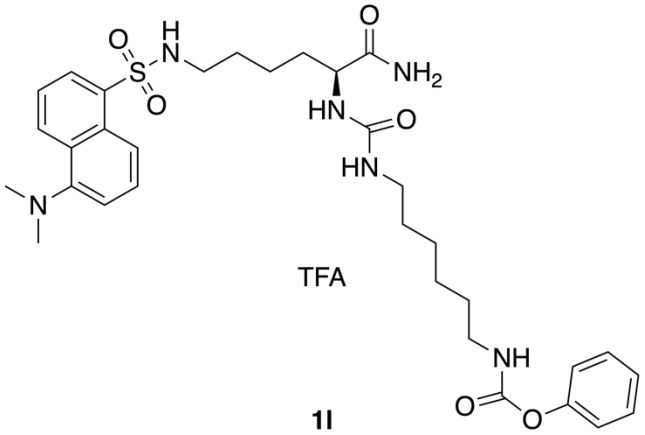
2-(pyridin-4-yl)ethyl (*S*)-(6-(3-(1-amino-6-((5-(dimethylamino)naphthalene)-1-sulfonamido)-1-oxohexan-2-yl)ureido)hexyl)carbamate (1k)



1k

1k was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (206.3 mg, 0.020 mmol, 1 eq.), hexamethylene diisocyanate (32.7 µL, 0.20 mmol, 10 eq.), TEA (14.2 µL, 0.1 mmol, 5 eq.), 2-(pyridin-4-yl)ethan-1-ol (11.5 µL, 0.1 mmol, 5 eq.), and LiHMDS (20 µL, 0.020 mmol, 1 eq.). Crude product had a calculated yield of 33% using a standard curve. The remaining solvent was removed under reduced pressure and purified by HPLC Method A to afford a pale yellow oil (3.6 mg, 0.002 mmol, 8%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.93 – 8.59 (bs, 2H), 8.57 (d, *J* = 8.5 Hz, 1H), 8.37 (d, *J* = 8.7 Hz, 1H), 8.20 (dd, *J* = 7.3, 1.0 Hz, 1H), 7.90 (bs, 2H), 7.68 – 7.54 (m, 2H), 7.31 (d, *J* = 7.6 Hz, 1H), 4.39 (t, *J* = 6.1 Hz, 1H), 4.08 (dd, *J* = 8.3, 4.8 Hz, 1H), 3.26 – 3.16 (m, 2H), 3.18 – 2.99 (m, 4H), 2.95 – 2.87 (m, 7H), 2.85 – 2.80 (m, 2H), 1.54 – 1.30 (m, 14H). ^{13}C NMR (126 MHz, CD_3OD) δ 177.0, 161.6, 159.2, 157.1, 151.6, 151.5, 135.7, 129.7, 129.6, 129.6, 128.8, 127.7, 123.0, 119.3, 115.6, 115.1, 62.6, 44.4, 42.3, 41.4, 40.2, 39.4, 32.0, 29.8, 29.3, 28.9, 26.0, 26.0, 22.4. HRMS (ESI) m/z: calcd. for $C_{33}H_{48}N_7O_6S$ $[M+H]^+$ 670.3381, found 670.3392; m/z: calcd. for $C_{33}H_{47}N_7NaO_6S$ $[M+Na]^+$ 692.3206, found 692.3212; calcd. for $C_{33}H_{47}KN_7O_6S$ $[M+K]^+$ 708.2946, found 708.2947.

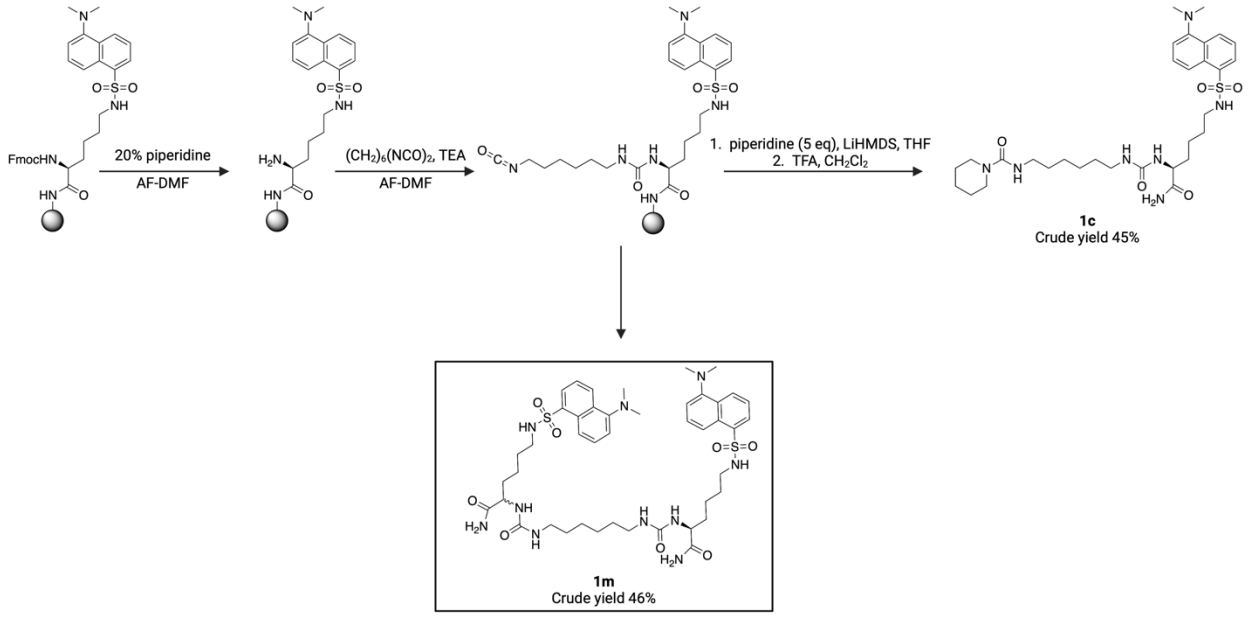
phenyl (*S*)-(6-(3-(1-amino-6-((5-(dimethylamino)naphthalene)-1-sulfonamido)-1-oxohexan-2-yl)ureido)hexyl)carbamate trifluoroacetic acid (1l)



1l

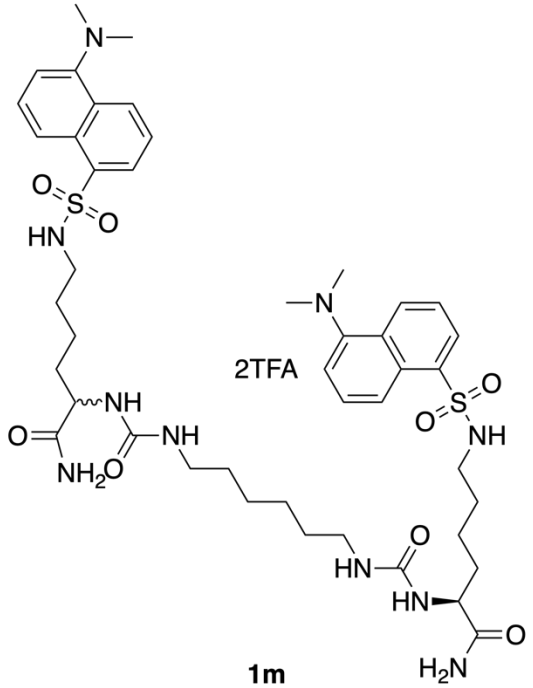
Crude product **1l** synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (206.8 mg, 0.024 mmol, 1 eq.), hexamethylene diisocyanate (38.1 µL, 0.24 mmol, 10 eq.), TEA (16.5 µL, 0.12 mmol, 5 eq.), phenol (11.2 mg, 0.12 mmol, 5 eq.), and LiHMDS (24 µL, 0.024 mmol, 1 eq.) had a calculated yield of 16% using a standard curve. To isolate pure product, additional **1l** was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (160.5 mg, 0.017 mmol, 1 eq.), hexamethylene diisocyanate (27.1 µL, 0.17 mmol, 10 eq.), TEA (11.7 µL, 0.085 mmol, 5 eq.), phenol (7.9 mg, 0.085 mmol, 5 eq.), and LiHMDS (17 µL, 0.017 mmol, 1 eq.). Purification by HPLC Method A yielded a pale yellow oil (2.4 mg, 0.003 mmol, 18%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.55 (d, *J* = 8.5 Hz, 1H), 8.49 (d, *J* = 8.7 Hz, 1H), 8.24 (dd, *J* = 7.3, 0.7 Hz, 1H), 7.71 – 7.60 (m, 2H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.36 (t, *J* = 7.9 Hz, 2H), 7.20 (t, *J* = 7.4 Hz, 1H), 7.09 (d, *J* = 7.7 Hz, 2H), 4.04 (dd, *J* = 8.5, 5.1 Hz, 1H), 3.20 – 3.09 (m, 4H), 3.02 (s, 6H), 2.85 (t, *J* = 6.8 Hz, 2H), 1.59 – 1.30 (m, 15H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.9, 159.1, 155.9, 151.3, 136.1, 129.5, 129.1, 128.9, 128.9, 128.8, 127.6, 124.8, 123.6, 121.4, 120.7, 115.8, 53.3, 44.8, 42.3, 40.4, 39.4, 32.0, 29.8, 29.3, 28.9, 26.1, 22.3. HRMS (ESI) m/z: calcd. for $C_{32}H_{45}N_6O_6S$ $[M+H]^+$ 641.3116, found 641.3125; m/z: calcd. for $C_{32}H_{44}N_6NaO_6S$ $[M+Na]^+$ 663.2941, found 663.2946; calcd. for $C_{32}H_{44}N_6KO_6S$ $[M+K]^+$ 679.2680, found 679.2693.

Side Product Formation and Optimization Experiments



Scheme S1. Isolation of major side product, **1m**, from synthesis of **1c**.

(*2S, 15S*)-2,15-bis(4-((5-(dimethylamino)naphthalene)-1-sulfonamido)butyl)-4,13-dioxo-3,5,12,14-tetraazahexadecanediamide trifluoracetic acid (1m)



1m

1m was a major side product isolated from the reactions depicted in Scheme 1B. Yield of **1m** was quantified in a reaction to generate **1c**. **1c** was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **1** (209 mg, 0.024 mmol, 1 eq.), hexamethylene diisocyanate (39 µL, 0.24 mmol, 10 eq.), TEA (16.7 µL, 0.12 mmol, 5 eq.), piperidine (11.8 µL,

0.12 mmol, 5 eq.), and LiHMDS (24 µL, 0.024 mmol, 1 eq.). From the crude reaction, **1m** was calculated to consume 46% of starting material using a standard curve. ^{1}H NMR (500 MHz, CD_3OD) δ 8.54 (d, *J* = 8.5 Hz, 2H), 8.49 (d, *J* = 8.7 Hz, 2H), 8.23 (d, *J* = 7.2 Hz, 2H), 7.70 – 7.60 (m, 4H), 7.46 (d, *J* = 7.6 Hz, 2H), 4.04 (dd, *J* = 8.6, 5.1 Hz, 2H), 3.14 – 3.09 (m, 4H), 3.03 (s, *J* = 5.1 Hz, 9H), 2.84 (t, *J* = 6.7 Hz, 4H), 1.55 – 1.27 (m, 20H). ^{13}C NMR (126 MHz, CD_3OD) δ 177.0, 159.1, 149.1, 136.1, 129.5, 129.0, 128.7, 127.6, 123.7, 120.9, 115.9, 53.3, 44.8, 42.3, 39.3, 32.0, 29.7, 28.9, 26.0, 22.4. HRMS (ESI) m/z: calcd. for $C_{44}H_{65}N_{10}O_8S_2$ $[M+H]^+$ 925.4423, found 925.4434; m/z: calcd. for $C_{44}H_{64}N_{10}NaO_8S_2$ $[M+Na]^+$ 947.4248, found 947.4254; calcd. for $C_{44}H_{64}KN_{10}O_8S_2$ $[M+K]^+$ 963.3987, found 963.4022.

Optimization Experiments to Reduce Side Product (1m)

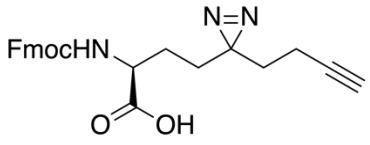
For conditions 1-5, crude product **1c** was synthesized according to **General Procedure B** with noted exceptions. Resin loading with **1** was conducted as in the original method (0.25 eq.) or halved (0.125 eq.). Resin was transferred to a larger reaction vessel before addition of the diisocyanate linker to prevent reactions between different beads. The ratio of hexamethylene diisocyanate was kept constant (10 eq.) or increased by 2.5 fold (25 eq.). For the reaction with hexamethylene diisocyanate, AF-DMF or a 3:1 solution of DCM to AF-DMF was used as a solvent. From the crude reactions, the amount of starting material consumed by **1c** and **1m** was calculated using standard curves generated from their respective purified products. There was resin lost during the transfer step, therefore results are reported as a ratio of starting material consumed by product **(1c)** to side product **(1m)**.

Condition	Eq. **1**	Eq. $(CH_2)_6(NCO)_2$	Solvent	Ratio of starting material consumed by **1c** to **1m**
Original	0.25	10	AF-DMF	45%/46%=1
1	0.25	25	AF-DMF	2.3
2	0.25	25	3:1 DCM:AF-DMF	2.7
3	0.125	10	AF-DMF	0.7
4	0.125	25	AF-DMF	2.0
5	0.125	25	3:1 DCM:AF-DMF	1.6

Table S1. Results from optimization experiments to reduce side product formation (**1m**) relative to product (**1c**).

Synthesis FKBP12 PAL Probes

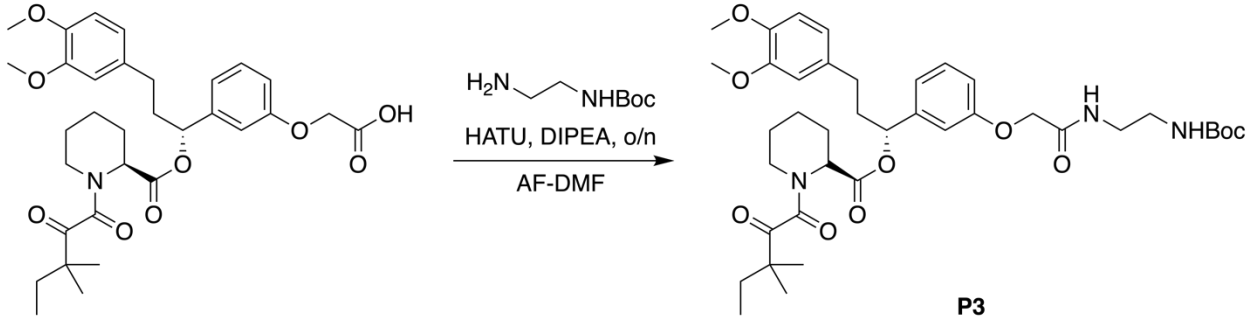
(*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)butanoic acid (2)



2

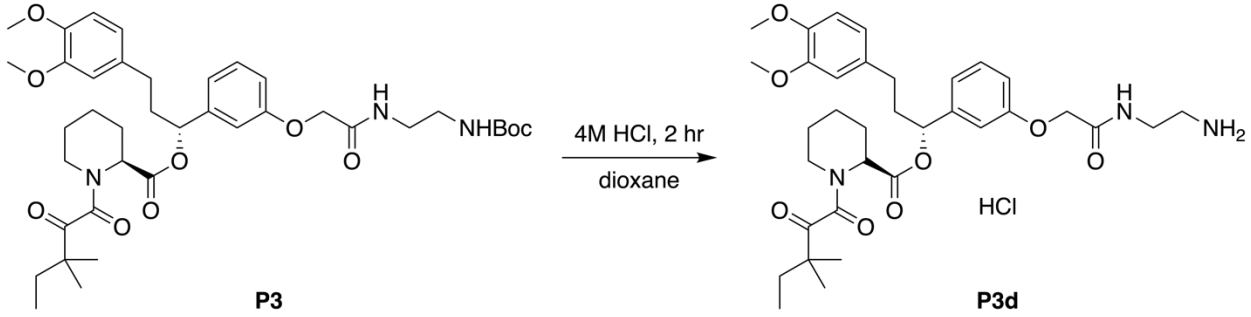
The photoaffinity moiety (**2**) was synthesized as previously described.[2,3]

(*R*)-1-(3-(2-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*R*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (P3)



2-(3-((*R*)-3-(3,4-dimethoxyphenyl)-1-((*S*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carbonyloxy)propyl)phenoxy)acetic acid (AP1497 acid) was synthesized as previously described.[4] The reaction was performed according to **General Procedure C** with AP1497 acid (100 mg, 0.17 mmol, 1 eq.), HATU (78.3 mg, 0.20 mmol, 1.2 eq), *N*-Boc-ethylenediamine (54.3 µL, 0.34 mmol, 2 eq.), and DIPEA (59.8 µL, 0.34 mmol, 2 eq.). Purification by HPLC Method C afforded a pale yellow oil (89.3 mg, 0.12 mmol, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.29 (m, 1H), 7.06 – 6.93 (m, 2H), 6.93 – 6.83 (m, 1H), 6.83 – 6.72 (m, 1H), 6.74 – 6.64 (m, 2H), 5.88 – 5.69 (m, 1H), 5.38 – 5.22 (m, 1H), 4.56 – 4.42 (m, 2H), 4.23 – 4.06 (m, 1H), 3.93 – 3.81 (m, 6H), 3.55 – 3.44 (m, 2H), 3.45 – 3.28 (m, 3H), 3.31 – 3.11 (m, 1H), 2.80 – 2.45 (m, 2H), 2.35 – 2.15 (m, 1H), 2.14 – 1.99 (m, 2H), 1.87 – 1.62 (m, 5H), 1.45 (s, *J* = 24.3 Hz, 9H), 1.32 – 1.13 (m, 7H), 0.96 – 0.78 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.9, 169.1, 167.6, 167.3, 157.4, 156.7, 148.9, 147.4, 142.3, 141.9, 133.4, 130.0, 120.2, 114.1, 113.4, 111.7, 111.3, 76.5, 67.2, 60.4, 55.9, 55.8, 51.3, 46.7, 44.2, 38.2, 32.5, 31.2, 28.3, 26.4, 25.0, 23.5, 23.2, 21.2, 21.0, 14.2, 8.8. HRMS (ESI) m/z: calcd. for $C_{39}H_{56}N_3O_{10}$ $[M+H]^+$ 726.3960, found 726.3966; m/z: calcd. for $C_{39}H_{55}N_3NaO_{10}$ $[M+Na]^+$ 748.3785, found 748.3789; calcd. for $C_{39}H_{59}N_4O_{10}$ $[M+NH_4]^+$ 743.4231, found 743.4232.

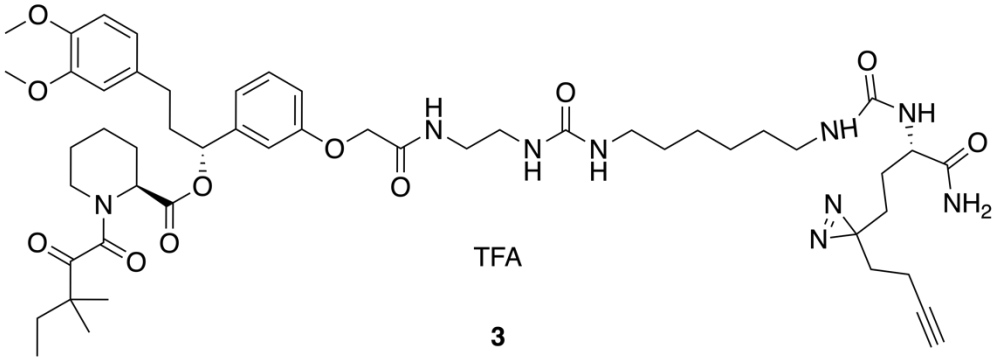
(*R*)-1-(3-(2-((2-aminoethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*R*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate hydrochloride (P3d)



4M HCl in dioxane (1 mL) was added to **P3** (85.7 mg, 0.12 mmol) and stirred for 2 hours at room temperature. The solvent was removed under reduced pressure yielding a white powder and was used in subsequent reactions without further purification. ^{1}H NMR (500 MHz, CD_3OD) δ 7.38 – 7.30 (m, 1H), 7.07 – 6.96 (m, 3H), 6.89 – 6.85 (m, 1H), 6.85 – 6.78 (m, 1H), 6.76 – 6.71 (m, 1H), 5.80 – 5.73 (m, 1H), 5.27 – 5.21 (m, 1H), 4.61 – 4.56 (m, 2H), 3.85 – 3.80 (m, 6H), 3.76 (t, *J* = 5.5 Hz, 1H), 3.60 (t, *J* = 5.2 Hz, 2H), 3.42 (d, *J* = 12.9 Hz, 1H), 3.23 (td, *J* = 13.2, 2.8 Hz, 1H), 3.14 (t, *J* = 5.7 Hz, 2H), 2.75 – 2.55 (m, 2H), 2.41 – 2.24 (m, 2H), 2.14 – 2.05 (m, 1H), 1.81 – 1.63 (m, 5H), 1.26 – 1.20 (m, 5H), 0.94 – 0.80 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ

208.0, 170.6, 169.8, 167.5, 157.5, 148.9, 147.4, 141.8, 133.4, 130.1, 120.2, 119.7, 114.2, 112.6, 111.8, 111.4, 72.3, 71.1, 67.5, 61.7, 55.9, 55.9, 46.7, 44.2, 42.9, 32.4, 31.3, 24.9, 23.4, 23.2, 8.8. HRMS (ESI) m/z: calcd. for $C_{34}H_{48}N_3O_8$ $[M+H]^+$ 626.3436, found 626.3443; m/z: calcd. for $C_{34}H_{47}N_3NaO_8$ $[M+Na]^+$ 648.3261, found 648.3260; calcd. for $C_{34}H_{51}N_4O_8$ $[M+NH_4]^+$ 643.3707, found 643.3618.

