A Remote Arene-binding Site on Prostate Specific Membrane Antigen Revealed by Antibody-Recruiting Small Molecules

Andrew X. Zhang,[a] Ryan P. Murelli,[a] Cyril Barinka,[b] Julien Michel,[a] Alexandra Cocleaza,[a] William L. Jorgensen,[a] Jacek Lubkowski,[b] and David A. Spiegel[a, c*]

[a]*Department of Chemistry, Yale University, New Haven, CT, 06520*
[b]*Macromolecular Crystallography Laboratory, National Cancer Institute at Frederick, Frederick, MD, 21702*
[c]*Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06520*

Supporting Information

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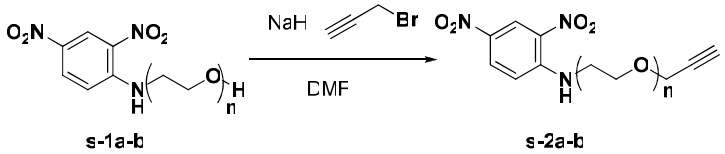
General Information

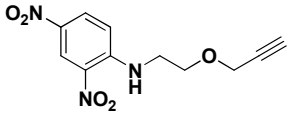
Synthesis: All starting materials and reagents were purchased from commercially available sources and used without further purification. ^{1}H NMR shifts are measured using the solvent residual peak as the internal standard ($CDCl_3$ δ 7.26, MeOD δ 3.31), and reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, dd = doublet of doublet, q = quartet, m = multiplet), coupling constant (Hz), integration. ^{13}C NMR shifts are measured using the solvent residual peak as the internal standard ($CDCl_3$ δ 77.20 or MeOD δ 49.00 or DMSO-d_6 δ 39.01), and reported as chemical shifts. Infrared (IR) spectral bands are characterized as broad (br), strong (s), medium (m), and weak (w).

Synthesis



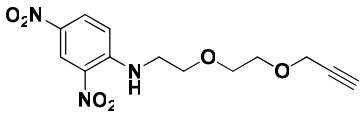
Scheme S-1. General synthesis of DNP-PEG-alkynes from the corresponding DNP-PEG-OH. (a, n = 1; b, n = 2).

2,4-dinitro-N-(2-(prop-2-ynyloxy)ethyl)aniline (s-2a).

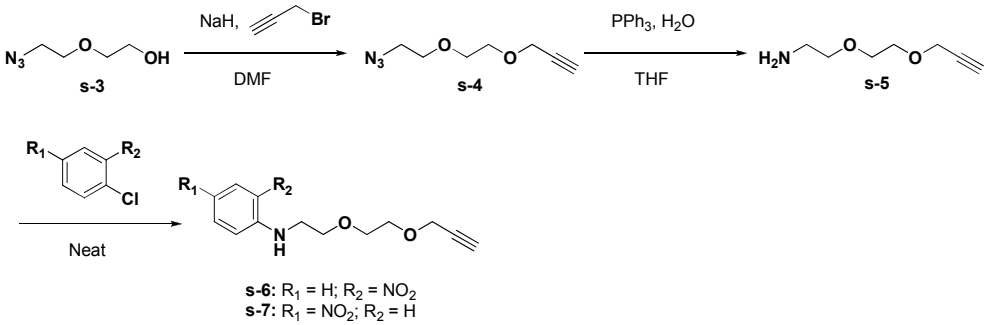


2-(2,4-dinitrophenylamino)ethanol **(s-1a)** (410 mg, 1.80 mmol, 1.0 equiv.) was dissolved in 3 mL of DMF and slowly added to a slurry of NaH (86.4 mg, 3.6 mmol, 2 equiv.) in 5 mL of DMF in a flame dried flask pre-cooled to 0 ^{o}C. To the resulting slurry, 80% propargyl bromide (0.240 mL, 2.16 mmol, 1.2 equiv.) in toluene, cooled to 0 ^{o}C, was added slowly. The ice bath was removed and the reaction was allowed to stir at room temperature for an additional 15 hours. The reaction was then re-cooled to 0 ^{o}C, quenched with saturated NH_4Cl, and extracted with diethyl ether (3x150 mL). The organic layers were combined, dried, concentrated under reduced pressure, and chromatographed (silica gel, 1x25 cm, 0% CH_3OH in $CHCl_3$, then 2.5% CH_3OH in $CHCl_3$) to yield 2,4-dinitro-N-(2-(prop-2-ynyloxy)ethyl)aniline **(s-2a)** as a dark yellow solid (310 mg, 64.8%). IR (thin film) 3356 (m), 3285 (m), 3105 (w), 2871 (w), 2117 (w), 1616 (s), 1584 (s), 1521 (s), 1499 (m), 1423 (m), 1274 (s), 1089 (s), 920 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (d, *J* = 2.6 Hz, 1H), 8.76 (bs, 1H), 8.28 (dd, *J* = 9.5, 2.7 Hz, 1H), 6.96 (d, *J* = 9.5 Hz, 1H), 4.25 (d, *J* = 2.4 Hz, 2H), 3.91 – 3.84 (m, 2H), 3.65 (d, *J* = 5.3 Hz, 1H), 2.50 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.5, 136.3, 130.7, 130.4, 124.4, 114.1, 78.9, 75.5, 67.3, 58.7, 43.3. HRMS (ES+) calc'd for $C_{11}H_{11}N_3O_5$ (M+H) *m/z* 266.0732 Found 266.0771.

2,4-dinitro-N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline (s-2b).

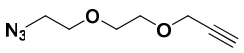


2-(2-(2,4-dinitrophenylamino)ethoxy)ethanol **(s-1b)** (100 mg, 0.369 mmol, 1.0 equiv.) was dissolved in 0.81 mL of DMF and slowly added to a slurry of NaH (17.71 mg, 0.738 mmol, 2.0 equiv.) in 0.81 mL of DMF in a flame dried flask pre-cooled to 0 ^{o}C . An 80% solution of propargyl bromide in toluene (0.049 μL, 0.443 mmol, 1.2 equiv.) was added slowly. The ice bath was then removed and the reaction was allowed to stir at room temperature for an additional 2 hours. The reaction was then re-cooled to 0 ^{o}C, quenched with saturated NH_4Cl, and then extracted with diethyl ether (3x50 mL). The organic layers were combined, dried with Na_2SO_4, concentrated under reduced pressure, and chromatographed (silica gel, 3x25 cm, 0% EtOAc in hexanes, then 10% EtOAc in hexanes, then 20% EtOAc in hexanes, then 30% EtOAc in hexanes) to yield 2,4-dinitro-N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline **(s-2b)** as a dark yellow solid (50 mg, 45% yield). IR (thin film) 3360 (m), 3285 (m), 3107 (w), 2872 (m), 1621 (s), 1588 (m), 1524 (m), 1425 (w), 1335 (s), 1305 (m), 1133 (m), 1101 (m), 920 (w), 832 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (d, *J* = 2.6 Hz, 1H), 8.81 (bs, 1H), 8.27 (dd, *J* = 9.4, 2.3 Hz, 1H), 6.96 (d, *J* = 9.5 Hz, 1H), 4.21 (d, *J* = 2.3 Hz, 2H), 3.84 (t, *J* = 5.2 Hz, 2H), 3.74 (m, 4H), 3.61 (q, *J* = 5.2 Hz, 2H), 2.44 (t, *J* = 2.3 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.6, 136.3, 130.7, 130.4, 124.4, 114.2, 79.6, 74.9, 70.7, 69.3, 68.9, 58.7, 43.4. HRMS (ES+) calc'd for $C_{13}H_{15}N_3O_6$ (M+H) *m/z* 310.0994 Found 310.1033.



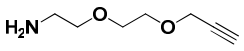
Scheme S-2. General synthesis of mononitro-PEG_2-alkynes.

3-(2-(2-azidoethoxy)ethoxy)prop-1-yne (s-4).



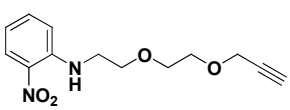
2-(2-azidoethoxy)ethanol[1] **(s-3)** (3 g, 22.89 mmol, 1.0 equiv.) was slowly added to a slurry of NaH (1.10 g, 45.78 mmol, 2 equiv.) in 102.64 mL of DMF in a flame dried flask pre-cooled to 0 °C. To the resulting slurry, 80% propargyl bromide in toluene (3.06 mL, 27.47 mmol, 1.2 equiv.), cooled to 0 °C, was added slowly. The ice bath was removed and the reaction was allowed to stir at room temperature for an additional 3 hours. The reaction was then re-cooled to 0 °C, and 3 mL of cold H_2O was added to quench the reaction, after which the reaction was concentrated under reduced pressure and taken up with saturated NH_4Cl. The reaction was extracted with diethyl ether, dried with Na_2SO_4, concentrated under reduced pressure, and chromatographed (silica gel, 3x25 cm, 0% CH_3OH in $CHCl_3$, then 10% MeOH in $CHCl_3$) to yield 3-(2-(2-azidoethoxy)ethoxy)prop-1-yne **(s-4)** as a dark brown oil (2.79 g, 72.1%). IR (thin film) 3291 (m), 2867 (m), 2099 (s), 1442 (w), 1347 (w), 1285 (m), 1101 (s), 1032 (w), 920 (w), 942 (w), 646 (m); ^{1}H NMR (125 MHz, $CDCl_3$) δ 4.21 (d, *J* = 2.4 Hz, 1H), 3.74 – 3.65 (m, 3H), 3.40 (t, *J* = 5.1 Hz, 1H), 2.43 (t, *J* = 2.4 Hz, 0H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 79.5, 74.6, 70.5, 70.0, 69.1, 58.5, 50.6. HRMS (ES+) calc'd for $C_7H_{11}N_3O_2$ (M+H) *m/z* 170.0885 Found 170.0924.

2-(2-(prop-2-ynyloxy)ethoxy)ethanamine (s-5).



3-(2-(2-azidoethoxy)ethoxy)prop-1-yne **(s-4)** (2 g, 11.83 mmol, 1.0 equiv) was dissolved in THF (30.5 mL). Triphenylphosphine (3.72 g, 14.20 mmol, 1.2 equiv.) and water (0.21 mL, 11.83 mol, 1.0 equiv.) were added to the solution and the reaction was allowed to stir at room temperature for 10 hours. The reaction was concentrated under reduced pressure and chromatographed (5% CH_3OH in $CHCl_3$, then 5% CH_3OH in $CHCl_3$ + 5% Et_3N) to yield 2-(2-(prop-2-ynyloxy)ethoxy)ethanamine **(s-5)** as a pale green oil (1.35 g, 80%). IR (thin film) 3250 (m), 2863 (m), 2112 (w), 1589 (w), 1443 (w), 1349 (m), 1291 (w), 1093 (s), 1037 (m), 918 (w), 840 (w), 673 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.21 (d, *J* = 2.3 Hz, 2H), 3.73 – 3.68 (m, 2H), 3.68 – 3.62 (m, 2H), 3.51 (t, *J* = 5.2 Hz, 2H), 2.87 (t, *J* = 5.2 Hz, 2H), 2.43 (dd, *J* = 2.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 79.7, 74.7, 73.7, 70.3, 69.2, 58.6, 41.9. HRMS (ES+) calc'd for $C_7H_{13}NO_2$ (M+H) *m/z* 144.0980 Found 144.1019.

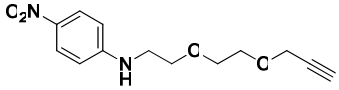
2-nitro-N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline (s-6).



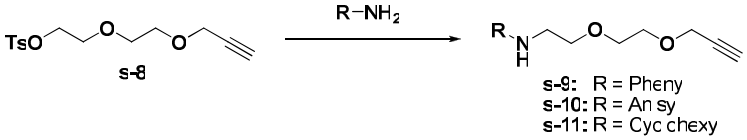
To **1**-chloro-2-nitrobenzene (152 mg, 0.97 mmol, 1.0 equiv.) was added neat 2-(2-(prop-2-ynyloxy)ethoxy)ethanamine **(s-5)** (900 mg, 6.31 mmol, 6.5 equiv.), and the resulting slurry was heated to 100 °C for 6 hours during which time the solid dissolved. At the end of this period, the heating bath was removed, the reaction content was mixed with water (50 mL), and

then extracted with CH_2Cl_2 (3x50 mL). The organic layers were combined, dried with Na_2SO_4, concentrated under reduced pressure, and chromatographed (silica gel, 1x25 cm, 0% CH_3OH in CH_2Cl_2, then 10% CH_3OH in CH_2Cl_2) to yield 2-nitro-N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline **(s-6)** as a dark yellow oil (76 mg, 30%). IR (thin film) 3378 (m), 3286 (m), 3085 (w), 2875 (m), 2114 (w), 1616 (s), 1570 (s), 1508 (s), 1417 (m), 1349 (m), 1228 (s), 1093 (s), 1034 (m), 740 (m), 670 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (bs, 1H), 8.18 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.43 (ddd, *J* = 8.6, 7.0, 1.6 Hz, 1H), 6.86 (d, *J* = 8.6 Hz, 1H), 6.65 (ddd, *J* = 8.3, 7.0, 1.2 Hz, 1H), 4.22 (d, *J* = 2.4 Hz, 2H), 3.80 (t, *J* = 5.5 Hz, 2H), 3.73 (d, *J* = 2.4 Hz, 4H), 3.52 (q, *J* = 5.4 Hz, 2H), 2.43 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.45, 136.13, 132.22, 126.94, 115.39, 113.78, 79.56, 74.61, 70.50, 69.20, 69.19, 58.52, 42.75; HRMS (ES+) calc'd for $C_{13}H_{16}N_2O_4$ (M+H) *m/z* 265.1144 Found 265.1181.

4-nitro-N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline (s-7).

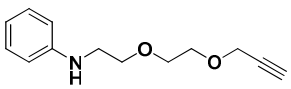


To 1-chloro-4-nitrobenzene (152 mg, 0.97 mmol, 1.0 equiv.) was added neat 2-(2-(prop-2-ynyloxy)ethoxy)ethanamine **(s-5)** (900 mg, 6.31 mmol, 6.5 equiv.), and the resulting slurry was heated to 100 °C for 19 hours. At the end of this period, the heating bath was removed, and the reaction content was mixed with water (50 mL) and then extracted with CH_2Cl_2 (3x50 mL). The organic layers were combined, dried with Na_2SO_4, concentrated under reduced pressure, and chromatographed (silica gel, 1x25 cm, 0% EtOAc in CH_2Cl_2, then 30% EtOAc in CH_2Cl_2 to yield 4-nitro-N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline **(s-7)** as a dark yellow oil (40 mg, 16%). IR (thin film) 3351 (m), 3239 (m), 2858 (w), 2112 (w), 1599 (s), 1535 (w), 1500 (w), 1467 (s), 1284 (s), 1086 (s), 1034 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 9.2 Hz, 2H), 6.53 (d, J = 9.2 Hz, 2H), 5.05 (bs, 1H), 4.19 (d, *J* = 2.4 Hz, 2H), 3.76 – 3.70 (m, 2H), 3.70 – 3.64 (m, 4H), 3.38 (q, *J* = 5.3 Hz, 2H), 2.45 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.4, 138.0, 126.4, 111.2, 79.5, 74.9, 70.3, 69.1, 69.0, 58.5, 42.9. HRMS (ES+) calc'd for $C_{13}H_{16}N_2O_4$ (M+H) *m/z* 265.1144 Found 265.1177.



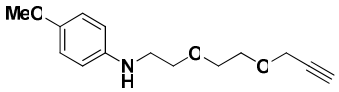
Scheme S-3: General synthesis of $(PEG)_2$-alkynes.

N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline (s-9).



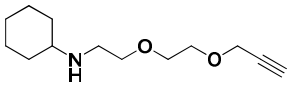
2-(2-(prop-2-ynyloxy)ethoxy)ethyl 4-methylbenzenesulfonate **(s-8)**[2] (100 mg, 0.335 mmol, 0.31 equiv.) was dissolved in aniline (102 mg, 1.10 mmol, 1 equiv.). The reaction was allowed to proceed at 100 °C in a sealed reaction vessel for 5 hours, after which time it was chromatographed (Silica Gel, 25g RediSep pre-packed column, 0% EtOAc:Hexanes→ 20% EtOAc:Hexanes) to yield N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline **(s-9)** as a brown oil (39.0 mg, 53.1%). IR (thin film) 3393 (m), 3278 (m), 3052 (w), 2865 (m), 2115 (w), 1603 (s), 1506 (s), 1461 (w), 1320 (w), 1277 (m), 1099 (s), 1030 (w), 750 (s), 693 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 – 7.16 (m, 2H), 6.72 (t, *J* = 7.3 Hz, 1H), 6.67 – 6.63 (m, 2H), 4.22 (d, *J* = 2.4 Hz, 2H), 4.12 (s, 1H), 3.74 – 3.70 (m, 4H), 3.70 – 3.66 (m, 2H), 3.32 (t, *J* = 5.2 Hz, 2H), 2.46 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.7, 129.6, 117.9, 113.5, 80.0, 75.1, 70.5, 70.1, 69.5, 58.9, 43.9. HRMS (ES+) calc'd for $C_{13}H_{17}NO_2$ (M+H) *m/z* 220.1293 Found 220.1327.

