Neurotransmitter-responsive nanosensors for T_2-weighted-magnetic resonance imaging

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Supporting Information

Table of contents **Page**

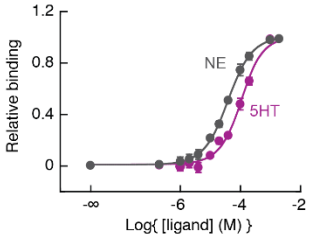


Figure S1. Specificity of ligand binding by 9D7*. Titration curves indicate binding of serotonin (5HT) and norepinephrine (NE) to the dopamine-selective BM3h derivative 9D7* (dopamine binding data in Figure 1d). Calculated K_d values for 5HT and NE are 79 ± 39 μM and 11.3 ± 3.2 μM, respectively. Error bars depict s.e.m. of three measurements per point. [9D7*] = 1 μM.

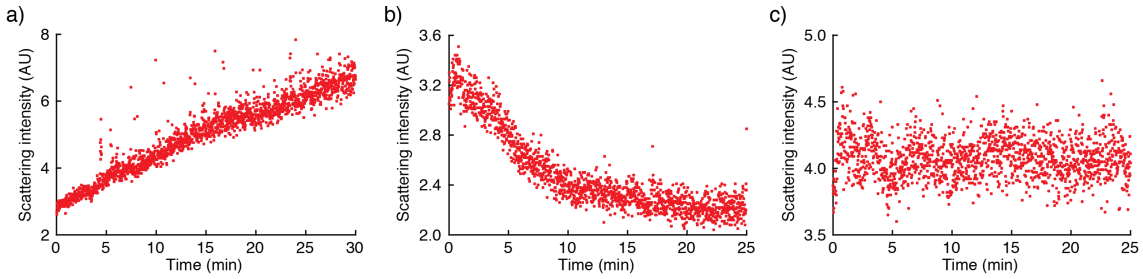


Figure S2. Time courses of DaReNa light scattering changes. (a) Increasing DLS scattering intensity as a function of time indicates clustering of DaReNas immediately after mixing Tyr-PEG-SPIO and 9D7*-SPIO solutions in a 1:1 ratio. (b) Dopamine-induced unclustering was observed immediately following addition of a dopamine solution into DaReNa solution. Final concentration of dopamine was 200 µM. (c) Unclustering was not observed by adding a solution without dopamine. All experiments were performed in phosphate buffered saline at pH 7.4, with an SPIO iron concentration of 1 mM.

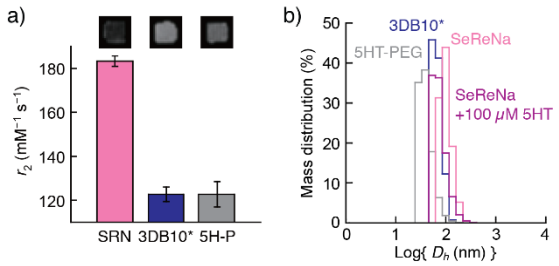
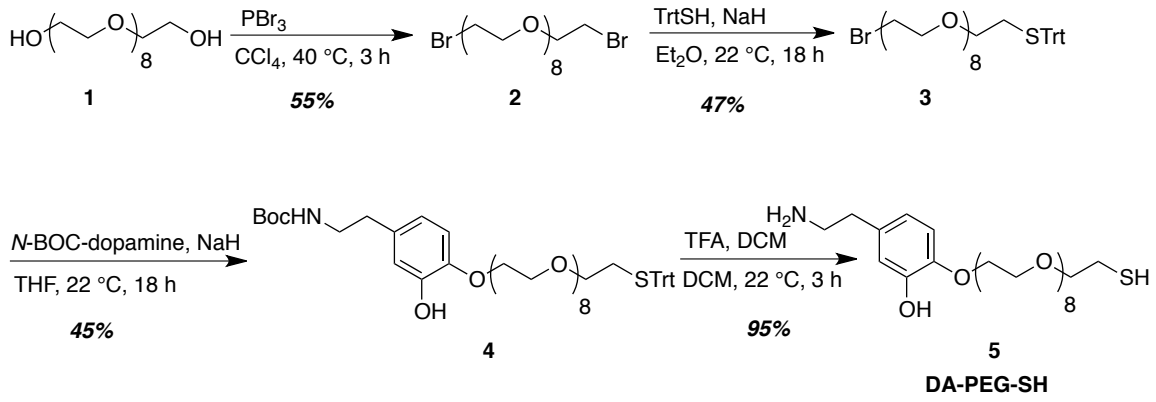
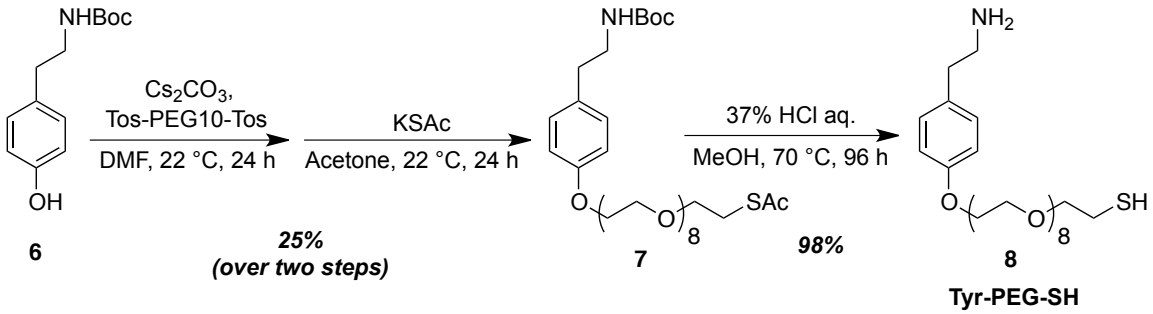


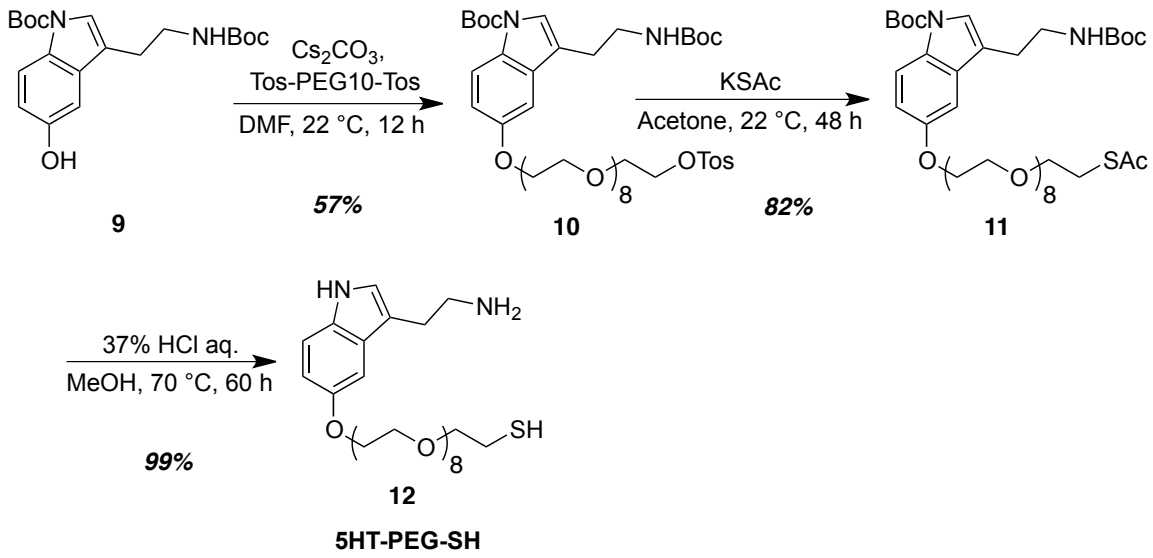
Figure S3. Values of r_2 and D_h for SeReNa components. (a) T_2-weighted MRI scans (top) and r_2 values (bars) corresponding to SeReNas (SRN), 3DB10*-functionalized SPIOs (3DB10*), and 5HT-PEG-functionalized SPIOs (5H-P). [Fe] = 100 μM. Error bars denote s.e.m. of three measurements each. (b) Hydrodynamic diameter histograms of SeReNa and control nanoparticles, as well as SeReNas in the presence of 100 μM serotonin (5HT), as measured by dynamic light scattering. The iron concentration in all conditions was 100 μM.



Scheme S1. Synthesis of DA-PEG-SH (**5**).



Scheme S2. Synthesis of Tyr-PEG-SH (**8**).



Scheme S3. Synthesis of 5HT-PEG-SH (**12**).