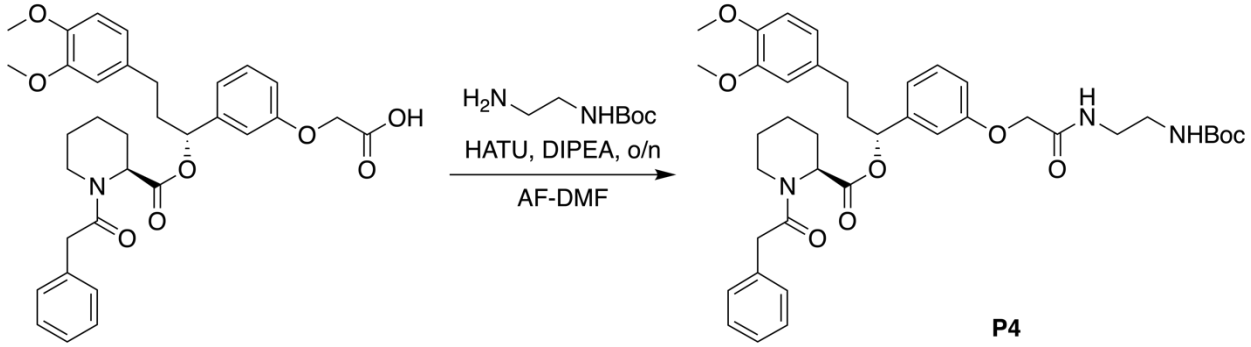
(*R*)-1-(3-(((*S*)-20-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)-18-carbamoyl-2,7,16-trioxo-3,6,8,15,17-pentaazaicosyl)oxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate trifluoroacetic acid (3)



3

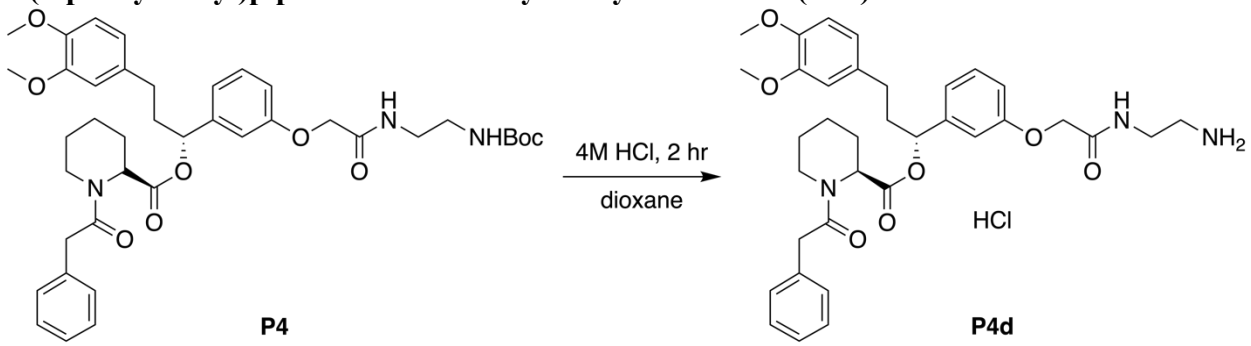
The molecule was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **2** (189.1 mg, 0.015 mmol, 1 eq.), hexamethylene diisocyanate (23.4 µL, 0.15 mmol, 10 eq.), TEA (10.2 µL, 0.075 mmol, 5 eq.), **P3d** (24.1 mg, 0.04 mmol, 2.5 eq.), and LiHMDS (15 µL, 0.015 mmol, 1 eq.). The reaction mixture was dissolved in 1 mL of methanol and 100 uL was removed for the quantification of crude product yield and generation of crude product DMSO stock solutions. Crude product yield was calculated using a standard curve (24%). The remaining sample was concentrated under reduced pressure and purified by HPLC Method B. Fractions containing product were lyophilized to afford a white powder (3.6 mg, 0.003 mmol, 25%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36 – 7.25 (m, 1H), 7.07 – 6.92 (m, 3H), 6.91 – 6.68 (m, 3H), 5.82 – 5.70 (m, 1H), 5.29 – 5.20 (m, 1H), 4.56 – 4.49 (m, 2H), 4.15 (dd, *J* = 8.1, 5.0 Hz, 1H), 3.89 – 3.77 (m, 6H), 3.45 – 3.34 (m, 3H), 3.31 – 3.27 (m, 2H), 3.12 – 3.04 (m, 4H), 2.73 – 2.56 (m, 2H), 2.41 – 2.25 (m, 3H), 2.14 – 2.06 (m, 1H), 2.03 (td, *J* = 7.4, 2.6 Hz, 2H), 1.80 – 1.57 (m, 8H), 1.54 – 1.31 (m, 13H), 1.28 – 1.20 (m, 5H), 0.93 – 0.78 (m, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 207.6, 176.1, 170.0, 169.6, 167.7, 160.0, 159.0, 157.9, 149.0, 147.5, 141.9, 133.8, 129.6, 120.3, 119.6, 114.2, 113.0, 112.2, 111.9, 82.2, 76.6, 69.0, 66.8, 56.9, 55.2, 55.0, 52.7, 51.4, 46.3, 44.3, 39.8, 39.5, 39.4, 38.9, 37.8, 32.2, 31.9, 30.9, 29.8, 29.7, 28.7, 27.4, 27.0, 26.1, 26.0, 24.5, 22.4, 22.2, 20.8, 20.7, 12.5, 7.7. HRMS (ESI) m/z: calcd. for $C_{51}H_{74}N_9O_{11}$ $[M+H]^+$ 988.5502, found 988.5513.

(*R*)-1-(3-(2-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*R*)-1-(2-phenylacetyl)piperidine-2-carboxylate (P4)



2-(3-((*R*)-3-(3,4-dimethoxyphenyl)-1-(((*S*)-1-(2-phenylacetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (AP1780 acid) was synthesized as previously described.[4] The reaction was performed according to **General Procedure C** with AP1780 acid (100 mg, 0.17 mmol, 1 eq.), HATU (79.3 mg, 0.20 mmol, 1.2 eq), *N*-Boc-ethylenediamine (55 µL, 0.34 mmol, 2 eq.), and DIPEA (60.5 µL, 0.34 mmol, 2 eq.). Purification by HPLC afforded a pale yellow oil (97.9 mg, 78% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.21 (m, 7H), 6.97 – 6.65 (m, 7H), 5.80 – 5.71 (m, 1H), 5.51 (s, 1H), 4.54 – 4.48 (m, 2H), 3.89 – 3.86 (m, 6H), 3.50 – 3.43 (m, 2H), 3.36 – 3.30 (m, 2H), 2.66 – 2.51 (m, 2H), 2.24 – 2.02 (m, 2H), 1.75 – 1.64 (m, 2H), 1.45 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.5, 170.5, 169.3, 157.3, 148.9, 147.4, 142.1, 134.5, 133.4, 130.0, 128.8, 128.6, 126.9, 120.2, 120.1, 114.0, 113.8, 113.1, 111.7, 111.4, 76.0, 67.1, 55.9, 55.9, 52.4, 52.3, 44.2, 40.9, 38.1, 31.3, 28.3, 26.6, 26.6, 25.1, 25.0, 20.8, 20.7. HRMS (ESI) m/z: calcd. for $C_{40}H_{52}N_3O_9$ $[M+H]^+$ 718.3698, found 718.3704; m/z: calcd. for $C_{40}H_{51}N_3NaO_9$ $[M+Na]^+$ 740.3523, found 740.3526; calcd. for $C_{40}H_{55}N_4O_9$ $[M+NH_4]^+$ 735.3969, found 735.3801.

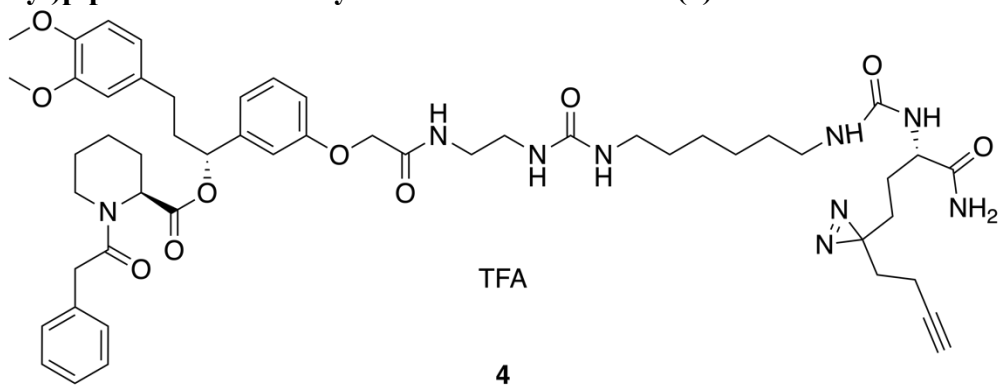
(*R*)-1-(3-(2-((2-aminoethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*R*)-1-(2-phenylacetyl)piperidine-2-carboxylate hydrochloride (P4d)



4M HCl in dioxane (1 mL) was added to **P4** (85 mg, 0.14 mmol) and stirred for 2 hours at room temperature. The solvent was removed under reduced pressure yielding a white powder and was used in subsequent reactions without further purification. ^{1}H NMR (500 MHz, CD_3OD) δ 7.33 – 7.18 (m, 5H), 6.99 – 6.91 (m, 2H), 6.80 – 6.67 (m, 2H), 5.70 (dt, J = 12.2, 6.1 Hz, 1H), 5.41 – 5.34 (m, 1H), 4.56 (d, J = 8.0 Hz, 2H), 3.83 – 3.80 (m, 5H), 3.70 – 3.67 (m, 2H), 3.60 – 3.55 (m, 2H), 3.12 – 3.07 (m, 2H), 2.69 – 2.51 (m, 2H), 2.36 – 2.16 (m, 2H), 2.11 – 1.99 (m, 1H), 1.76 – 1.59 (m, 3H), 1.46 – 1.19 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.8, 171.5, 170.7, 170.3, 157.6, 148.9, 147.4, 142.0, 134.7, 134.6, 133.4, 129.9, 128.9, 128.8, 128.8, 127.0, 120.2, 111.7, 111.4, 76.4, 67.4, 55.9, 52.5, 44.1, 40.8, 38.3, 36.8, 31.4, 26.7, 25.0, 20.9. HRMS (ESI)

m/z: calcd. for $C_{35}H_{44}N_3O_7$ $[M+H]^+$ 618.3174, found 618.3182; m/z: calcd. for $C_{35}H_{43}N_3NaO_7$ $[M+Na]^+$ 640.2999, found 640.3000; calcd. for $C_{35}H_{47}N_3O_7$ $[M+NH_4]^+$ 635.3445, found 635.3326.

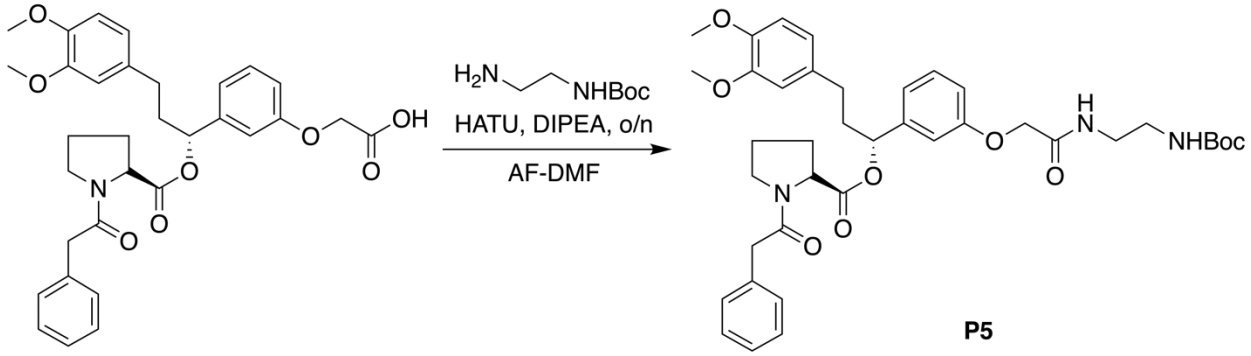
(*R*)-1-(3-(((*S*)-20-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)-18-carbamoyl-2,7,16-trioxo 3,6,8,15,17-pentaazaicosyl)oxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-1-(2-phenylacetyl)piperidine-2-carboxylate trifluoroacetic acid (4)



4

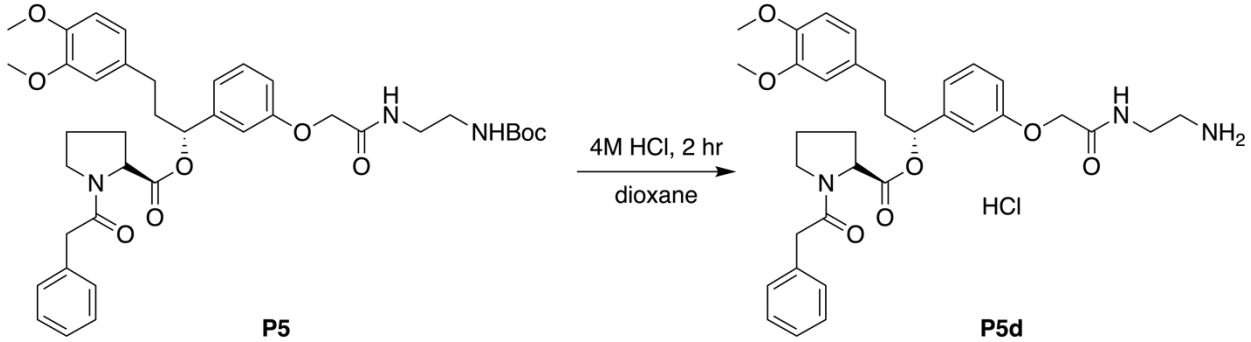
Crude product synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **2** (199.9 mg, 0.015 mmol, 1 eq.), hexamethylene diisocyanate (24.7 µL, 0.15 mmol, 10 eq.), TEA (10.7 µL, 0.075 mmol, 5 eq.), **P4d** (25.2 mg, 0.039 mmol, 2.5 eq.), and LiHMDS (15 µL, 0.015 mmol, 1 eq.) had an estimated yield of 48% using a standard curve. To isolate pure product, **4** was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **2** (100 mg, 0.015 mmol, 1 eq.), hexamethylene diisocyanate (25.2 µL, 0.15 mmol, 10 eq.), TEA (10.5 µL, 0.075 mmol, 5 eq.), **P4d** (16.1 mg, 0.02 mmol, 1.6 eq.), and LiHMDS (16 µL, 0.015 mmol, 1 eq.). The reaction was concentrated under reduced pressure and purified by HPLC Method B. Fractions containing product were lyophilized to afford a white powder (2 mg, 0.002 mmol, 12%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.41 – 7.16 (m, 6H), 7.06 – 6.83 (m, 4H), 6.82 – 6.66 (m, 2H), 5.76 – 5.66 (m, 1H), 5.43 – 5.31 (m, 1H), 4.50 (d, J = 12.8 Hz, 2H), 4.15 (dd, J = 8.1, 5.0 Hz, 1H), 3.98 (d, J = 13.1 Hz, 1H), 3.84 – 3.80 (m, 6H), 3.38 – 3.34 (m, 2H), 3.30 – 3.26 (m, 2H), 3.11 – 3.05 (m, 4H), 2.66 – 2.53 (m, 2H), 2.32 (d, J = 13.3 Hz, 1H), 2.28 (t, J = 2.6 Hz, 1H), 2.24 – 2.17 (m, 3H), 2.07 – 1.99 (m, 3H), 1.61 – 1.32 (m, 19H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.1, 172.2, 170.5, 170.0, 160.0, 159.0, 157.8, 149.0, 147.5, 142.1, 134.8, 133.8, 129.5, 128.5, 128.4, 126.5, 120.3, 119.5, 114.1, 112.9, 112.2, 111.9, 82.2, 76.2, 69.0, 66.8, 55.2, 55.0, 52.7, 52.4, 44.2, 40.1, 39.8, 39.5, 39.4, 38.8, 37.8, 31.9, 30.9, 29.8, 29.7, 29.4, 28.7, 27.4, 27.0, 26.1, 24.6, 20.5, 16.9, 12.5. HRMS (ESI) m/z: calcd. for $C_{52}H_{70}N_9O_{10}$ $[M+H]^+$ 980.5240, found 980.5250.

(*R*)-1-(3-(2-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (2-phenylacetyl)-*L*-prolinate (P5)



2-(3-((*R*)-3-(3,4-dimethoxyphenyl)-1-(((*S*)-1-(2-phenylacetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (ProAP1780 acid) was synthesized as previously described.[4] The reaction was performed according to **General Procedure C** with ProAP1780 acid (100 mg, 0.18 mmol, 1 eq.), HATU (81.2 mg, 0.21 mmol, 1.2 eq), *N*-Boc-ethylenediamine (56.5 µL, 0.36 mmol, 2 eq.), and DIPEA (62 µL, 0.36 mmol, 2 eq.). Purification by HPLC afforded a pale yellow oil (80.2 mg, 0.11 mmol, 64% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.20 (m, 7H), 6.97 – 6.78 (m, 4H), 6.71 – 6.69 (m, 1H), 5.79 – 5.71 (m, 1H), 4.66 (dd, *J* = 8.5, 3.0 Hz, 1H), 4.51 – 4.45 (m, 2H), 3.92 – 3.85 (m, 6H), 3.77 – 3.70 (m, 2H), 3.72 – 3.65 (m, 1H), 3.59 – 3.50 (m, 1H), 3.45 – 3.36 (m, 2H), 3.34 – 3.22 (m, 2H), 2.68 – 2.50 (m, 2H), 2.30 – 2.18 (m, 2H), 2.09 – 1.98 (m, 3H), 1.45 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.52, 170.30, 169.27, 157.45, 156.60, 148.89, 147.34, 142.13, 134.11, 133.6, 129.8, 129.0, 128.7, 127.0, 120.2, 119.7, 114.1, 112.7, 111.8, 111.3, 75.7, 67.2, 59.3, 55.9, 55.9, 47.5, 41.8, 38.1, 31.2, 29.4, 28.4, 24.9. HRMS (ESI) m/z: calcd. for $C_{39}H_{50}N_3O_9$ $[M+H]^+$ 704.3542, found 704.3550; m/z: calcd. for $C_{39}H_{49}N_3NaO_9$ $[M+Na]^+$ 726.3366, found 726.3336; calcd. for $C_{39}H_{53}N_4O_9$ $[M+NH_4]^+$ 721.3813, found 721.3774.

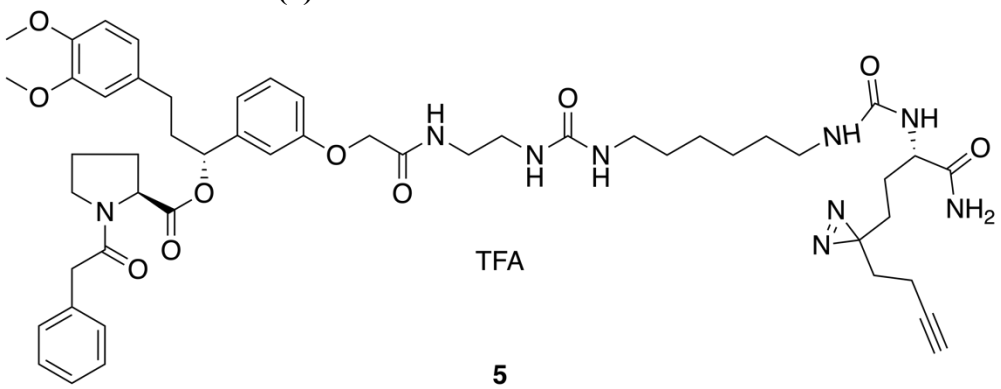
(*R*)-1-(3-(2-((2-aminoethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (2-phenylacetyl)-*L*-prolinate hydrochloride (P5d)



4M HCl in dioxane (1 mL) was added to **P5** (48.6 mg, 0.07 mmol) and stirred for 2 hours at room temperature. The solvent was removed under reduced pressure yielding a light yellow powder and was used in subsequent reactions without further purification. ^{1}H NMR (500 MHz, CD_3OD) δ 7.37 – 7.18 (m, 6H), 6.99 – 6.92 (m, 3H), 6.90 – 6.85 (m, 1H), 6.81 – 6.77 (m, 1H), 6.74 – 6.68 (m, 1H), 5.72 – 5.64 (m, 1H), 4.60 – 4.49 (m, 3H), 3.83 – 3.81 (m, 6H), 3.77 – 3.68 (m, 3H), 3.58 – 3.53 (m, 2H), 3.13 – 3.03 (m, 2H), 2.70 – 2.47 (m, 2H), 2.33 – 2.14 (m, 2H), 2.08 – 1.93 (m, 4H). ^{13}C NMR (126 MHz, CD_3OD) δ 172.2, 171.0, 170.5, 157.7, 149.0, 147.5, 142.2, 134.8,

133.8, 129.6, 128.5, 128.4, 126.5, 120.3, 119.6, 114.0, 112.8, 112.2, 111.9, 76.1, 66.8, 55.2, 52.4, 44.2, 40.1, 39.6, 37.8, 36.5, 30.9, 26.2, 24.6, 22.8, 20.5. HRMS (ESI) m/z: calcd. for $C_{34}H_{42}N_3O_7$ $[M+H]^+$ 604.3017, found 604.3026; m/z: calcd. for $C_{34}H_{41}N_3NaO_7$ $[M+Na]^+$ 626.2842, found 626.2845; calcd. for $C_{34}H_{41}KN_3O_7$ $[M+K]^+$ 642.2582, found 642.2583.

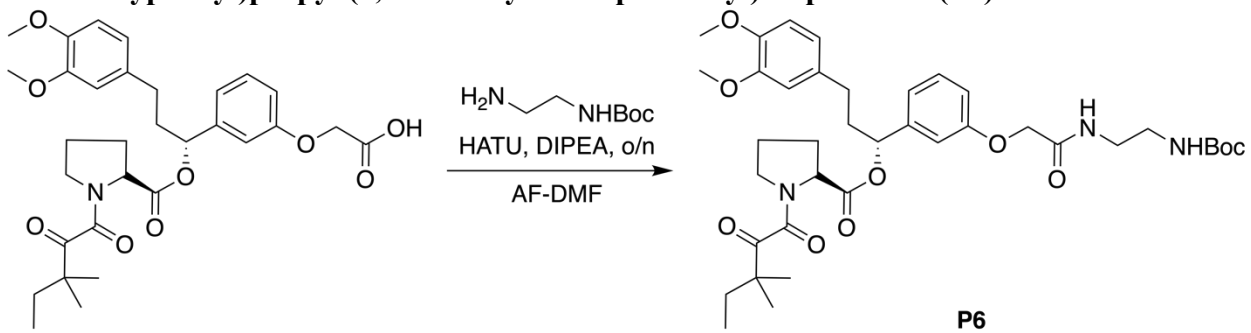
(*R*)-1-(3-(((*S*)-20-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)-18-carbamoyl-2,7,16-trioxo-3,6,8,15,17-pentaazaicosyl)oxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (2-phenylacetyl)-*L*-prolinate trifluoroacetic acid (5)



5

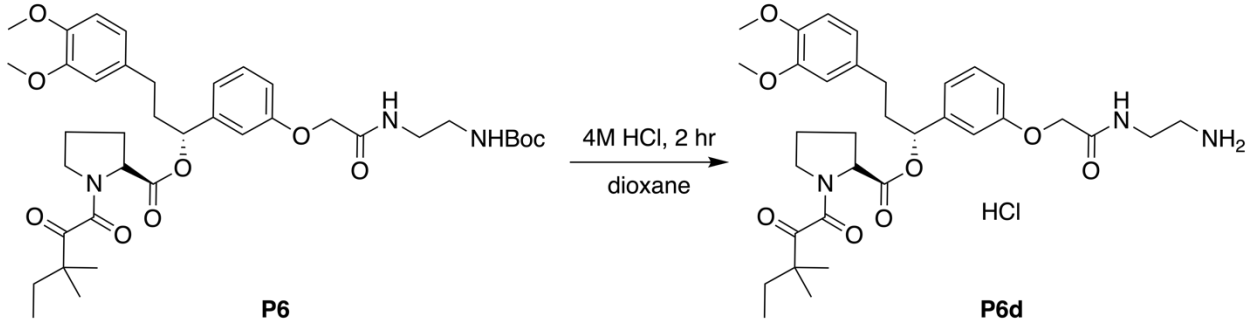
The molecule was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **2** (128.5 mg, 0.019 mmol, 1 eq.), hexamethylene diisocyanate (31.1 µL, 0.19 mmol, 10 eq.), TEA (13.5 µL, 0.09 mmol, 5 eq.), **P5d** (24.8 mg, 0.04 mmol, 2 eq.), and LiHMDS (19 µL, 0.019 mmol, 1 eq.). The reaction mixture was dissolved in 1 mL of methanol and 100 uL was removed for the quantification of crude product yield and generation of crude product DMSO stock solutions. Crude product yield was calculated using a standard curve (43%). The remaining material was concentrated under reduced pressure, purified by HPLC Method B, and lyophilized to afford a white powder (2.3 mg, 0.002 mmol, 14%). Purity was calculated by EIC (84%) and yields were adjusted accordingly (1.9 mg, 0.00168, 12%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.35 – 7.18 (m, 6H), 7.00 – 6.91 (m, 3H), 6.89 – 6.86 (m, 1H), 6.80 – 6.77 (m, 1H), 6.74 – 6.69 (m, 1H), 5.73 – 5.67 (m, 1H), 4.56 (dd, J = 8.7, 4.1 Hz, 1H), 4.15 (dd, J = 8.0, 5.1 Hz, 1H), 3.85 – 3.80 (m, 7H), 3.76 – 3.69 (m, 2H), 3.35 (d, J = 5.4 Hz, 2H), 3.30 – 3.24 (m, 2H), 3.14 – 3.04 (m, 5H), 2.66 – 2.48 (m, 2H), 2.35 – 2.25 (m, 2H), 2.23 – 2.12 (m, 2H), 2.06 – 1.92 (m, 6H), 1.62 – 1.30 (m, 17H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.1, 171.7, 171.1, 170.1, 160.0, 159.0, 157.8, 149.0, 147.4, 142.1, 134.5, 134.0, 129.5, 129.0, 128.9, 128.3, 126.5, 120.3, 119.3, 114.0, 112.6, 112.3, 111.8, 82.2, 75.7, 69.0, 66.8, 59.5, 55.2, 55.1, 52.7, 41.0, 39.7, 39.5, 39.4, 38.8, 38.0, 31.9, 30.8, 29.8, 29.7, 29.0, 28.7, 27.4, 27.0, 26.1, 24.5, 12.5. HRMS (ESI) m/z: calcd. for $C_{51}H_{68}N_9O_{10}$ $[M+H]^+$ 966.5084, found 966.5081; m/z: calcd. for $C_{51}H_{67}NaN_9O_{10}$ $[M+Na]^+$ 988.4909, found 988.4898.