4-methoxy-N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline (s-10).

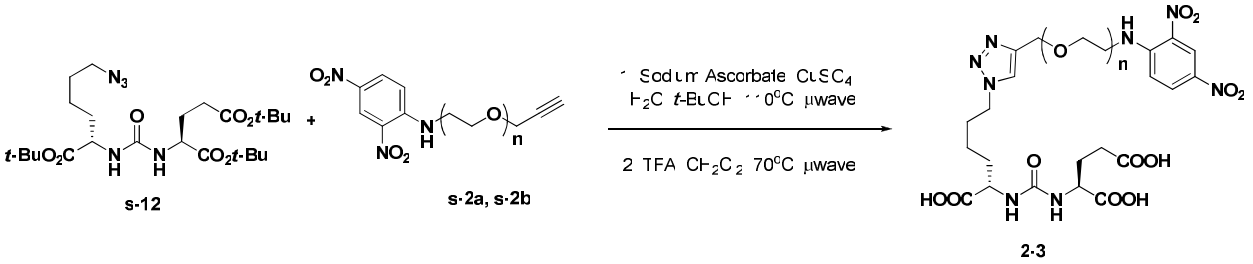


K_2CO_3 (380 mg, 2.76 mmol, 2.5 equiv.) was added to a solution of 4-methoxyaniline (136 mg, 1.1 mmol, 1 equiv.) in 1 mL of DMF, and the resulting slurry was heated to 100 °C. 2-(2-(prop-2-ynyloxy)ethoxy)ethyl 4-methylbenzenesulfonate **(s-8)** (100 mg, 0.276 mmol, 0.25 equiv.), dissolved in DMF (1 mL), was then added to the reaction via syringe-pump over 5 hours. The reaction was stirred for an additional 12 hours, after which time it was concentrated under reduced pressure and partially purified (Silica Gel, 12 g RediSep pre-packed column, 0% EtOAc:Hexanes → 20% EtOAc:Hexanes → 50% EtOAc:Hexanes, followed by EtOAc flush). The material obtained after chromatography was carried directly on to the next step without further purification.

N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)cyclohexanamine (s-11).

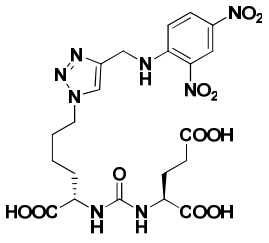


Cyclohexylamine (150 mg, 1.5 mmol, 1 equiv.) and 2-(2-(prop-2-ynyloxy)ethoxy)ethyl 4-methylbenzenesulfonate **(s-8)** (100 mg, 0.335 mmol, 0.2 equiv.) were dissolved in 1 mL of ethanol. The reaction was allowed to proceed under microwave irradiation at 80 °C for 10 minutes, after which it was concentrated under reduced pressure and chromatographed (Silica Gel, 25g RediSep pre-packed column, 10% EtOAc:Hexanes → 50% EtOAc:Hexanes, followed by EtOAc flush) to give N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)cyclohexanamine **(s-10)** as a clear oil (28 mg, 37%). IR (thin film) 3253 (w), 2924 (s), 2852 (s), 2113 (w), 1449 (m), 1349 (m), 1263 (w), 1102 (s), 919 (w), 839 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (d, *J* = 2.4 Hz, 2H), 3.70 – 3.65 (m, 2H), 3.65 – 3.60 (m, 2H), 3.59 (t, *J* = 5.4 Hz, 2H), 2.80 (t, *J* = 5.4 Hz, 2H), 2.42 (t, *J* = 2.3 Hz, 1H), 2.40 – 2.34 (m, 1H), 1.90-1.84 (m, 2H), 1.72-1.69 (m, 2H), 1.64 – 1.54 (m, 1H), 1.30 – 1.15 (m, 3H), 1.15 – 0.99 (m, 2H), 0.92-0.81 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 79.6, 74.6, 70.9, 70.1, 69.0, 58.4, 56.8, 42.3, 33.4, 26.2, 25.1. HRMS (ES+) calc'd for $C_{13}H_{23}NO_2$ (M+H) *m/z* 226.1762 Found 226.1797.



***Scheme S-4**:* General synthesis of **2** and **3**. (**2**, n = 1; **3**, n = 2).

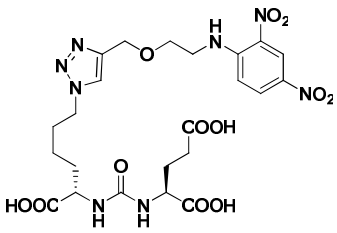
(S)-2-(3-((S)-1-carboxy-5-(4-((2,4-dinitrophenylamino)methyl)-1H-1,2,3-triazol-1-yl)pentyl)ureido)pentanedioic acid (1).



2,4-dinitro-N-(prop-2-ynyl)aniline[3] (48.75 mg, 0.220 mmol, 1.1 equiv.) and azide **s-12**[4] (100 mg, 0.194 mmol, 1 equiv.) were added to a mixture of water (0.694 mL) and *t*-BuOH (0.694 mL). The slurry was placed in a microwave reaction tube, to which a 0.1 M solution of sodium ascorbate in water (0.388 mL, 0.039 mmol, 0.2 equiv.) and a 0.1 M solution of copper (II) sulfate in water (0.078 mL, 0.008 mmol, 0.04 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 °C for 20 minutes. The crude mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3

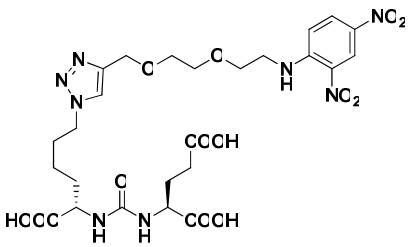
mL). The tube was capped and subjected to microwave irradiation at 70 °C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **1** (47.7 mg, 43.6% over two steps) as a yellow solid. IR (thin film) 3367 (br), 2946 (br), 1720 (m), 1619 (s), 1589 (m), 1524 (w), 1425 (w), 1338 (m), 1203 (m), 1137 (m); ^{1}H NMR (400 MHz, MeOD) δ 9.05 (d, *J* = 2.6 Hz, 1H), 8.29 (dd, *J* = 9.5, 2.6 Hz, 1H), 8.00 (s, 1H), 7.24 (d, *J* = 9.6 Hz, 1H), 4.82 (s, 2H), 4.41 (t, *J* = 7.0 Hz, 2H), 4.29 (ddd, *J* = 18.6, 8.5, 4.9 Hz, 2H), 2.50 – 2.33 (m, 2H), 2.20-2.10 (m, 1H), 2.03 – 1.80 (m, 4H), 1.72-1.62 (m, 1H), 1.48 – 1.36 (m, 2H). ^{13}CNMR (125 MHz, DMSO-d_6) δ 174.3, 174.1, 173.7, 157.2, 147.8, 143.1, 135.2, 130.1, 130.0, 123.5, 123.0, 115.7, 52.0, 51.5, 49.3, 38.4, 29.8, 29.4, 27.4, 22.1. HRMS (ES+) calc'd for $C_{21}H_{26}N_8O_{11}$ (M+H) *m/z* 567.1755 Found 567.1796.

(S)-2-(3-((S)-1-carboxy-5-(4-((2-(2,4-dinitrophenylamino)ethoxy)methyl)-1H-1,2,3-triazol-1-yl)pentyl)ureido)pentanedioic acid (2).



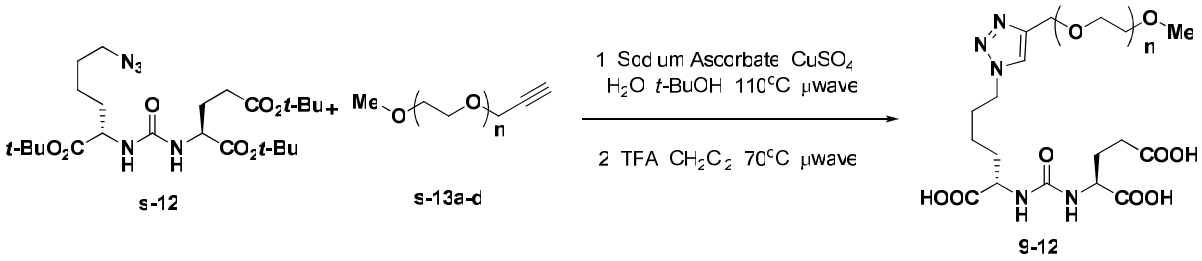
2,4-dinitro-N-(2-(prop-2-ynyloxy)ethyl)aniline **(s-2a)** (51.4 mg, 0.194 mmol, 1 equiv.) and azide **s-12** (100 mg, 0.194 mmol, 1 equiv.) were added to a mixture of water (0.694 mL) and *t*-BuOH (0.694 mL). This slurry was placed in a microwave reaction tube, to which a 0.1 M solution of sodium ascorbate in water (0.388 mL, 0.039 mmol, 0.2 equiv.) and 0.1 M solution of copper (II) sulfate in water (0.078 mL, 0.008 mmol, 0.04 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 °C for 20 minutes. The crude mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave irradiation at 70 °C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **2** (38.0 mg, 32.2% over two steps), as a yellow oil. IR (thin film) 3356 (m), 2938 (m), 1731 (s), 1621 (s), 1586 (m), 1525 (m), 1426 (w), 1336 (s), 1306 (w), 1137 (w), 1087 (m), 833 (w); ^{1}H NMR (500 MHz, MeOD) δ 9.00 (d, *J* = 2.2 Hz, 1H), 8.25 (dd, *J* = 9.6, 2.3 Hz, 1H), 7.98 (s, 1H), 7.18 (d, *J* = 9.6 Hz, 1H), 4.68 (s, 2H), 4.41 (t, *J* = 6.9 Hz, 2H), 4.28 (ddd, *J* = 18.1, 8.2, 5.0 Hz, 2H), 3.82 (t, *J* = 5.0 Hz, 2H), 3.67 (t, *J* = 5.0 Hz, 2H), 2.48 – 2.33 (m, 2H), 2.17-2.10 (m, 1H), 2.01 – 1.81 (m, 4H), 1.71-1.64 (m, 1H), 1.46 – 1.35 (m, 2H). ^{13}CNMR (125 MHz, DMSO-d_6) δ 174.4, 174.1, 173.7, 157.2, 148.3, 143.6, 134.9, 129.9, 129.7, 123.8, 123.6, 115.6, 67.3, 63.4, 52.1, 51.6, 49.1, 42.6, 31.5, 29.9, 29.4, 27.5, 22.1. HRMS (ES+) calc'd for $C_{23}H_{30}N_8O_{12}$ (M+H) *m/z* 611.2017 Found 611.2074.

(S)-2-(3-((S)-1-carboxy-5-(4-((2-(2-(2,4-dinitrophenylamino)ethoxy)ethoxy)methyl)-1H-1,2,3-triazol-1-yl)pentyl)ureido)pentanedioic acid (3).



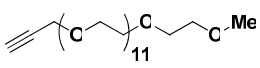
2,4-dinitro-N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline **(s-2b)** (60 mg, 0.194 mmol, 1 equiv.) and azide **s-12** (100 mg, 0.194 mmol, 1 equiv.) were added to a mixture of water (0.694 mL) and *t*-BuOH (0.694 mL). This slurry was placed in a microwave reaction tube, to which a 0.1 M solution of sodium ascorbate in water (0.388 mL, 0.039 mmol, 0.2 equiv.) and 0.1 M solution of copper (II) sulfate in water (0.078 mL, 0.008 mmol, 0.04 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 °C for 20 minutes. The crude

mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave irradiation at 70 °C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **3**(31.0 mg, 24.6% over two steps) as a yellow oil. IR (thin film) 3360 (m), 2933 (m), 1726 (s), 1621 (s), 1587 (m), 1525 (w), 1425 (w), 1337 (s), 1306 m), 1136 (m); ^{1}H NMR (400 MHz, MeOD) δ 9.01 (d, J = 2.7 Hz, 1H), 8.26 (dd, J = 9.6 Hz, 2.7 Hz, 1H), 7.97 (s, 1H), 7.20 (d, J = 9.6 Hz, 1H), 4.63 (s, 2H), 4.41 (t, J = 7.0 Hz, 2H), 4.33-4.23 (m, 2H), 3.80 (t, J = 5.2 Hz, 2H), 3.72-3.68 (m, 4H), 3.66 (t, J = 5.2, 2H), 2.48-2.32 (m, 2H), 2.19-2.08 (m, 1H), 2.00-1.80 (m, 4H), 1.73-163 (m, 1H), 1.46-1.35 (m, 2H). ^{13}CNMR (125 MHz, DMSO-d_6) δ 174.3, 174.1, 173.6, 157.2, 148.2, 143.8, 134.9, 129.9, 129.6, 123.6, 115.6, 69.7, 68.9, 68.2, 63.6, 52.0, 51.5, 49.1, 42.5, 31.4, 29.8, 29.4, 27.4, 22.1. HRMS (ES+) calc'd for $C_{25}H_{34}N_8O_{13}$ (M+H) *m/z* 655.2279 Found 655.2275.



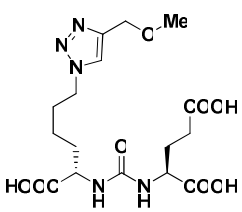
***Scheme S-5*:** General synthesis of **8-12** (**8**, n = 0; **9**, n = 2; **10**, n = 4; **11**, n = 8; **12**, n = 12) from **s-13a-e** (a, n = 0; b, n = 2; c, n = 4; d, n = 8; e, n = 12).

2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaoxahentetracont-40-yne (s-13e).



2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaheptatriacontan-37-ol (150 mg, 22.89 mmol, 1.0 equiv.) was slowly added to a slurry of NaH (48 mg, 45.78 mol, 8 equiv.) in DMF (1.4 mL) in a flame dried flask pre-cooled to 0 °C. 80% propargyl bromide in toluene (36 μL, 27.47 mmol, 1.2 equiv.), cooled to 0 °C, was added slowly. The ice bath was removed and the reaction was allowed to stir at room temperature for an additional 2 hours. The reaction was then re-cooled to 0 °C, 0.5 mL of ice cold water was added, and the reaction was concentrated under reduced pressure. The remaining residue was redissolved in dichloromethane, then partially purified via silica gel chromatography (10% MeOH in DCM) to remove all the salts. After partial purification, 2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaoxahentetracont-40-yne **(s-13e)** was obtained as a brown oil that was taken on to the next step without full purification.

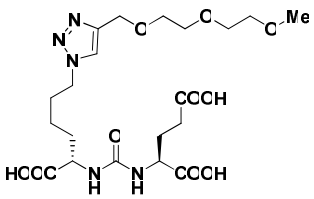
(S)-2-(3-((S)-1-carboxy-5-(4-(methoxymethyl)-1H-1,2,3-triazol-1-yl)pentyl)ureido)pentanedioic acid (8).



Methyl propargyl ether (**s-13a**) (81.8 mg, 1.16 mmol, 6 equiv.) and azide **s-12** (100 mg, 0.194 mmol, 1 equiv.) were added to a mixture of water (0.694 mL) and *t*-BuOH (0.694 mL). This slurry was placed in a microwave reaction tube to which 0.1 M solution of sodium ascorbate in water (0.388 mL, 0.039 mmol, 0.2 equiv.) and 0.1 M solution of copper (II) sulfate in water (0.078 mL, 0.008 mmol, 0.04 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 °C for 20 minutes. The crude mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave irradiation at 70 °C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified

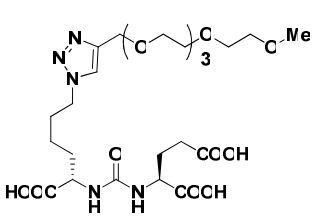
via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **8**(32.9 mg, 41.4% over two steps) as a clear oil. IR (thin film) 3368 (br), 2936 (m), 1670 (s), 1564 (m), 1437 (w), 1193 (s), 1139 (s), 1064 (m), 839 (w), 800 (w), 723 (w). ^{1}H NMR (500 MHz, MeOD) δ 8.00 (s, 1H), 4.55 (s, 2H), 4.43 (t, *J* = 7.0 Hz, 2H), 4.36 – 4.25 (m, 2H), 3.38 (s, 3H), 3.18 (s, 0H), 2.48 – 2.35 (m, 2H), 2.19-2.12 (m, 1H), 2.02 – 1.82 (m, 4H), 1.75 – 1.64 (m, 1H), 1.48 – 1.36 (m, 2H). ^{13}CNMR (125 MHz, MeOD) δ 176.5, 176.2, 175.8, 160.1, 145.6, 125.2, 66.2, 58.4, 53.7, 53.5, 51.3, 32.8, 31.1, 30.7, 28.8, 23.4. HRMS (ES+) calc'd for $C_{16}H_{25}N_5O_8$ (M+H) *m/z* 416.1737 Found 415.2019.

