Supporting Information

Graphene-based biosensor for On-chip detection of Bio-orthogonally Labeled Proteins to Identify the Circulating Biomarkers of Aging during Heterochronic Parabiosis.

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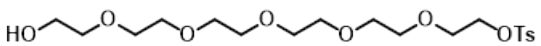
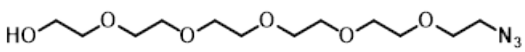
Synthesis of PEG azide



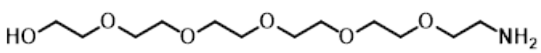
6

17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (***6***). Hexaethylene glycol (2.6 g, 9.15 mmol, 1 equiv), TsCl (1.93 g, 11.1 mmol, 1.1 equiv), Ag_2O (3.20 g, 13.8 mmol, 1.5 equiv), and KI (0.306 g, 1.8 mmol, 0.2 equiv) were suspended in dichloromethane (90 mL) and stirred under N_2 overnight at room temperature. The reaction was concentrated and purified via column chromatography on silica gel eluting at 2:3 hexanes: acetone to yield 2.14 g of clear yellow oil (53.6%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.75 (d, J = 8.3 Hz, 2H), 7.31 (d, J = 8.8 Hz, 2H), 4.08-4.15 (m, 2H), 3.46-3.73 (m, 25H), 2.72 (s, 1H), 2.41 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 144.9, 133.0, 129.9, 128.0, 72.6, 70.8, 70.7, 70.6, 70.4, 69.3, 68.7, 61.8, 21.7. HRMS (ESI) m/z calculated for: $[C_{19}H_{32}O_9S]^+$: 437.516. Found: 437.1832 (Δ 0.3328).



7

17-azido-3,6,9,12,15-pentaoxaheptadecan-1-ol (***7***). To a solution of compound **6** (5.68 g, 13.06 mmol, 1 equiv) in DMF (60 mL) was added NaN_3 (21.0 g, 326.5 mmol, 1 equiv). The reaction mixture stirred at 50 °C overnight under N_2, and was then filtered through celite to yield 1.13 g of a clear yellow oil (28%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 3.49 (t, J =4.5 Hz, 1H), 2.70-2.95 (m, 22H), 2.56 (t, J =4.9 Hz, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 162.6 (DMF), 72.6, 70.6, 70.4, 70.4, 70.2, 70.0, 36.6 (DMF), 31.4 (DMF). HRMS (ESI) m/z calculated for: $[C_{12}H_{25}N_3O_6Na]^+$: 330.3470. Found: 330.1630 (Δ 0.1840).



8

17-amino-3,6,9,12,15-pentaoxaheptadecan-1-ol (**8**). To a solution of compound **7** (1.23 g, 4.00 mmol, 1 equiv) in MeOH (20 mL) was added 7N NH_3 (572 μL, 4.00 mmol, 1 equiv) and 10%

Pd/C (0.085 g, 0.800 mmol, 0.2 equiv). The reaction mixture was stirred under 800 PSI overnight, and then was filtered through celite and concentrated to yield 0.815 g of a clear yellow oil (72.4%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 3.13-3.63 (m, 20H), 2.34-2.75 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$, ppm) δ 72.9, 72.7, 70.3, 70.1, 70.0, 60.9, 41.3. HRMS (EI) m/z calculated for: $C_{12}H_{27}NO_6$: 281.3490. Found: 282.1907. (Δ 0.8417).