(*R*)-1-(3-(2-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (3,3-dimethyl-2-oxopentanoyl)-*D*-prolinate (P6)



2-(3-((*R*)-3-(3,4-dimethoxyphenyl)-1-(((3,3-dimethyl-2-oxopentanoyl)-*L*-prolyl)oxy)propyl)phenoxy)acetic acid (ProAP1497 acid) was synthesized as previously described.[4] The reaction was performed according to **General Procedure C** with ProAP1497 acid (100 mg, 0.18 mmol, 1 eq.), HATU (80.1 mg, 0.22 mmol, 1.2 eq), N-Boc-ethylenediamine (55.6 µL, 0.36 mmol, 2 eq.), and DIPEA (61.3 µL, 0.36 mmol, 2 eq.). Purification by HPLC afforded a pale yellow oil (48.7 mg, 0.07 mmol, 39%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 – 7.28 (m, 1H), 7.03 – 6.92 (m, 2H), 6.90 – 6.84 (m, 1H), 6.83 – 6.78 (m, 1H), 6.74 – 6.66 (m, 2H), 5.81 – 5.68 (m, 1H), 4.72 – 4.60 (m, 1H), 4.56 – 4.52 (m, 2H), 3.90 – 3.85 (m, 6H), 3.73 – 3.53 (m, 2H), 3.48 (dd, *J* = 11.5, 5.7 Hz, 2H), 3.33 (s, 2H), 2.67 – 2.52 (m, 2H), 2.30 – 2.16 (m, 2H), 2.10 – 1.94 (m, 4H), 1.79 – 1.61 (m, 2H), 1.44 (s, 9H), 1.25 – 1.15 (m, 5H), 0.89 – 0.69 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.0, 170.7, 169.3, 165.2, 157.3, 156.8, 148.9, 147.3, 142.0, 133.5, 129.9, 120.2, 120.0, 114.1, 113.0, 111.8, 111.3, 76.1, 67.2, 59.8, 58.6, 55.9, 55.9, 47.2, 46.9, 38.1, 32.7, 32.2, 31.1, 29.0, 28.3, 24.9, 23.6, 23.2, 8.9. HRMS (ESI) m/z: calcd. for $C_{38}H_{54}N_3O_{10}$ $[M+H]^+$ 712.3804, found 712.3803; m/z: calcd. for $C_{38}H_{57}N_4O_{10}$ $[M+NH_4]^+$ 729.4075, found 729.4103.

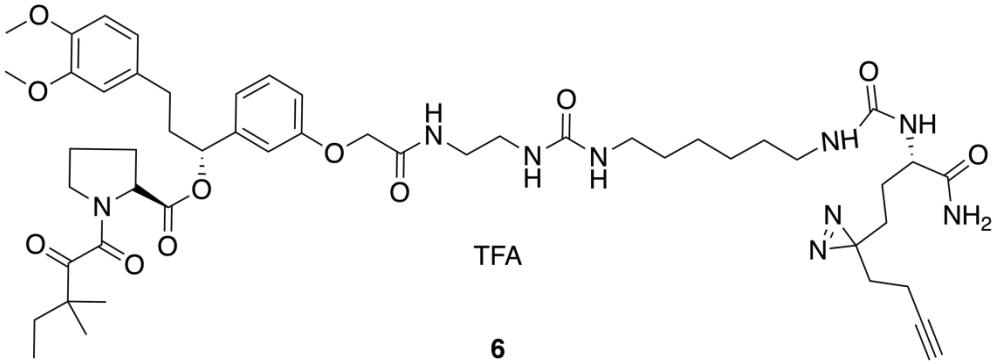
(*R*)-1-(3-(2-((2-aminoethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (3,3-dimethyl-2-oxopentanoyl)-*D*-prolinate hydrochloride (P6d)



4M HCl in dioxane (1 mL) was added to **P6** (45.4 mg, 0.06 mmol) and stirred for 2 hours at room temperature. The mixture was concentrated under reduced pressure yielding a white powder and was used in subsequent reactions without further purification. ^{1}H NMR (500 MHz, CD_3OD) δ 7.34 (t, *J* = 7.9 Hz, 1H), 7.04 – 6.95 (m, 3H), 6.90 – 6.86 (m, 1H), 6.84 – 6.78 (m, 1H), 6.76 – 6.70 (m, 1H), 5.75 – 5.68 (m, 1H), 4.64 – 4.54 (m, 3H), 3.86 – 3.79 (m, 6H), 3.78 – 3.74 (m, 1H), 3.70 – 3.67 (m, 2H), 3.61 – 3.58 (m, 3H), 3.16 – 3.07 (m, 2H), 2.72 – 2.56 (m, 2H), 2.29 – 2.16 (m, 1H), 2.07 – 1.92 (m, 3H), 1.75 – 1.60 (m, 2H), 1.24 – 1.22 (m, 4H), 0.92 – 0.68 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.2, 170.8, 165.5, 157.4, 148.9, 147.9, 147.3, 141.9, 133.5, 130.0, 120.2, 119.6, 114.2, 113.4, 111.8, 111.3, 76.4, 72.2, 67.4, 61.7, 58.6, 55.9, 46.9, 38.2, 32.2, 31.2, 29.1, 24.9, 23.5, 23.2, 8.9. HRMS (ESI) m/z: calcd. for $C_{33}H_{46}N_3O_8$ $[M+H]^+$ 612.3279, found

612.3287; m/z: calcd. for $C_{33}H_{45}N_3NaO_8$ $[M+Na]^+$ 634.3104, found 634.3104; calcd. for $C_{33}H_{49}N_4O_8$ $[M+NH_4]^+$ 629.3550, found 629.3365.

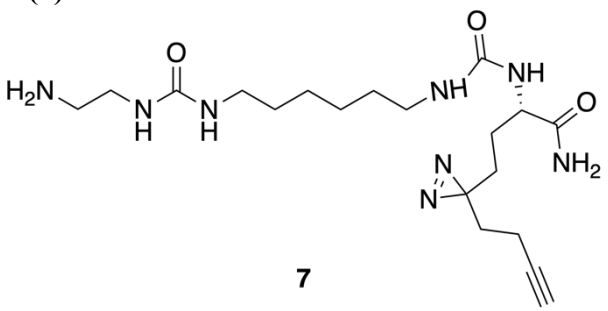
(*R*)-1-(3-(((S)-20-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)-18-carbamoyl-2,7,16-trioxo-3,6,8,15,17-pentaazaicosyl)oxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (3,3-dimethyl-2-oxopentanoyl)-*D*-prolinate trifluoroacetic acid (6)



6

Crude product **6** synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **2** (146.5 mg, 0.011 mmol, 1 eq.), hexamethylene diisocyanate (18.1 µL, 0.11 mmol, 10 eq.), TEA (7.9 µL, 0.06 mmol, 5 eq.), **P6d** (17.3 mg, 0.03 mmol, 2.5 eq.), and LiHMDS (11 µL, 0.011 mmol, 1 eq.) had an estimated yield of 28% using a standard curve. To isolate pure product, **6** was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **2** (191.3 mg, 0.015 mmol, 1 eq.), hexamethylene diisocyanate (23.7 µL, 0.15 mmol, 10 eq.), TEA (10.3 µL, 0.08 mmol, 5 eq.), **P6d** (14.5 mg, 0.02 mmol, 1.5 eq.), and LiHMDS (15 µL, 0.016 mmol, 1 eq.). Solvent was removed under reduced pressure. Sample was purified by HPLC Method B and lyophilized to yield a white powder (1.8 mg, 0.002 mmol, 11%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.32 (t, J = 7.9 Hz, 1H), 7.04 – 6.94 (m, 3H), 6.90 – 6.85 (m, 1H), 6.85 – 6.77 (m, 1H), 6.76 – 6.69 (m, 1H), 5.76 – 5.68 (m, 1H), 4.60 – 4.52 (m, 3H), 4.15 (dd, J = 8.0, 5.1 Hz, 1H), 3.86 – 3.81 (m, 6H), 3.64 – 3.51 (m, 2H), 3.36 (t, J = 5.2 Hz, 2H), 3.31 – 3.27 (m, 2H), 3.13 – 3.07 (m, 4H), 2.71 – 2.55 (m, 2H), 2.39 – 2.31 (m, 1H), 2.28 (t, J = 2.6 Hz, 1H), 2.17 – 1.95 (m, 6H), 1.75 – 1.57 (m, 5H), 1.52 – 1.38 (m, 8H), 1.38 – 1.31 (m, 5H), 1.23 (d, J = 2.6 Hz, 4H), 0.89 – 0.68 (m, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 206.8, 176.1, 170.9, 170.1, 159.0, 157.9, 149.0, 147.4, 142.1, 134.0, 129.5, 120.3, 119.6, 119.4, 114.3, 114.1, 112.5, 112.3, 111.8, 82.2, 76.6, 76.0, 69.0, 66.8, 60.0, 58.8, 55.2, 55.1, 52.7, 46.4, 39.8, 39.5, 39.4, 38.9, 38.1, 32.0, 31.9, 30.8, 29.8, 29.7, 28.7, 28.7, 27.4, 27.0, 26.1, 24.6, 22.5, 22.3, 12.5, 7.9. HRMS (ESI) m/z: calcd. for $C_{50}H_{72}N_9O_{11}$ $[M+H]^+$ 974.5346, found 974.5358; m/z: calcd. for $C_{50}H_{71}N_9NaO_{11}$ $[M+Na]^+$ 996.5171, found 996.5176.

(*S*)-16-amino-2-(2-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)ethyl)-4,13-dioxo-3,5,12,14-tetraazahexadecanamide (7)



7

Crude product **7** was synthesized according to **General Procedure B** using resin functionalized with carboxylic acid **2** (154.8 mg, 0.031 mmol, 1 eq.), hexamethylene diisocyanate (50.2 µL, 0.31 mmol, 10 eq.), TEA (21.8 µL, 0.155 mmol, 5 eq.), N-Boc-ethylenediamine (24.7 µL, 0.155 mmol, 5 eq.), and LiHMDS (31 µL, 0.031 mmol, 1 eq.). LCMS (ESI) m/z: calcd. for $C_{19}H_{35}N_8O_3$ $[M+H]^+$ 423.3, found 423.2. No further purification was conducted. Stock solutions were calculated based on bead loading.

Reactivities Analysis

Small molecule SMILES were downloaded from the respective suppliers, and duplicate entries were removed from each list separately. Compounds were de-salted, sanitized, and standardized using the RDKit library. Functional group detection was performed within RDKit using SMARTS (Table S2). Amine queries exclude amide groups, and alcohol queries exclude enols. SMARTS definitions for reactive groups are given below.

Functional Group	SMARTS query
Primary Aliphatic Amine	[ND1][C;!$(C=O)]
Secondary Aliphatic Amine	[ND2]([C;!$(C=O)])[C;!$(C=O)]
Secondary Alkyl/Aryl Amine	[ND2](-[c])[C;!$(C=O)]
Primary Alcohol	[OD1][CX4H2,CX4H3]
Aromatic Alcohol	[OD1]-[c]
Primary Thiol	[SD1][CH2,CH3]

Table S2**.** SMARTS queries for functional groups.

The BindingDB set of compounds represents a comprehensive collection of known non-covalent compound-protein interactions.[5] ChEMBL is a manually annotated set collection of drug-like, bioactive compounds.[6] The Enamine REAL Diverse set includes chemical structures intended to broadly sample the drug-like chemical space with relaxed constraints.[7] The Molecular Libraries Probe Center Network (MLPCN) library contains multiple sources of drug-like small molecules and has been extensively assayed for various bioactivities.[8]

Biological Methods

Pure Protein Target Engagement Assay

Pure and crude **3, 4, 5**, **6,** and rapamycin were each prepared as DMSO stocks with a fixed concentration of active compound (1 mM). Crude **7** was also prepared as a DMSO stock (10 mM) based on resin loading. AP1497, ProAP1497, AP1780, and ProAP1780 acid were used as parent inhibitors for **3**, **6**, **4**, and **5** (10 mM stocks), respectively.

Recombinant FKBP12 (abcam85840) was diluted to 10 ng/µL in ice cold HEPES buffered saline (50 mM HEPES, 150 mM NaCl, pH 7.4) with 1% NP-40 and supplemented with protease inhibitor (1:100 dilution, Sigma P8340). For competition experiments, the FKBP12 solution was either pretreated with rapamycin (10 µM final) or parent inhibitor (100 µM final) at 4 ºC for 1 hour. Samples (50 µL total) treated with **3, 4, 5**, **6** (10 µM final), crude **7** (10 µM or 40 µM), or DMSO (2% final DMSO concentration) were protected from light and rotated at 4 ºC for 1 hour. UV irradiation was performed via 365 nm lamp for 20 minutes, with corresponding negative controls incubated without irradiation. The DMSO control and competition samples were all UV irradiated. Alexa Fluor 647 (AF-647) picolyl azide was installed on probe-labeled proteins with a copper mediated click chemistry kit (Invitrogen, Cat# C10643, 50 µL reaction). Excess fluorophore was removed by chloroform-methanol precipitation.[9] Dried samples were resuspended in 1% SDS in PBS and boiled with 4x Laemmli Sample Buffer (Bio-Rad). AF-647 and FKBP12 (abcam2918, 1:2,000; LiCor 925-32211, 1:15,000) were analyzed via fluorescence imaging of a Western blot (LI-COR Odyssey CLx). Western blot images were analyzed with Image Studio Lite Software Version 5.2.5.

Compound		**3**			**4**			**5**			**6**			**DMSO**
Condition	UV	+	-	+	+	-	+	+	-	+	+	-	+	+
	R	-	-	+	-	-	+	-	-	+	-	-	+	-
	Affinity	++++			++			+			+++			
Channel	700	58800	1080	21.6	12400	329	104	6710	264	577	54600	782	291	147
	800	43200	22800	22800	25000	20900	22700	23700	20500	18100	40500	19900	20800	20900
	700/800	1.361	0.047	0.001	0.496	0.016	0.005	0.283	0.013	0.032	1.348	0.039	0.014	0.007

Table S3. Quantification of Figure 1B Western blot with HPLC purified PAL probes.

Compound		**3**			**4**			**5**			**6**			**DMSO**
Condition	UV	+	-	+	+	-	+	+	-	+	+	-	+	+
	R	-	-	+	-	-	+	-	-	+	-	-	+	-
	Affinity	++++			++			+			+++			
Channel	700	7030	63.7	778	4450	96.5	1000	2890	108	1050	13000	173	945	101
	800	32700	12900	19000	26600	17300	17800	20000	20800	17100	36300	19100	14400	16200
	700/800	0.215	0.005	0.041	0.167	0.006	0.056	0.145	0.005	0.061	0.358	0.009	0.066	0.006

Table S4. Quantification of Figure 1B Western blot with crude reaction PAL probes.

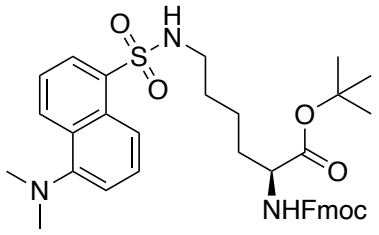
Compound		**3**								**DMSO**	**7**	
Condition	Conc (µM)	10	2	0.4	0.08	0.016	$3.2x10^{-3}$	$6.4x10^{-4}$	$1.28x10^{-4}$	2%	10	40
Channel	700	51899	39400	16900	4420	586	158	68.5	46.1	307	1910	1780
	800	9960	7750	7680	7680	6250	6440	7900	7290	9530	8630	9600
	700/800	5.211	5.084	2.201	0.576	0.094	0.025	0.009	0.006	0.032	0.221	0.185

Table S5. Quantification of Figure 2B Western blot with dose-response treatment (**3)**.

NMR Spectra

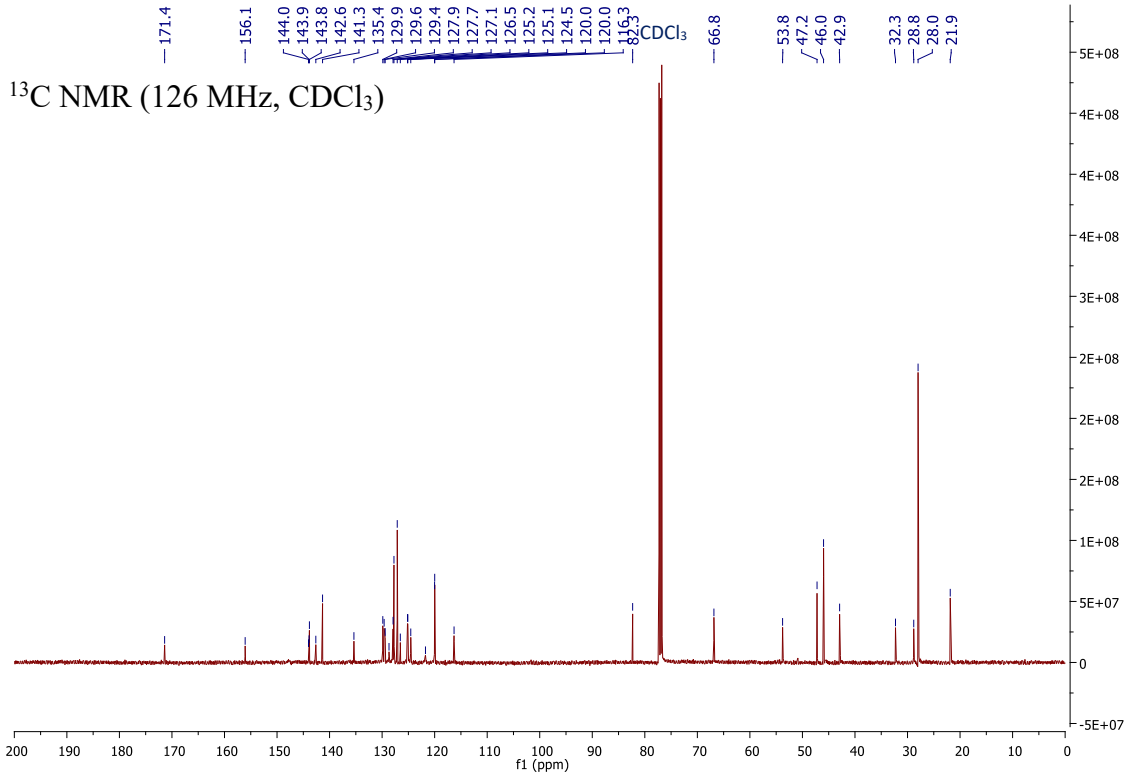
tert-butyl N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^6-((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-*L*-lysinate (P1)

^{1}H NMR (500 MHz, $CDCl_3$)



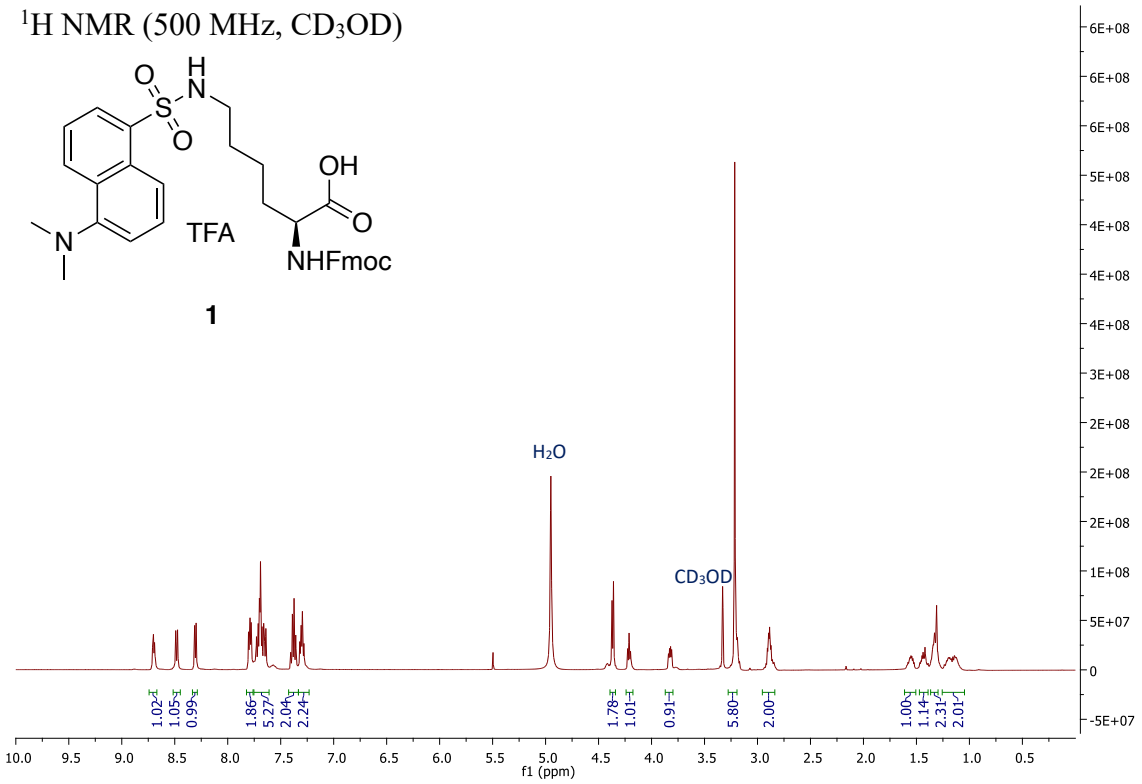
P1

^{13}C NMR (126 MHz, $CDCl_3$)



***N*-(((9H-Fluoren-9-yl)methoxy)carbonyl)-*N*-((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-L-lysine trifluoroacetic acid (1)**

^{1}H NMR (500 MHz, CD_3OD)