(S)-2-(3-((S)-1-carboxy-5-(4-((2-(2-methoxyethoxy)ethoxy)methyl)-1H-1,2,3-triazol-1-yl)pentyl)ureido)pentanedioic acid (9).



3-(2-(2-methoxyethoxy)ethoxy)prop-1-yne (**s-13b**)[5] (30.7 mg, 0.194 mmol, 1 equiv.) and azide **s-12** (100 mg, 0.194 mmol, 1 equiv.) were added to a mixture of water (0.694 mL) and *t*-BuOH (0.694 mL). This slurry was placed in a microwave reaction tube, to which a 0.1 M solution of sodium ascorbate in water (0.388 mL, 0.039 mmol, 0.2 equiv.) and 0.1 M solution of copper (II) sulfate in water (0.078 mL, 0.008 mmol, 0.04 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 °C for 20 minutes. The crude mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave irradiation at 70 °C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **9** (30.6 mg, 31.5% over two steps) as a clear oil. IR (thin film) 3346 (br), 2931 (m), 1734 (s), 1642 (m), 1562 (s), 1451 (w), 1201 (s), 1087 (s); ^{1}H NMR (400 MHz, MeOD) δ 8.01 (s, 1H), 4.65 (s, 2H), 4.43 (t, *J* = 7.0 Hz, 2H), 4.33 – 4.24 (m, 2H), 3.69 – 3.64 (m, 4H), 3.64-3.60 (m, 2H), 3.56 – 3.49 (m, 2H), 3.33 (s, 3H), 2.46 – 2.34 (m, 2H), 2.16-2.11 (m, 1H), 2.00 – 1.82 (m, 4H), 1.71-1.64 (m, 1H), 1.48 – 1.31 (m, 2H). ^{13}CNMR (125 MHz, CD_3OD) δ 176.4, 176.1, 175.8, 160.1, 145.8, 125.3, 72.9, 71.5, 71.3, 70.8, 64.8, 59.1, 53.7, 53.5, 51.3, 32.8, 31.1, 30.7, 28.8, 23.4. HRMS (ES+) calc'd for $C_{20}H_{33}N_5O_{10}$ (M+H) *m/z* 504.2261 Found 504.2590.

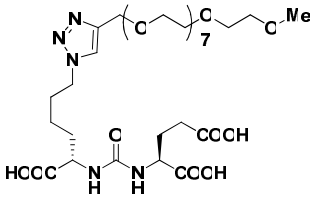
(S)-2-(3-((S)-5-(4-2,5,8,11,14-pentaoxapentadecyl-1H-1,2,3-triazol-1-yl)-1-carboxypentyl)ureido)pentanedioic acid (10).



2,5,8,11,14-pentaoxaheptadec-16-yne (**s-13c**)[5] (52 mg, 0.194 mmol, 1 equiv.) and azide **s-12** (100 mg, 0.194 mmol, 1 equiv.) were added to a mixture of water (0.694 mL) and *t*-BuOH (0.694 mL). This slurry was placed in a microwave reaction tube, to which a 0.1 M solution of sodium ascorbate in water (0.388 mL, 0.039 mmol, 0.2 equiv.) and 0.1 M solution of copper (II) sulfate in water (0.078 mL, 0.008 mmol, 0.04 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 °C for 20 minutes. The crude mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave irradiation at 70 °C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **10** (19.8 mg, 15.9% over two steps)as a clear oil. IR (thin film) 3323 (br), 2921 (m),

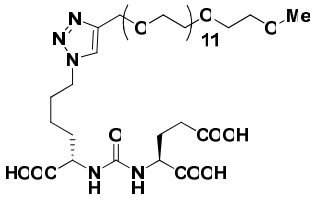
1734 (s), 1642 (w), 1562 (m), 1452 (w), 1201 (m), 1089 (m), 845 (w); ^{1}H NMR (500 MHz, MeOD) δ 8.04 (s, 1H), 4.65 (s, 2H), 4.44 (t, *J* = 7.0 Hz, 2H), 4.34 – 4.24 (m, 2H), 3.70 – 3.65 (m, 4H), 3.65 – 3.58 (m, 12H), 3.52 (dd, *J* = 5.6, 3.6 Hz, 2H), 3.34 (s, 3H), 2.47 – 2.35 (m, 2H), 2.19 – 2.09 (m, 1H), 2.02 – 1.82 (m, 4H), 1.72-1.65 (m, 1H), 1.45 – 1.36 (m, 2H). ^{13}CNMR (125 MHz, DMSO-d_6) δ 176.4, 176.1, 160.1, 145.7, 125.3, 72.9, 71.5, 71.3, 70.8, 64.8, 59.1, 53.7, 53.5, 51.3, 32.8, 31.1, 30.7, 28.8, 23.4. HRMS (ES+) calc'd for $C_{24}H_{41}N_5O_{12}$ (M+H) *m/z* 592.2785 Found 592.2783.

(S)-2-(3-((S)-5-(4-2,5,8,11,14,17,20,23,26-nonaoxaheptacosyl-1H-1,2,3-triazol-1-yl)-1-carboxypentyl)ureido)pentanedioic acid (11).



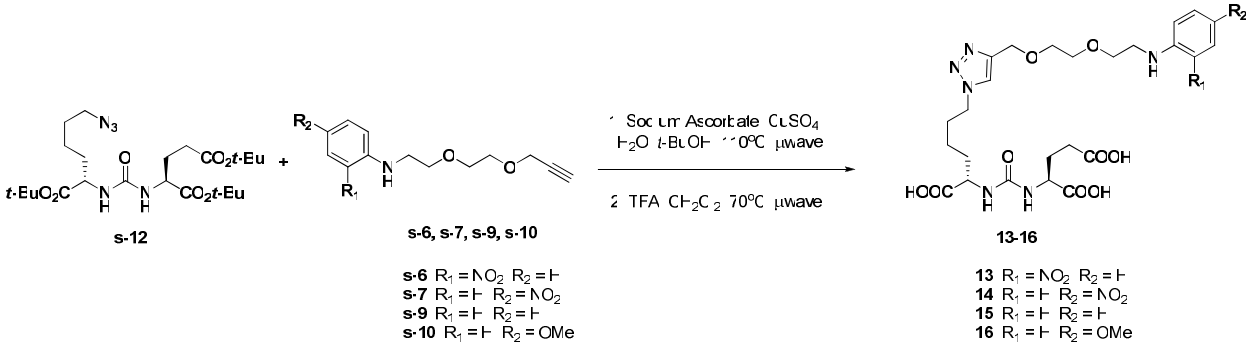
2,5,8,11,14,17,20,23,26-nonaoxanonacos-28-yne **(s-13d)**[6] (41 mg, 0.097 mmol, 1 equiv.) and azide **s-12** (50 mg, 0.097 mmol, 1 equiv.) were added to a mixture of water (0.350 mL) and *t*-BuOH (0.350 mL). This slurry was placed in a microwave reaction tube, to which a 0.1 M solution of sodium ascorbate in water (0.194 mL, 0.019 mmol, 0.2 equiv.) and 0.1 M solution of copper (II) sulfate in water (0.039 mL, 0.004 mmol, 0.04 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 ^{o}C for 20 minutes. The crude mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave irradiation at 70 ^{o}C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **11** (9.6 mg, 21.4% over two steps) as aclear oil. IR (thin film) 3332 (br), 2919 (m), 2875 (m), 1734 (s), 1557 (m), 1452 (w), 1203 (s), 1088 (s), 946 (w), 850 (w); ^{1}H NMR (400 MHz, MeOD) δ 8.00 (s, 1H), 4.64 (s, 2H), 4.42 (t, *J* = 6.9 Hz, 2H), 4.35 – 4.23 (m, 2H), 3.66 (s, 4H), 3.62 (m, *J* = 5.5, 1.1 Hz, 26H), 3.56 – 3.52 (m, 2H), 3.36 (d, *J* = 1.1 Hz, 3H), 2.45-2.41 (m, 2H), 2.22 – 2.07 (m, 1H), 1.98-1.82 (m, 4H), 1.72-1.64 (m, 1H), 1.44-1.35 (m, 2H). ^{13}CNMR (125 MHz, MeOD) δ 176.4, 176.1, 175.8, 160.1, 145.9, 125.2, 73.0, 71.5, 71.3, 70.8, 64.9, 59.1, 53.7, 53.5, 51.2, 32.8, 31.1, 30.7, 28.8, 23.4. HRMS (ES+) calc'd for $C_{32}H_{57}N_5O_{16}$ (M+H) *m/z* 768.3834 Found 768.3865.

(S)-2-(3-((S)-5-(4-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaoxanonatriacontyl-1H-1,2,3-triazol-1-yl)-1-carboxypentyl)ureido)pentanedioic acid (12).



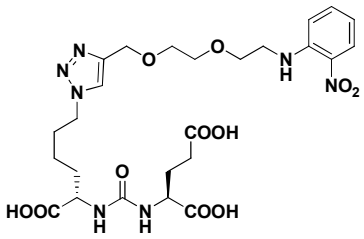
Crude 2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaoxahentetracont-40-yne **(s-13e)** (58 mg, 0.097 mmol, 1 equiv.) and azide **s-12** (50 mg, 0.097 mmol, 1 equiv.) were added to a mixture of water (0.350 mL) and *t*-BuOH (0.350 mL). This slurry was placed in a microwave reaction tube, to which a 0.1 M solution of sodium ascorbate in water (0.194 mL, 0.019 mmol, 0.2 equiv.) and 0.1 M solution of copper (II) sulfate in water (0.039 mL, 0.004 mmol, 0.04 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 ^{o}C for 20 minutes. The crude mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave irradiation at 70 ^{o}C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **12** (13.0 mg, 9.6%, 3 steps) as a clear oil. IR (thin film) 3369 (br), 2878 (s), 1673 (s), 1561 (w), 1456 (w), 1351 (w), 1200 (s), 1105 (s), 950 (w), 836 (w), 800

(w), 721 (w); ^{1}H NMR (500 MHz, MeOD) δ 8.00 (s, 1H), 4.64 (s, 2H), 4.42 (t, *J* = 7.0 Hz, 2H), 4.33 – 4.24 (m, 2H), 3.69 – 3.59 (m, 46H), 3.56-3.52 (m, 2H), 2.48 – 2.34 (m, 2H), 2.19 – 2.09 (m, 1H), 2.02 – 1.81 (m, 4H), 1.71-1.64 (m, 1H), 1.45 – 1.33 (m, 2H). ^{13}CNMR (125 MHz, MeOD) δ 176.4, 176.1, 175.8, 160.1, 145.9, 125.2, 72.9, 71.5, 71.3, 70.7, 65.0, 59.1, 53.7, 53.5, 51.1, 32.9, 31.1, 30.8, 28.9, 23.4. LCMS (ES+) calc'd for $C_{40}H_{73}N_5O_{20}$ (M+H) *m/z* 944.49 Found 944.72.



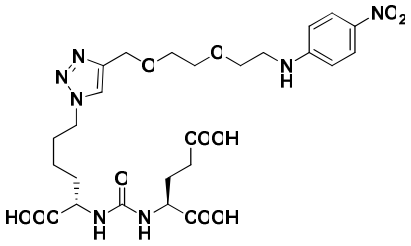
Scheme S-6: General synthesis of **13**-**16**.

(S)-2-(3-((S)-1-carboxy-5-(4-((2-(2-(2-nitrophenylamino)ethoxy)ethoxy)methyl)-1H-1,2,3-triazol-1-yl)pentyl)ureido)pentanedioic acid (13).



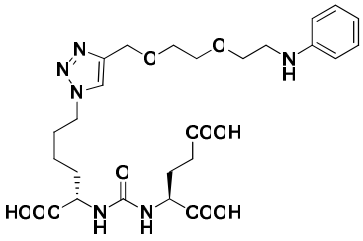
2-nitro-N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline **(s-6)** (51.2 mg, 0.194 mmol, 1 equiv.) and azide **s-12** (100 mg, 0.194 mmol, 1 equiv.) were added to a mixture of water (0.694 mL) and *t*-BuOH (0.694 mL). This slurry was placed in a microwave reaction tube, to which a0.1 M solution of sodium ascorbate in water (0.388 mL, 0.039 mmol, 0.2 equiv.) and 0.1 M solution of copper (II) sulfate in water (0.078 mL, 0.008 mmol, 0.04 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 °C for 20 minutes. The crude mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave irradiation at 70 °C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **13** (36.5 mg, 31.0% over two steps) as a dark yellow oil. IR (thin film) 3335 (br), 2923 (m), 1722 (s), 1668 (m), 1602 (s), 1561 (w), 1470 (w), 1308 (s), 1187 (m), 1111 (w), 998 (w), 836 (w); ^{1}H NMR (500 MHz, MeOD) δ 8.12 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.98 (s, 1H), 7.49 (ddd, *J* = 8.6, 6.9, 1.6 Hz, 1H), 7.02 (dd, *J* = 8.7, 0.9 Hz, 1H), 6.67 (ddd, *J* = 8.3, 6.9, 1.2 Hz, 1H), 4.65 (s, 2H), 4.41 (t, *J* = 7.1 Hz, 2H), 4.30 (ddd, *J* = 17.8, 8.4, 5.0 Hz, 2H), 3.78 (t, *J* = 5.3 Hz, 2H), 3.71 (m, 4H), 3.53 (t, *J* = 5.3 Hz, 2H), 2.44-2.40 (m, 2H), 2.21 – 2.10 (m, 1H), 2.00 – 1.81 (m, 4H), 1.72-1.65 (m, 1H), 1.47 – 1.35 (m, 2H). ^{13}CNMR (125 MHz, DMSO-d_6) δ 174.4, 174.1, 173.7, 157.2, 145.2, 143.9, 136.6, 131.0, 126.2, 123.6, 115.4, 114.7, 69.6, 68.9, 68.4, 63.6, 52.1, 51.6, 49.1, 42.0, 31.5, 29.9, 29.4, 27.5, 22.1. HRMS (ES+) calc'd for $C_{25}H_{35}N_7O_{11}$ (M+H) *m/z* 610.2428 Found 610.2471.

(S)-2-(3-((S)-1-carboxy-5-(4-((2-(2-(4-nitrophenylamino)ethoxy)ethoxy)methyl)-1H-1,2,3-triazol-1-yl)pentyl)ureido)pentanedioic acid (14).



4-nitro-N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline **(s-7)** (34.2 mg, 0.129 mmol, 1 equiv.) and azide **s-12** (66.7 mg, 0.129 mmol, 1 equiv.) were added to a mixture of water (0.600 mL) and *t*-BuOH (0.600 mL). This slurry was placed in a microwave reaction tube, to which a 0.1 M solution of sodium ascorbate in water (0.260 mL, 0.026 mmol, 0.2 equiv.) and a 0.1 M solution of copper (II) sulfate in water (0.051 mL, 0.005 mmol, 0.04 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 °C for 20 minutes. The crude mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave radiation at 70 °C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **14** (21.7 mg, 27.6% over two steps)as a yellow oil. IR (thin film) 3335 (br), 2924 (m), 2870 (m), 1722 (s), 1668 (m), 1602 (s), 1561 (m), 1505 (w), 1470 (w), 1308 (s), 1187 (m), 1118 (m), 837 (w); ^{1}H NMR (500 MHz, MeOD) δ 8.01 (d, J = 10.4 Hz, 1H), 7.95 (s, 1H), 6.64 (d, J = 10.4 Hz, 1H) 4.63 (s, 2H), 4.39 (t, *J* = 7.0 Hz, 2H), 4.29 (ddd, *J* = 16.4, 8.4, 5.0 Hz, 2H), 3.71 – 3.62 (m, 6H), 3.38 (t, *J* = 5.4 Hz, 2H), 2.47 – 2.34 (m, 2H), 2.19 – 2.08 (m, 1H), 1.99 – 1.80 (m, 4H), 1.71-1.64 (m, 1H), 1.42-1.36 (m, 2H). ^{13}CNMR (125 MHz, DMSO-d_6) δ 174.4, 174.1, 173.7, 157.3, 154.6, 143.8, 135.6, 126.2, 123.7, 110.8, 69.6, 68.9, 68.6, 63.5, 52.1, 51.6, 49.1, 42.3, 31.5, 29.9, 29.4, 27.5, 22.1. HRMS (ES+) calc'd for $C_{25}H_{35}N_7O_{11}$ (M+H) *m/z* 610.2428 Found 610.2468.