Protein variant	Dopamine	DA-PEG-SH
9D7-S450C	1.3 ± 0.1	108 ± 1
L17A	2.0 ± 0.2	17.2 ± 1.8
Q189S	1.7 ± 0.2	15.5 ± 2.0
Q189T	2.2 ± 0.2	14.8 ± 2.7
L17A Q189S	2.1 ± 0.1	13.3 ± 2.9
L17A Q189T (9D7*)	2.1 ± 0.6	14.5 ± 1.2

Table S1. K_d values for ligand binding to BM3h-9D7 mutants. Data are obtained from titration analysis as in Figure 1d, where error margins denote s.e.m. of three measurements.

SUPPLEMENTAL METHODS

General methods. Thin layer chromatography (TLC) was performed on TLC-aluminum sheets (Silica gel 60 F254). Flash column chromatography was performed with VWR (Radnor, PA) silica gel (230–400 mesh). Semi-preparative reversed phase HPLC was run on a Waters (Milford, MA) 2487 system equipped with a dual λ absorbance detector for product visualization using a Vydac C18 column (Hichrom, Berkshire, UK) with 10 μm particle size, 10 × 250 mm. Buffer A: 0.1% w/v trifluoroacetic acid (TFA) in H_2O. Buffer B: 0.1% w/v TFA acetonitrile. Typical gradients ran from 5% to 95% B within 40 minutes with 3.5 mL/min flow. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 22 °C at the Massachusetts Institute of Technology Department of Chemistry Instrumentation Facility (DCIF) on a Bruker (Ettlingen, Germany) DPX 400 spectrometer (^{1}H NMR frequency 400.13 MHz), with chemical shifts (δ) reported in ppm relative to the solvent residual signals of $CDCl_3$ (7.26 ppm) or CD_3OD (3.31 ppm), and coupling constants reported in Hz. Mass spectra were recorded on an Agilent (Santa Clara, CA) 1100 Series LC/MSD system. High resolution mass spectra (HRMS) were measured on a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS), with electrospray ionization.

Materials and reagents. Photocrosslinkable lipid was custom-ordered from Avanti Polar Lipids (Alabaster, AL), with product number 791127. 25 mg/mL iron oxide nanoparticle solution (10 nm with oleic acid coating in chloroform) was purchased from Ocean NanoTech (San Diego, CA). Standard chemicals were purchased from Sigma-Aldrich (St. Louis, MO), Broadpharm (San Diego, CA), Toronto Research

Chemicals (Toronto, Canada), and Alfa Aesar (Tewksbury, MA), and were used without further purification. Anhydrous solvents from Sigma-Aldrich were used without further treatment and distillation. Standard molecular biology supplies were purchased from Bio-Rad Laboratories (Hercules, CA), Qiagen (Hilden, German), and Thermo Fisher Scientific (Waltham, MA).

1,26-dibromo-3,6,9,12,15,18,21,24-octaoxahexacosane, 2. OH-PEG8-OH (**1**) (1 g, 2.4 mmol) was dissolved in anhydrous tetrachloromethane (10 mL) and phosphorus-tribromide (520 mg, 1.9 mmol) was added dropwise while stirring at room temperature (rt). After stirring for an additional 30 min the reaction mixture heated to 40 °C and stirred for 3 h. The resulting solution was poured over cold sodium hydroxide solution (1 mM, 20 mL, 4 °C) and extracted with dichloromethane (2 x 50 mL). The organic phase was dried over $MgSO_4$ and after removal of all volatiles the resulting oily residue was purified by column chromatography (silica, hexane-acetone 2:1). Yield: 715 (55%). ^{1}H NMR (400 MHz, Acetone-d_6): δ 3.80 (t, J = 6.0 Hz, 1H), 3.65 – 3.57 (m, 6H), 3.55 (t, J = 6.0 Hz, 1H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 206.26, 71.98, 71.41, 71.23, 32.11, 30.56, 30.37, 30.18, 29.98, 29.79, 29.60, 29.41. HRMS (ESI+) *m/z* calc. 563.2711 for $C_{18}H_{36}Br_2NaO_8^+$, found 563.27 $[M+Na]^+$.

28-bromo-1,1,1-triphenyl-5,8,11,14,17,20,23,26-octaoxa-2-thiaoctacosane, 3. Triphenylmethanethiol (270 mg, 1 mmol) and sodium hydride (72 mg, 3 mmol) were

suspended in anhydrous diethylether (50 mL) and stirred under argon at rt for 1 h. The resulting mixture was filtered to remove the excess of unreacted sodium hydride and the filtrate was added dropwise to a solution of **2** (540 mg, 1 mmol) in anhydrous diethylether (50 mL) and stirred for 18 h at rt. The reaction mixture was diluted with diethylether (100 mL) and washed with water. After drying the organic phase over $MgSO_4$ and removing all volatiles in vacuum the oily residue was purified by column chromatography (silica, DCM:EtOAc, 1:1) to afford **3** as colorless oil. Yield: 340 mg (47%). ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.46 – 7.37 (m, 6H), 7.29 (t, J = 7.6 Hz, 6H), 7.25 – 7.19 (m, 3H), 3.79 (t, J = 6.0 Hz, 2H), 3.68 – 3.56 (m, 24H), 3.57 – 3.52 (m, 2H), 3.50 (t, J = 6.0 Hz, 2H), 3.44 (dd, J = 5.8, 3.5 Hz, 2H), 3.28 (d, J = 6.7 Hz, 1H), 2.39 (t, J = 6.7 Hz, 2H). 13C NMR (101 MHz, Methanol-d_4): δ 146.28, 130.80, 128.92, 127.81, 72.35, 71.54, 71.41, 71.21, 70.59, 67.77, 49.64, 49.42, 49.21, 49.00, 48.79, 48.57, 48.36, 32.85, 31.41. HRMS (ESI+) *m/z* calc. 758.7555 for $C_{37}H_{51}BrNaO_8S^+$, found 758.7592 $[M+Na]^+$.

tert-butyl (3-hydroxy-4-((triphenyl-5,8,11,14,17,20,23,26-octaoxa-2-thiaoctaco-san-28-yl)oxy)phenethyl)carbamate, 4. *N*-BOC-dopamine (117 mg, 0.5 mmol) and sodium hydride (13 mg, 0.6 mmol) were suspended in anhydrous

tetrahydrofuran (50 mL) under argon atmosphere and at rt. After stirring the reaction mixture for 1 h, solution of **3** (340 mg, 0.5 mmol) in anhydrous tetrahydrofuran (30 mL) was added dropwise and stirred for 18 h. The mixture was filtered over a Celite pad and the filtrated was diluted with diethylether (100 mL), washed with water (2 x 50 mL) and dried over $MgSO_4$. After removal of all volatiles the resulting residue was purified by column chromatography (silica, DCM:MeOH, 97:3) to afford **4** as colorless oil that solidified at -10 °C. Yield: 190 mg (45%). ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.44 – 7.36 (m, 5H), 7.29 (t, J = 7.6 Hz, 8H), 7.25 – 7.17 (m, 4H), 6.67 (d, J = 8.0 Hz, 1H), 6.63 (d, J = 2.0 Hz, 1H), 6.51 (dd, J = 8.0, 2.1 Hz, 1H), 3.79 (t, J = 6.0 Hz, 3H), 3.65 – 3.57 (m, 30H), 3.57 – 3.40 (m, 8H), 3.27 (d, J = 6.7 Hz, 2H), 3.18 (dd, J = 8.4, 6.6 Hz, 2H), 2.59 (dd, J = 8.3, 6.6 Hz, 2H), 2.39 (t, J = 6.7 Hz, 3H), 1.42 (s, 10H). ^{13}C NMR (101 MHz, Methanol-d_4): δ 146.28, 132.18, 130.80, 128.92, 127.81, 121.03, 116.87, 116.32, 72.35, 71.59, 71.54, 71.41, 71.21, 70.59, 49.64, 49.42, 49.28, 49.21, 49.00, 48.79, 48.58, 48.36, 43.37, 36.62, 32.85, 31.41, 28.77. HRMS (ESI+) *m/z* calc. 931.1388 for $C_{50}H_{69}NNaO_{12}S^+$, found 931.1405 $[M+Na]^+$.