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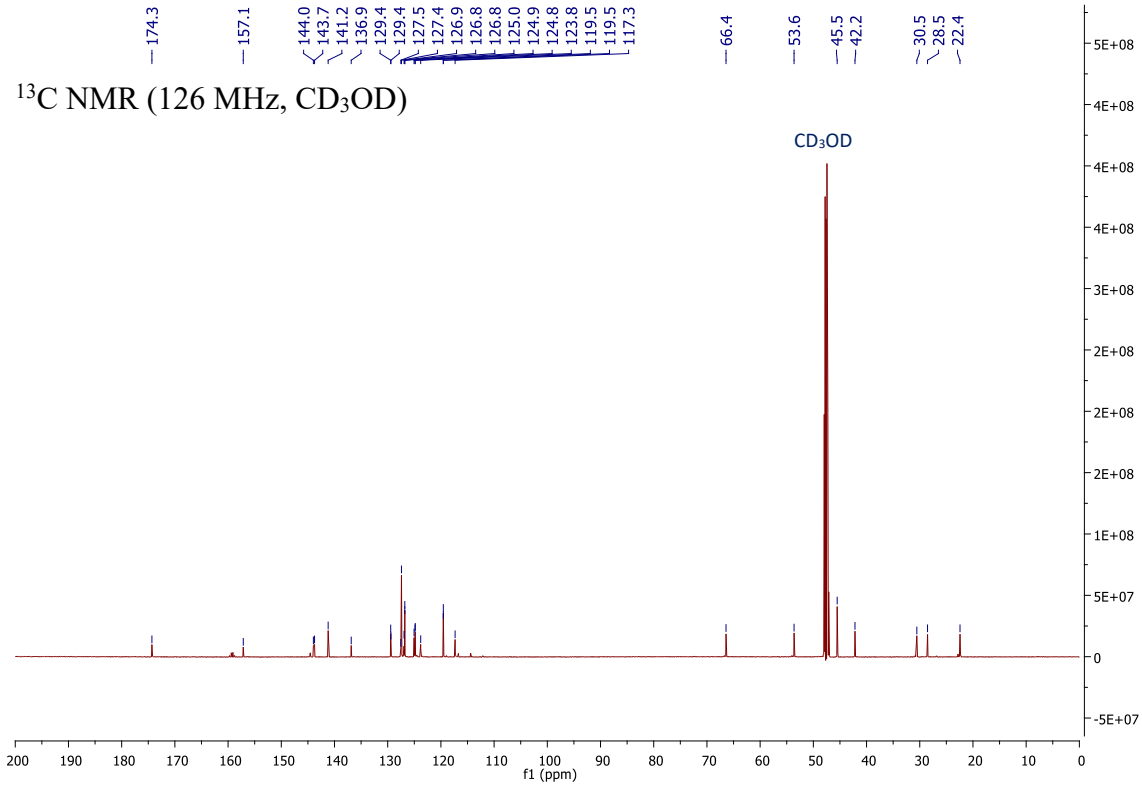
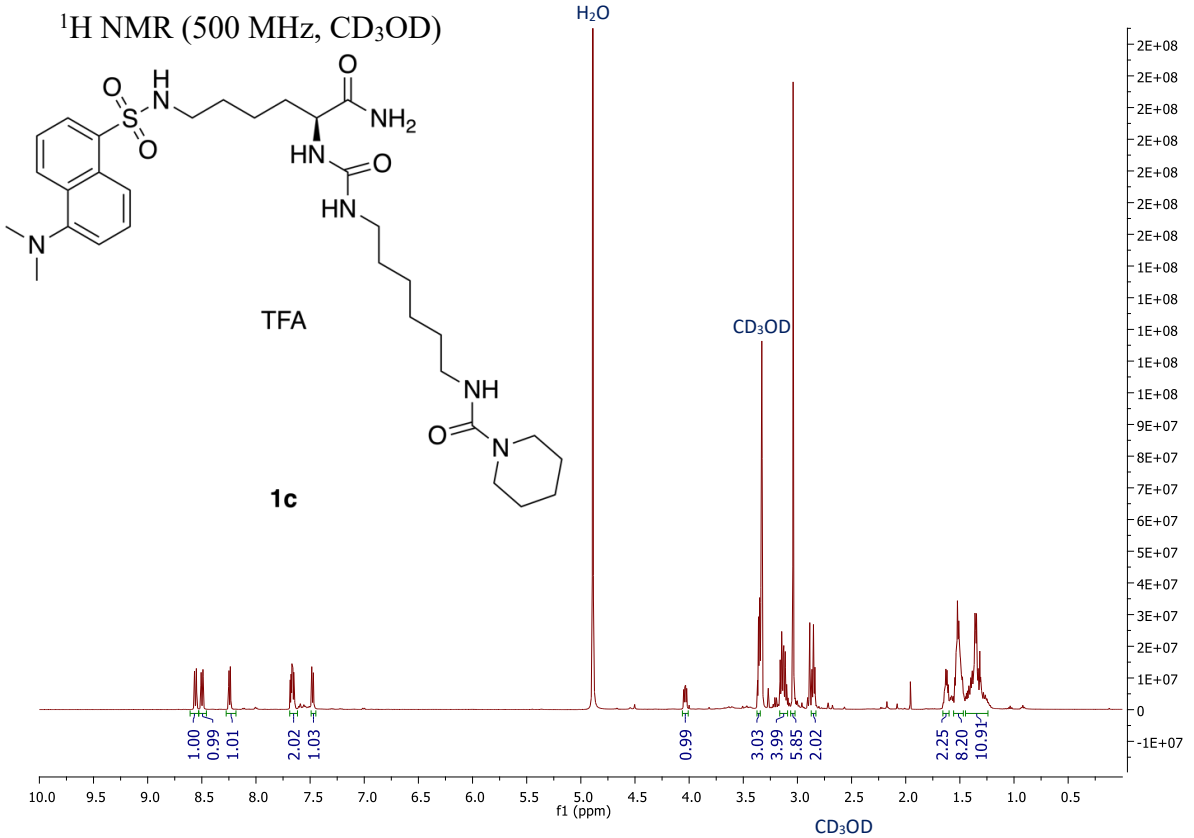


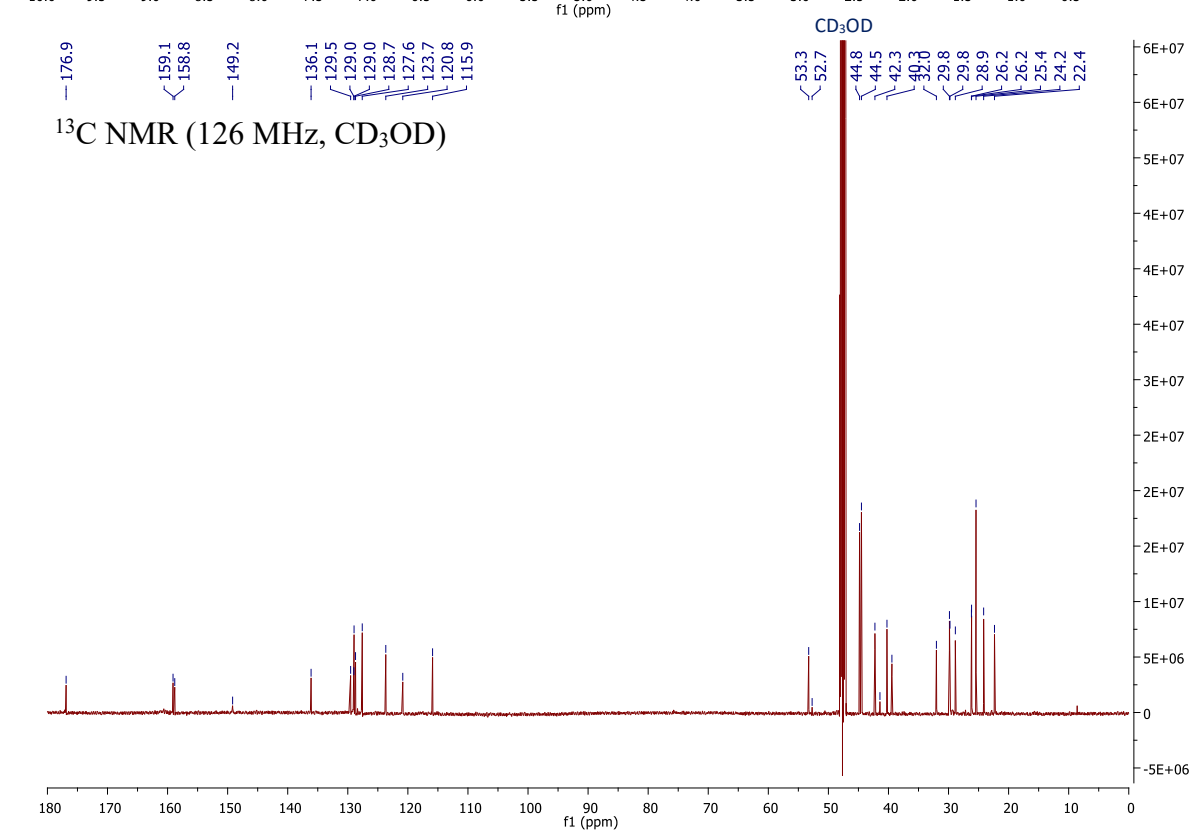
^{13}C NMR (126 MHz, CD_3OD)

(S)-*N*-(6-(3-(1-amino-6-((5-(dimethylamino)naphthalene)-1-sulfonamido)-1-oxohexan-2-yl)ureido)hexyl)piperidine-1-carboxamide trifluoroacetic acid (1c)

^{1}H NMR (500 MHz, CD_3OD)

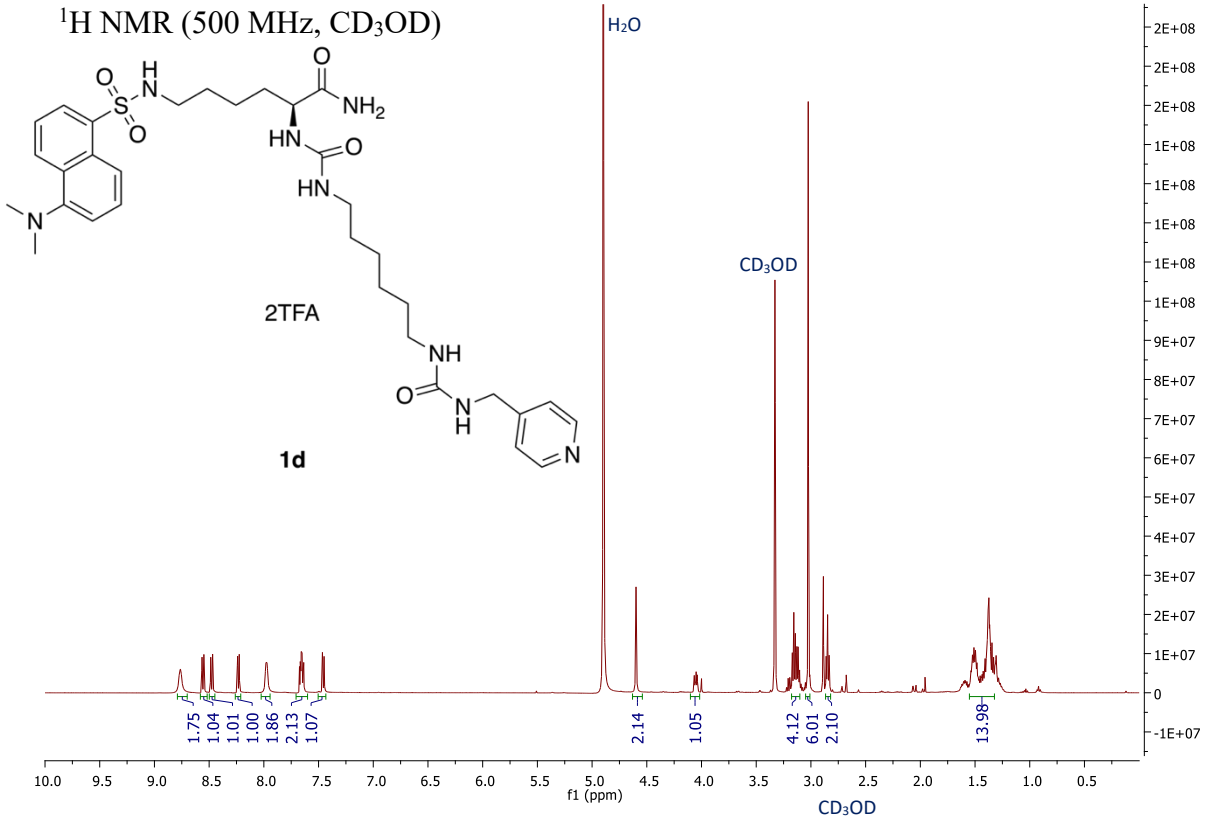


^{13}C NMR (126 MHz, CD_3OD)

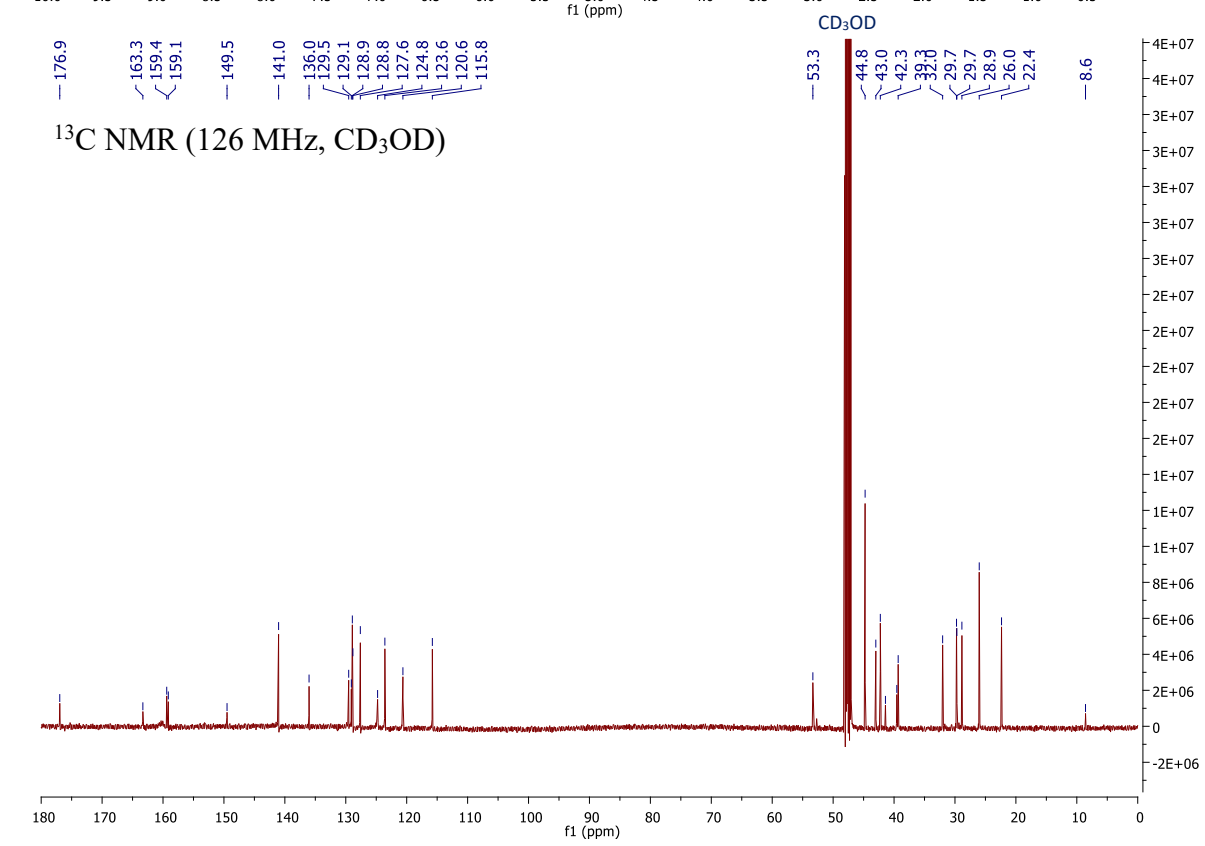


(*S*)-14-(4-((5-(dimethylamino)naphthalene)-1-sulfonamido)butyl)-3,12-dioxo-1-(pyridin-4-yl)-2,4,11,13-tetraazapentadecan-15-amide trifluoroacetic acid (1d)

^{1}H NMR (500 MHz, CD_3OD)

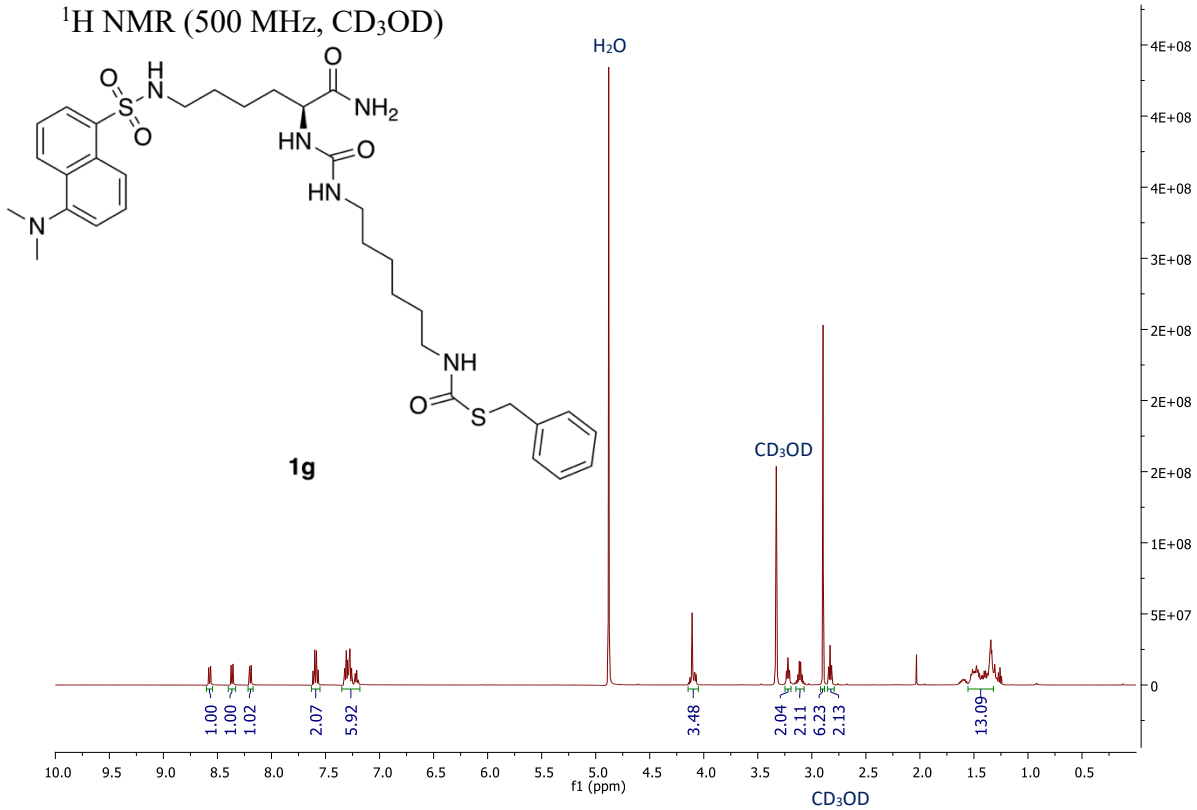


^{13}C NMR (126 MHz, CD_3OD)

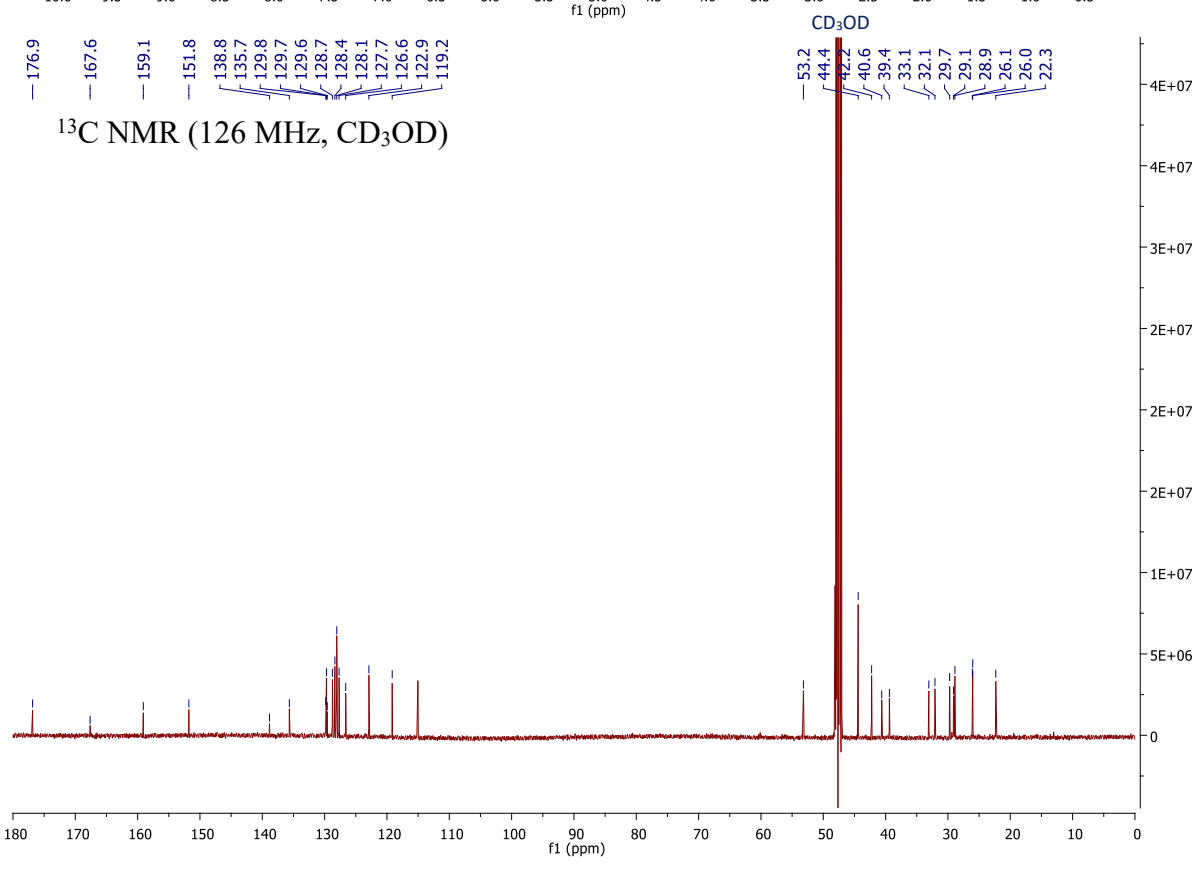


***S*-benzyl (*S*)-(6-(3-(1-amino-6-((5-(dimethylamino)naphthalene)-1-sulfonamido)-1-oxohexan-2-yl)ureido)hexyl)carbamothioate (1g)**

^{1}H NMR (500 MHz, CD_3OD)