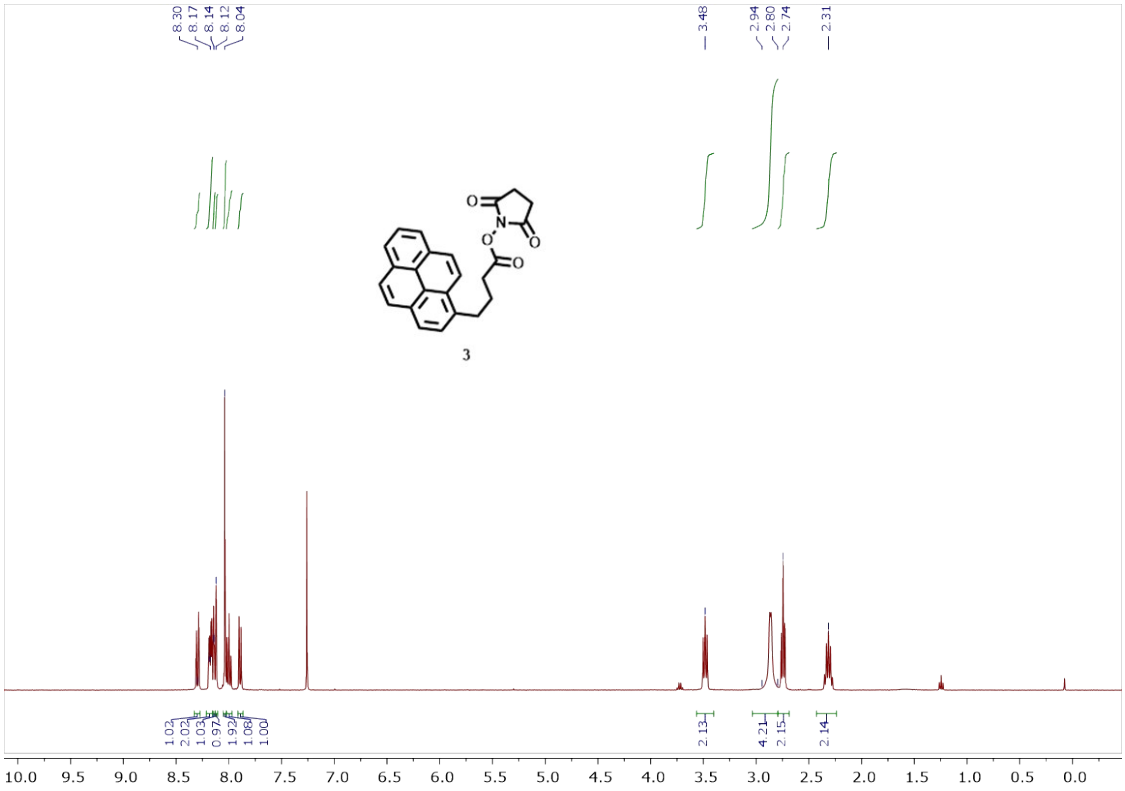


Figure S1: ^{1}H NMR spectrum of 2,5-dioxopyrrolidin-1-yl 4-(pyren-1-yl)butanoate (***3***) in $CDCl_3$.

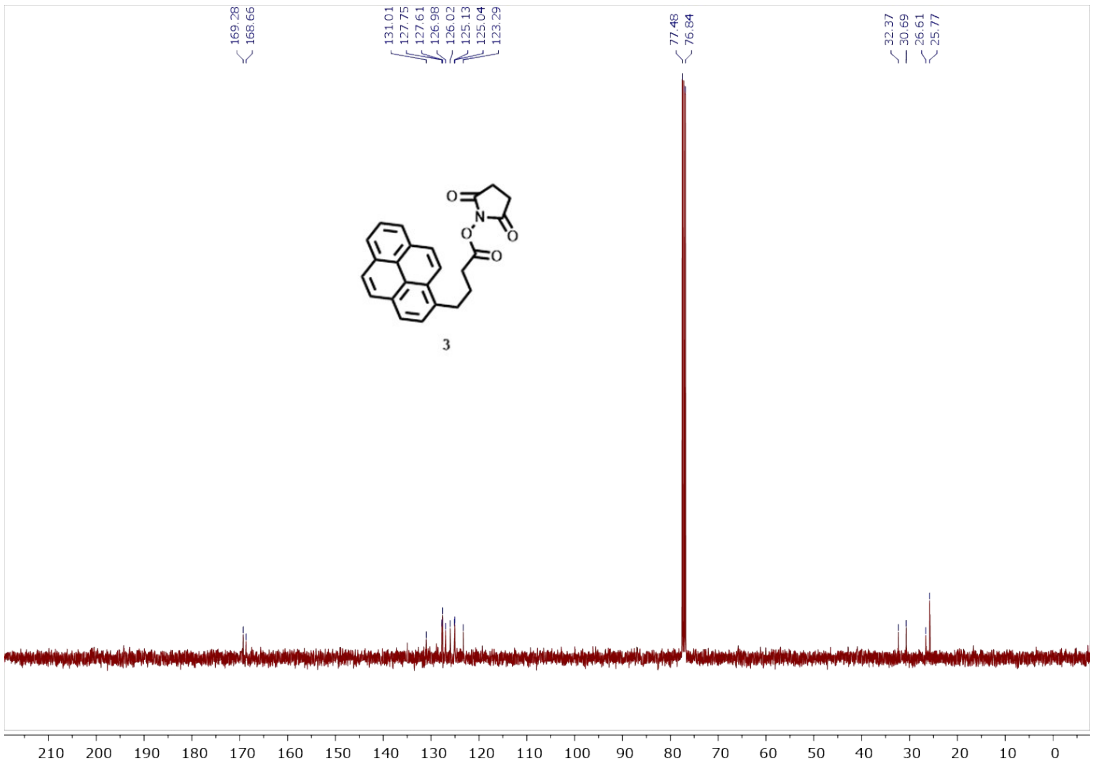


Figure S2: ^{13}C NMR spectrum of 2,5-dioxopyrrolidin-1-yl 4-(pyren-1-yl)butanoate (***3***) in $CDCl_3$.