5-(2-aminoethyl)-2-((26-mercapto-3,6,9,12,15,18,21,24-octaoxahexacosyl)oxy) phenol, 5. Solution of **4** (190 mg, 0.2 mmol) in dichloromethane (10 mL) was treated with trifluoroacetic acid (1.5 mL) and deionized water (0.1 mL) and stirred under

argon atmosphere at room temperature (rt) for 3 h. All volatiles were removed in vacuum and the residue was purified by high performance liquid chromatography (HPLC) (C18 coated silica, H_2O (0.1%v TFA):MeCN (0.1%v TFA), 95:5 to 5:95 in 27 min, R_t = 18 min). Yield:110 mg (95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.67 (d, J = 8.0 Hz, 1H), 6.61 (d, J = 2.1 Hz, 1H), 6.48 (dd, J = 8.0, 2.1 Hz, 1H), 3.72 (t, J = 5.8 Hz, 2H), 3.62 – 3.45 (m, 30H), 3.43 (dd, J = 5.9, 3.6 Hz, 2H), 3.34 (dd, J = 5.8, 3.6 Hz, 2H), 3.23 (t, J = 6.6 Hz, 2H), 2.66 (dd, J = 9.3, 6.4 Hz, 2H), 2.28 (t, J = 6.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 144.44, 127.75, 127.51, 126.72, 119.25, 116.00, 115.66, 70.37, 69.77, 69.61, 69.56, 69.49, 68.54, 32.47, 32.25, 31.38. HRMS (ESI+) *m/z* calc. 566.7258 for $C_{26}H_{48}NO_{10}S^+$, found 566.7298 $[M+H]^+$.

***S*-(26-(4-(2-((*tert*-butoxycarbonyl)amino)ethyl)phenoxy)-3,6,9,12,15,18,21,24-octaoxahexacosyl) ethanethioate, 7.** *N*-BOC-Tyramine (**6**) (150 mg, 0.63 mmol) and cesium carbonate (205 mg, 0.63 mmol) were dissolved in anhydrous DMF (3 mL) and stirred for 30 min under argon. Then, Tos-PEG10-Tos (457 mg, 0.63 mmol) in anhydrous DMF (1 mL) was added and the reaction mixture was stirred at rt for 24 h. The solvent was removed on vacuum and the residue purified by column chromatography (silica, hexane-acetone 2:1). The fraction containing the product (400 mg) was treated with KSAc (150 mg, 1.3 mmol) and stirred at rt for 24 h. Afterwards, the solvent was removed on vacuum and the residue purified by silica gel column chromatography (hexane-acetone 3:1 to 1:1) to give compound **7** as a colorless oil (110 mg, 25% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11 (d, J = 8.2 Hz, 2H),

6.91 – 6.84 (m, 2H), 4.13 (t, *J* = 4.8 Hz, 2H), 3.87 (t, *J* = 4.8 Hz, 2H), 3.79 – 3.63 (m, 28H), 3.62 (t, *J* = 6.5 Hz, 2H), 3.35 (s, 2H), 3.11 (t, *J* = 6.5 Hz, 2H), 2.74 (t, *J* = 7.0 Hz, 2H), 2.36 (s, 3H), 1.48 – 1.43 (m, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.63, 157.39, 129.70, 114.75, 77.33, 77.22, 77.01, 76.70, 70.74, 70.54, 70.52, 70.46, 70.46, 70.44, 70.25, 69.76, 67.42, 67.42, 30.56, 30.56, 28.77, 28.38. MS (ESI+) *m/z* calc. 692.3380 for $C_{33}H_{58}NO_{12}S^+$, found 692.3596 $[M+H]^+$. HRMS (ESI+) *m/z* calc. 709.3945 for $C_{33}H_{61}N_2O_{12}S^+$, found 709.3 $[M+NH_4]^+$.

26-(4-(2-aminoethyl)phenoxy)-3,6,9,12,15,18,21,24-octaoxahexacosane-1-thiol, Tyr-PEG-SH, 8. A solution of **7** (80 mg, 0.12 mmol) in anhydrous methanol (10 mL) was degassed by bubbling with argon for 10 min. Afterwards, HCl_{conc} (37%, 56 µL, 0.68 mmol) was added and the reaction mixture was stirred at 70 ºC under argon for 96 h. The solvent was removed on vacuum to give **Tyr-PEG-SH** (**8**) (64 mg, 98%). HPLC: 1 single peak at R_t = 11 min (3 mg injected, solvent A: H_2O 0.1 % TFA and solvent B: CH_3CN 0.1% TFA. Gradient from 5% to 95% B within 40 minutes). ^{1}H NMR (400 MHz, CD_3OD): δ 7.24 – 7.16 (m, 2H), 6.91 (dt, *J* = 8.6, 2.1 Hz, 2H), 4.16 – 4.07 (m, 2H), 3.86 – 3.75 (m, 2H), 3.75 – 3.66 (m, 3H), 3.71 – 3.52 (m, 28H), 3.35 (s, 5H), 3.16 (ddd, *J* = 8.3, 6.1, 2.7 Hz, 2H), 2.95 – 2.84 (m, 3H), 2.64 (t, *J* = 6.5 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 159.36, 159.33, 131.09, 130.94, 130.91, 130.19, 130.13, 129.01, 116.07, 73.96, 71.61, 71.59, 71.46, 71.44, 71.40, 71.37, 71.18, 71.03, 71.00, 70.94, 70.79, 70.32, 69.68, 68.61, 49.85, 49.71, 49.64, 49.50, 49.43, 49.28, 49.21, 49.07, 49.00, 48.79, 48.57, 48.36, 42.30, 42.23, 42.20, 39.42, 33.67, 30.68, 30.43, 24.77, 24.64. HRMS (ESI+) *m/z* calc. 550.3050 for $C_{26}H_{48}NO_9S^+$, found 550.3044 $[M+H]^+$.

tert-butyl 3-(2-((tert-butoxycarbonyl)amino)ethyl)-5-((26-(tosyloxy)-3,6,9,12,15,18,21,24-octaoxahexacosyl)oxy)-1H-indole-1-carboxylate, 10. *N*,*N'*-di-(tert-butyloxycarbonyl) serotonin (**9**) (238 mg, 0.63 mmol) and cesium carbonate (205 mg, 0.63 mmol) were dissolved in anhydrous DMF (3 mL) and stirred for 10 min under argon. Then, Tos-PEG10-Tos (457 mg, 0.63 mmol) in anhydrous DMF (2 mL) was added and the reaction mixture was stirred at rt for 12 h. After this time, the solvent was removed under vacuum and the residue purified by silica gel column chromatography (hexane-acetone 3:1 to 1:1) to give compound **10** as a colorless oil (335 mg, 57%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (d, *J* = 9.1 Hz, 1H), 7.82 – 7.62 (m, 2H), 7.37 – 7.20 (m, 3H), 6.97 (d, *J* = 2.5 Hz, 1H), 6.89 (dd, *J* = 9.0, 2.5 Hz, 1H), 4.76 (s, 1H), 4.20 – 4.04 (m, 4H), 3.88 – 3.79 (m, 2H), 3.74 – 3.67 (m, 2H), 3.67 – 3.54 (m, 23H), 3.53 (d, *J* = 2.2 Hz, 4H), 3.38 (q, *J* = 6.6, 5.5 Hz, 3H), 2.90 (s, 1H), 2.83 (d, *J* = 0.7 Hz, 1H), 2.79 (s, 1H), 2.39 (s, 3H), 2.36 – 2.22 (m, 3H), 1.60 (s, 8H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.03, 162.56, 155.93, 154.96, 149.62, 144.81, 132.98, 131.23, 130.42, 129.84, 127.96, 123.81, 117.58, 115.94, 113.49, 103.09, 83.35, 79.20, 77.48, 77.36, 77.16, 76.84, 70.78, 70.70, 70.60, 70.58, 70.56, 70.52, 70.47, 69.86, 69.28, 68.65, 68.10, 40.04, 36.49, 31.57, 31.42, 30.91, 28.41, 28.22, 27.77, 25.61, 22.64, 21.63, 14.12. HRMS (ESI+) *m/z* calc. 949.4344 for $C_{45}H_{70}N_2NaO_{16}S^+$, found 949.4351 $[M+Na]^+$.

tert-butyl 3-(2-((tert-butoxycarbonyl)amino)ethyl)-5-((28-oxo-3,6,9,12,15,18,21,24-octaoxa-27-thianonacosyl)oxy)-1H-indole-1-carboxylate, 11. A mixture of **10** (78 mg, 0.08 mmol) and KSAc (12 mg, 0.11 mmol) in acetone (3 mL) was stirred

at rt for 48 h. After this time, the solvent was removed under vacuum and the residue purified by silica gel column chromatography (hexane-acetone 2:1) to give compound **11** as a colorless oil (54 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (t, *J* = 10.4 Hz, 1H), 7.34 (d, *J* = 12.1 Hz, 1H), 7.15 – 6.84 (m, 2H), 4.69 (s, 1H), 4.16 (dd, *J* = 5.7, 4.2 Hz, 2H), 3.86 (dd, *J* = 5.7, 4.1 Hz, 2H), 3.77 – 3.50 (m, 33H), 3.41 (q, *J* = 5.5, 4.9 Hz, 2H), 3.06 (t, *J* = 6.5 Hz, 2H), 2.82 (t, *J* = 6.8 Hz, 2H), 2.31 (s, 3H), 1.63 (s, 9H), 1.41 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.01, 195.58, 162.61, 155.97, 155.05, 149.69, 131.28, 130.50, 129.89, 128.40, 128.05, 123.90, 117.61, 116.03, 114.15, 113.56, 103.16, 83.42, 79.31, 77.48, 77.36, 77.16, 76.84, 70.90, 70.81, 70.71, 70.70, 70.64, 70.58, 70.46, 70.39, 70.34, 70.23, 70.09, 69.96, 69.83, 69.70, 69.32, 68.74, 68.19, 40.10, 39.39, 38.46, 36.56, 31.99, 31.51, 31.00, 30.64, 29.76, 29.58, 29.43, 29.35, 29.23, 28.91, 28.55, 28.51, 28.49, 28.31, 27.86, 25.69, 23.24, 22.76, 21.72, 14.20. MS (ESI+) *m/z* calc. 869.39 for $C_{40}H_{66}KN_2O_{14}S^+$, found 869.81 $[M+K]^+$.