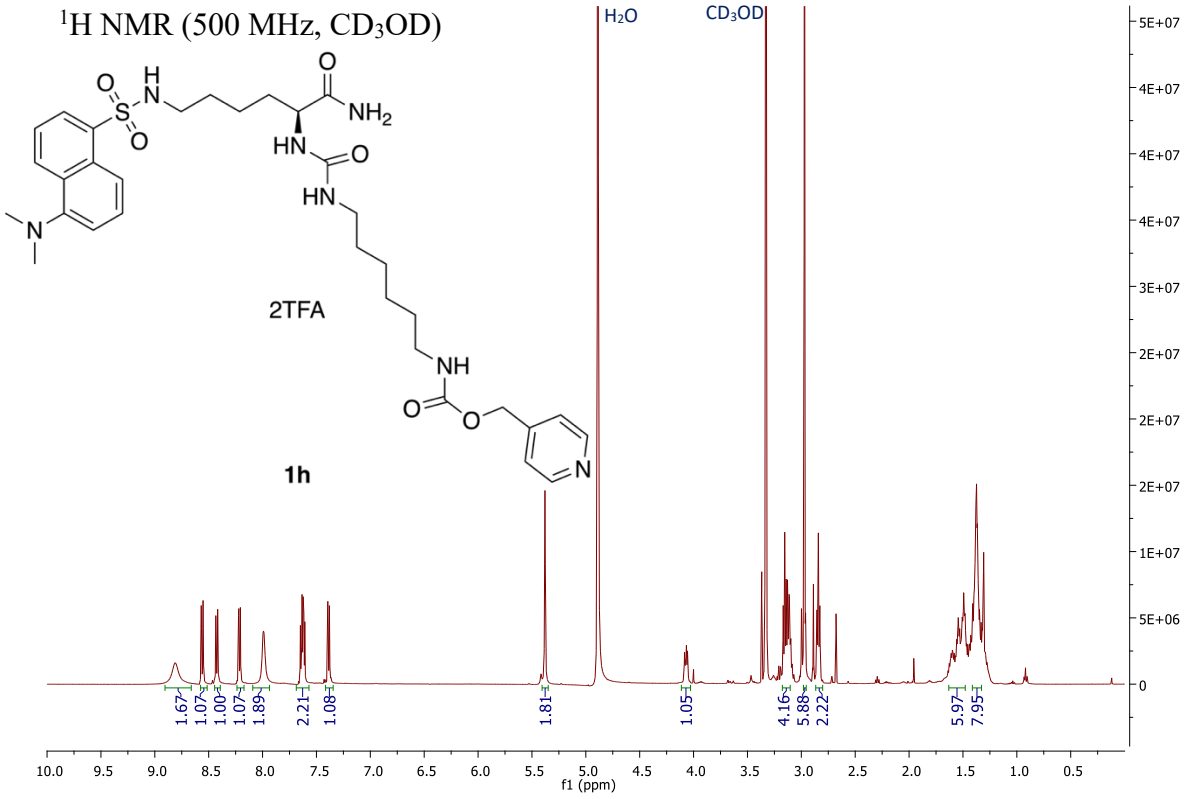


^{13}C NMR (126 MHz, CD_3OD)

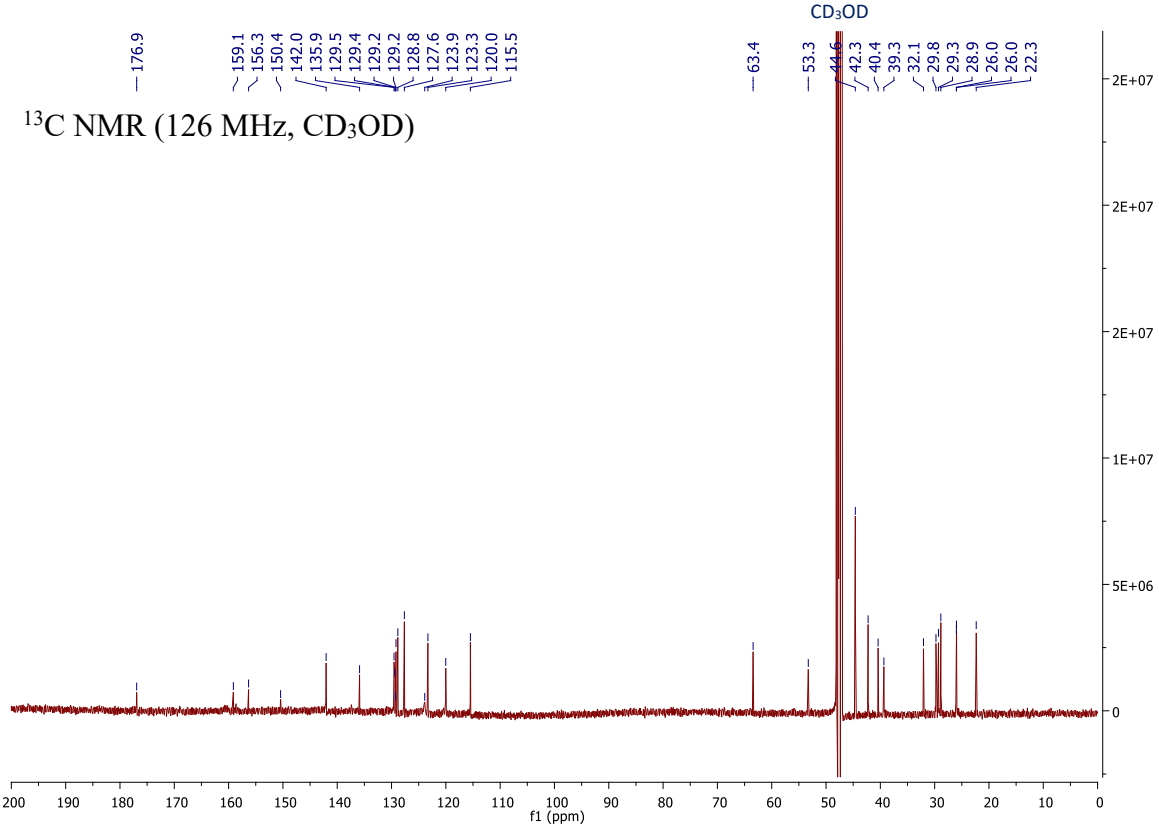


Pyridin-4-ylmethyl (S)-(6-(3-(1-amino-6-((5-(dimethylamino)naphthalene)-1-sulfonami-do)-1-oxohexan-2-yl)ureido)hexyl)carbamate trifluoroacetic acid (1h)

^{1}H NMR (500 MHz, CD_3OD)

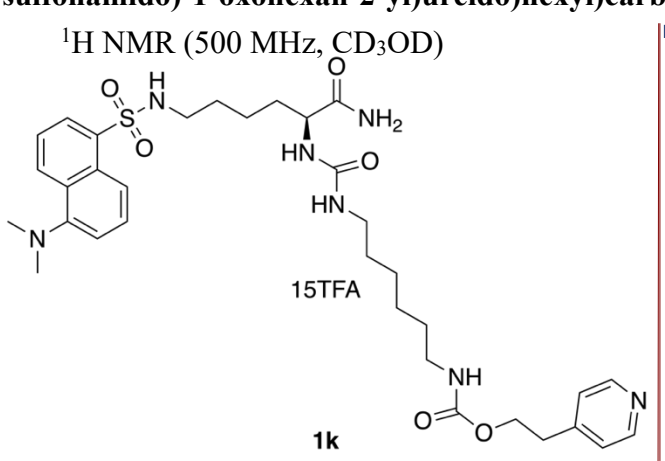


^{13}C NMR (126 MHz, CD_3OD)

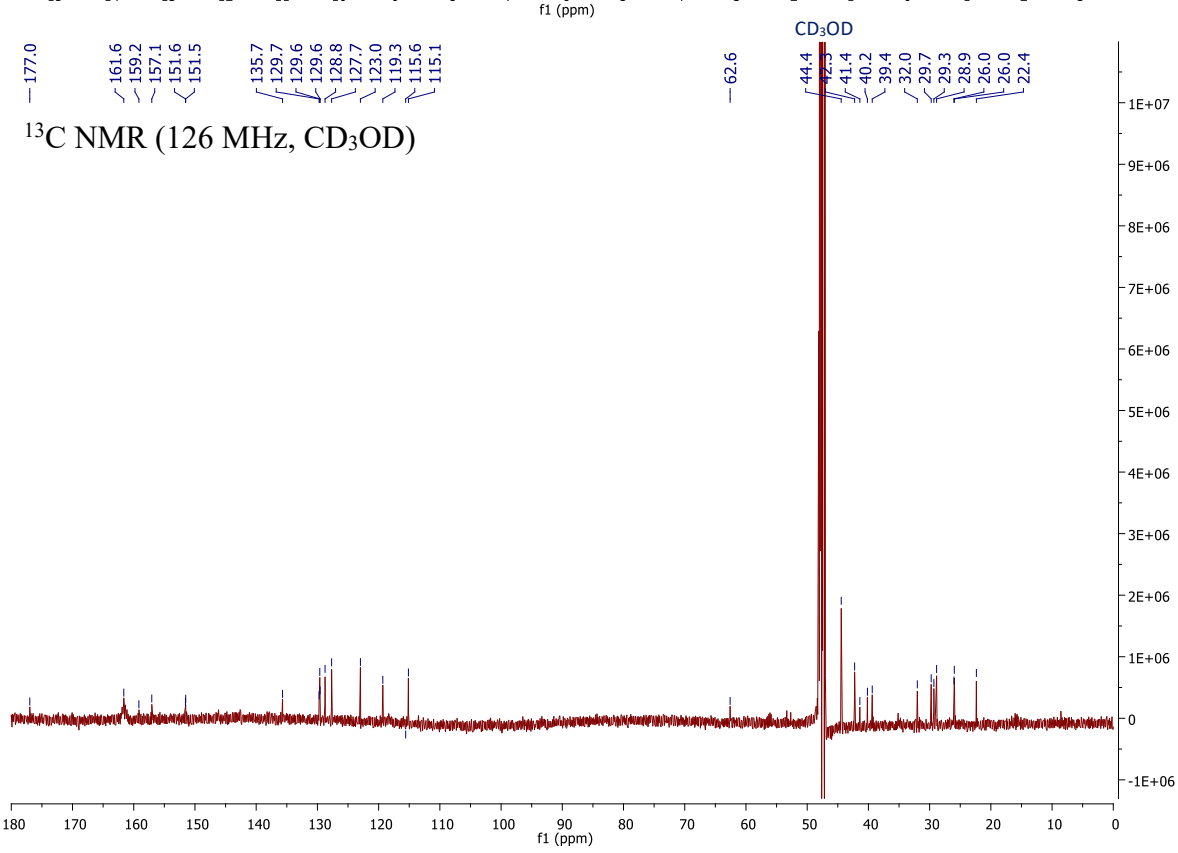


2-(pyridin-4-yl)ethyl (*S*)-(6-(3-(1-amino-6-((5-(dimethylamino)naphthalene)-1-sulfonamido)-1-oxohexan-2-yl)ureido)hexyl)carbamate trifluoroacetic acid (1k)

^{1}H NMR (500 MHz, CD_3OD)

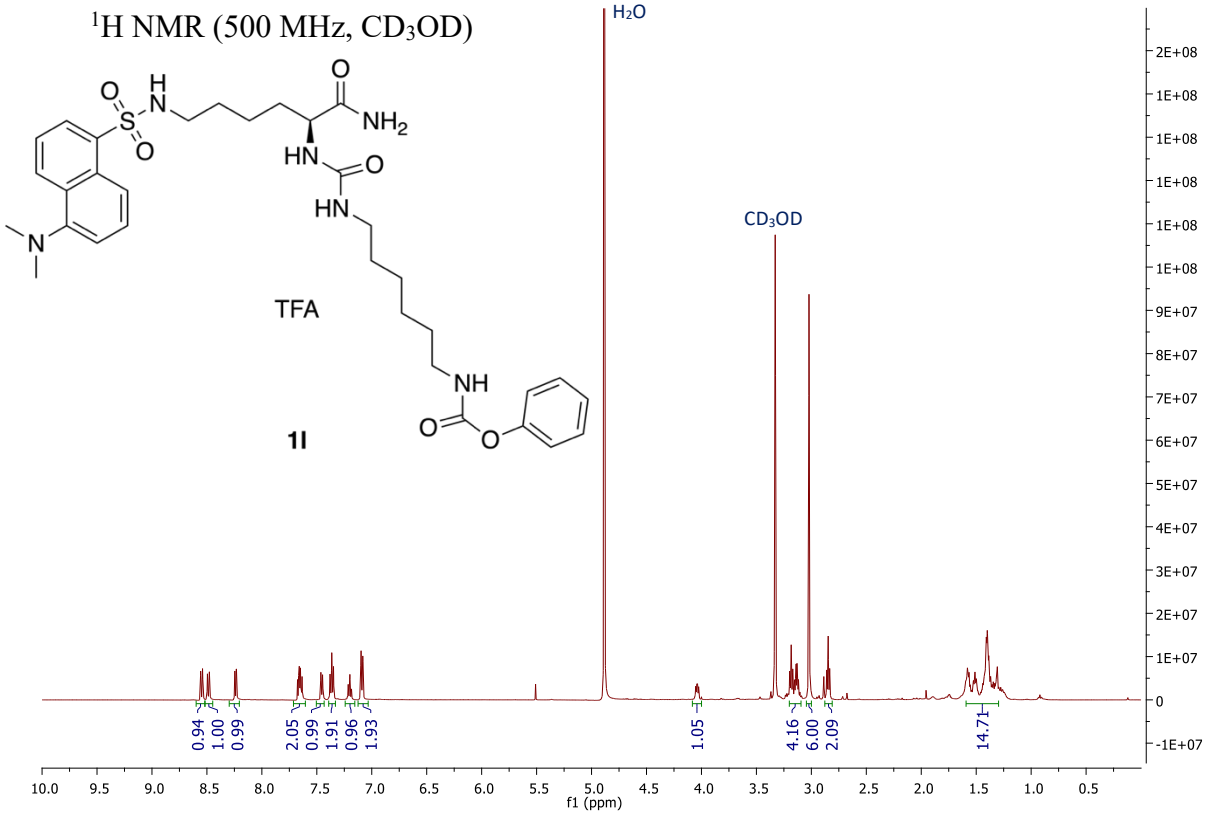


^{13}C NMR (126 MHz, CD_3OD)

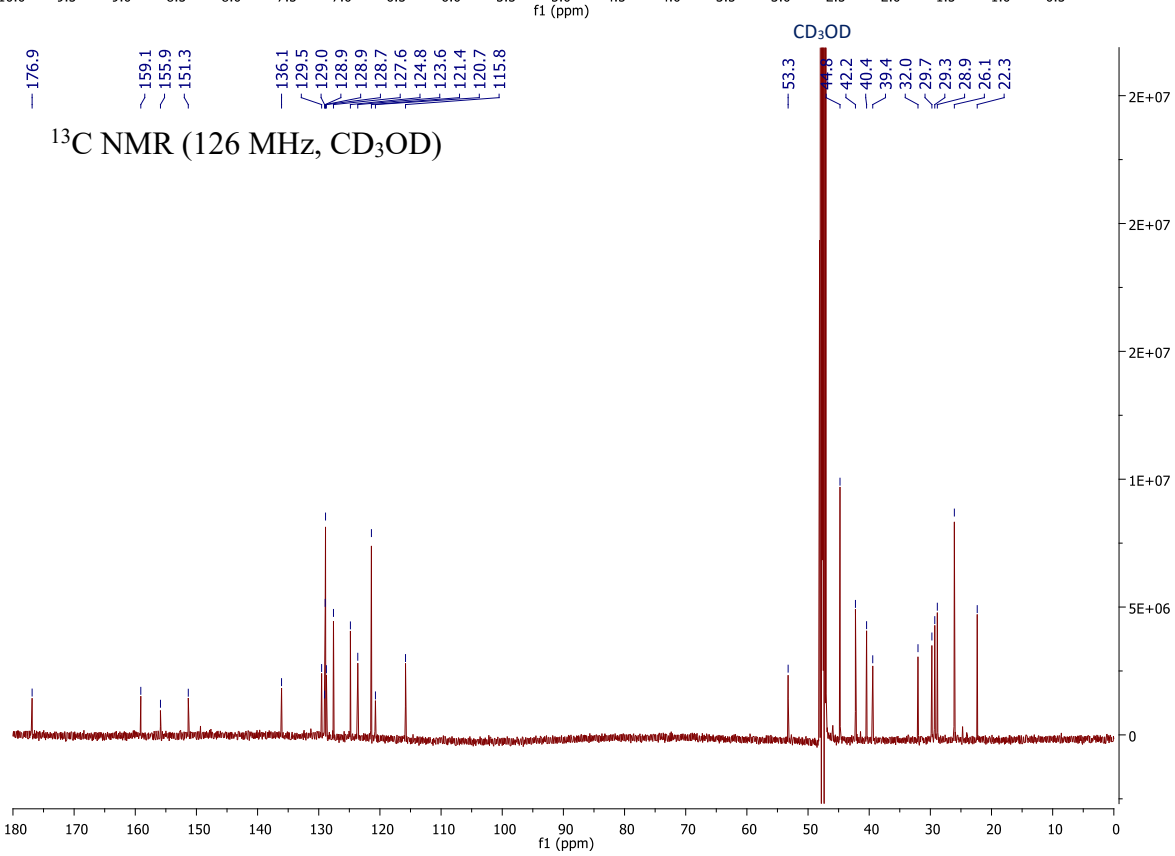


phenyl (*S*)-(6-(3-(1-amino-6-((5-(dimethylamino)naphthalene)-1-sulfonamido)-1-oxohexan-2-yl)ureido)hexyl)carbamate trifluoroacetic acid trifluoroacetic acid (1l)

^{1}H NMR (500 MHz, CD_3OD)

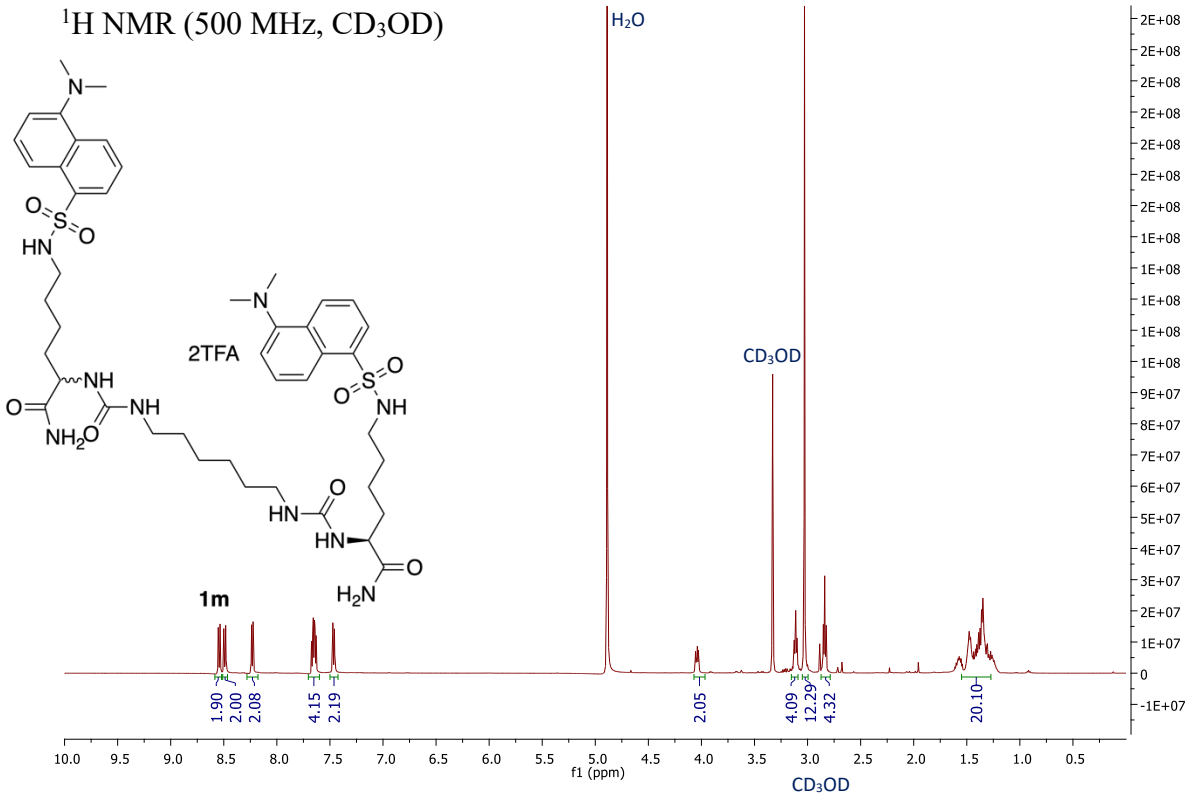


^{13}C NMR (126 MHz, CD_3OD)

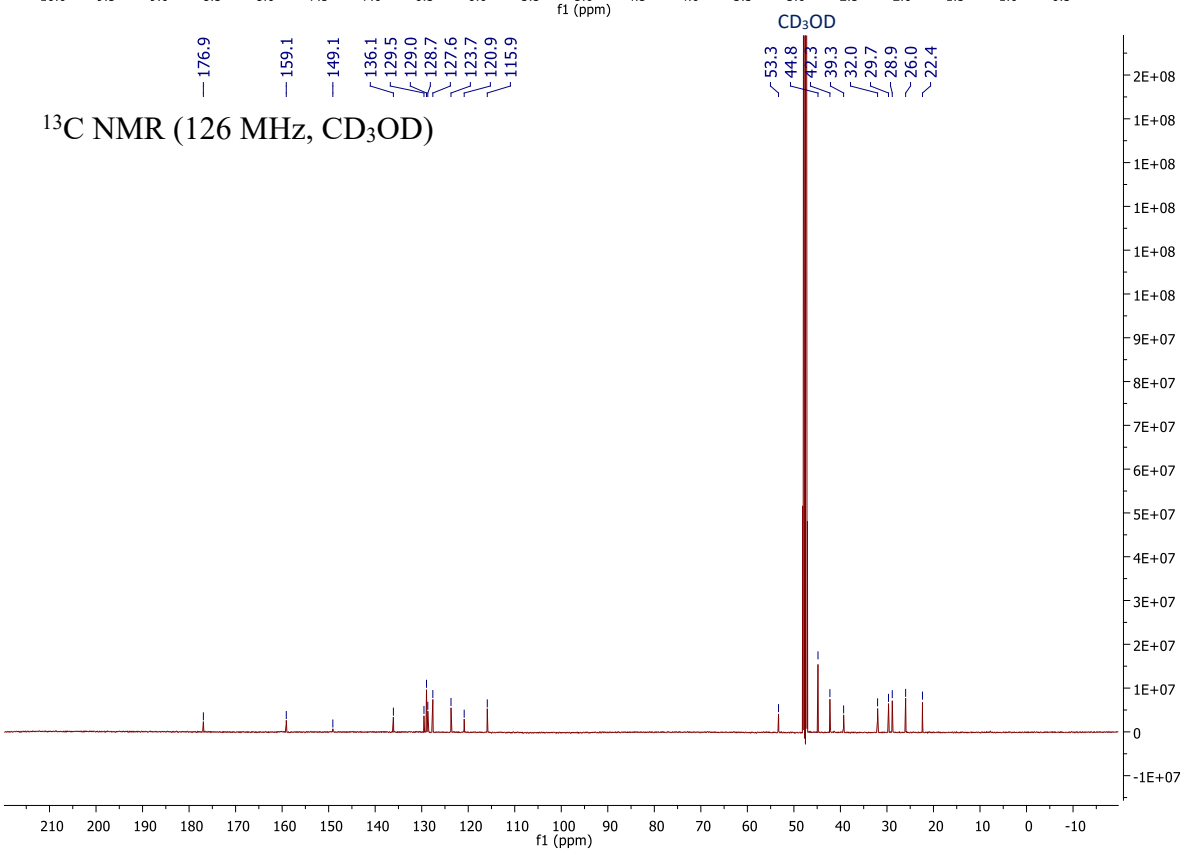


(*2S*, *15S*)-2,15-bis(4-((5-(dimethylamino)naphthalene)-1-sulfonamido)butyl)-4,13-dioxo-3,5,12,14-tetraazahexadecanediamide trifluoracetic acid (1m)

^{1}H NMR (500 MHz, CD_3OD)