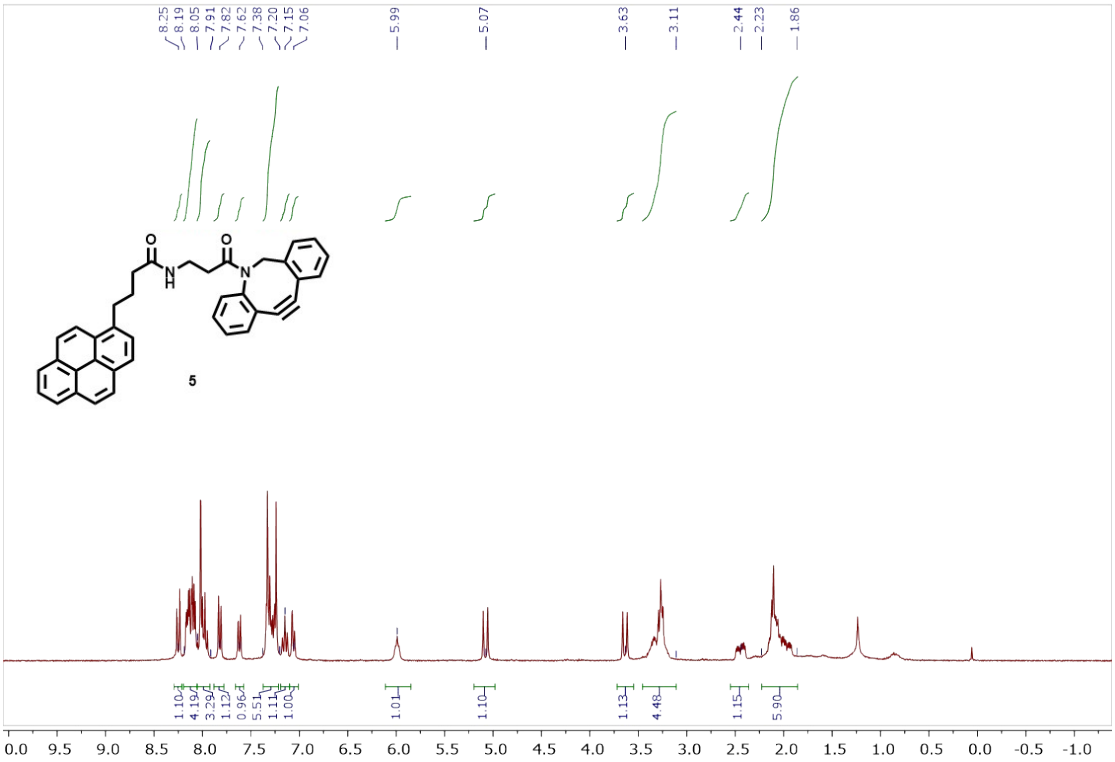


Figure S3: ^{1}H NMR spectrum of dibenzocyclooctyne-amine (***5***) in $CDCl_3$.