26-((3-(2-aminoethyl)-1H-indol-5-yl)oxy)-3,6,9,12,15,18,21,24-octaoxahexacosane-1-thiol, 5HT-PEG-SH, 12. A solution of **11** (62 mg, 0.07 mmol) in anhydrous methanol (10 mL) was degassed by bubbling with argon for 10 min. Afterwards, HCl_{conc} (37%, 60 μL, 0.72 mmol) was added and the reaction mixture was stirred at 70 ºC under argon for 60 h. The solvent was removed on vacuum to give **5HT-PEG-SH** (**12**) (44 mg, quantitative). HPLC: 1 single peak at R_t = 4 min (2 mg injected, H_2O-CH_3CN 0.1% TFA 50:50). ^{1}H NMR (400 MHz, CD_3OD): δ 7.31 (m, <1H), 7.20 (d, *J* = 2.5 Hz, 1H), 7.15 (dd, *J* = 6.5, 2.4 Hz, 1H), 6.84 (dt, *J* = 8.8, 2.3 Hz, 1H), 4.28 – 4.11 (m, 2H), 3.94 – 3.81 (m, 2H), 3.81 – 3.54 (m, 32H), 3.33 (p, *J* = 1.7 Hz, 2H),

3.28 (t, J = 7.4 Hz, 1H), 3.17 (t, J = 5.3 Hz, 1H), 3.11 (t, J = 7.5 Hz, 2H), 2.72 (s, 1H), 2.68 – 2.59 (m, 1H), 1.30 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 154.22, 133.76, 128.58, 125.30, 113.71, 113.33, 110.17, 103.12, 73.97, 71.58, 71.52, 71.48, 71.43, 71.38, 71.35, 71.30, 71.25, 71.16, 71.01, 70.98, 69.80, 67.84, 49.64, 49.42, 49.21, 49.00, 48.79, 48.57, 48.36, 40.65, 39.40, 35.41, 30.71, 24.62. MS (ESI+) *m/z* calc. 589.32 for $C_{28}H_{49}N_2O_9S^+$, found 589.02 $[M+H]^+$.

Preparation of photocrosslinked lipid-coated iron oxide (pcLCIO). 5 mg of 1,2-bis(10,12-tricosadiynoyl)-*sn*-glycero-3-phosphoethanolamine-*N*-[maleimide(poly-ethylene glycol)-2000] was dissolved in 200 μL of chloroform. 50 μL of 10 nm iron oxide nanoparticle solution (5 mg Fe/mL) was mixed with the lipid solution and the mixture was thoroughly evaporated to obtain an even brown lipid film. 200 μL of 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer with 150 mM NaCl (pH 7.4) was added into the lipid film and sonicated until the entire brown layer was homogenously dispersed into the buffer, yielding a brown transparent solution. The solution was transferred into a 1 mm quartz cell and flushed with argon gas for 5–10 min. The lipids were photocrosslinked by irradiation of UV light at 254 nm for 40 min using an 8 W UV lamp placed 10 cm from the cuvette. The solution was applied into a magnetic μ column placed into a μMACS magnetic separator (Miltenyi Biotec, Cambridge, MA), and washed five times with 50 μL buffer aliquots. Purified solution was eluted by removing the column from the magnet and adding 500 μL of the buffer. The eluate was centrifuged at 6000*g* for 10 min to remove large aggregates and then the supernatant was filtered using a 0.1 μm syringe top filter. The final resulting concentration of pcLCIOs was quantified from absorbance at 450

nm using a molar absorbance coefficient ε_{450} = 690 $(M\ Fe)^{-1}\ cm^{-1}$, which in turn was determined by a colorimetric assay using bathophenanthrolinedisulfonic acid (Tamarit, J. et al. *Anal. Biochem.* **2006**, *351*, 149–151). The estimated nanoparticle concentrations are 14 nM/(mM Fe), as calculated from the magnetite density of 5.17 g/cm^3 and 15 nm core diameter, as indicated by transmission electron microscopy after magnetic separation, despite the fact that iron oxide cores with ~10 nm mean diameter were used for synthesis. The pcLCIO solution was freshly used for conjugation with ligands and proteins just after preparation.

Preparation of pcLCIO functionalized with neurotransmitter analogs. To reduce disulfide bonds formed by thiol-functionalized ligands prior to conjugation to pcLCIOs, 50 µL of 10 mM of each ligand in MeOH (final concentration = 1 mM) and 6 µL of 100 mM tris(2-carboxyethyl)phosphine (TCEP) hydrochloride were mixed into 444 µL of 10 mM HEPES buffer (pH 7.4). The 1 mM analog mixture was incubated at rt for 1–2 h and then added into freshly prepared pcLCIO solution at ratios of 10,000 ligand molecules per nanoparticle. Thiol-maleimide condensation was performed at rt for 3 h and then 20 mM cystamine (final concentration = 2 mM) was added to quench free maleimide groups. After incubating overnight at rt, the nanoparticles were washed four times with 450 µL of 10 mM HEPES buffer with 150 mM NaCl (pH 7.4) by using 100K Amicon Ultra 0.5 mL filter (MilliporeSigma, Burlington, MA) at 14,000*g* for 3 min. The final resulting concentrations of the nanoparticles were quantified by the same method as used for pcLCIOs. The number of analogs conjugated per nanoparticle was estimated from the absorbance at 275 nm of the first flow-through fraction obtained during Amicon purification.

Preparation of pcLCIO functionalized with BM3h variants. All of the following procedures were performed at 4 °C. 1 mM of BM3h solution was added into freshly prepared pcLCIO solution at ratios of 10,000 proteins per nanoparticle and incubated for 3 h. 20 mM cystamine (final conc. = 2 mM) was added to quench free maleimide groups. After incubating overnight, the nanoparticle solution was applied into a magnetic μ column placed into a μMACS magnetic separator, and washed five times with 20 μL aliquots of phosphate buffered saline (PBS). Purified solution was eluted by removing the column from the magnet and adding 100 μL of PBS. The final resulting concentration of the nanoparticles was quantified by the same method as pcLCIO. The functionalized number of the proteins per nanoparticle was estimated using the Pierce 660 nm Protein Assay (Thermo Fisher Scientific). For the assay, a standard curve was made using bovine serum albumin solutions including the same amount of non-functionalized pcLCIO as in the samples.

Expression and purification of recombinant BM3h proteins. BM3h proteins were expressed and purified following established methods. Briefly, BM3h plasmids were transformed into BL21(DE3) *E. coli* cells. Colonies were incubated overnight in terrific broth (TB) containing 100 μg/mL carbenicillin at 30°C in a shaking incubator. Overnight cultures were diluted 1:100 in TB with 100 μg/mL carbenicillin and incubated at 37°C with shaking until the cultures were induced at late log phase (OD_{600} ≈ 0.8–1.0) with 1.0 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 0.6 mM of the heme precursor δ-aminolevulinic acid. Cultures were shaken for an additional 18–24 h at 30°C. The cells were then pelleted and lysed using Bugbuster reagent with

Lysonase and an EDTA-free protease inhibitor cocktail set (MilliporeSigma) diluted 1:200. BM3h was affinity purified from the soluble fraction of the lysate using Ni-NTA agarose (Qiagen, Germantown, MD). The eluted protein was desalted and exchanged into PBS over Sephadex G-25 columns (GE Healthcare Life Sciences, Marlborough, MA). Protein concentration was determined using a carbon monoxide binding assay.

Site-directed mutagenesis of BM3h. Mutations were introduced using the Agilent QuikChange Lightning Multi Site-Directed Mutagenesis kit. The parental plasmid used for polymerase chain reaction (PCR) was either pCWori cytochrome P450-BM3h 3DB10 or -BM3h 9D7, with the latter available from Addgene (Cambridge, MA). Mutagenesis primers were designed using the QuikChange Primer Design online tool and ordered from Integrated DNA Technologies (Skokie, IL). PCR mixtures and thermal cycling were set up according to the manufacturer protocol, using an extension time of 3.25 minutes. Parental DNA was removed by digestion with Dpn I restriction enzyme supplied in the kit. XL10-Gold competent cells (Agilent) were transformed with Dpn I-treated DNA from the mutagenesis reactions and plated onto lysogeny broth (LB) agar plates with 100 µg/mL carbenicillin for selection.