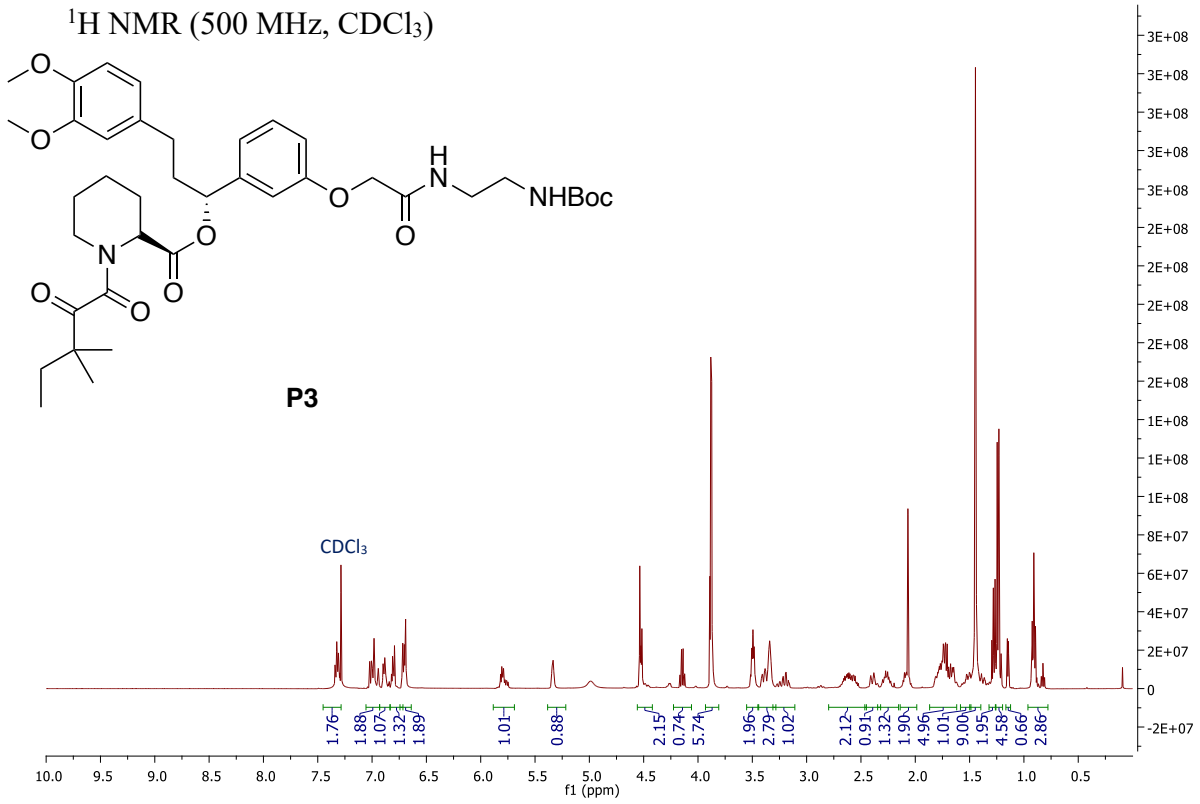


^{13}C NMR (126 MHz, CD_3OD)

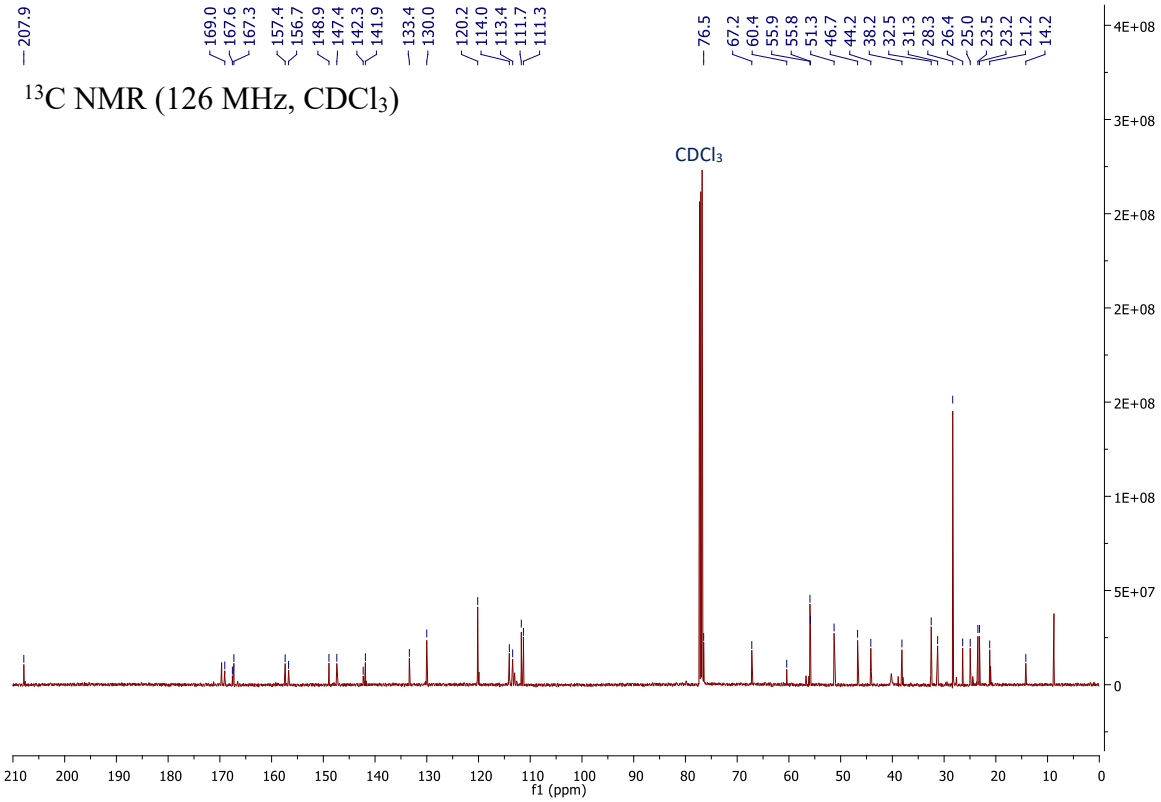


(*R*)-1-(3-(2-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*R*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (P3)

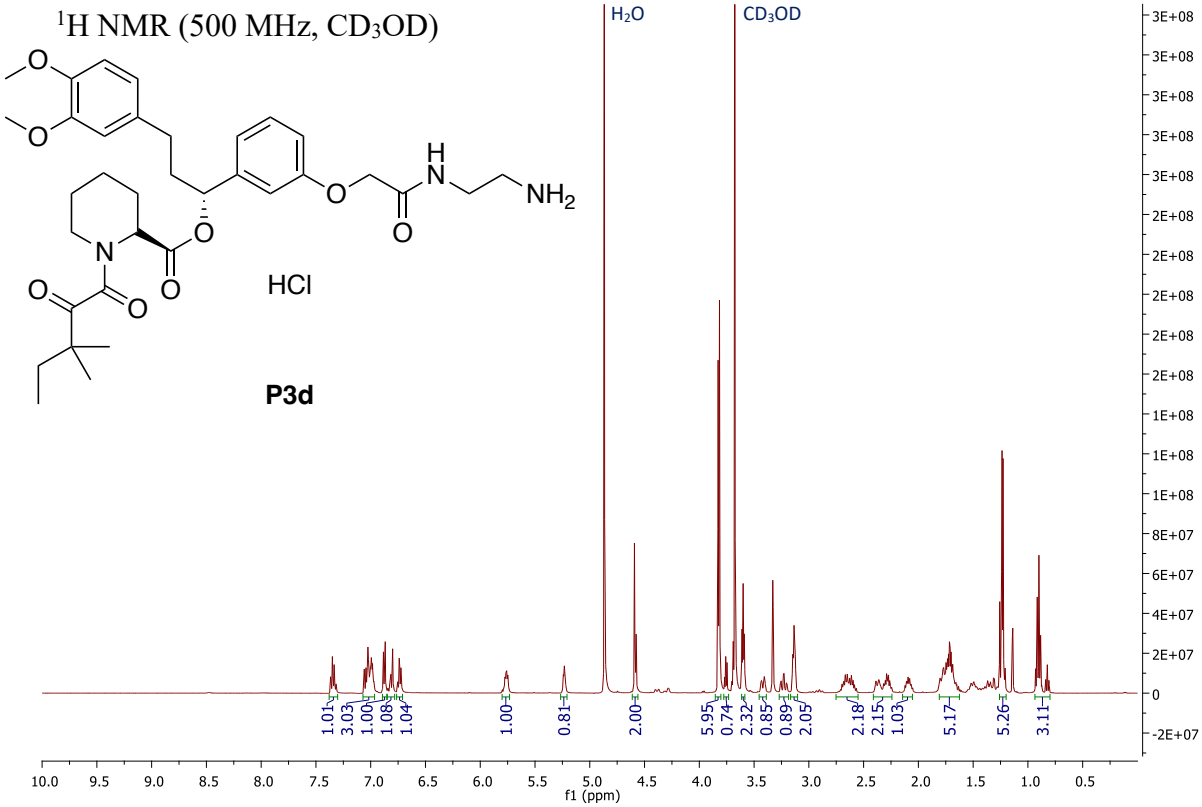
^{1}H NMR (500 MHz, $CDCl_3$)

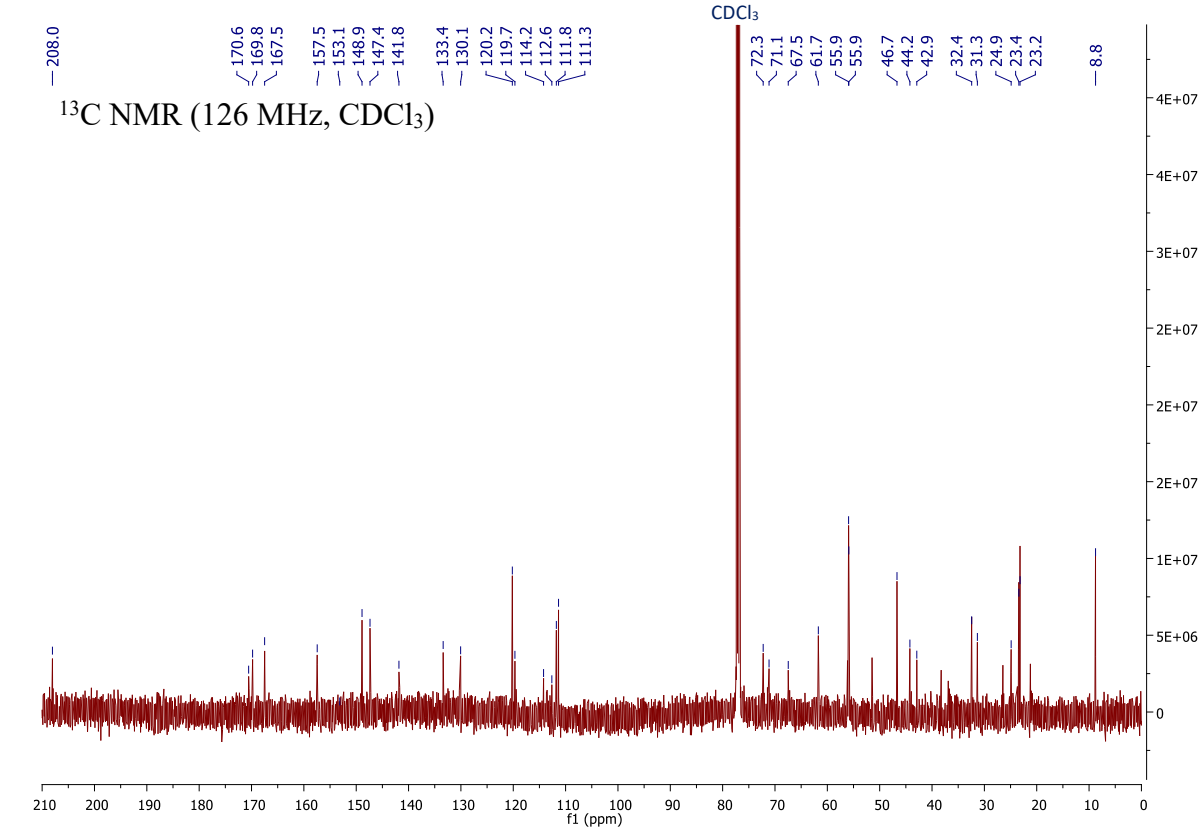


^{13}C NMR (126 MHz, $CDCl_3$)



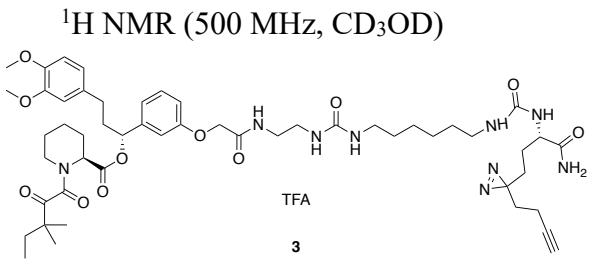
(*R*)-1-(3-(2-((2-aminoethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*R*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate hydrochloride (P3d)



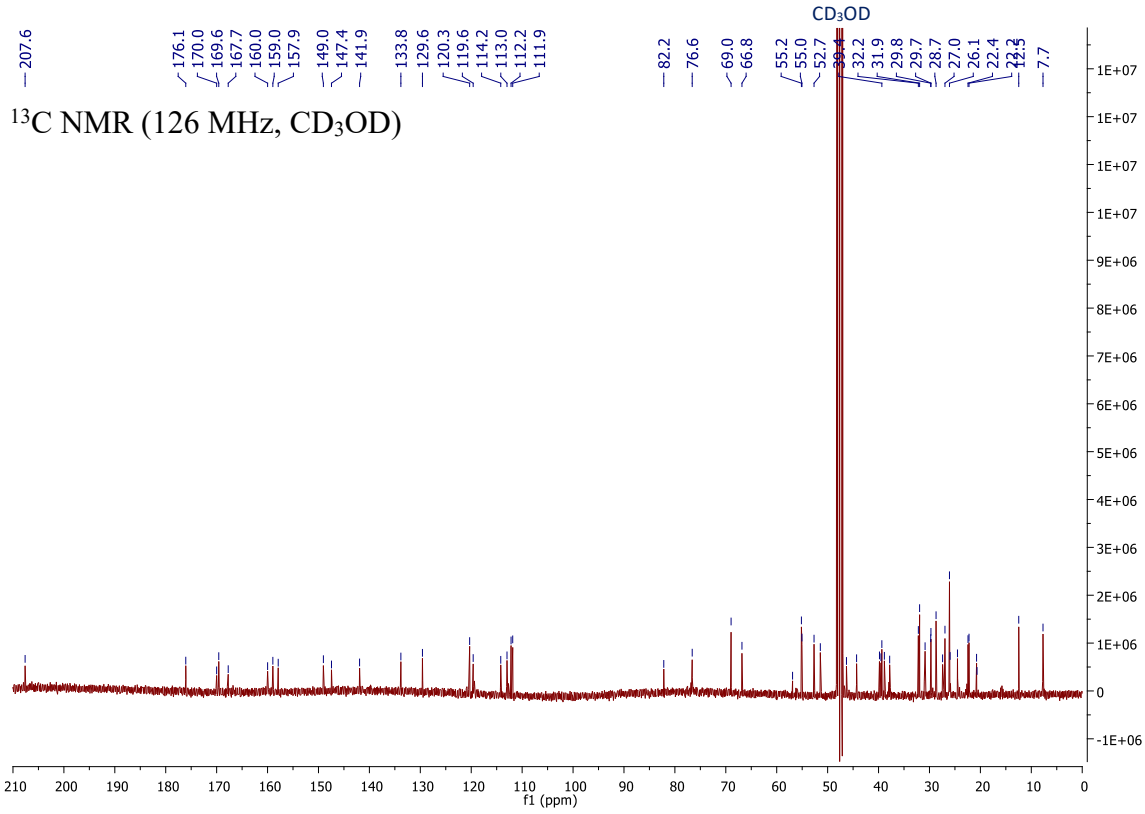


(*R*)-1-(3-(((*S*)-20-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)-18-carbamoyl-2,7,16-trioxo-3,6,8,15,17-pentaazaicosyl)oxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate trifluoroacetic acid (3)

^{1}H NMR (500 MHz, CD_3OD)

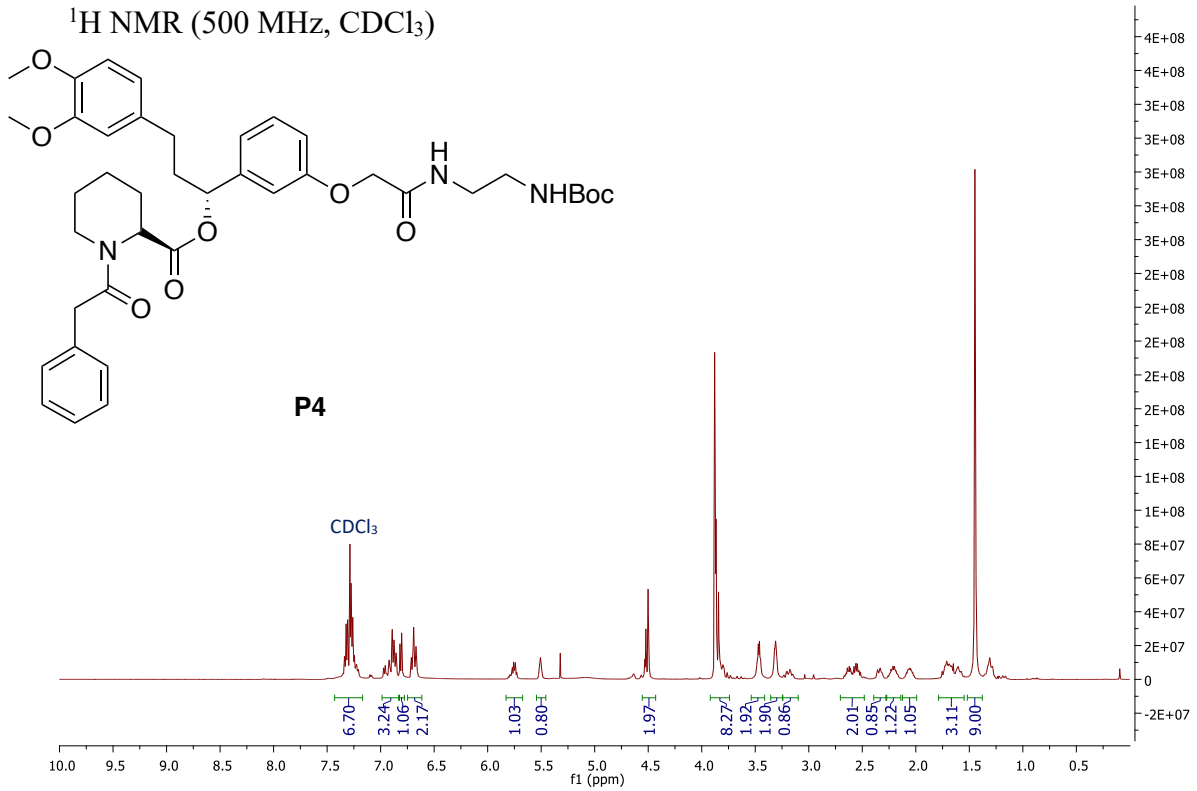


^{13}C NMR (126 MHz, CD_3OD)

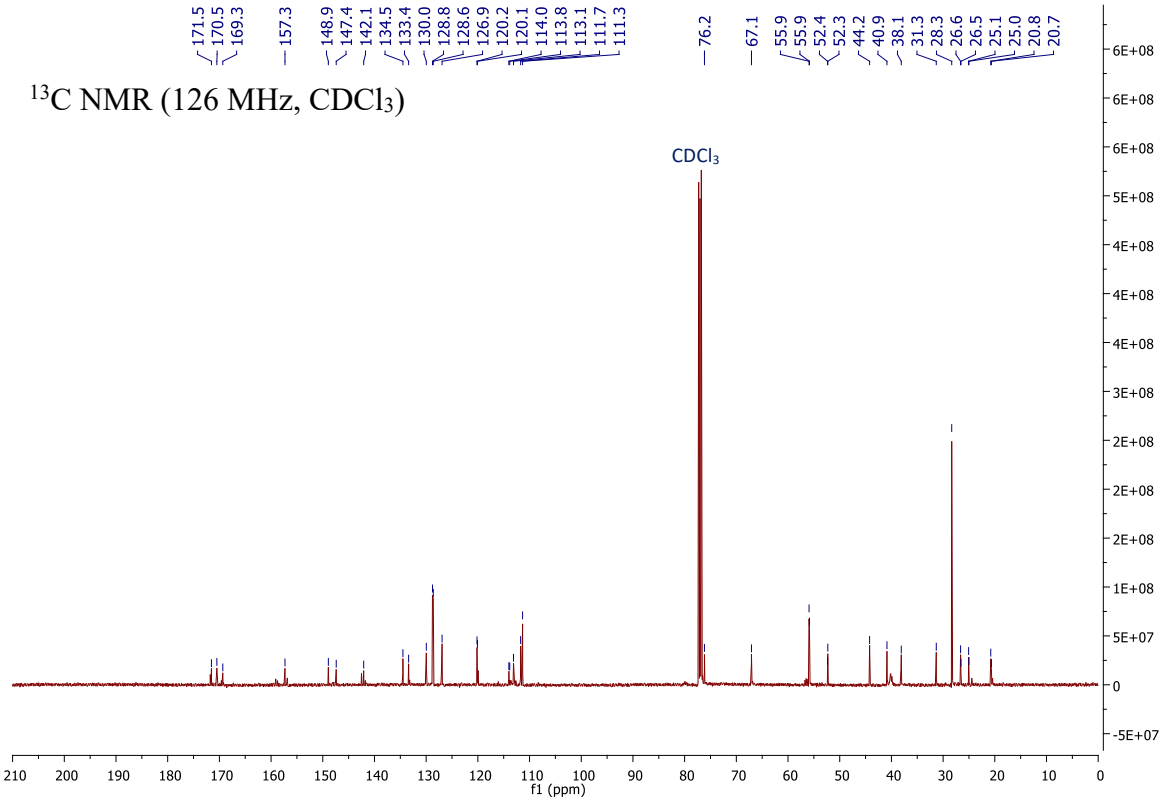


(*R*)-1-(3-(2-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*R*)-1-(2-phenylacetyl)piperidine-2-carboxylate (P4)

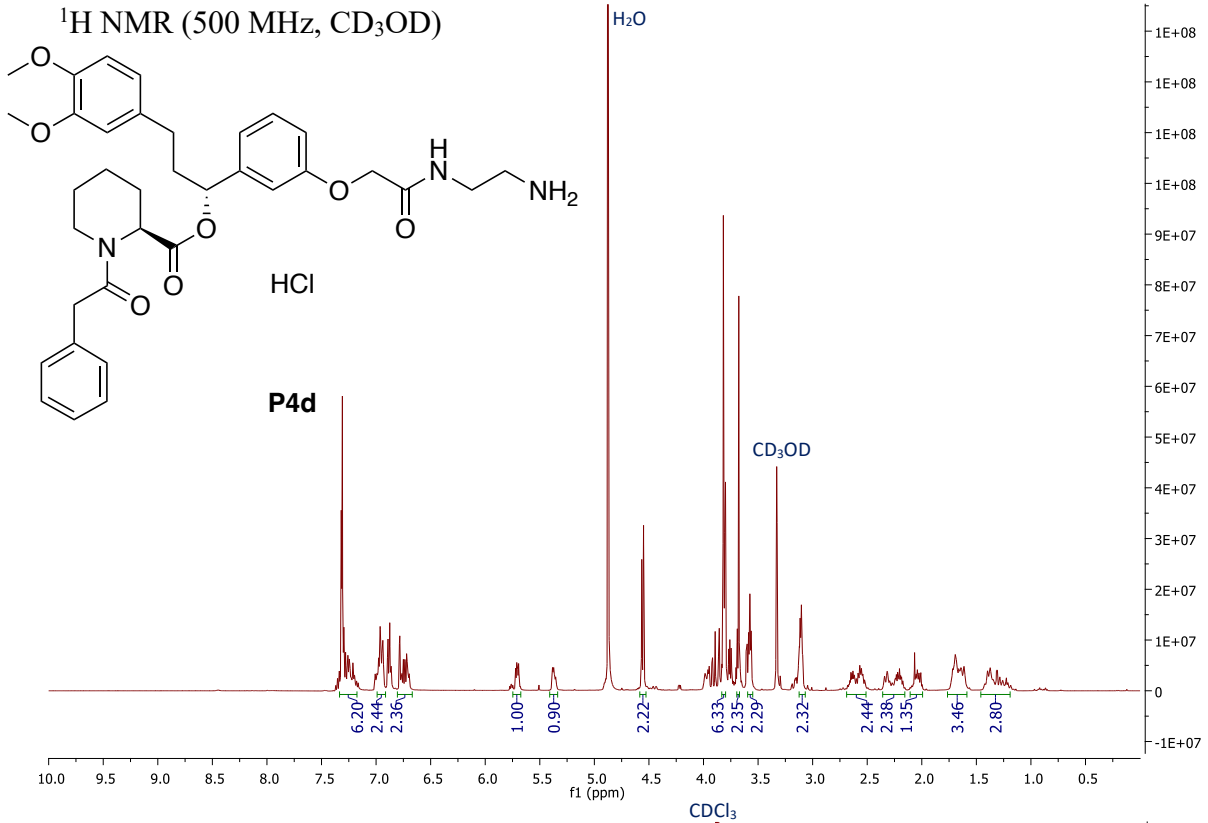
^{1}H NMR (500 MHz, $CDCl_3$)