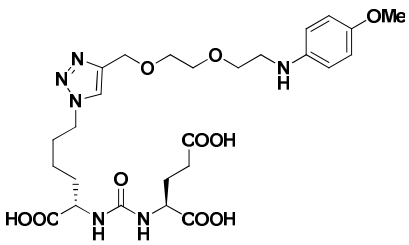
(S)-2-(3-((S)-1-carboxy-5-(4-((2-(2-(phenylamino)ethoxy)ethoxy)methyl)-1H-1,2,3-triazol-1-yl)pentyl)ureido)pentanedioic acid (15).



N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline **(s-9)** (35 mg, 0.160 mmol, 1 equiv.) and azide **31** (82 mg, 0.160 mmol, 1 equiv.) were added to a mixture of water (1 mL) and *t*-BuOH (1 mL). This slurry was placed in a microwave reaction tube, to which a 0.1 M solution of sodium ascorbate in water (7.8 mg, 0.04 mmol, 0.25 equiv.) and a 0.1 M solution of copper (II) sulfate in water (0.080 mL, 0.008 mmol, 0.05 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 °C for 20 minutes. The crude mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave irradiation at 70 °C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **15** (13.7 mg, 15.3% over two steps) as a light brown oil. IR (thin film) 3369 (br), 2939 (m), 1664 (s), 1563 (m), 1497 (w), 1438 (w), 1188 (s), 1134 (s), 837 (w), 798 (w), 753 (w), 721 (w); ^{1}H NMR (400 MHz, MeOD) δ 8.02 (s, 1H), 7.58-7.49 (m, 5H), 4.64 (s, 2H), 4.37 (t, *J* = 7.0 Hz, 2H), 4.33 – 4.24 (m, 2H), 3.70 – 3.63 (m, 6H), 3.27 (t, *J* = 5.4 Hz, 2H), 2.46 – 2.34 (m, 2H), 2.19 – 2.08 (m, 1H), 1.98 – 1.79 (m, 4H), 1.71-1.62 (m, 1H), 1.44 – 1.32 (m, 2H). ^{13}CNMR (125 MHz, DMSO-d_6) δ 174.4, 174.1, 173.7, 157.3, 146.2, 143.8, 129.1, 123.7, 118.3, 114.2, 69.6, 68.9, 68.2, 63.6,

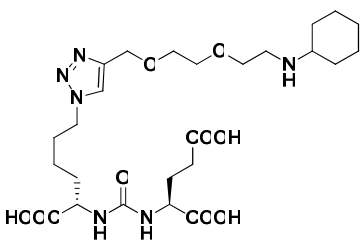
52.1, 51.7, 49.1, 44.1, 31.5, 29.9, 29.4, 27.5, 22.1. HRMS (ES+) calc'd for $C_{25}H_{36}N_6O_9$ (M+H) *m/z* 565.2577 Found 565.2621.

(S)-2-(3-((S)-1-carboxy-5-(4-((2-(2-(4-methoxyphenylamino)ethoxy)ethoxy)methyl)-1H-1,2,3-triazol-1-yl)pentyl)ureido)pentanedioic acid (16).



4-methoxy-N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)aniline **(s-10)** (34 mg, 0.137 mmol, 1 equiv.) and azide **s-12** (70 mg, 0.137 mmol, 1 equiv.) were added to a mixture of water (1 mL) and *t*-BuOH (1 mL). This slurry was placed in a microwave reaction tube, to which a 0.1 M solution of sodium ascorbate (6 mg, 0.034 mmol, 0.25 equiv.) and a 0.1 M solution of copper (II) sulfate in water (0.068 mL, 0.007 mmol, 0.05 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 °C for 20 minutes. The crude mixture was concentrated under reduced pressure, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave irradiation at 70 °C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **16** (9.3 mg, 11.8% over three steps) as a light brown oil. IR (thin film) 3347 (br), 2956 (m), 1728 (s), 1670 (s), 1564 (m), 1513 (m), 1443 (w), 1259 (w), 1200 (s), 1137 (m), 1029 (w), 837 (w); ^{1}H NMR (400 MHz, MeOD) δ 8.01 (s, 1H), 7.45 – 7.39 (m, 2H), 7.09 – 7.04 (m, 2H), 4.68 (s, 2H), 4.42 (t, *J* = 7.0 Hz, 2H), 4.30 (dd, *J* = 8.6, 5.0 Hz, 1H), 4.22 (dd, *J* = 8.5, 4.9 Hz, 1H), 3.87 (s, 3H), 3.75-3.73 (m, 2H), 3.72 – 3.68 (m, 2H), 3.68 – 3.64 (m, 2H), 3.57 – 3.50 (m, 2H), 2.49 – 2.33 (m, 2H), 2.17-2.12 (m, 1H), 2.01 – 1.79 (m, 4H), 1.72 – 1.57 (m, 1H), 1.45 – 1.32 (m, 2H). ^{13}CNMR (125 MHz, DMSO-d_6) δ 174.4, 174.1, 173.7, 157.2, 143.7, 126.5, 123.7, 121.6, 117.4, 114.9, 69.7, 68.8, 65.9, 63.5, 55.4, 52.1, 51.6, 49.1, 48.4, 31.5, 29.9, 29.4, 27.5, 22.1. HRMS (ES+) calc'd for $C_{26}H_{38}N_6O_{10}$ (M+H) *m/z* 595.2683 Found 595.2722.

(S)-2-(3-((S)-1-carboxy-5-(4-((2-(2-(cyclohexylamino)ethoxy)ethoxy)methyl)-1H-1,2,3-triazol-1-yl)pentyl)ureido)pentanedioic acid (17).



N-(2-(2-(prop-2-ynyloxy)ethoxy)ethyl)cyclohexanamine **(s-11)** (16 mg, 0.071 mmol, 1 equiv.) and azide **s-12** (16 mg, 0.071 mmol, 1 equiv.) were added to a mixture of water (0.500 mL) and *t*-BuOH (0.500 mL). This slurry was placed in a microwave reaction tube, to which a 0.1 M solution of sodium ascorbate in water (3.4 mg, 0.018 mol, 0.25 equiv.) and a 0.1 M solution of copper (II) sulfate in water (0.0355 mL, 0.00355 mmol, 0.05 equiv.) were added. The tube was capped and subjected to microwave irradiation at 110 °C for 20 minutes. The crude mixture was concentrated, and taken up in 67% trifluoroacetic acid in CH_2Cl_2 (3 mL). The tube was capped and subjected to microwave irradiation at 70 °C for 2 minutes. The crude mixture was concentrated under reduced pressure, purified via HPLC, and the pure fractions were collected and concentrated under reduced pressure to yield **17** (7.9 mg, 19.7% over two steps) as a clear oil. IR (thin film) 3344 (w), 2939 (m), 2865 (w), 1732 (m), 1670 (s), 1453 (w), 1201 (s), 1136 (m), 1088 (w), 799 (w); ^{1}H NMR (400 MHz, MeOD) δ 8.02 (s, 1H), 4.65 (s, 2H), 4.43 (t, *J* = 7.0 Hz, 2H), 4.34 – 4.21 (m, 3H), 3.76 – 3.64 (m, 7H), 3.26 – 3.19 (m, 2H), 3.08 (s, 1H), 2.50 – 2.34 (m, 2H), 2.16-2.10 (m, 3H), 2.03 – 1.81

(m, 6H), 1.70-1.63 (m, 2H), 1.46 – 1.28 (m, 7H), 1.28 – 1.14 (m, 1H). ^{13}CNMR (125 MHz, DMSO-d_6) δ 174.2, 173.9, 173.5, 157.1, 143.7, 123.7, 69.7, 69.5, 68.7, 68.3, 65.8, 63.4, 56.0, 49.1, 43.1, 31.4, 29.8, 29.3, 28.3, 27.4, 24.6, 23.8, 22.1. HRMS (ES+) calc'd for $C_{25}H_{42}N_6O_9$ (M+H) *m/z* 571.3047 Found 571.3091.

Biological Assays and Crystallographic Data

Measurement of PSMA K_m:

A 10 mM solution of N-acetyl-aspartylglutamate (NAAG) in 40 mM NaOH, and was then diluted in Reaction Buffer (100 mM Tris-HCl, pH 7.5) to a final NAAG concentration of 40 μM. The solution was added to a 384 well plate (20 μL per well). For K_m measurements and controls the NAAG solution was serially diluted 2-fold in Reaction Buffer to obtain final NAAG concentrations ranging from 40 μM – 312.5 nM. rhPSMA (20 ng/mL in Reaction Buffer, 20 μL, R&D Research) was then added to each well. Reaction Buffer (20 μL) was added to the K_m control series. The plate was incubated at room temperature for 15 min, and then heated to 95 ^{o}C for 3 minutes. The plate was allowed to cool to room temperature, and glutamic acid levels were measured using a comercially available Amplex®- Red Glutamic Acid / Glutamate Oxidase Assay Kit (Invitrogen). Fluorescence intensities were measured using a Synergy 2 multiwell plate reader (Biotek), fitted with excitation and emission filters of 545 nm and 590 nm, respectively. The K_m was calculated using nonlinear least-squares regression algorithms contained in the GraphPad Prism software package to provide an average K_m value for this enzymatic reaction of 0.925 μM. This value is consistent with that reported in the literature[7] and was employed in subsequent K_i calculations (see below).

PSMA Inhibition Assay:

For IC_{50} measurements, inhibitors were dissolved in Reaction Buffer containing containing 40 μM NAAG to a final volume of 100 μL. Then, 25 μL of this solution was transferred to each of three wells in a microtiter plate, and 5 μL aliquots were serially diluted into 20 μL of solution containing 40 μM NAAG over 10 wells (5-fold dilutions). Inhibitor concentration therefore ranged over 6 orders of magnitude in these experiments. rhPSMA (20 ng/mL in Reaction Buffer, 20 μL, R&D Research) was then added to each well. The plate was incubated at room temperature for 15 min, and then heated to 95 ^{o}C for 3 minutes. The plate was allowed to cool to room temperature, and glutamic acid levels were measured using a comercially available Amplex®-Red Glutamic Acid / Glutamate Oxidase Assay Kit (Invitrogen). Fluorescence intensities were measured using a Synergy 2 multiwell plate reader (Biotek), fitted with excitation and emission filters of 545 nm and 590 nm, respectively. The concentration of inhibitors giving 50% inhibition of enzyme activity (IC_{50}) was calculated from the least-squares regression line of the residual enzymatic activity plotted as a function of logarithmic inhibitor concentrations using algorithms contained in the GraphPad Prism software package. K_i values were obtained from IC_{50} values using the Cheng-Prusoff equation, a substrate concentration of 20 μM, and a K_m value of 0.925 μM. All reported values in Tables 1 and 2 represent the average of at least three replicates ± standard deviation.

rhPSMA expression and purification

The extracellular domain of human PSMA (amino acids 44-750) was expressed and purified as described previously and we designate this construct rhPSMA[8]. For crystallization experiments, rhPSMA was dialyzed against 20 mM MOPS, 20 mM NaCl, pH 7.4, and concentrated to 10 mg/mL.

Crystallization, data collection and processing

The stock solutions of individual inhibitors at 50mM were prepared in 25% (v/v) acetonitrile in water. Diffraction quality crystals of rhPSMA/ARMs complexes were grown at 293 K by vapor diffusion in hanging drops. The stock solution of rhPSMA was mixed in a 10:1 ratio with an ARM and hanging drops formed by mixing equal volumes of the protein and reservoir solutions (33% (v/v) pentaerythritol propoxylate PO/OH 5/4 (Hampton Research), 0.5 % (w/v) PEG 3350, and 100 mM Tris-HCl, pH 8.0). Prior to the data collection, the crystals were flash-frozen in liquid nitrogen directly from the hanging drop. Each of the four datasets was collected from a single crystal at 100 K using synchrotron radiation at the SER-CAT sector 22 beamlines of the Advanced Photon Source (Argonne, IL, USA) equipped with MAR225 or MAR300 CCD detectors. Data were integrated and scaled with the HKL2000 package[9].

Electron map density fit

Individual compounds were fit into the positive electron density in the final stages of the refinement. For all four inhibitors, clear interpretable densities were observed for the C-terminal part encompassing the P1' glutarate, the urea linkage, the lysine linker, and the triazole ring

Structure solution and refinement

Structure determination of rhPSMA/ARMs complexes was carried out using difference Fourier methods with the ligand-free rhPSMA (PDB code 2OOT;[10]) as a starting model. Calculations were performed with the program Refmac 5.1[11], and the refinement protocol was interspersed with manual corrections to the model employing the program Coot[12]. Library and PDB-format files of individual inhibitors were prepared using the PRODRG server[13] and the inhibitors were fitted into the positive electron density map in the final stages of the refinement. During the refinement process, ~ 1% of the randomly selected reflections were kept aside for cross-validation (R_{free}). The quality of the final model was evaluated using the MOLPROBITY server[14]. The data collection and refinement statistics are summarized in Table S1.

PDB Accession Numbers

Atomic coordinates of the present structures together with the experimental structure factor amplitudes will be deposited in the RCSB Protein Data Bank.

Table S1: Data calculations and refinement statistics

	GCPII/ARM-P2	**GCPII/ARM-P4**	**GCPII/ARM-P8**	**GCPII/ARM-M4**
PDB code	TBD	TBD	TBD	TBD
Data Collection Statistics				
Wavelength (Å)	1.0000	1.0000	1.0000	1.0000
Temperature (K)	100			
Space group	I222			
Unit-cell parameters: a, b, c (Å)	*a* = 101.5; *b* = 130.0; *c* = 158.6	*a* = 101.7; *b* = 130.0; *c* = 159.0	*a* = 101.8; *b* = 130.0; *c* = 158.8	*a* = 101.5; *b* = 130.0; *c* = 159.2

Resolution limits (Å)	30.0 – 1.69 (1.75 – 1.69)*	30.0 – 1.59 (1.65 – 1.59)*	30.0 – 1.59 (1.63 – 1.59)*	30.0 – 1.78 (1.84 – 1.78)*
Number of unique reflections	114,649 (9,759)	137,271 (11,088)	137,748 (10,972)	100,565 (9,717)
Redundancy	5.8 (2.5)	7.0 (5.0)	6.6 (3.8)	7.1 (5.6)
Completeness (%)	97.8 (84.1)	97.6 (79.9)	96.9 (77.9)	99.7 (97.3)
I / σ(I)	18.4 (2.1)	27.8 (2.0)	15.4 (2.6)	21.3 (2.5)
R_{merge}	0.086 (0.492)	0.058 (0.501)	0.078 (0.438)	0.086 (0.520)
Refinement Statistics				
Resolution limits (Å)	30.0 – 1.69 (1.73 – 1.69)*	30.0 – 1.59 (1.63 – 1.59)*	30.0 – 1.59 (1.63 – 1.59)*	20.0 – 1.78 (1.82 – 1.78)*
Total number of reflections	112,878 (6,994)	135,823 (7,887)	135,631 (7,827)	99,006 (6,972)
Number of reflections in working set	111,178 (6,872)	134,450 (7,815)	133,594 (7,726)	97,519 (6,871)
Number of reflections in test set	1,700 (122)	1,373 (72)	2,037 (101)	1,487 (101)
R factor	16.0 (23.6)	16.8 (25.3)	16.1 (24.6)	15.7 (26.6)
Free-R	18.5 (29.3)	19.1 (33.0)	18.3 (28.7)	18.5 (29.1)
Total number of non-H atoms	6,491	6,618	6,687	6,546
Number of inhibitor atoms	92[#]	52	128[#]	41
Number of ions	4	4	4	4
Number of water molecules	505	612	581	563
Average B factor ($Å^2$)				
Protein atoms	28.0	25.9	24.5	18.7
Water molecules	38.4	36.3	37.4	27.4
Ligand atoms	40.3	48.5	51.8	48.8
r.m.s.d.				
Bond lengths (Å)	0.021	0.018	0.017	0.021
Bond angles (°)	1.85	1.71	1.69	1.72
Planarity (Å)	0.011	0.010	0.011	0.010
Chiral centers ($Å^3$)	0.14	0.13	0.12	0.14
Ramachandran plot (%)**				
Most favored	97.7	97.7	97.7	97.8
Allowed	2.3	2.3	2.3	2.2
Disallowed	0	0	0	0
Missing residues	44-54; 654-655	44-54; 654-655	44-54; 654-655	44-54; 654-655