Optical titration measurements. 9D7* and 3DB10* proteins were formulated as 1 µM solutions in PBS, pH 7.4. Absorbance measurements were made using a SpectraMax M2e microplate reader (Molecular Devices, San Jose, CA). DA, DA-PEG-SH, Tyr-PEG-SH, 5HT, 5HT-PEG-SH, and NE ligands were added separately into the buffered protein solutions, at concentrations indicated in the figures. Spectroscopic

data were obtained and used to calculate the absorbance difference between 416 nm and 436 nm as a function of ligand concentration. These data were median filtered and fit to a standard bimolecular binding model, accounting for ligand depletion, in order to yield dissociation constants (K_d values). Display and analysis of titration data were performed using Kaleidagraph (Synergy Software, Reading, PA).

Dynamic light scattering and biolayer interferometry. Dynamic light scattering was performed using a DynaPro DLS system (Wyatt Technology, Goleta, CA) by collecting 60 acquisitions with 1 s acquisition time at 22 °C. The laser power was adjusted so that all the samples gave scattering light intensity the order of 10^5 counts s^{-1}. Biolayer interferometry (BLI) was performed using a ForteBio Octet system (Pall ForteBio LLC, Fremont, CA). The tip was pre-coated with Ni-NTA and functionalized by addition of 100 μM 9D7* protein for 400 s. Kinetic measurements were obtained using rapid translocation of the BLI probe tip into conditions noted, followed by recording with acquisition at a rate of 5 Hz, where the binding thickness of the tip was measured in solutions maintained at 30 °C and vibrated at 1,000 cycles per minute to promote mixing.

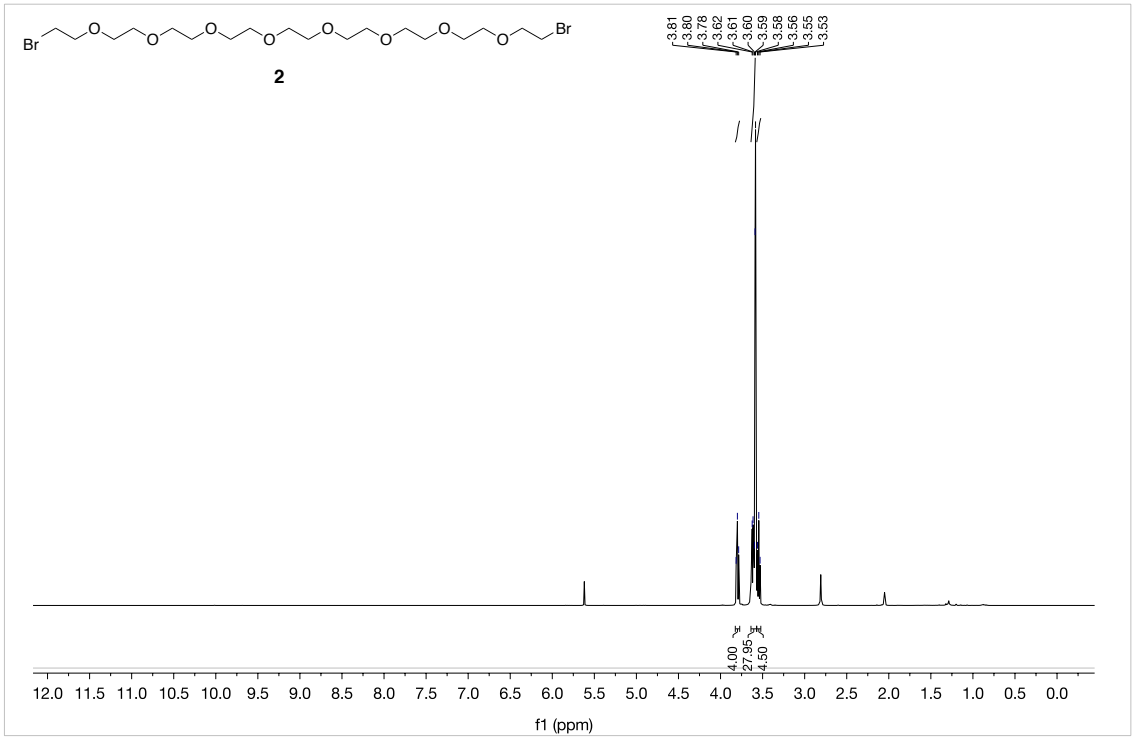
Transmission electron microscopy. 9D7*-SPIO, Tyr-PEG-SPIO, DaReNa, and DaReNa plus 100 μM dopamine (all 1 mM Fe) were separately drop-cast onto transmission electron microscopy (TEM) copper grids, followed by drying under vacuum. TEM images of the samples were then obtained using a JEOL 2010 (Peabody, MA) electron microscope operated at 200 kV with a magnification of 25,000.

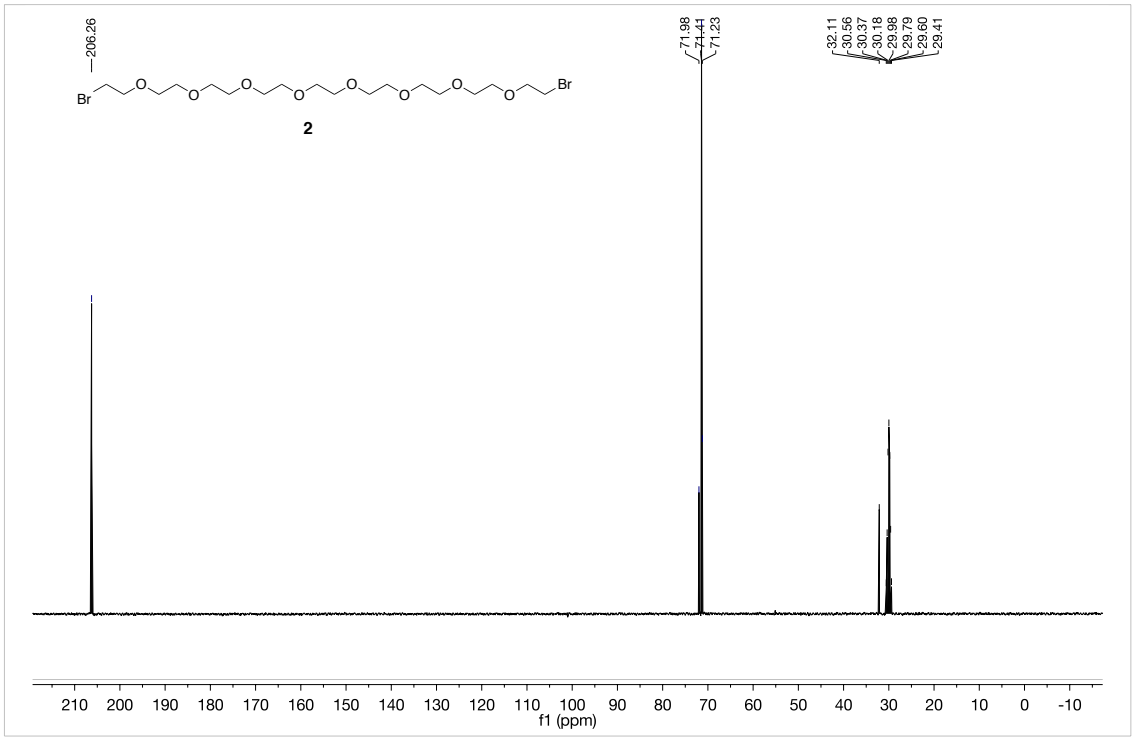
***In vitro* MRI measurements.** Magnetic resonance imaging (MRI) was performed with a 7 T Bruker Biospec MRI scanner. Samples were arrayed into bisected 384-well microtiter plates and imaged using a volume transceiver coil. A multislice multiecho (MSME) pulse sequence was used to obtain T_2-weighted images, with typical parameters including matrix size = 256 × 256, field of view (FOV) = 5 cm × 5 cm, slice thickness = 2 mm, repetition time (*TR*) = 2000 ms, echo spacing with echo time (*TE*) = 8 ms, and number of echoes = 30. T_2 relaxation time values were obtained by exponential fitting to MRI intensities as a function of echo time. Relaxivity (r_2) values were defined by two-point measurements of the slopes between $1/T_2$ of PBS without nanoparticles and $1/T_2$ of PBS containing 100 μM Fe nanoparticles, as quantified by OD_{450}, and are reported as the mean and s.e.m. of measurements performed in triplicate. Relaxivity titrations were performed in PBS with varying concentrations of monoamine neurotransmitters.