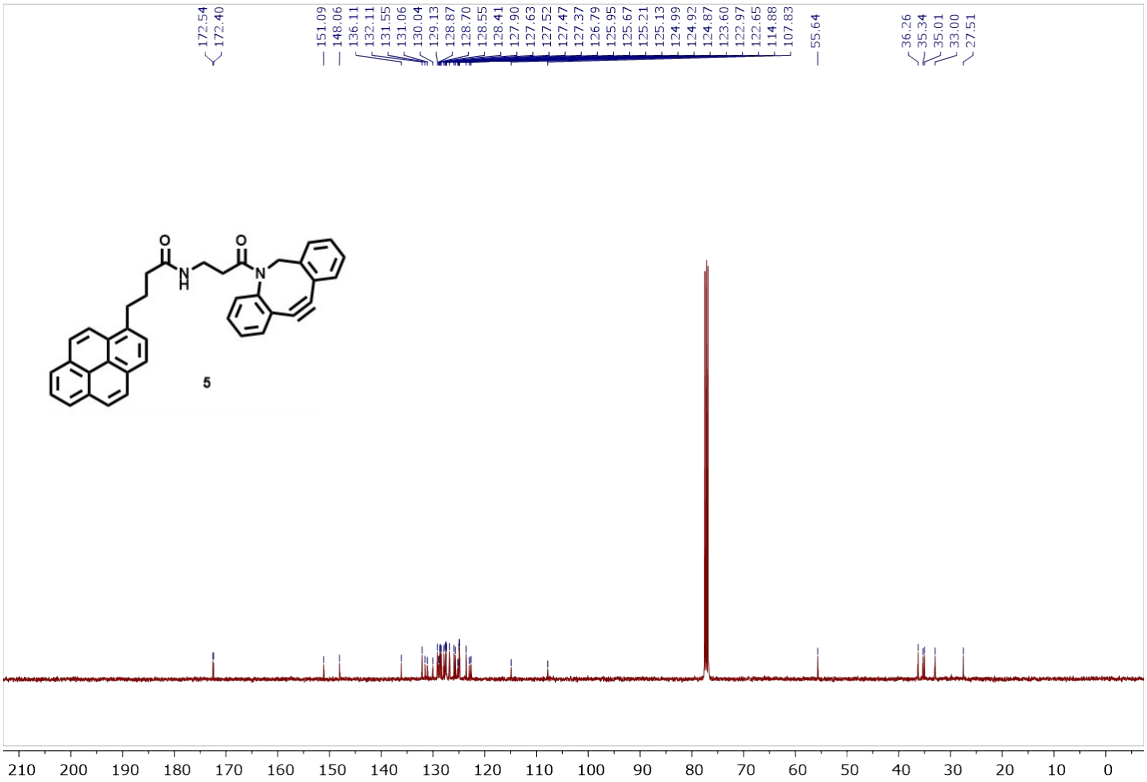


Figure S4: ^{13}C NMR spectrum of dibenzocyclooctyne-amine (***5***) in $CDCl_3$.

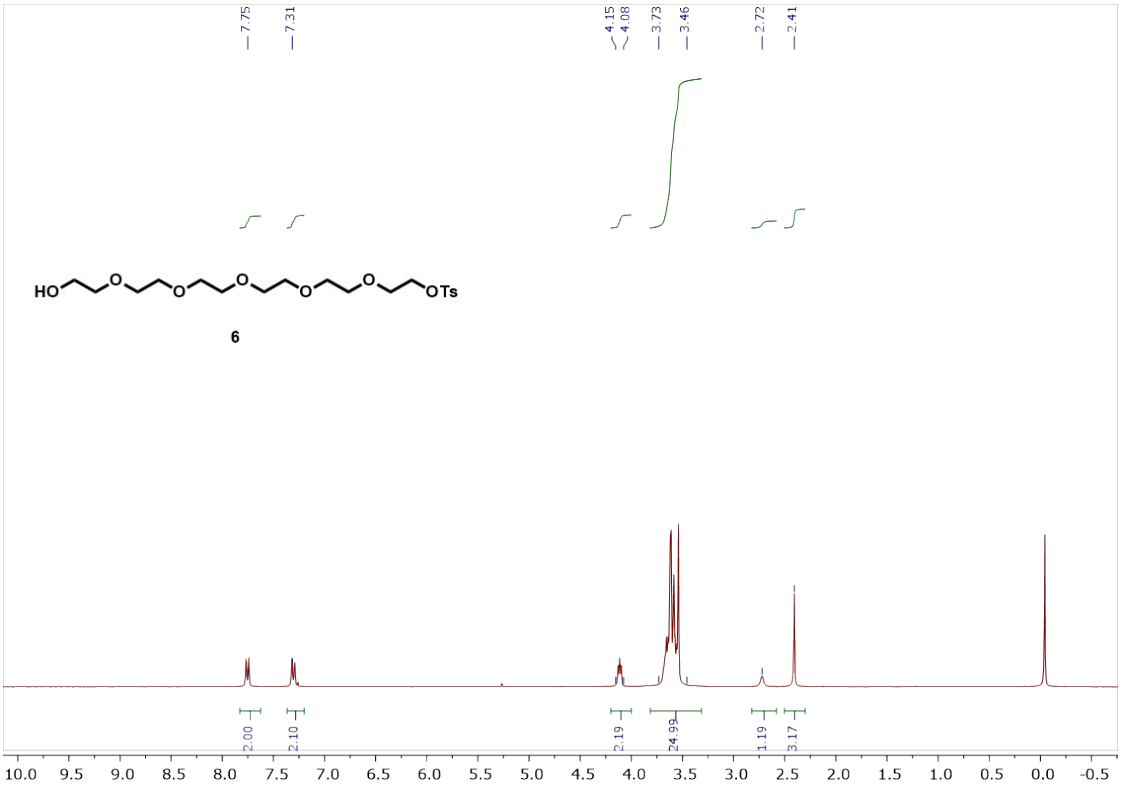


Figure S5: ^{1}H NMR spectrum of 17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (***6***) in $CDCl_3$.