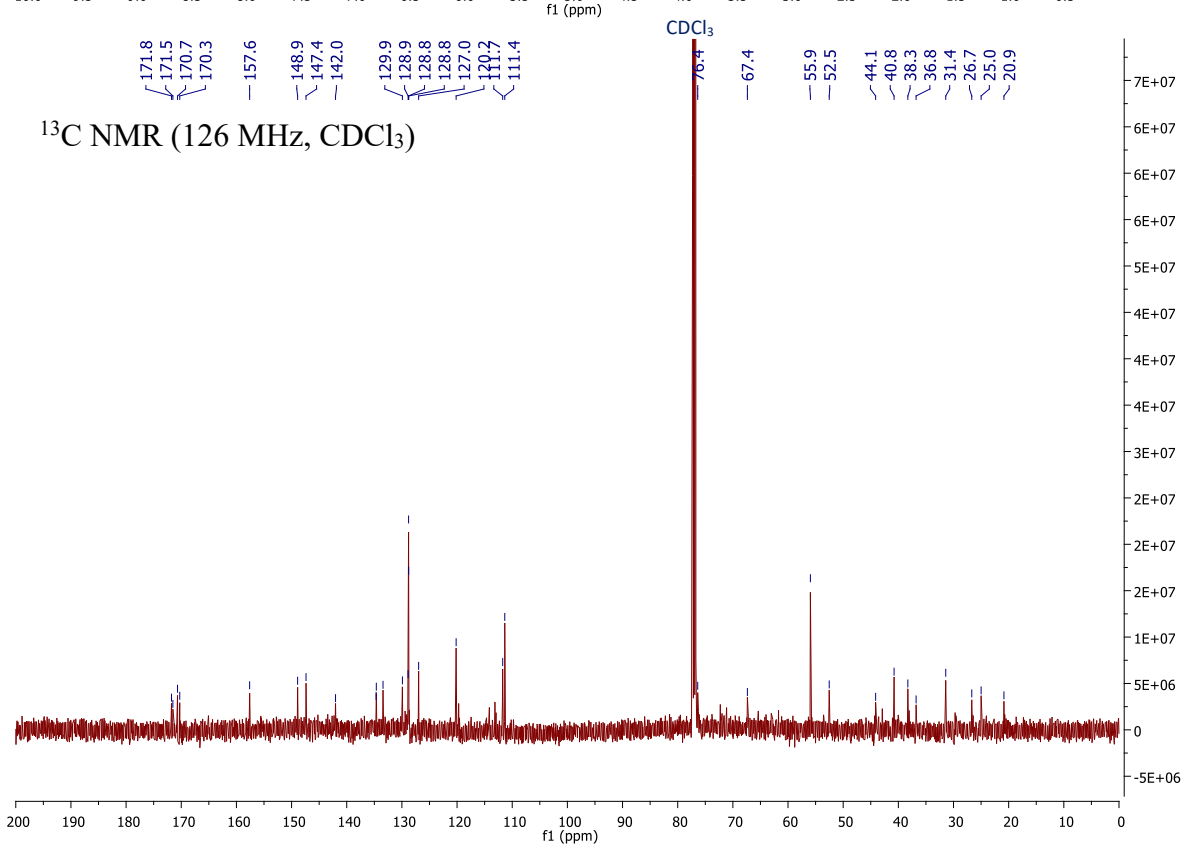


^{13}C NMR (126 MHz, $CDCl_3$)



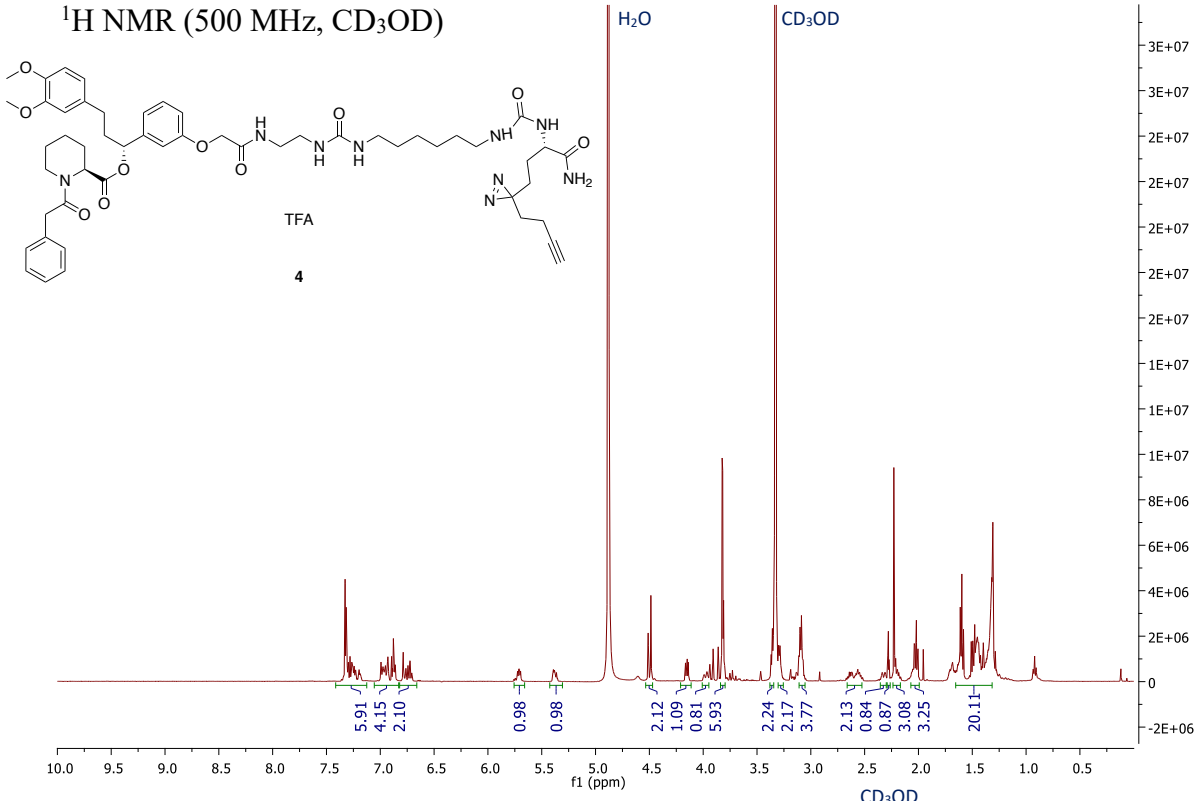
(*R*)-1-(3-(2-((2-aminoethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*R*)-1-(2-phenylacetyl)piperidine-2-carboxylate hydrochloride (P4d)



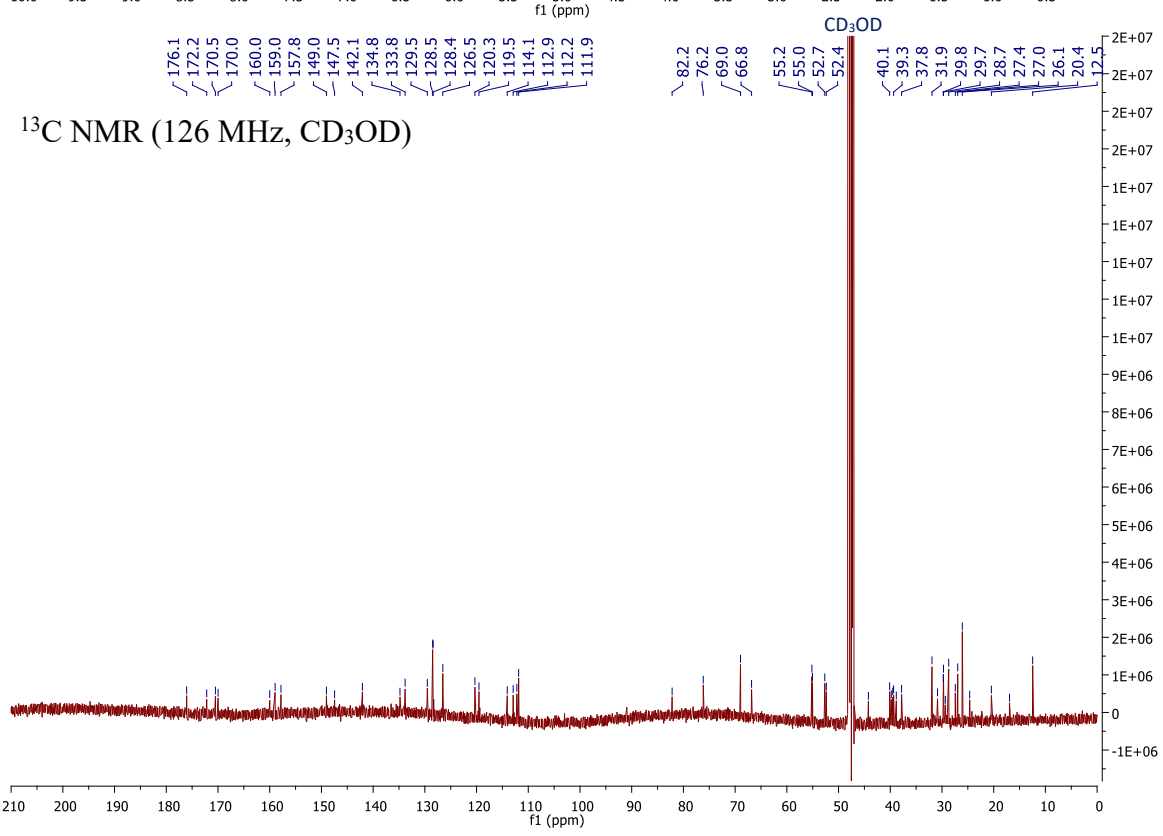


(*R*)-1-(3-(((*S*)-20-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)-18-carbamoyl-2,7,16-trioxo 3,6,8,15,17-pentaazaicosyl)oxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-1-(2-phenylacetyl)piperidine-2-carboxylate trifluoroacetic acid (4)

^{1}H NMR (500 MHz, CD_3OD)

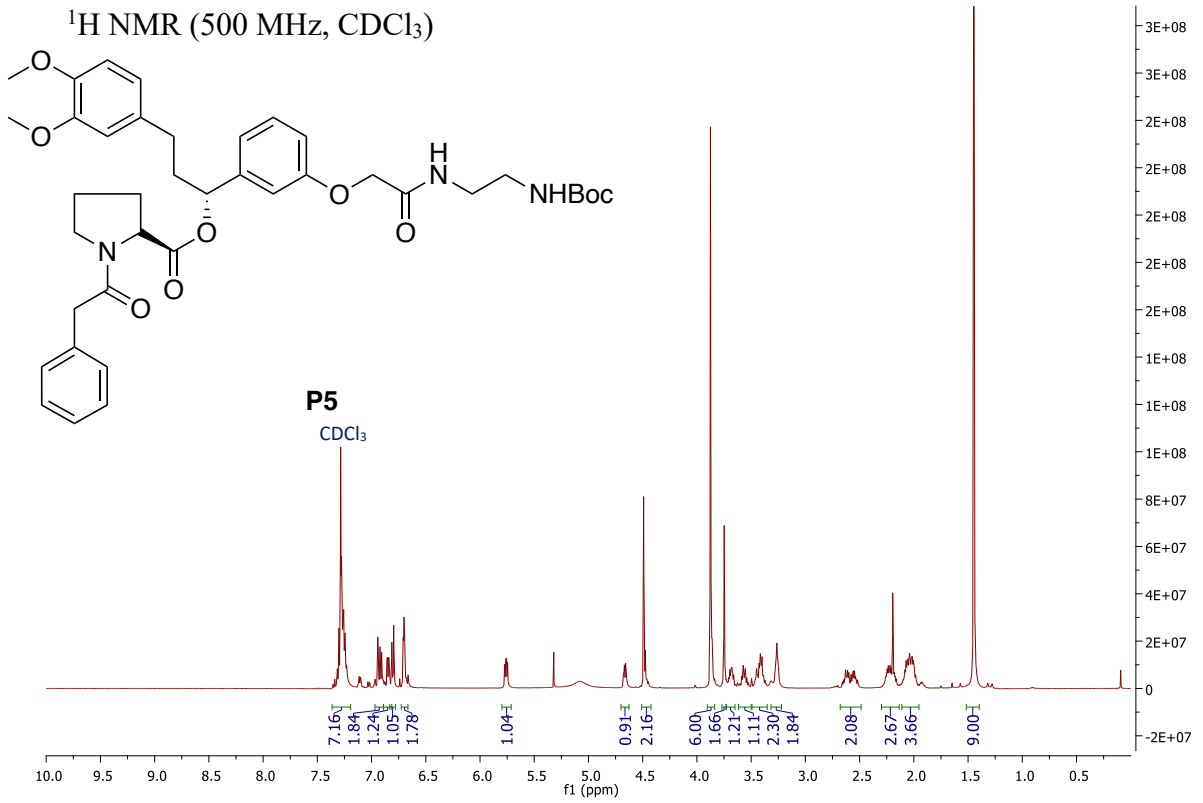


^{13}C NMR (126 MHz, CD_3OD)

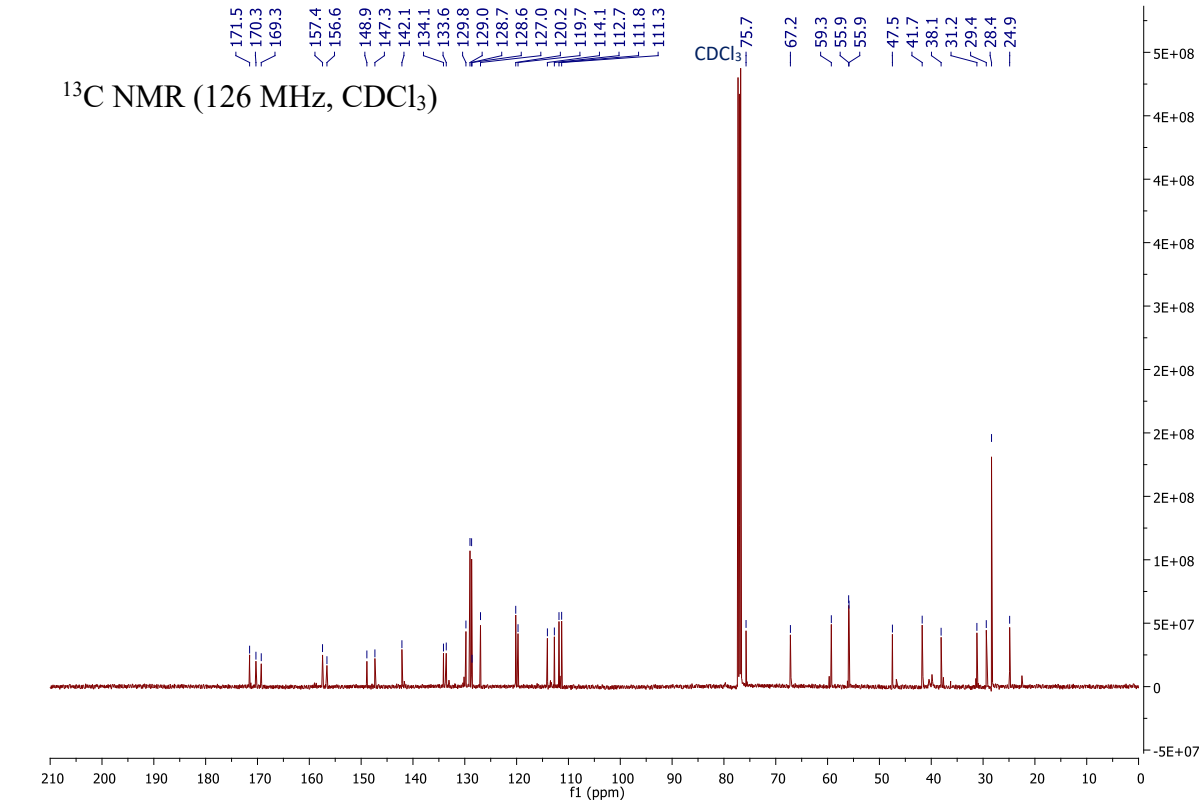


(*R*)-1-(3-(2-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (2-phenylacetyl)-*L*-prolinate (P5)

^{1}H NMR (500 MHz, $CDCl_3$)

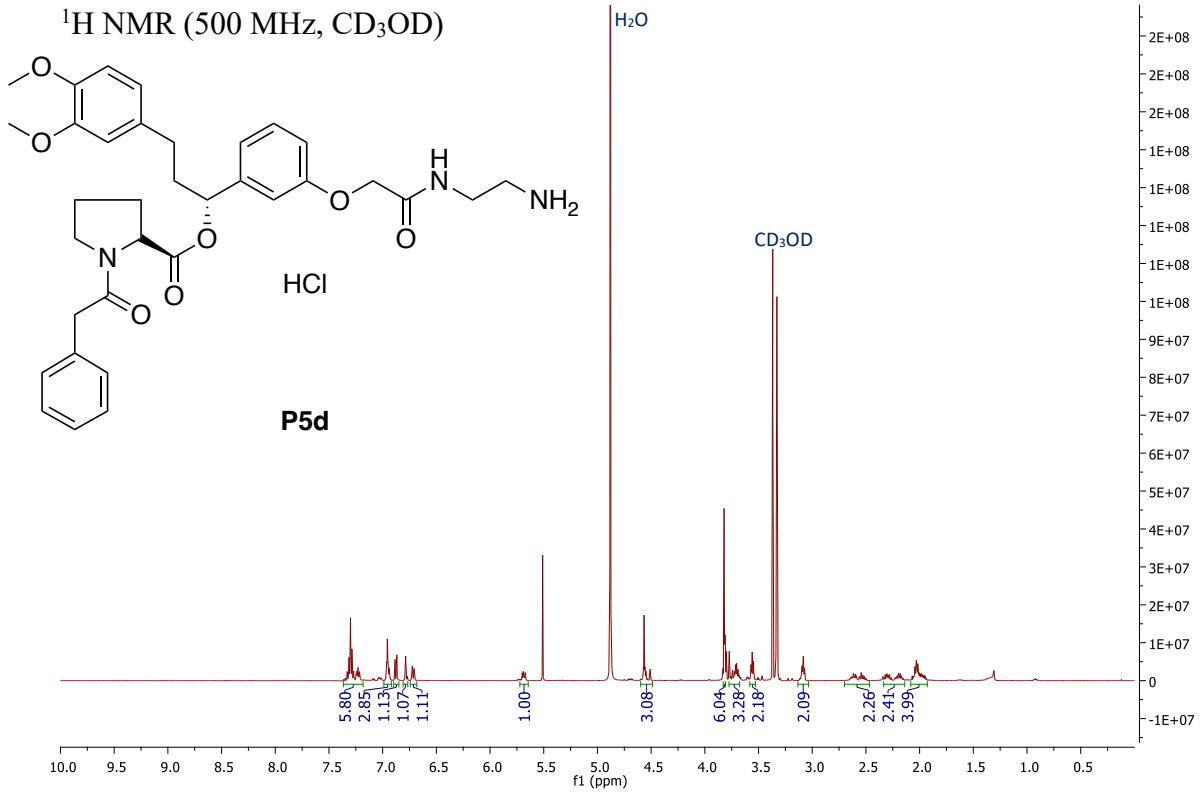


^{13}C NMR (126 MHz, $CDCl_3$)

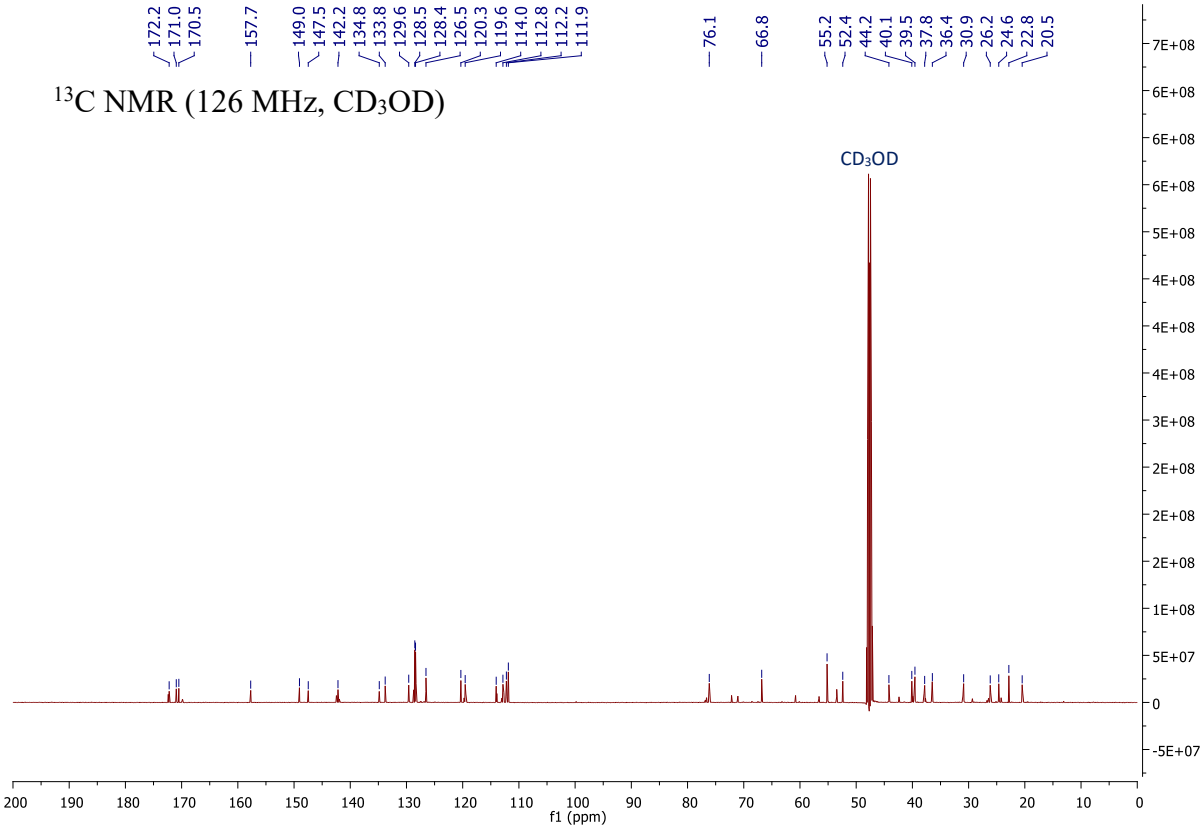


(*R*)-1-(3-(2-((2-aminoethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (2-phenylacetyl)-*L*-prolinate hydrochloride (P5d)