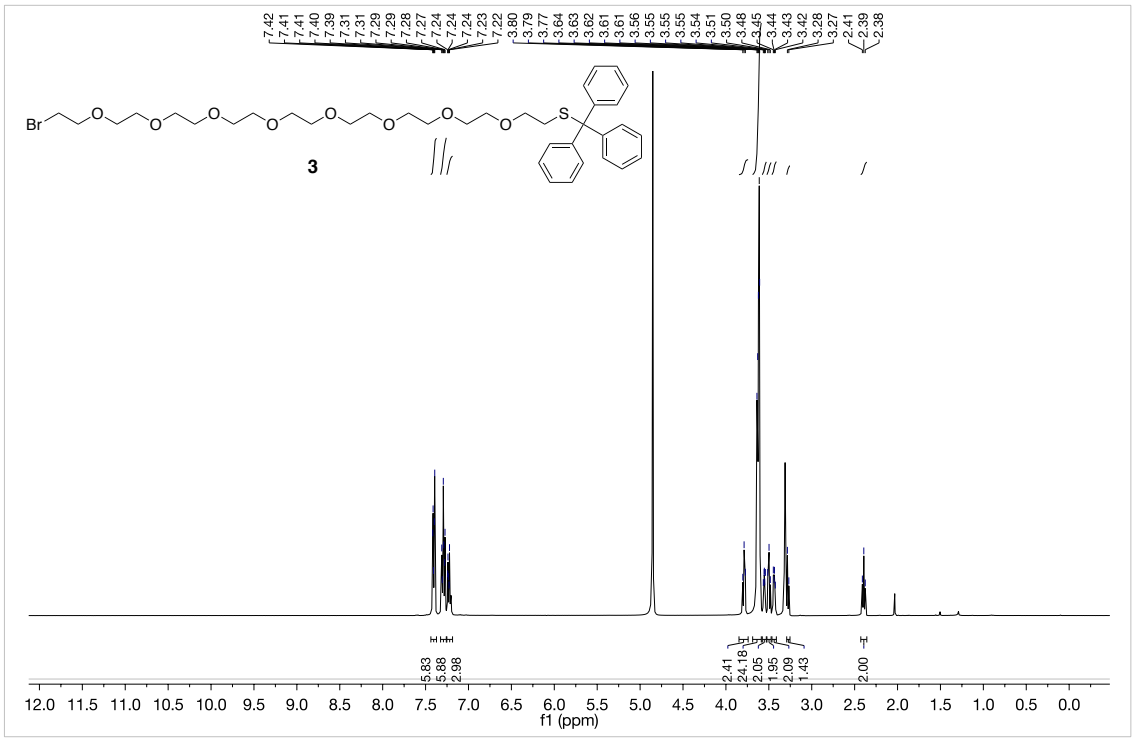
***In vivo* MRI.** Animal procedures were conducted in accordance with National Institutes of Health guidelines and with the approval of the MIT Committee on Animal Care (protocol number 0718-068-21). Male Sprague-Dawley rats, weight ~350 g, were supplied by Charles River Laboratories (Wilmington, MA). Animals were anesthetized with 2% isoflurane and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). The scalp was retracted and craniotomies drilled over right and left striatum with injection coordinates: 1 mm anterior and 3 mm lateral to bregma. DaReNa (1 mM Fe) and artificial cerebrospinal fluid were infused into opposite hemispheres at a rate of 0.12 μL/min, via 33 G metal injection cannulae lowered to a depth of 5.5 mm. Upon the completion of injection, the cannulae were removed and

rats were then transferred into a 7 T Biospec MRI scanner (Bruker). *In vivo* MRI measurements were acquired using a cross coil volume transmit and surface receive configuration, with rapid acquisition with refocused echoes (RARE) pulse sequences that were employed to obtain T_2-weighted anatomical images and R_2 maps. Imaging parameters for anatomical images were: number of averages = 4, matrix size = 300 × 175, FOV = 3 cm × 1.75 cm, slice thickness = 1 mm, *TR* = 3500 ms, effective *TE* = 10 ms, RARE factor = 2. Imaging parameters for R_2 maps were: number of averages = 2, matrix size = 150 × 87, FOV = 3 cm × 1.75 cm, slice thickness = 1 mm, *TR* = 4600 ms, effective *TE* = 10, 30, 50, 70, 90, 110, 130, and 150 ms, RARE factor = 2.