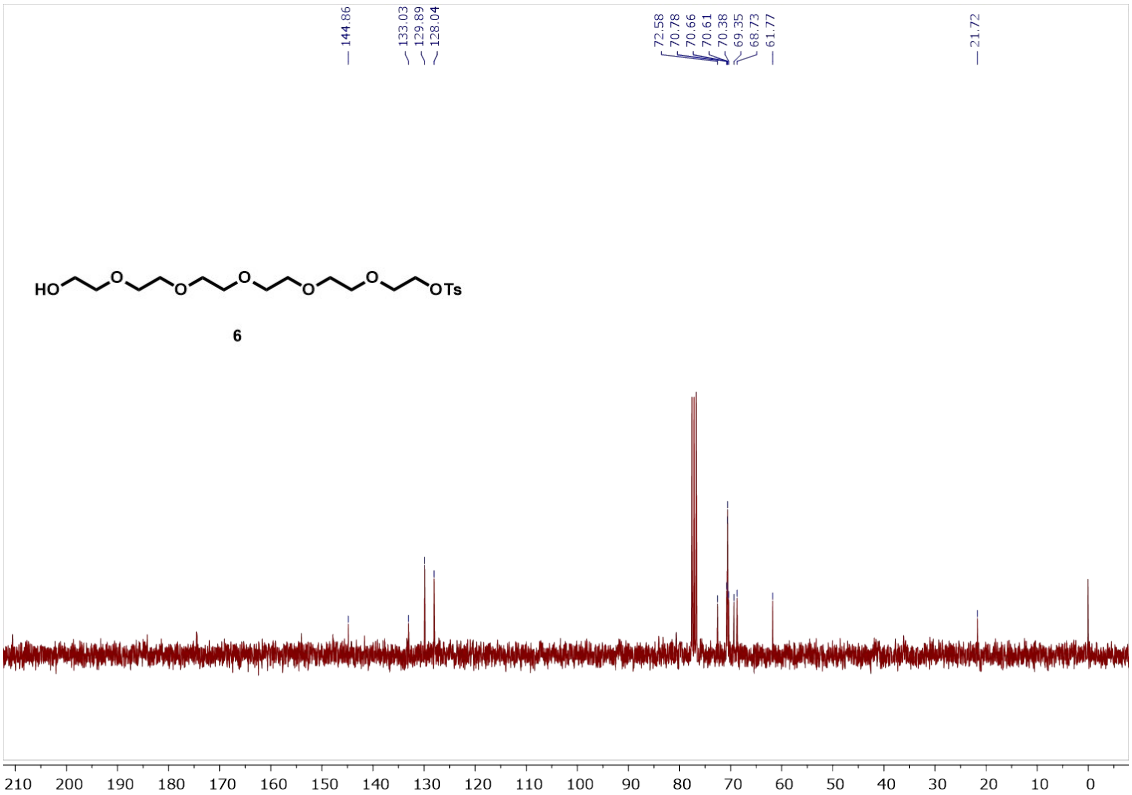


Figure S6: ^{13}C NMR spectrum of 17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (***6***) in $CDCl_3$.