* Values in parentheses correspond to the highest resolution shells

** Calculated with MOLPROBITY[14]

[#] inhibitor modeled in two conformations

Computational Studies

Quantum chemical computations

Models of substituted methyl-amino-phenyls were constructed using the software Maestro.[15] All calculations were carried out using the software BOSS.[16] Geometry optimization was performed using the semiempirical method PPDG/PM3.[17]

Molecular dynamics simulations

The crystal structure of ARM-P2-DNP in complex with PSMA (695 residues) was used to setup all the protein-ligand complexes. MeO-P0, ARM-P0, ARM-P2, ARM-P4 and ARM-P8 were modeled in the same protein structure on the basis of available experimental data. The LigPrep module of the software Maestro was used to add missing hydrogen atoms, choose the protonation state of protein side chains ,and minimize the energy of the protein-ligand complex.[18] The 2005 update of the OPLS force field was used throughout.[19] The resulting structure was then embedded in a triclinic box of circa 13300 TIP3P water molecules,[20] the dimension of the box was circa 96x87x94 Å. The net charge of the system was neutralized by addition of one sodium ion to the solvent box. The total number of atoms was circa 53,000 atoms. The simulations were performed with the Desmond molecular dynamics package[21]. All bond lengths to hydrogen atoms were constrained using MM-SHAKE.[22] Van der Waals and short range electrostatic interactions were cut off at 9 Å. Long range electrostatic interactions were computed using the particle mesh Ewald method using a 32x32x32 grid with σ=2.18 Å and fifth-order B-splines for interpolation.[23] A RESPA integrator was used with a time-step of 2 fs, and the long range electrostatic interactions were computed every 6 fs.[24] Each system was initially energy minimized with steepest decent and then subjected to the following equilibration protocol: 12 ps of dynamics at 10 K in the NVT ensemble (Berendsen thermostat)[25] and harmonic restraints (50 kcal/mol/A^2) on the solutes heavy atoms, followed by 12 ps in the NPT ensemble (Berendsen thermostat and barostat) at 10 K and retaining harmonic restraints on the solutes heavy atoms, followed by 24 ps in the NPT ensemble (Berendsen thermostat and barostat) at 300 K and retaining harmonic restraints on the solutes heavy atoms, followed by 24 ps in the NPT ensemble (Berendsen thermostat and barostat) at 300 K without harmonic restraints on the solutes heavy atoms, followed by 100 ps of dynamics at 300 K in the NPT ensemble (Martyna-Tobias-Klein barostat and Nose-Hoover thermostat).[26,27] The production simulations were run for 50 nanoseconds in the NPT ensemble (300 K, 1 bar, Martyna-Tobias-Klein barostat and Nose-Hoover thermostat). Coordinates were saved every 10 ps and analyzed using the software Visual Molecular Dynamics.[28]

References:

(1) Sinha, J.; Sahoo, R.; Kumar, A. *Macromolecules* **2009**, *42*, 2015-2022.

(2) Diot, J.; Garcia-Moreno, M. I.; Gouin, S. G.; Ortiz Mellet, C.; Haupt, K.; Kovensky, J. *Organic & Biomolecular Chemistry* **2009**, *7*, 357-363.

(3) Knapp, S.; Ziv, J.; Rosen, J. D. *Tetrahedron* **1989**, *45*, 1293-8.

(4) Murelli, R. P.; Zhang, A. X.; Michel, J.; Jorgensen, W. L.; Spiegel, D. A. *J. Am. Chem. Soc.* **2009**, 17090-17092.

(5) Scates, B. A.; Lashbrook, B. L.; Chastain, B. C.; Tominaga, K.; Elliott, B. T.; Theising, N. J.; Baker, T. A.; Fitch, R. W. *Bioorg. Med. Chem.* **2008**, *16*, 10295-10300.

(6) Maeda, H.; Maeda, T.; Mizuno, K.; Fujimoto, K.; Shimizu, H.; Inouye, M. *Chemistry--A European Journal* **2006**, *12*, 824-831.

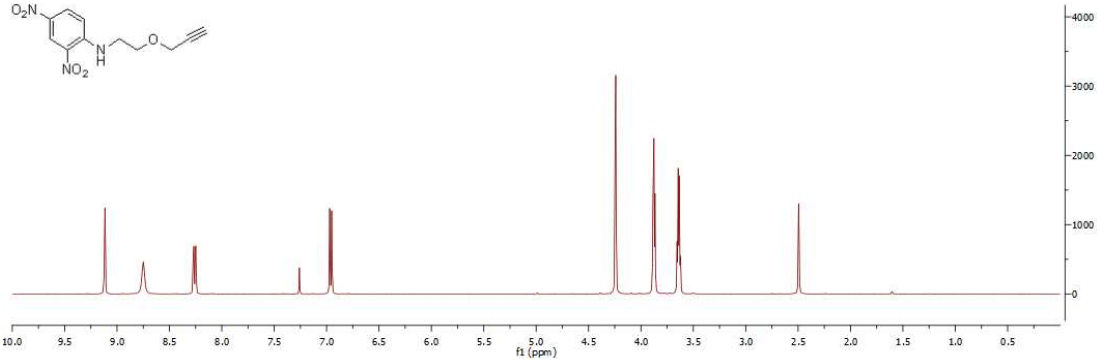
(7) Barinka, C.; Hlouchova, K.; Rovenska, M.; Majer, P.; Dauter, M.; Hin, N.; Ko, Y.-S.; Tsukamoto, T.; Slusher, B. S.; Konvalinka, J.; Lubkowski, J. *J. Mol. Biol.* **2008**, *376*, 1438-1450.
(8) Barinka, C.; Rinnova, M.; Sacha, P.; Rojas, C.; Majer, P.; Slusher, B. S.; Konvalinka, J. *Journal of Neurochemistry* **2002**, *80*, 477-487.
(9) Otwinowski, Z.; Minor, W. *Methods in Enzymology* **1997**, *276*, 307-326.
(10) Barinka, C.; Starkova, J.; Konvalinka, J.; Lubkowski, J. *Acta Crystallographica, Section F: Structural Biology and Crystallization Communications* **2007**, *F63*, 150-153.
(11) Murshudov, G. N.; Vagin, A. A.; Lebedev, A.; Wilson, K. S.; Dodson, E. J. *Acta Crystallographica, Section D: Biological Crystallography* **1999**, *D55*, 247-255.
(12) Emsley, P.; Cowtan, K. *Acta Crystallographica, Section D: Biological Crystallography* **2004**, *D60*, 2126-2132.
(13) Schuttelkopf, A. W.; van Aalten Daan, M. F. *Acta crystallographica. Section D, Biological crystallography* **2004**, *60*, 1355-63.
(14) Davis, I. W.; Leaver-Fay, A.; Chen, V. B.; Block, J. N.; Kapral, G. J.; Wang, X.; Murray, L. W.; Arendall, W. B., 3rd; Snoeyink, J.; Richardson, J. S.; Richardson, D. C. *Nucleic Acids Res.* **2007**, *35*, W375-83.
(15) *Maestro*, Schrodinger, LLC, New York, NY.
(16) Jorgensen, W. L.; Tirado-Rives, J. *J. Comput. Chem.* **2005**, *26*, 1689-1700.
(17) Repaski, M. P.; Chandrasekhar, J.; Jorgensen, W. L. *J. Comput. Chem*. **2002**, *23*, 1601-1622.
(18) *LigPrep*, Schrodinger, LLC, New York, NY.
(19) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *Journal of the American Chemical Society* **1996**, *118*, 11225-11236.
(20) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *Journal of Chemical Physics* **1983**, *79*, 926-35.
(21) Bowers, K. J.; Chow, E.; Xu, H.; Dror, R. O.; Eastwood, M. P.; Gregersen, B. A.; Klepeis, J. L.; Kolossvary, I.; Moraes, M. A.; Sacerdoti, F. D.; Salmon, J. K.; Shan, Y.; Shaw, D. E. Proceedings of the ACM/IEEE Conference on Supercomputing (SC06), New York, 2006.
(22) Krautler, V.; Van Gunsteren, W. F.; Hunenberger, P. H. *Journal of Computational Chemistry* **2001**, *22*, 501-508.
(23) Darden, T.; York, D.; Pedersen, L. *Journal of Chemical Physics* **1993**, *98*, 10089-92.
(24) Tuckerman, M.; Berne, B. J.; Martyna, G. J. *Journal of Chemical Physics* **1992**, *97*, 1990-2001.
(25) Berendsen, H. J. C.; Postma, J. P. M.; Van Gunsteren, W. F.; DiNola, A.; Haak, J. R. *Journal of Chemical Physics* **1984**, *81*, 3684-90.
(26) Martyna, G. J.; Tobias, D. J.; Klein, M. L. *Journal of Chemical Physics* **1994**, *101*, 4177-89.
(27) Evans, D. J.; Holian, B. L. *Journal of Chemical Physics* **1985**, *83*, 4069-74.
(28) Humphrey, W.; Dalke, A.; Schulten, K. *Journal of Molecular Graphics* **1996**, *14*, 33-8, plates, 27-28.

Extended Reference List:

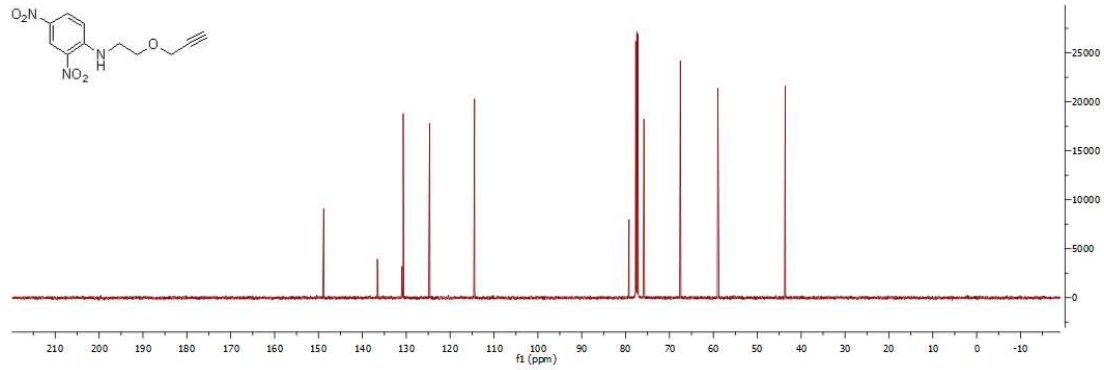
(15) Majer, P.; Jackson, P. F.; Delahanty, G.; Grella, B. S.; Ko, Y.-S.; Li, W.; Liu, Q.; Maclin, K. M.; Polakova, J.; Shaffer, K. A.; Stoermer, D.; Vitharana, D.; Wang, E. Y.; Zakrzewski, A.; Rojas, C.; Slusher, B. S.; Wozniak, K. M.; Burak, E.; Limsakun, T.; Tsukamoto, T. *J. Med. Chem.* **2003**, *46*, 1989-1996.

Spectral Data

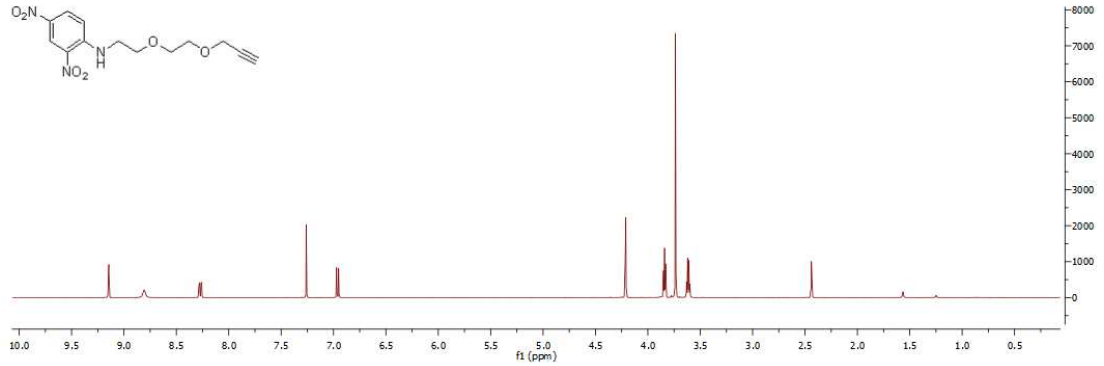
^{1}H-NMR spectrum of **s-2a** ($CDCl_3$)



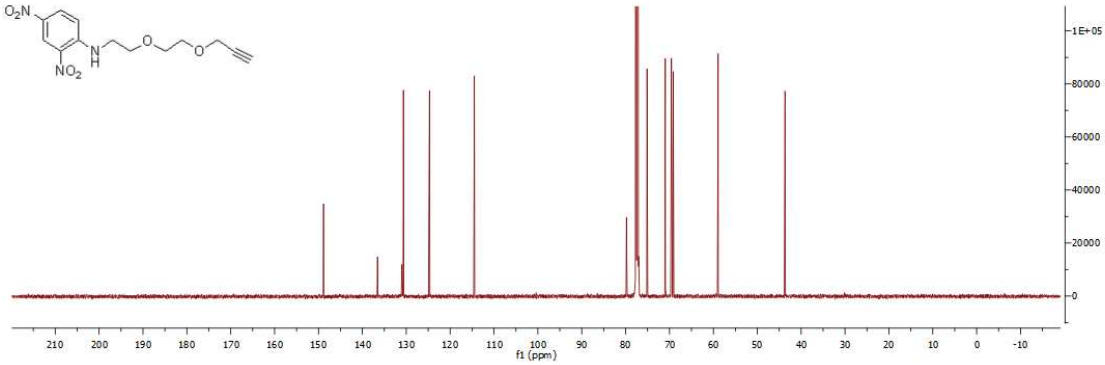
^{13}C-NMR spectrum of **s-2a** ($CDCl_3$)



^{1}HNMR spectrum of **s-2b** ($CDCl_3$)



^{13}CNMR spectrum of **s-2b** ($CDCl_3$)



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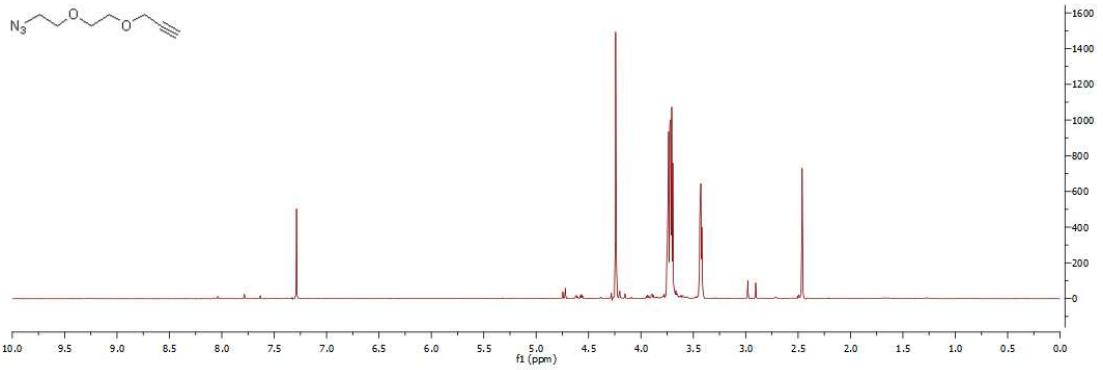
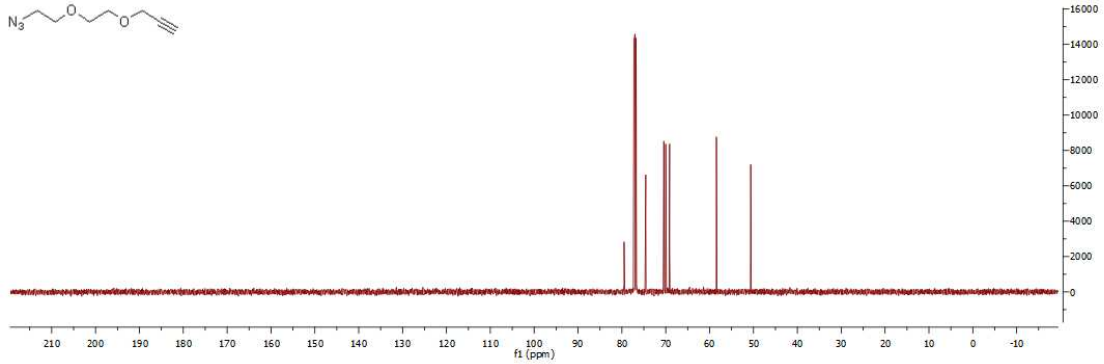