NMR spectra of synthetic compounds



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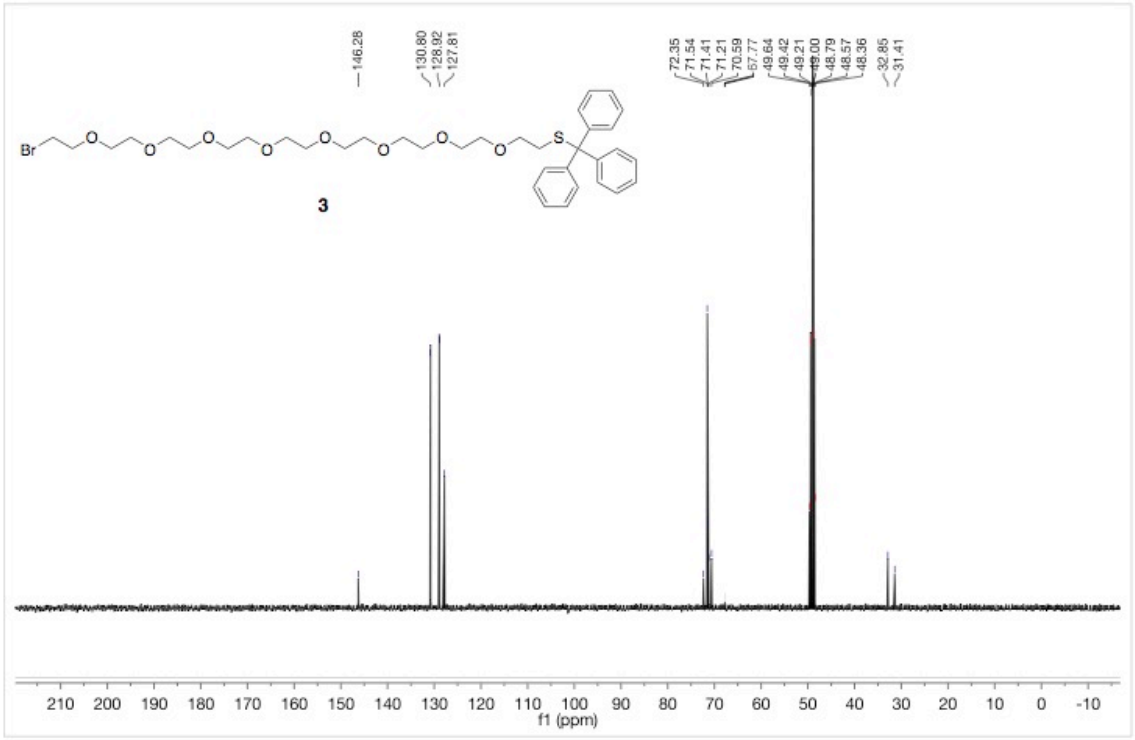
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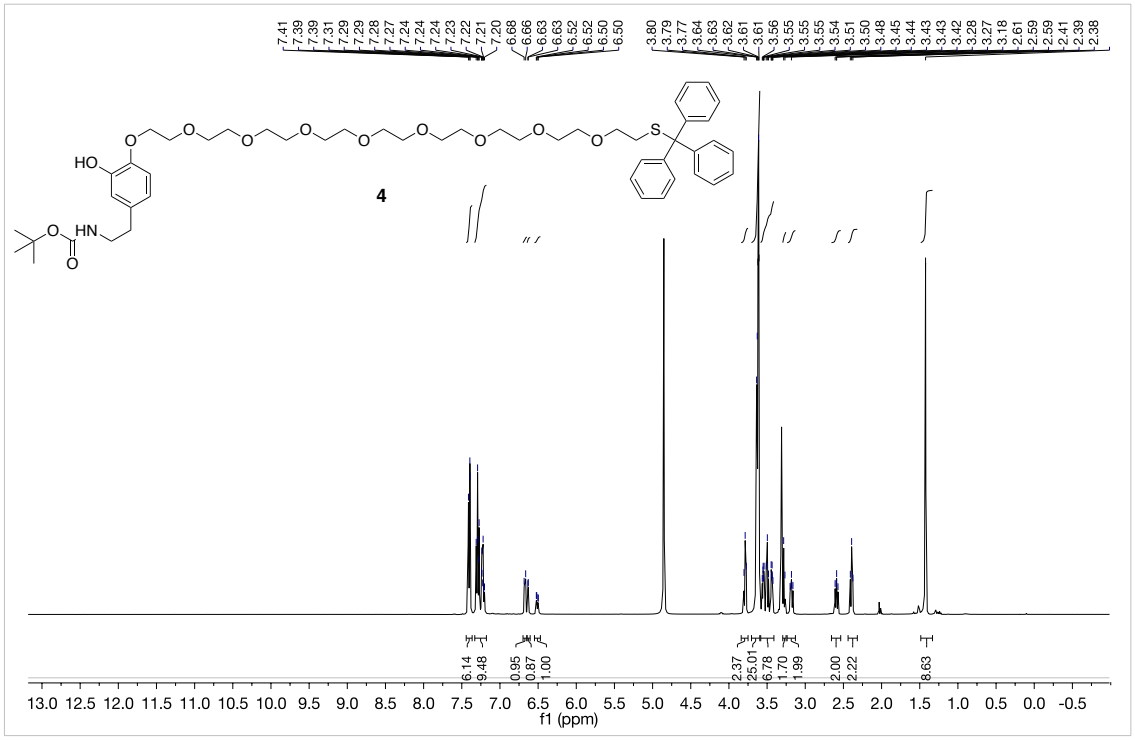
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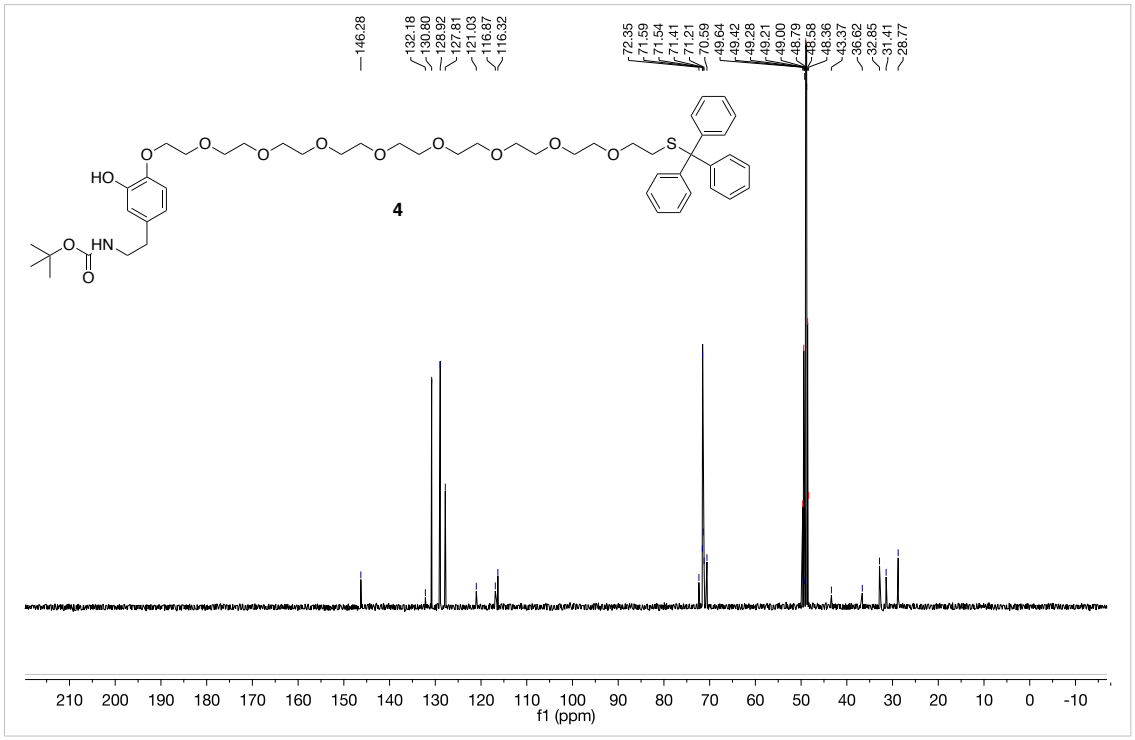
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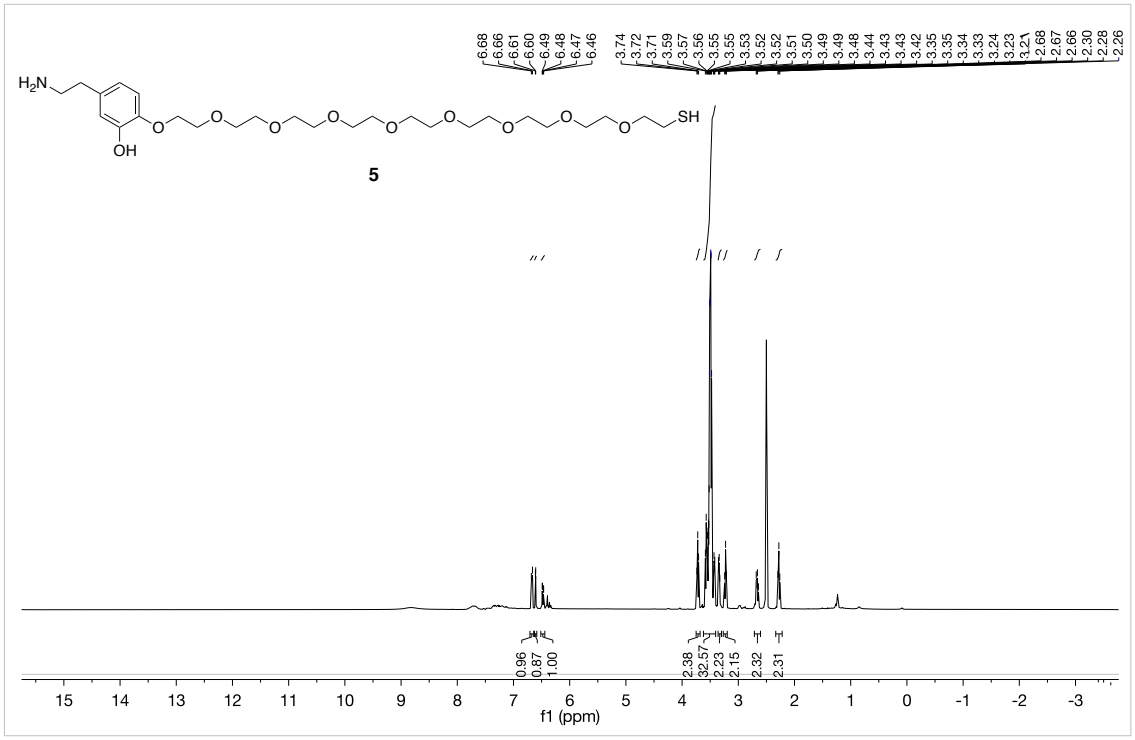