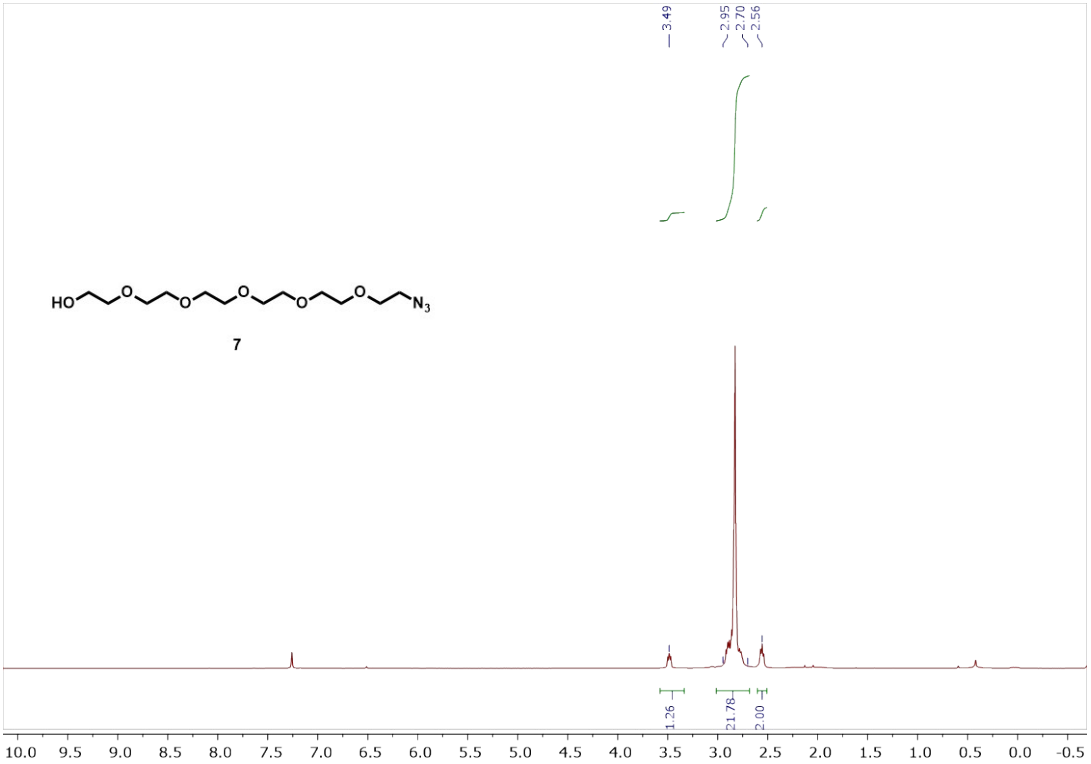


Figure S7: ^{1}H NMR spectrum of 17-azido-3,6,9,12,15-pentaoxaheptadecan-1-ol (*7*) in $CDCl_3$.

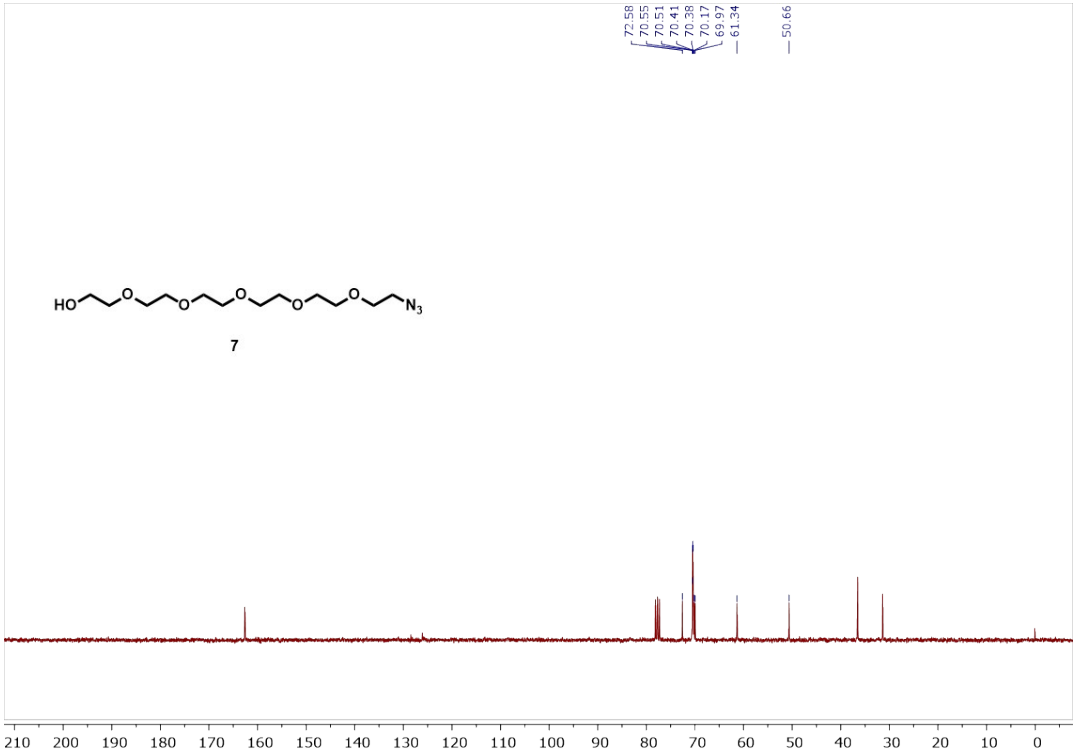


Figure S8: ^{13}C NMR spectrum of 17-azido-3,6,9,12,15-pentaoxaheptadecan-1-ol (*7*) in $CDCl_3$.