^{13}CNMR spectrum of **s-4** ($CDCl_3$)



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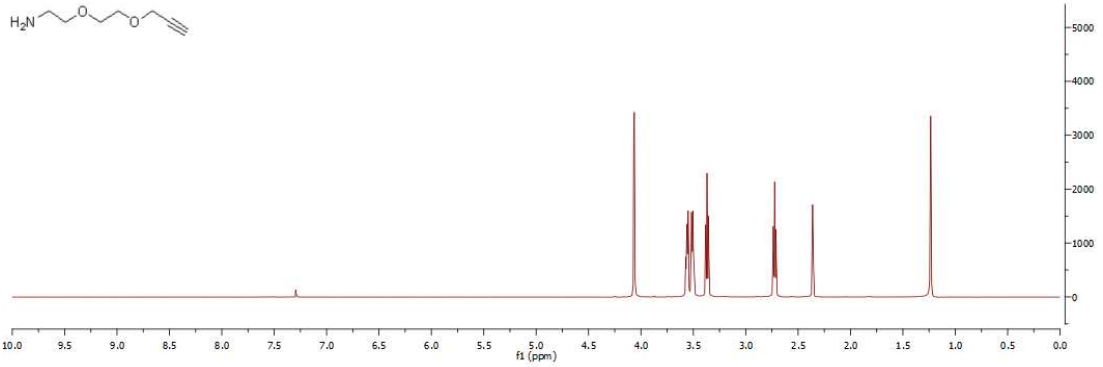

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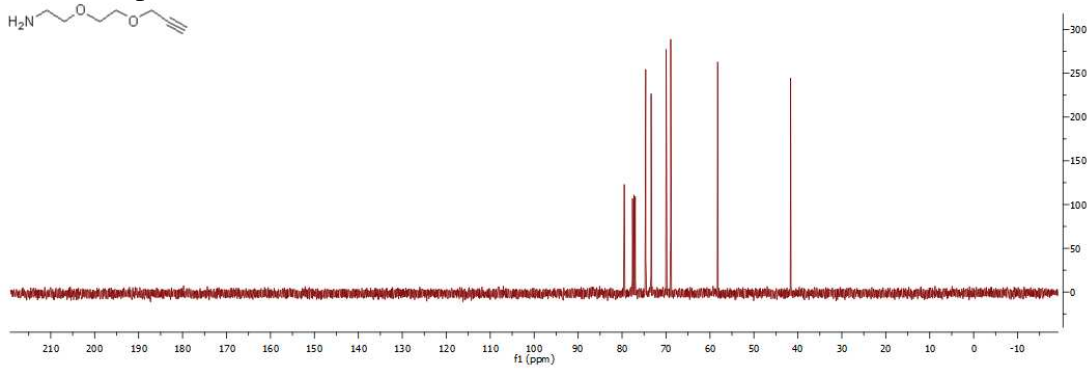
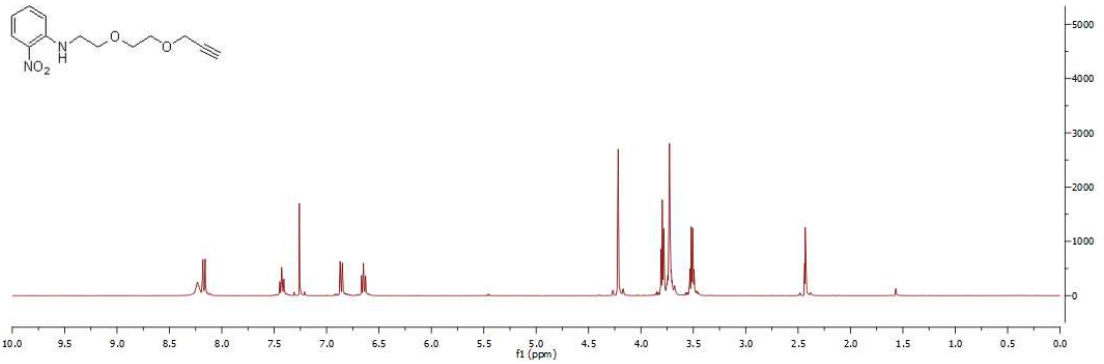
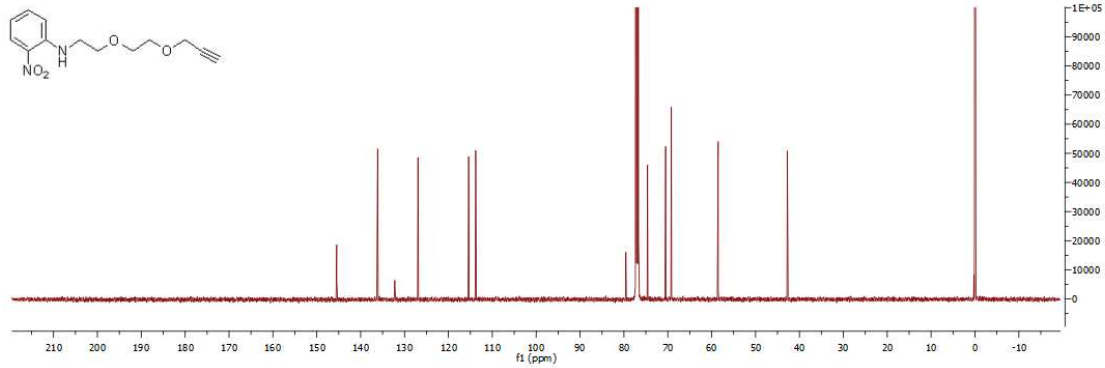


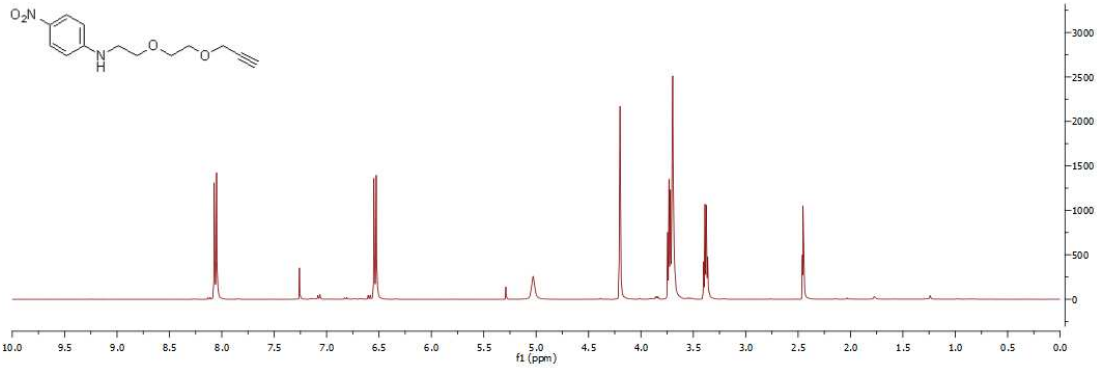
^{1}HNMR spectrum of **s-6** ($CDCl_3$)



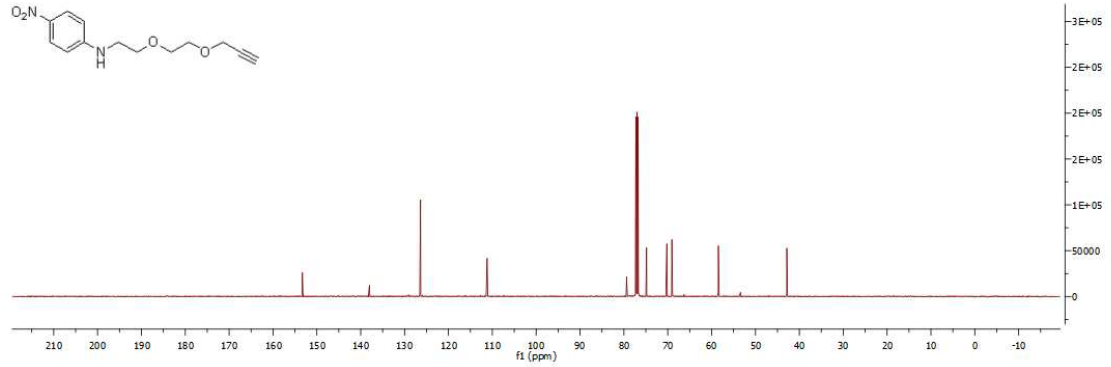
^{13}CNMR spectrum of **s-6** ($CDCl_3$ + TMS)



^{1}HNMR spectrum of **s-7** (CDCl3)



^{13}CNMR spectrum of **s-7** ($CDCl_3$)



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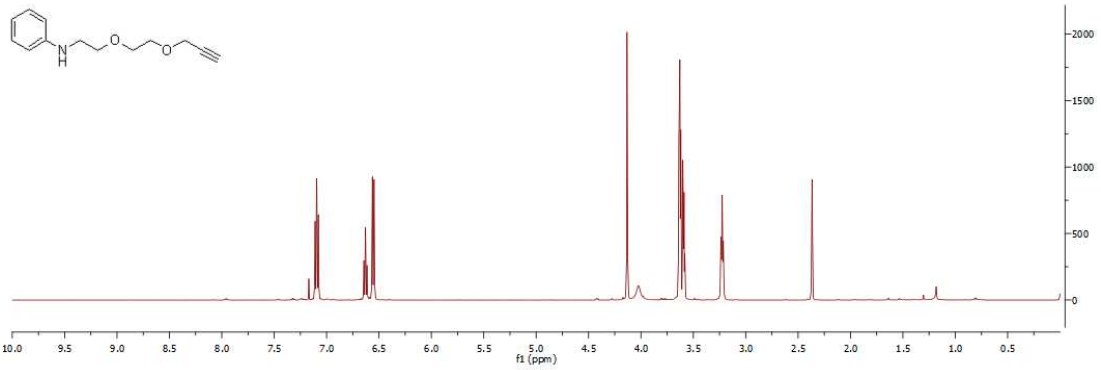
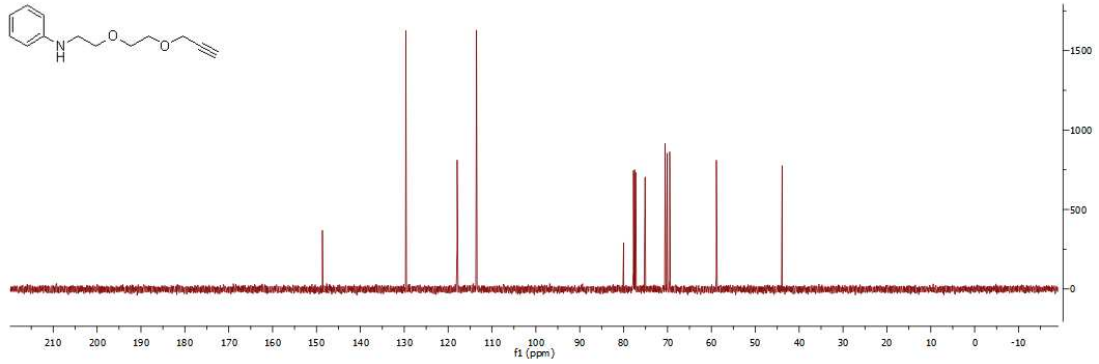
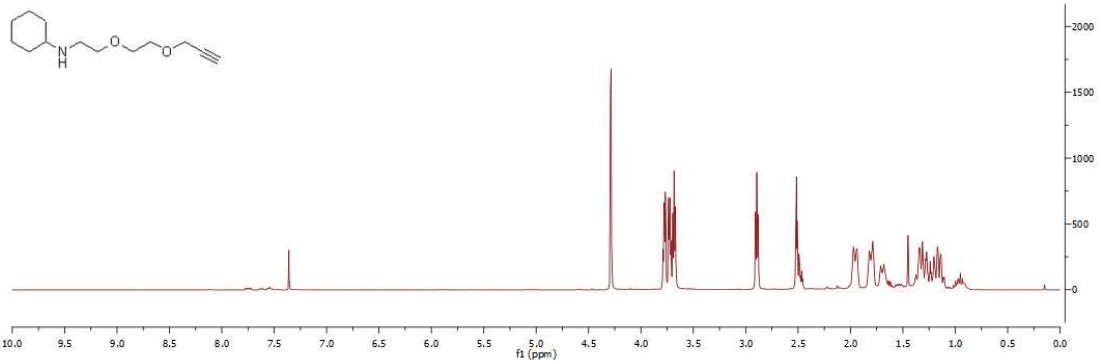


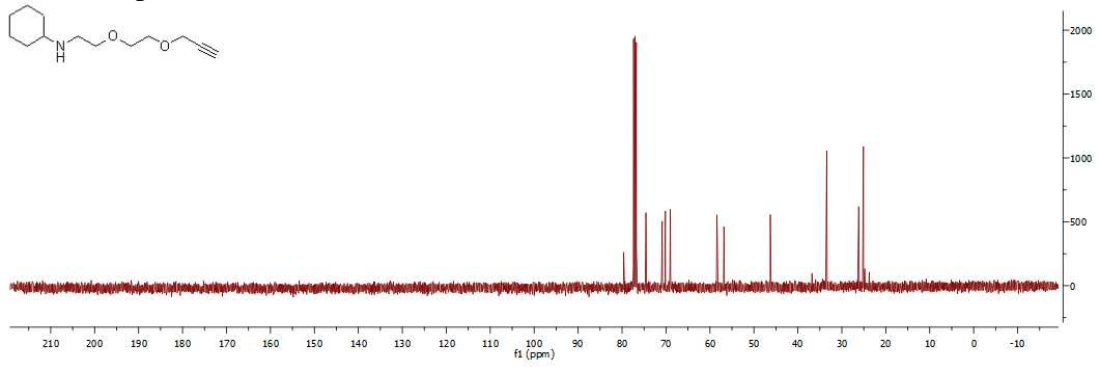
^{13}CNMR spectrum of **s-9** ($CDCl_3$)



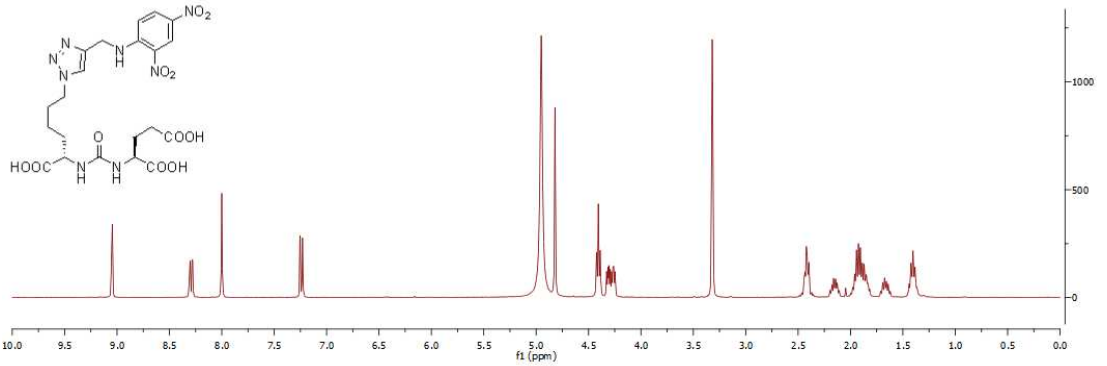
^{1}HNMR spectrum of **s-11** ($CDCl_3$)



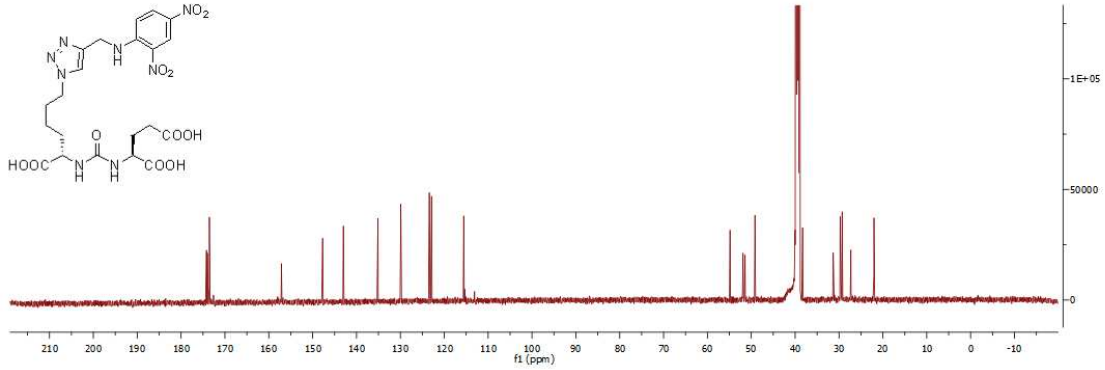
^{13}CNMR spectrum of **s-11** ($CDCl_3$)