^{1}H NMR (500 MHz, CD_3OD)

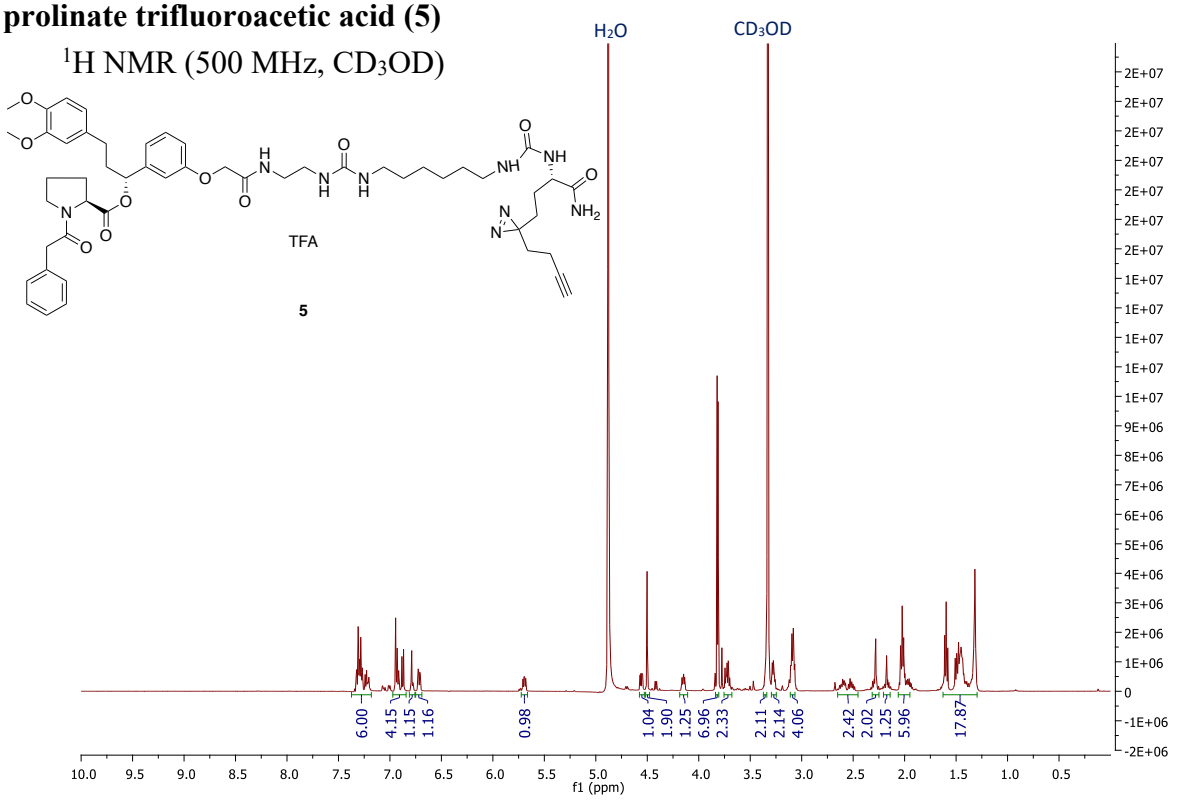


^{13}C NMR (126 MHz, CD_3OD)

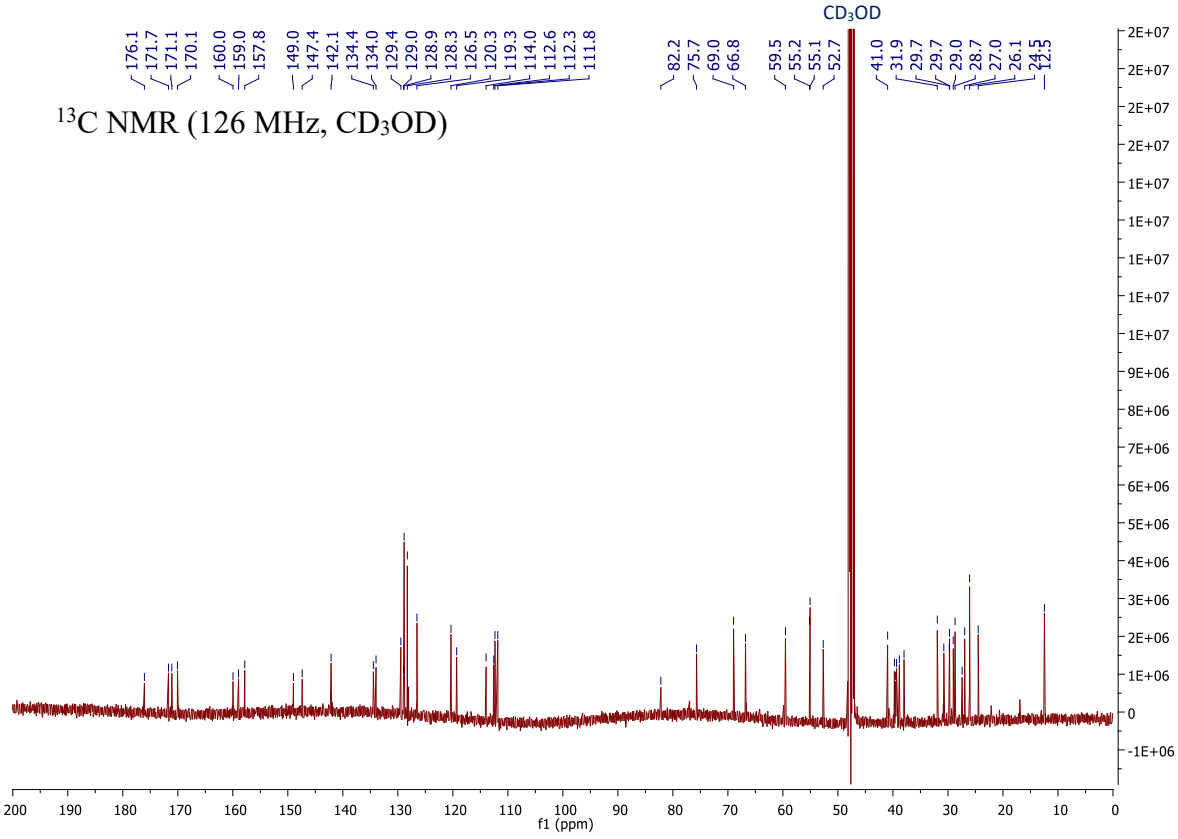


(*R*)-1-(3-(((*S*)-20-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)-18-carbamoyl-2,7,16-trioxo-3,6,8,15,17-pentaazaicosyl)oxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (2-phenylacetyl)-*L*-prolinate trifluoroacetic acid (5)

^{1}H NMR (500 MHz, CD_3OD)

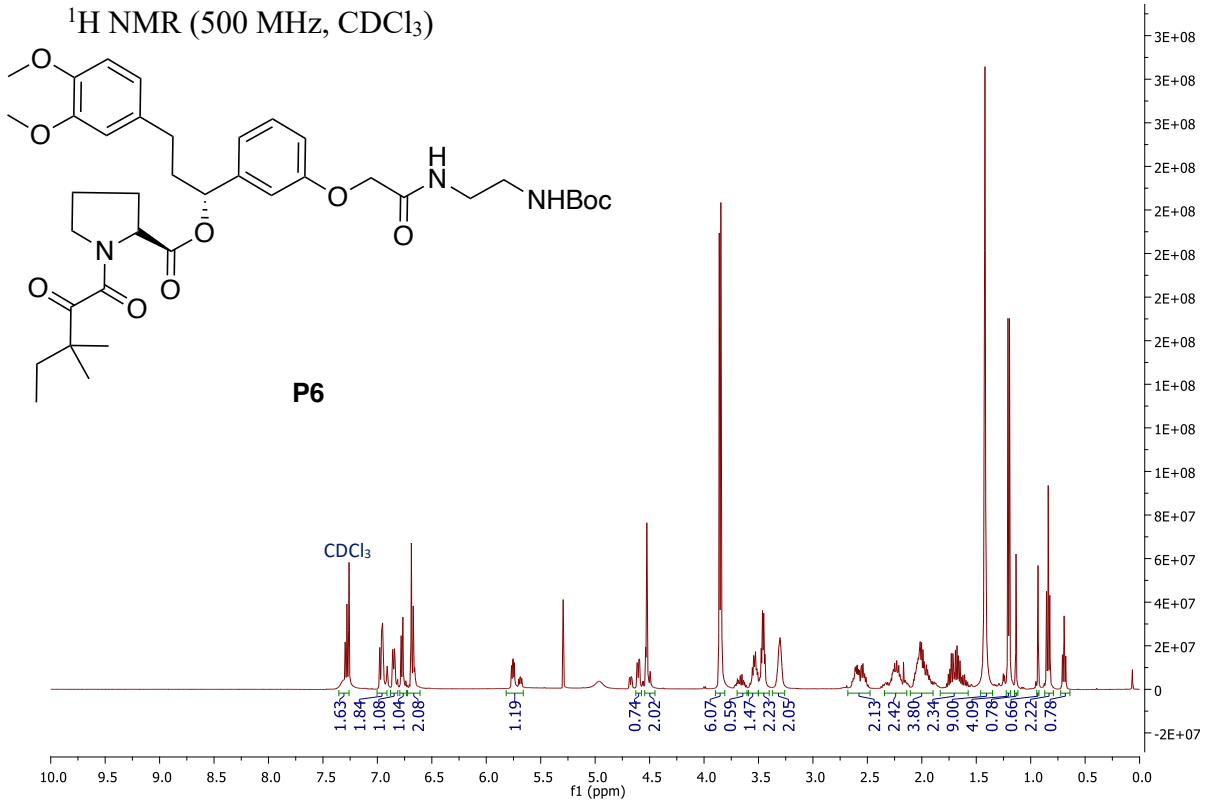


^{13}C NMR (126 MHz, CD_3OD)

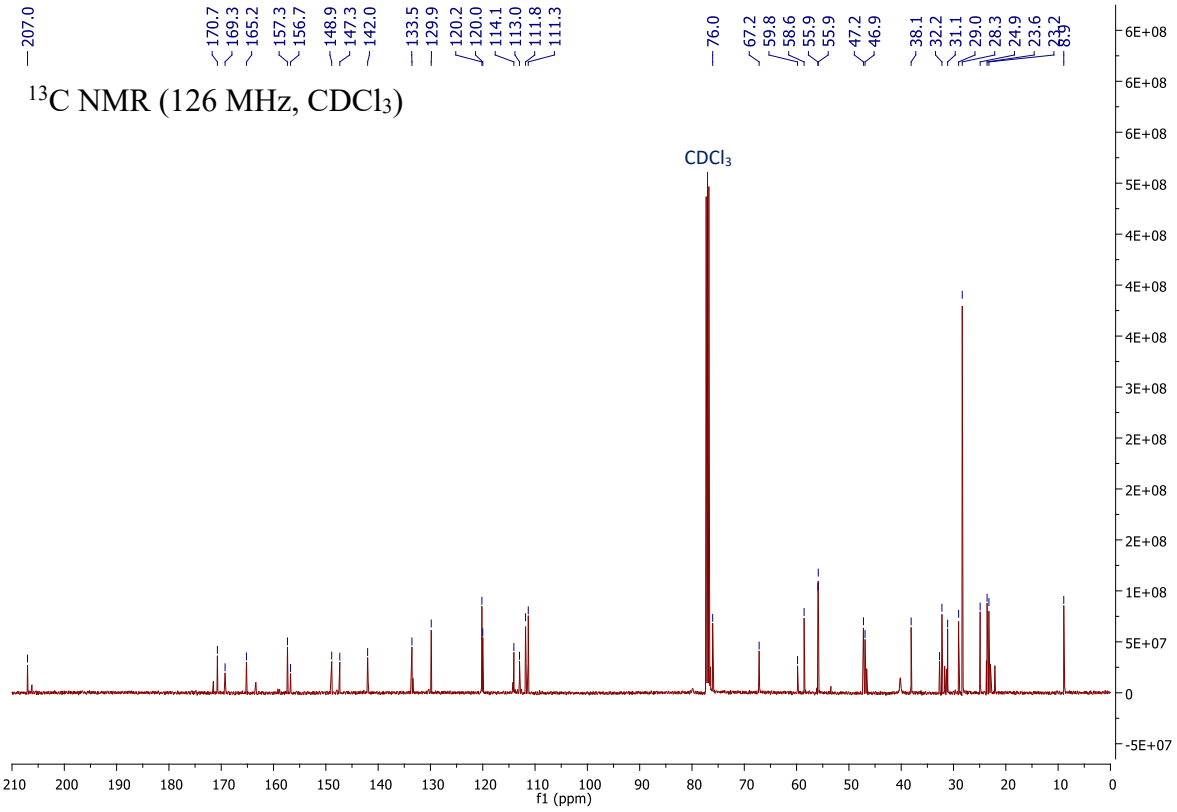


(*R*)-1-(3-(2-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (3,3-dimethyl-2-oxopentanoyl)-*D*-prolinate (P6)

^{1}H NMR (500 MHz, $CDCl_3$)

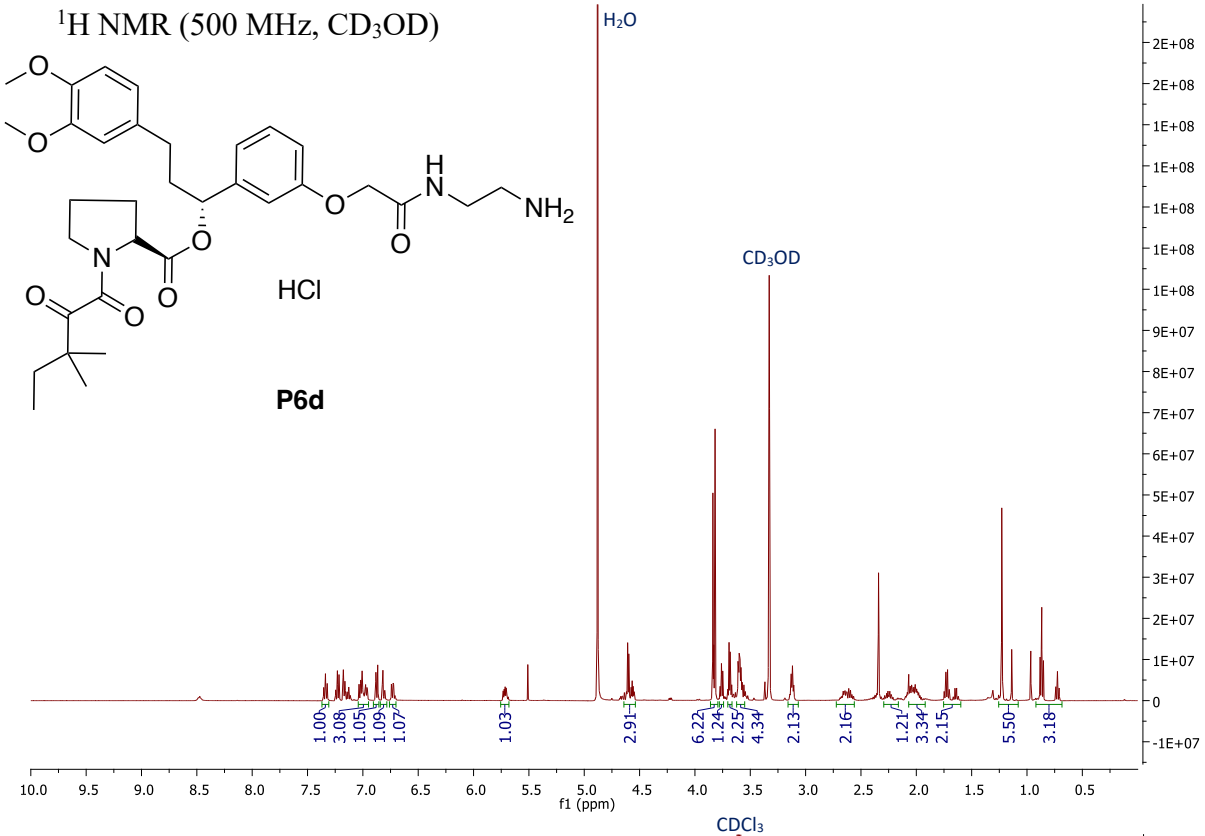


^{13}C NMR (126 MHz, $CDCl_3$)

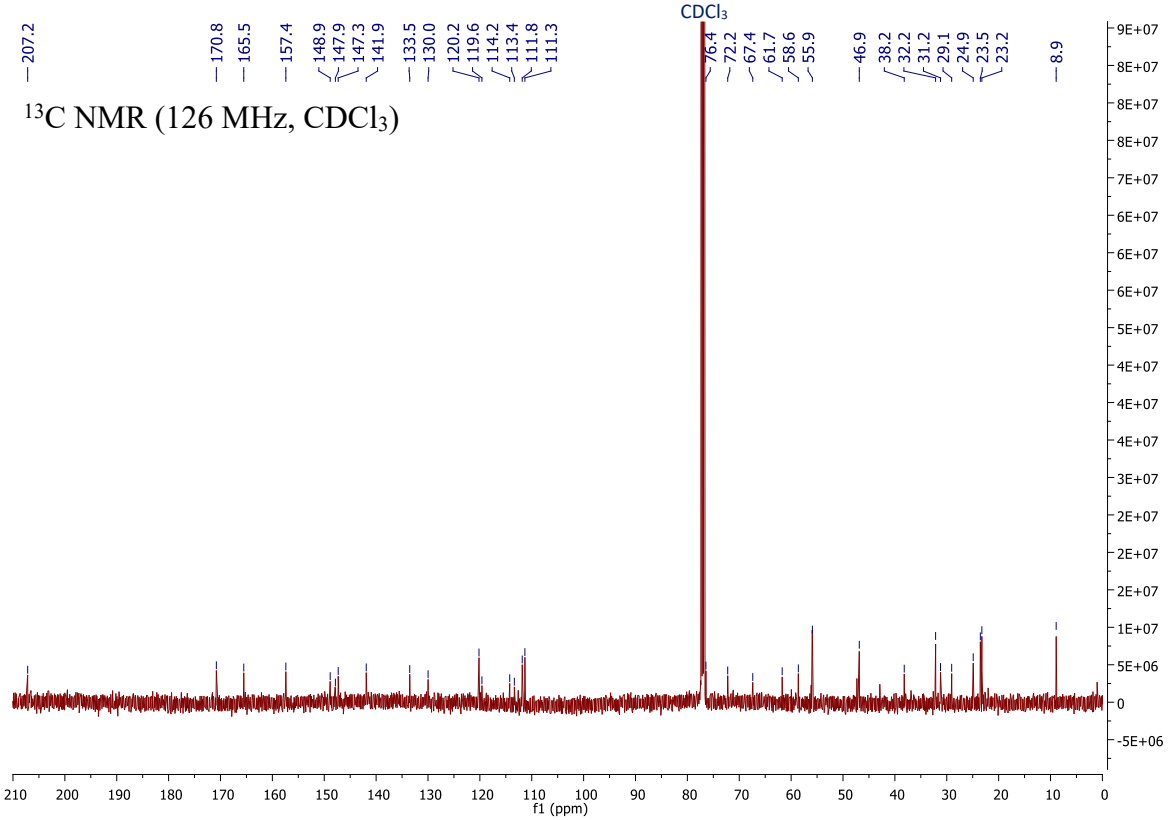


(*R*)-1-(3-(2-((2-aminoethyl)amino)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (3,3-dimethyl-2-oxopentanoyl)-*D*-prolinate hydrochloride (P6d)

^{1}H NMR (500 MHz, CD_3OD)

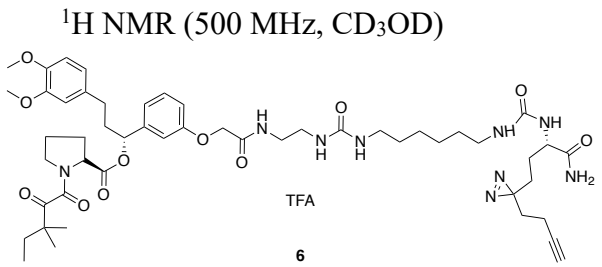


^{13}C NMR (126 MHz, $CDCl_3$)

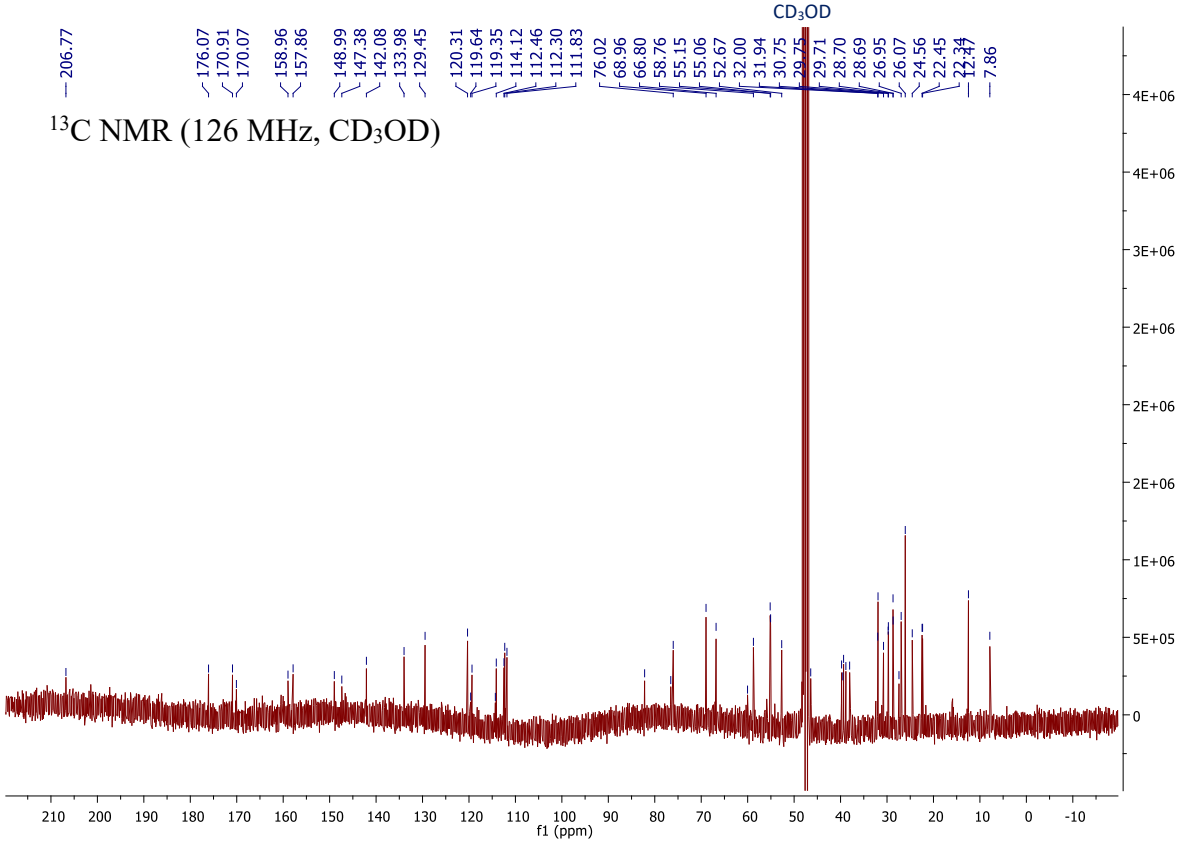


(*R*)-1-(3-(((S)-20-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)-18-carbamoyl-2,7,16-trioxo-3,6,8,15,17-pentaazaicosyl)oxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (3,3-dimethyl-2-oxopentanoyl)-*D*-prolinate trifluoroacetic acid (6)

^{1}H NMR (500 MHz, CD_3OD)



^{13}C NMR (126 MHz, CD_3OD)



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