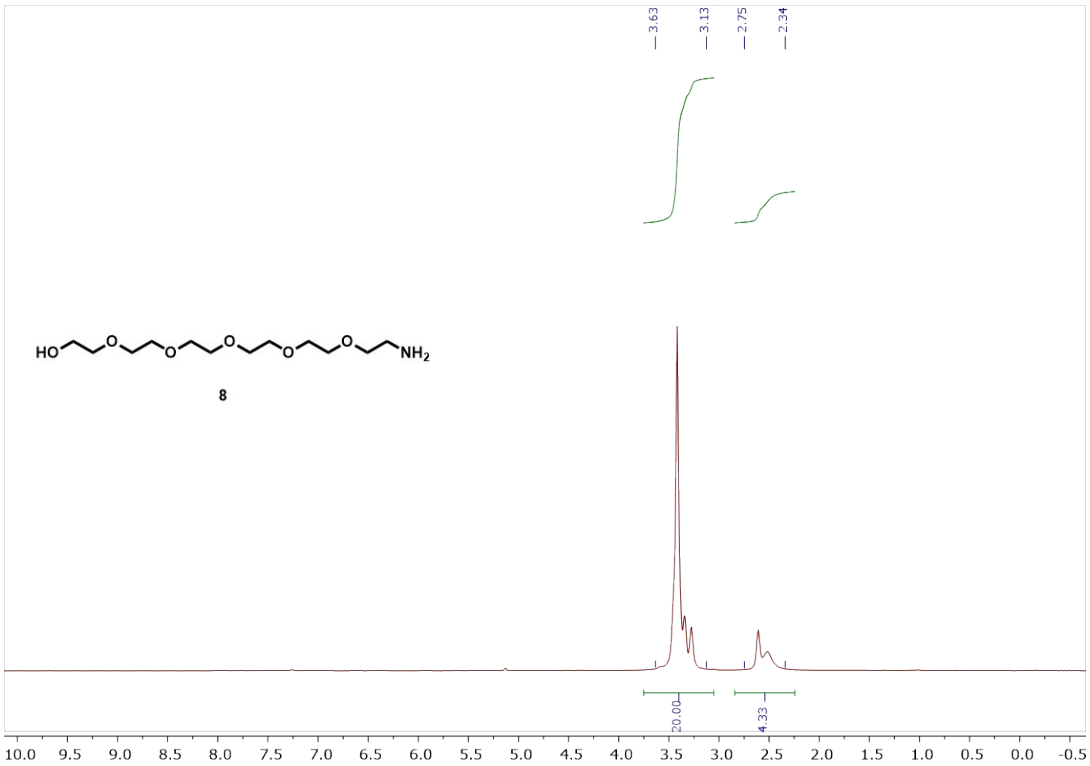


Figure S9: ^{1}H NMR spectrum of 17-amino-3,6,9,12,15-pentaoxaheptadecan-1-ol (***8***) in $CDCl_3$.