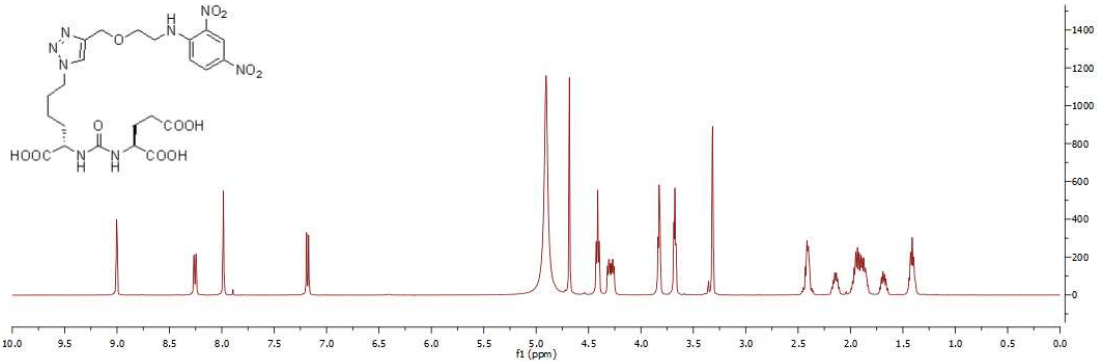
^{1}HNMR spectrum of **1** (MeOD)



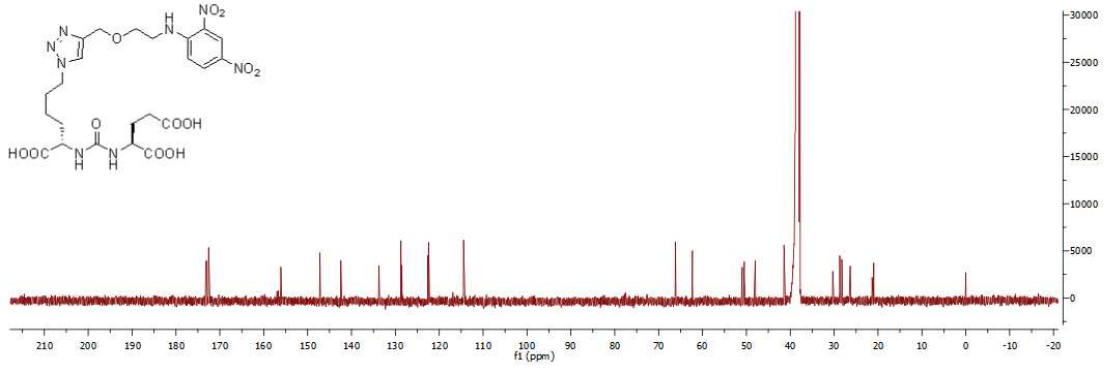
^{13}CNMR spectrum of **1** (DMSO–d_6)



^{1}HNMR spectrum of **2** (MeOD)



^{13}CNMR spectrum of **2** (DMSO–d_6)



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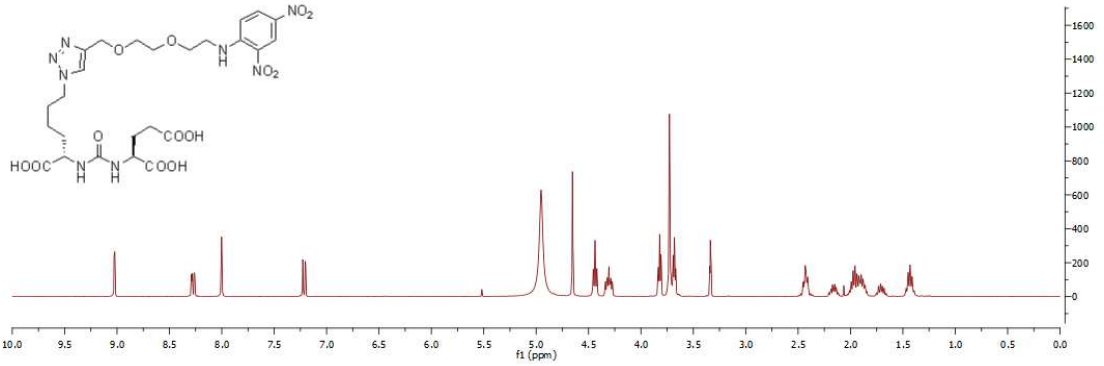
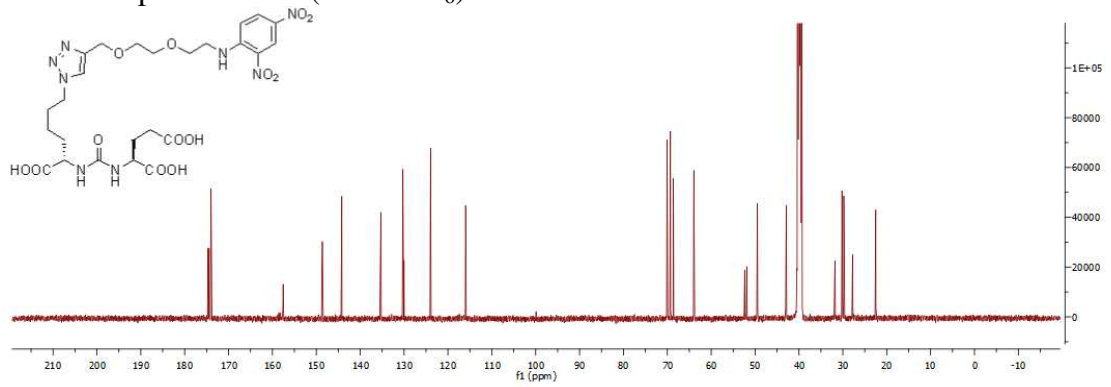
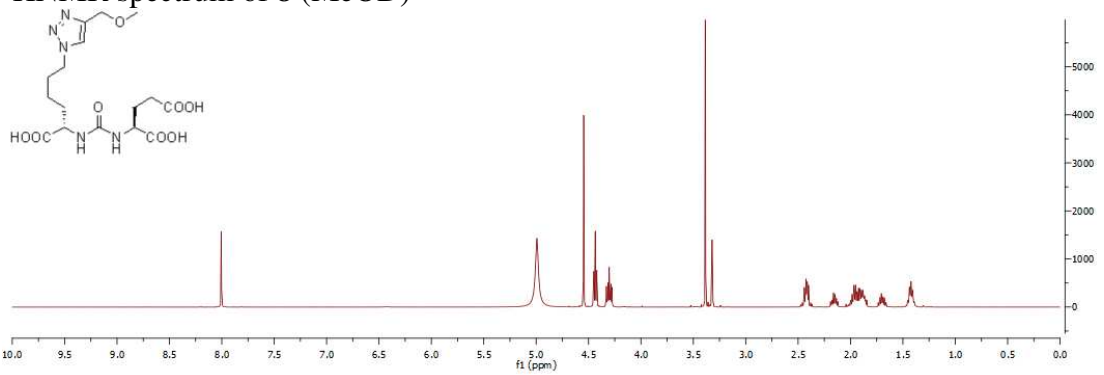


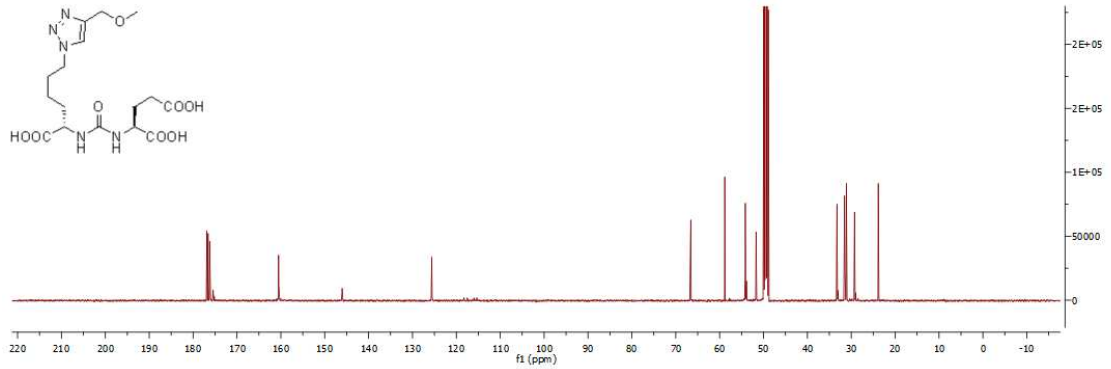
^{13}CNMR spectrum of **3** (DMSO–d_6)



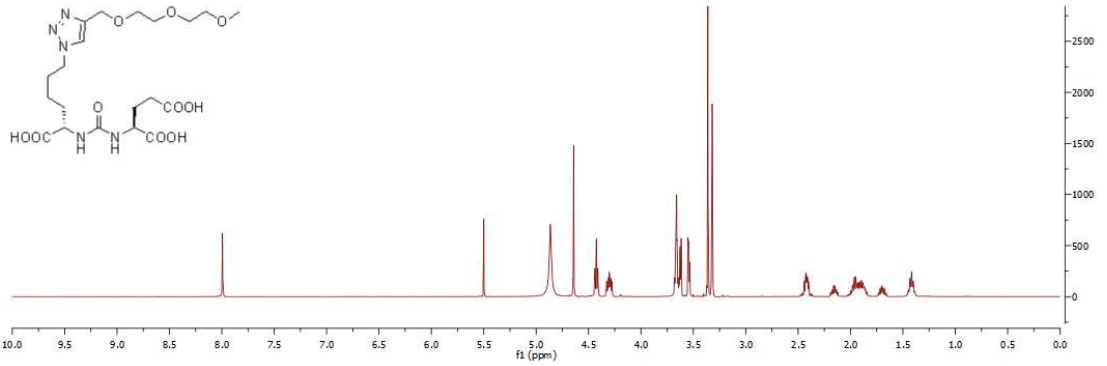
^{1}HNMR spectrum of **8** (MeOD)



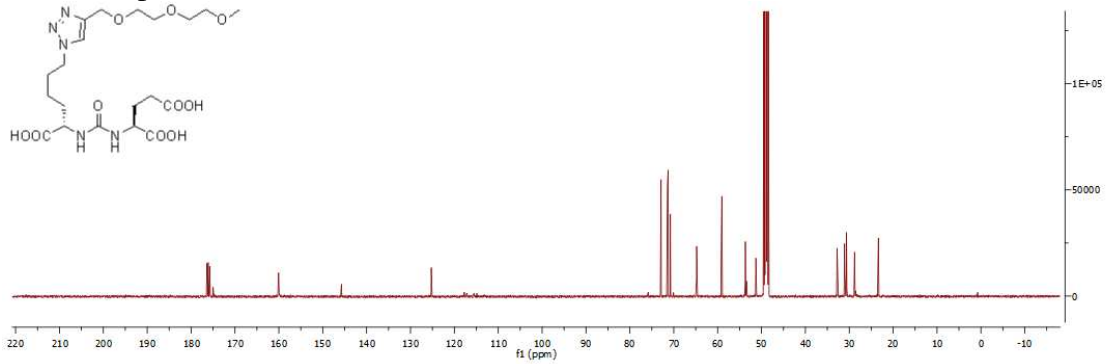
^{13}CNMR spectrum of **8** (MeOD)



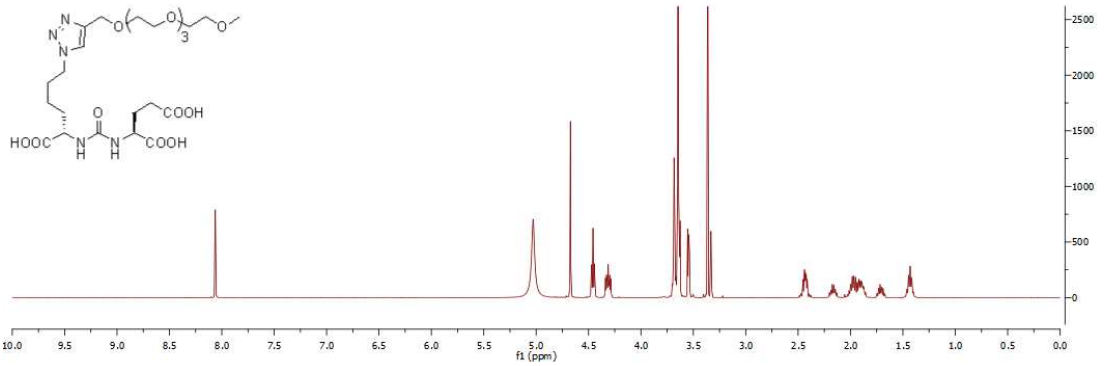
^{1}HNMR spectrum of **9** (MeOD)



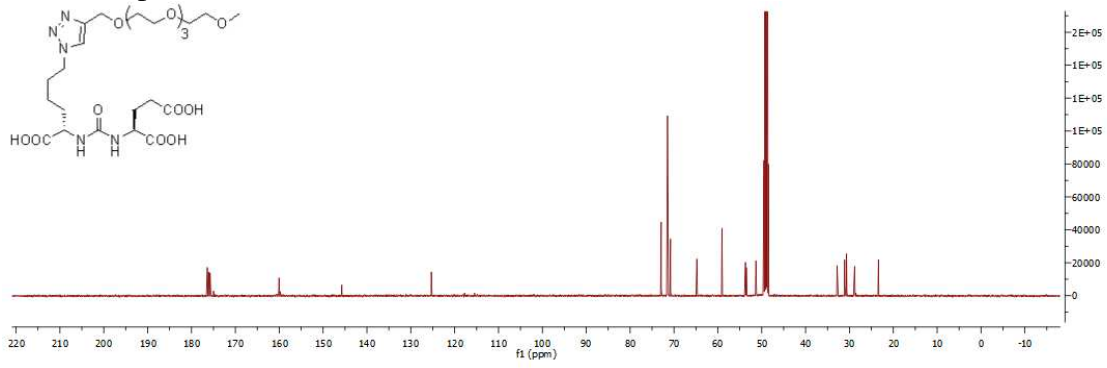
^{13}CNMR spectrum of **9** (MeOD)



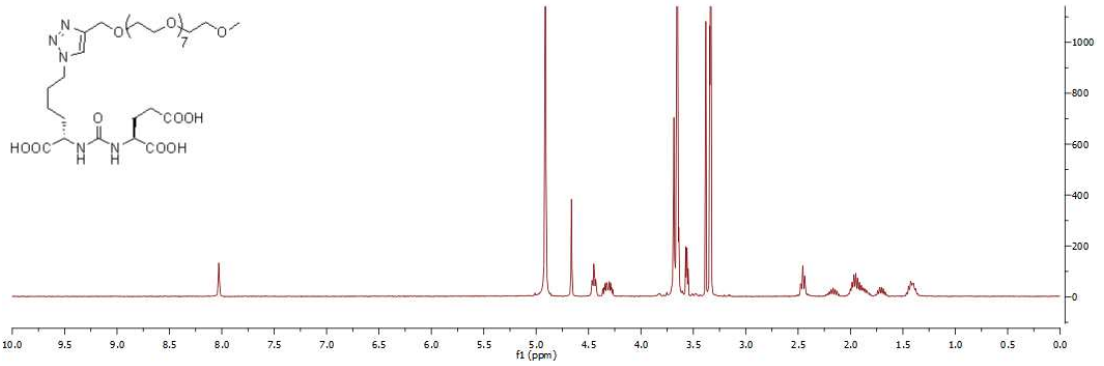
^{1}HNMR spectrum of **10** (MeOD)