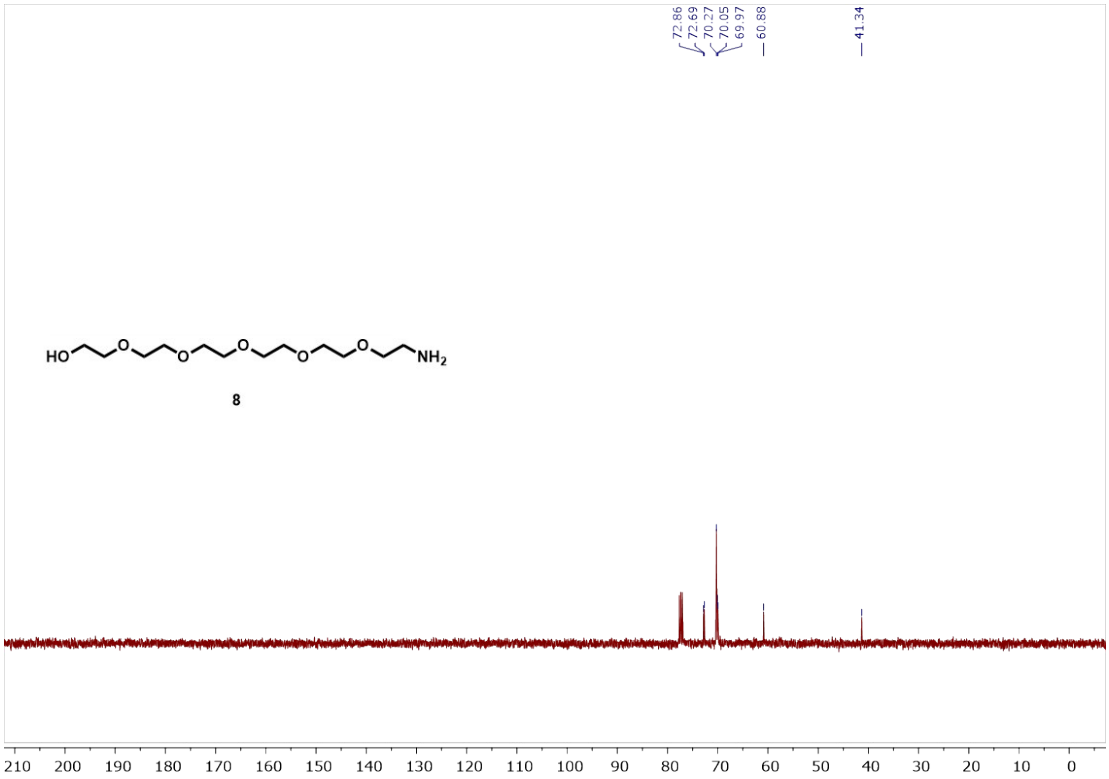


Figure S10: ^{13}C NMR spectrum of 17-amino-3,6,9,12,15-pentaoxaheptadecan-1-ol (***8***) in $CDCl_3$.

	Sample	M(23-ANL) count	M count	Putative ANL tag rate
Serum samples	MetRS^{L274G}	9	2469	0.0036452
	C57.Bl/6 untreated mouse1	6	5032	0.001192369
	C57.Bl/6 untreated mouse2	56	6573	0.008519702

Serum sample with 95% filter	Old C57.Bl/6 partner of young MetRSL274G mouse	55	6466	0.008506032
	Old C57.Bl/6 partner of young C57.Bl/6 mouse	4	1647	0.002428658
	MetRS^{L274G} parabiont	3	3760	0.000797872
	C57.Bl/6 female mouse single	4	444	0.009009009
	Young C57.Bl/66 partner of old C57.Bl/6 parabiont	1	4001	0.000249938
	MetRS^{L274G}	3	2360	0.001271186
	YFP transgeing mouse, untreated	33	6298	0.005239759
	C57.Bl/6 mouse, untreated	3	2264	0.001325088

Muscle samples	Old C57.Bl/6 partner of young MetRS^{L274G} mouse	117	6997	0.016721452
	Old C57.Bl/6 partner of young C57.Bl/6 mouse	31	2016	0.015376984
	MetRS ^{L274G} parabiont	3	3760	0.000797872
	C57.Bl/6B6 female mouse	12	529	0.02268431
	Young C57.Bl/66 mouse parabiont	1	4001	0.000249938
	C57.Bl/6 untreated mouse, sample 1 GA	4	3564	0.001122334
	C57.Bl/6 untreated mouse, sample 2 TA	34	18285	0.001859448
	C57.Bl/6 untreated mouse, sample 3 GA	4	3683	0.001086071
	C57.Bl/6 untreated mouse, sample 4 TA	61	25585	0.002384209

Muscle samles with 95% filter	C57.Bl/6 untreated mouse, sample GA	4	3863	0.001035465
	C57.Bl/6 untreated mouse, sample TA	61	25585	0.002384209
	MetRS ^{L274G}	177	7690	0.023016905
	Floxed MetRS	1	252	0.003968254

Table S1: Discovery of false positive results in Mass Spectrometry downstream of BONCAT. Tissue samples (blood serum and skeletal muscles Tibialis Anterior (TA) and Gastrocneimus (GA)) were isolated from MetRS^{L274G} mice, which are capable of incorporating ANL, as well as Floxed MetRS^{L274G} and C57.Bl/6 mice, which cannot metabolically label their proteomes with ANL. Additionally, the tissues of old C57.Bl/6 mice, which were joined in heterochronic parabiosis with young MetRS^{L274G} animals, were examined and compared to those of old C57.Bl/6 mice joined to young C57.Bl/6 mice. The ANL label was detected by Mass Spectrometry as M23. Upon the typical resolution of 1000s of Methionine-containing (M) peptides (e.g. successful assays) a number of putative M23 – ANL containing peptides were detected, not only in the tissues of MetRS^{L274G} mice and their old wild type parabiotic partners (*blue outlines*), but also unexpectedly, in the tissues of Floxed mice and C57.Bl/6 isogenic parabionts treated with ANL. Even the C57.Bl6 mouse tissues not exposed to ANL, which were derived and stored prior to any ANL presence in our laboratory (*red outlines*) showed a number of putative M23 – ANL containing peptides. These false positive results persisted in multiple Mass Spectrometry runs, even when using a high stringency 95% filter. Unless indicated otherwise (e.g. untreated), all tissues were derived from animals and pairs that were administered with ANL in vivo, as detailed in[8].