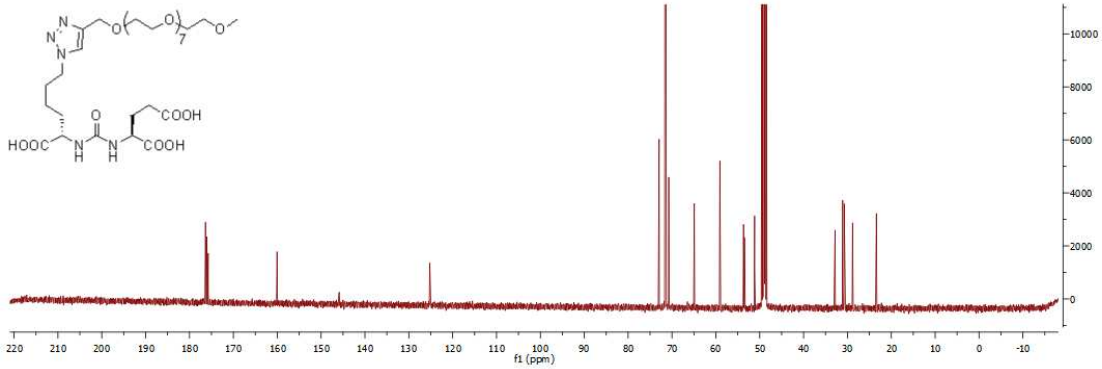
^{13}CNMR spectrum of **10** (MeOD)



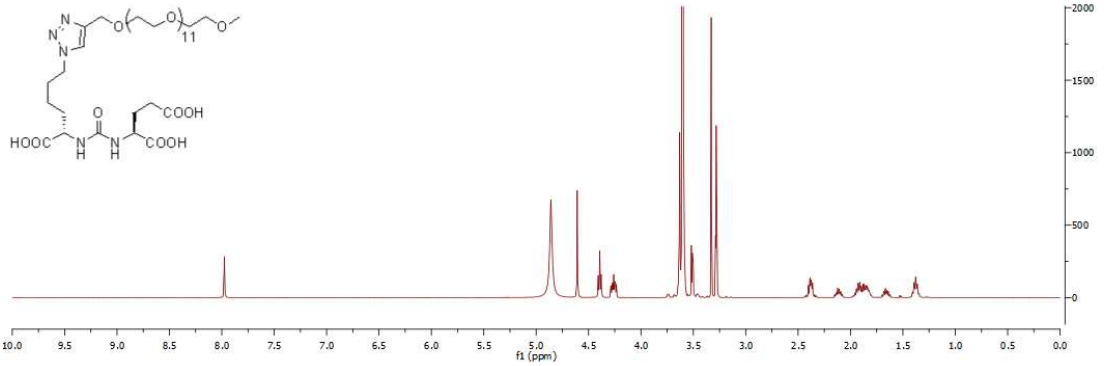
^{1}HNMR spectrum of **11** (MeOD)



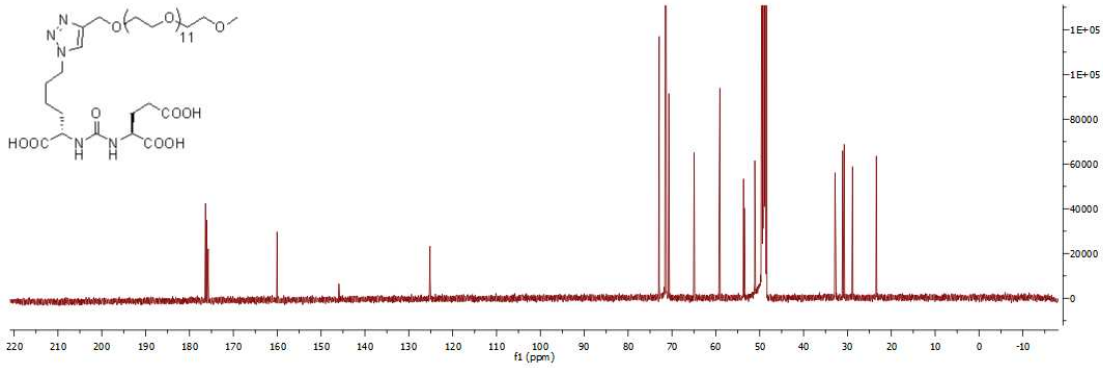
^{13}CNMR spectrum of **11** (MeOD)



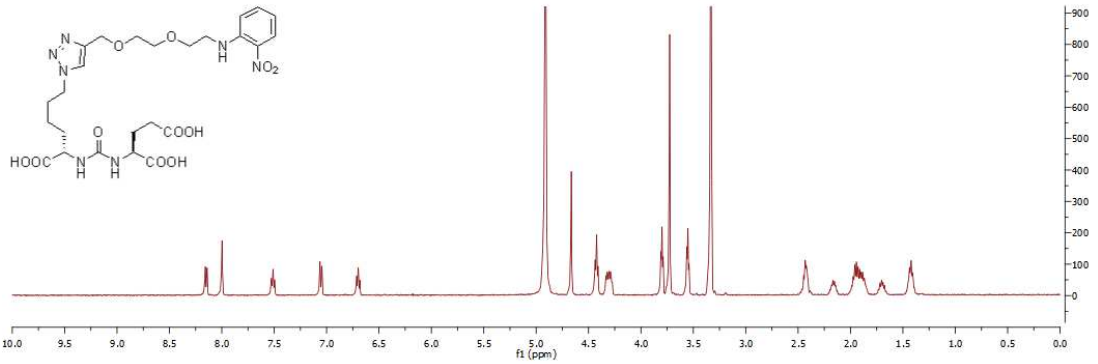
^{1}HNMR spectrum of **12** (MeOD)



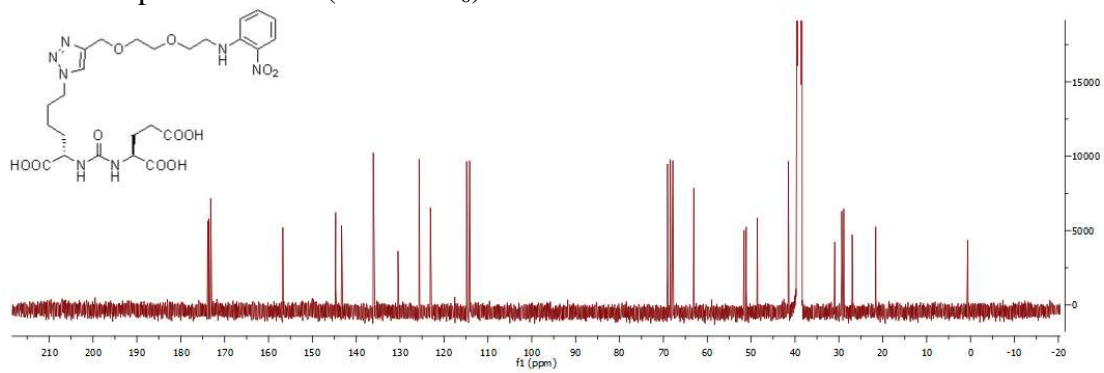
^{13}CNMR spectrum of **12** (MeOD)



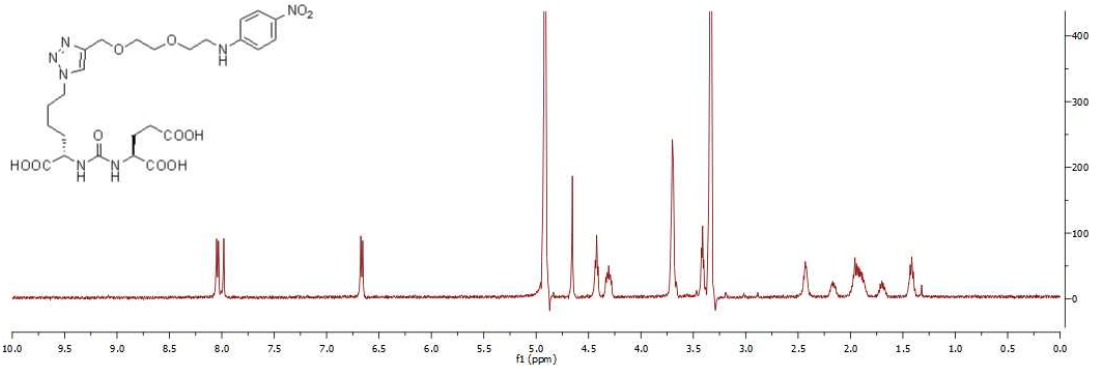
^{1}HNMR spectrum of **13** (MeOD)



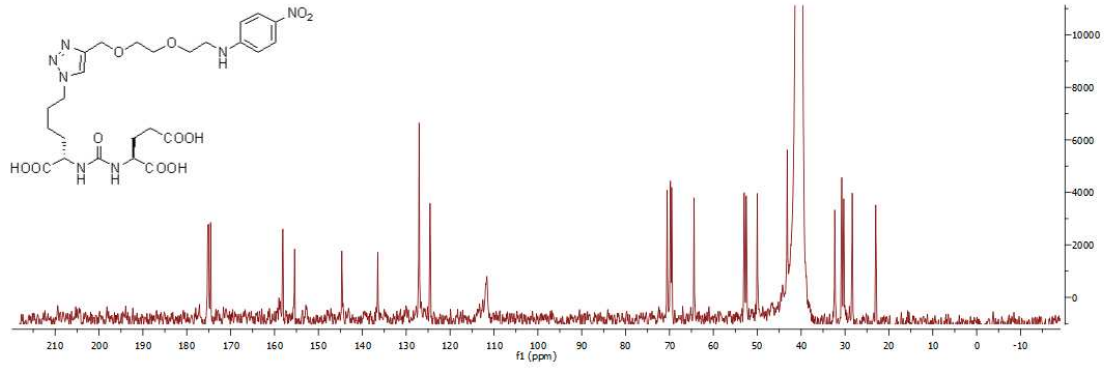
^{13}CNMR spectrum of **13** (DMSO–d_6)



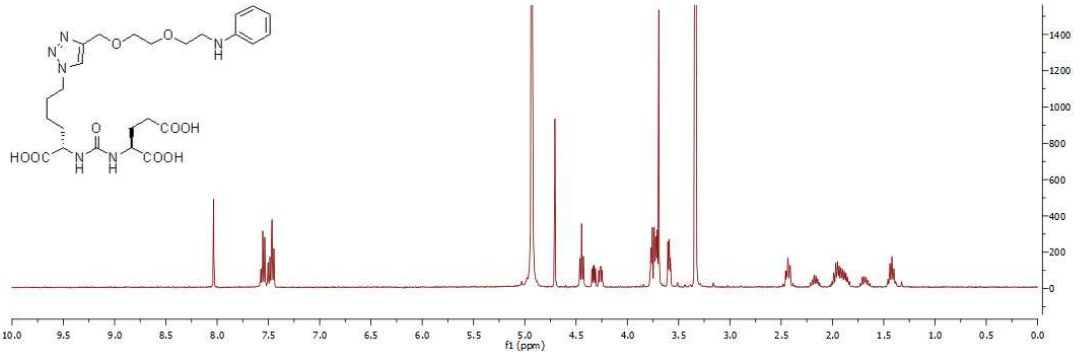
^{1}HNMR spectrum of **14** (MeOD)



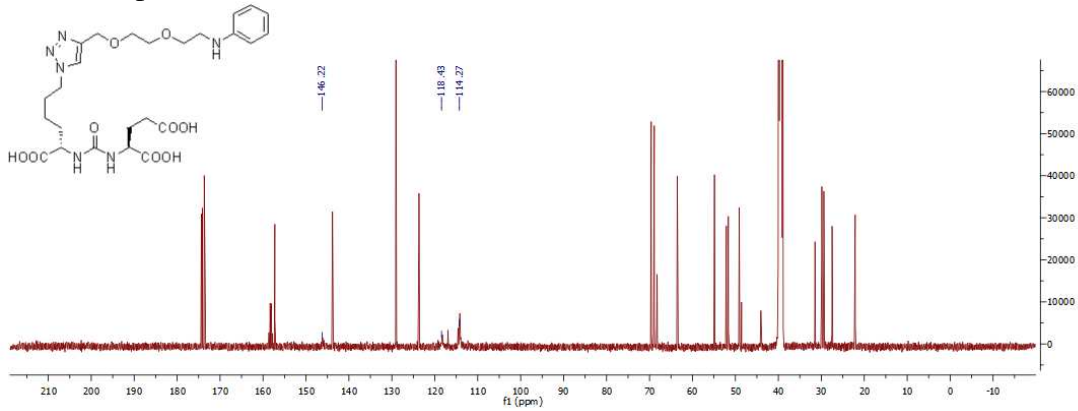
^{13}CNMR spectrum of **14** (DMSO–d_6)



^{1}HNMR spectrum of **15** (MeOD)



^{13}CNMR spectrum of **15** (DMSO–d_6)



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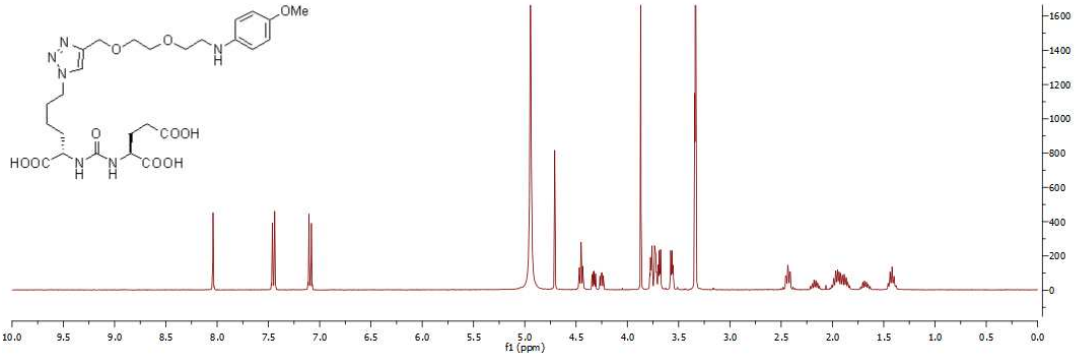
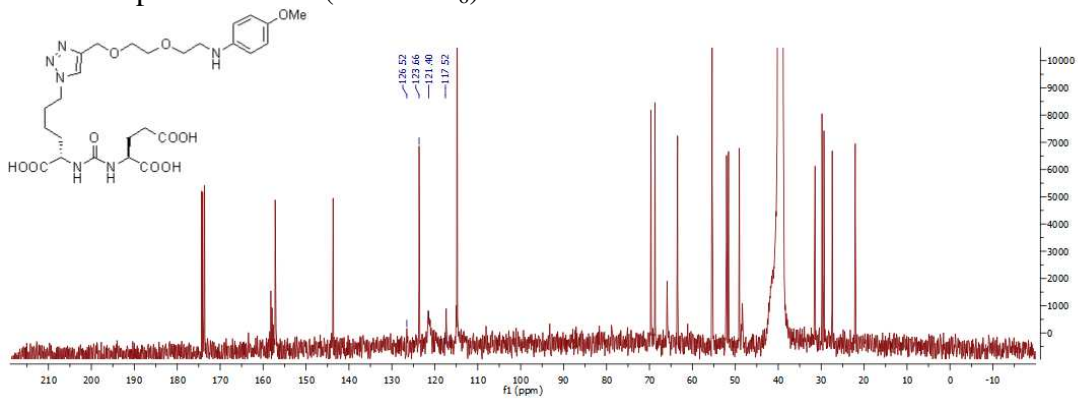
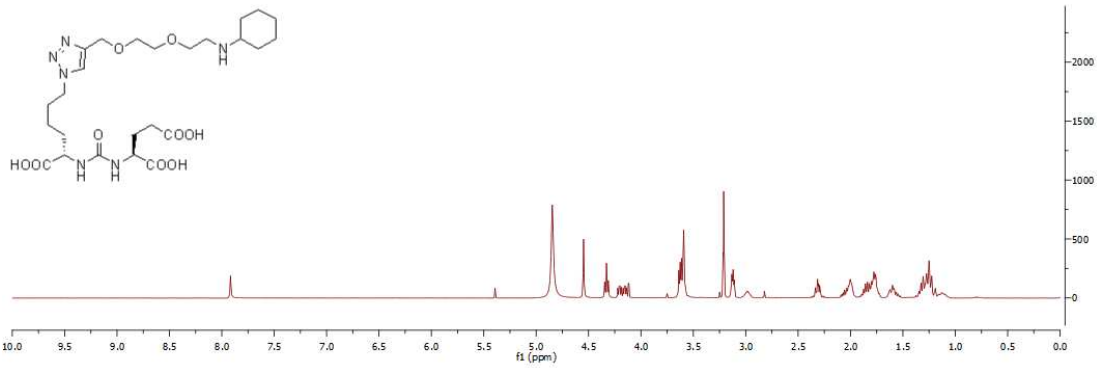


^{13}CNMR spectrum of **16** (DMSO–d_6)



^{1}HNMR spectrum of **17** (MeOD)



^{13}CNMR spectrum of **17** (DMSO–d_6)