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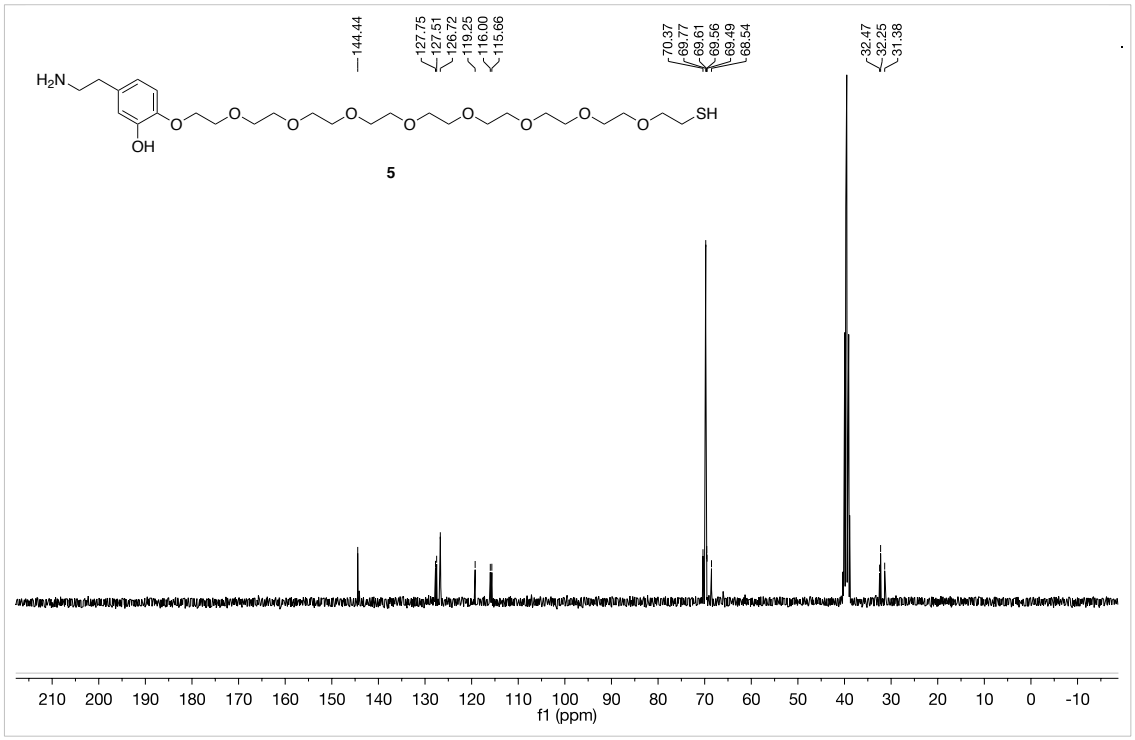
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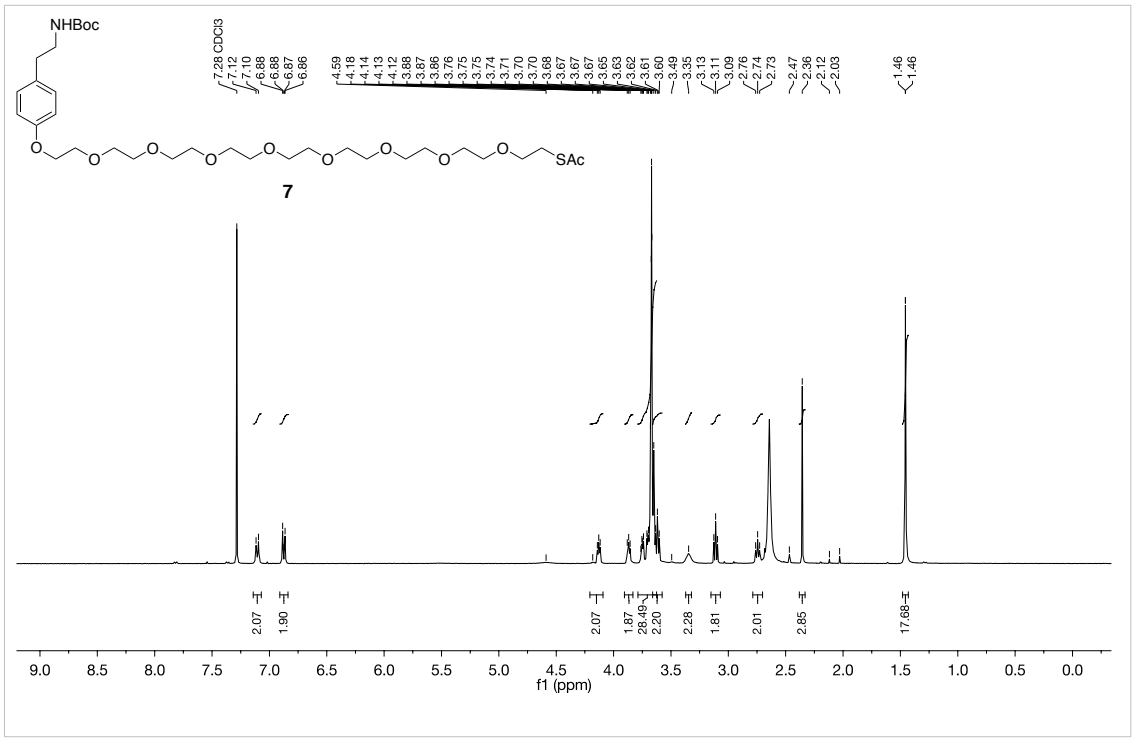
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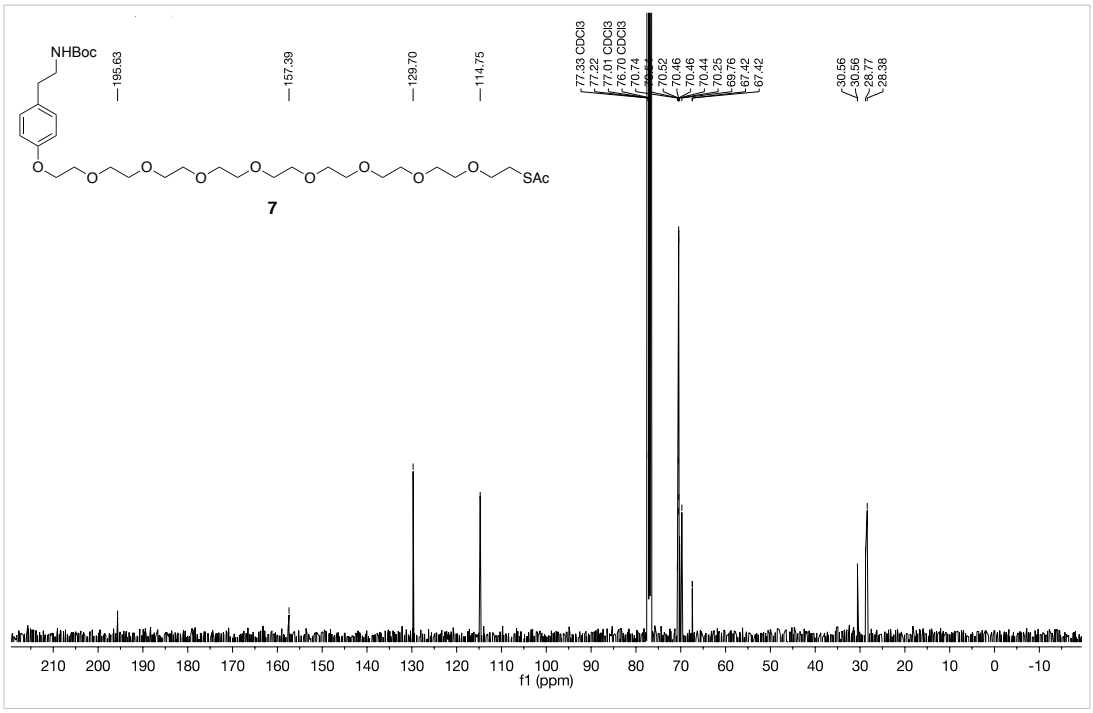
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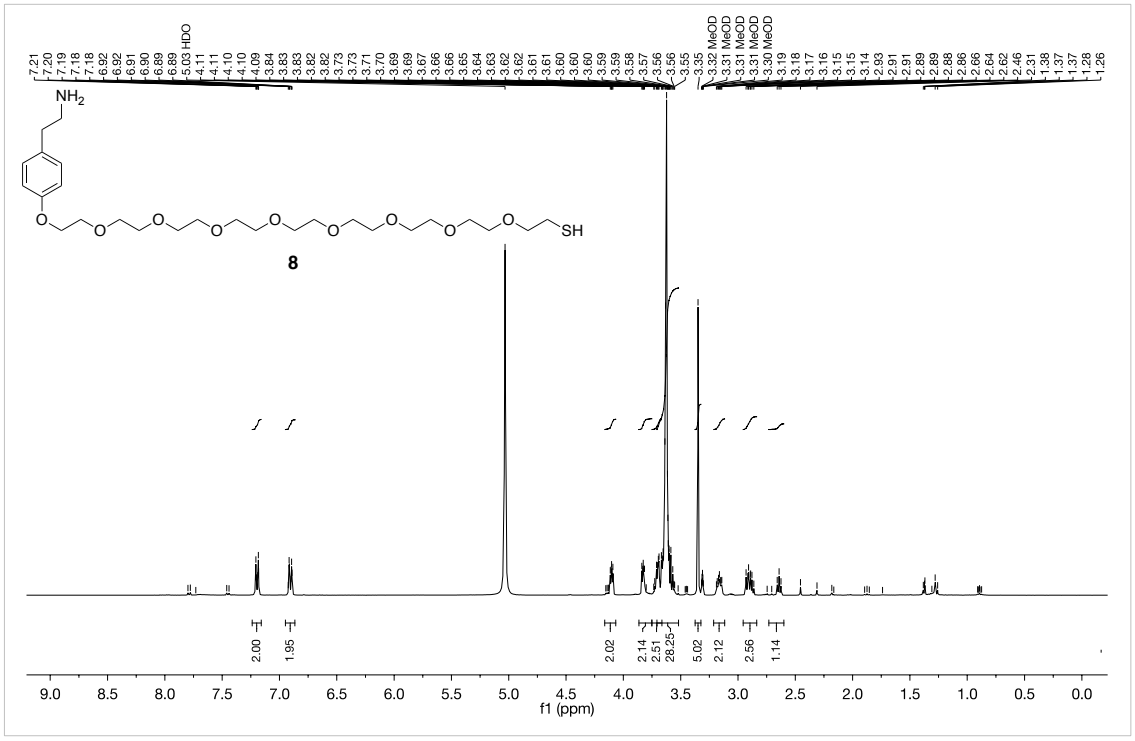
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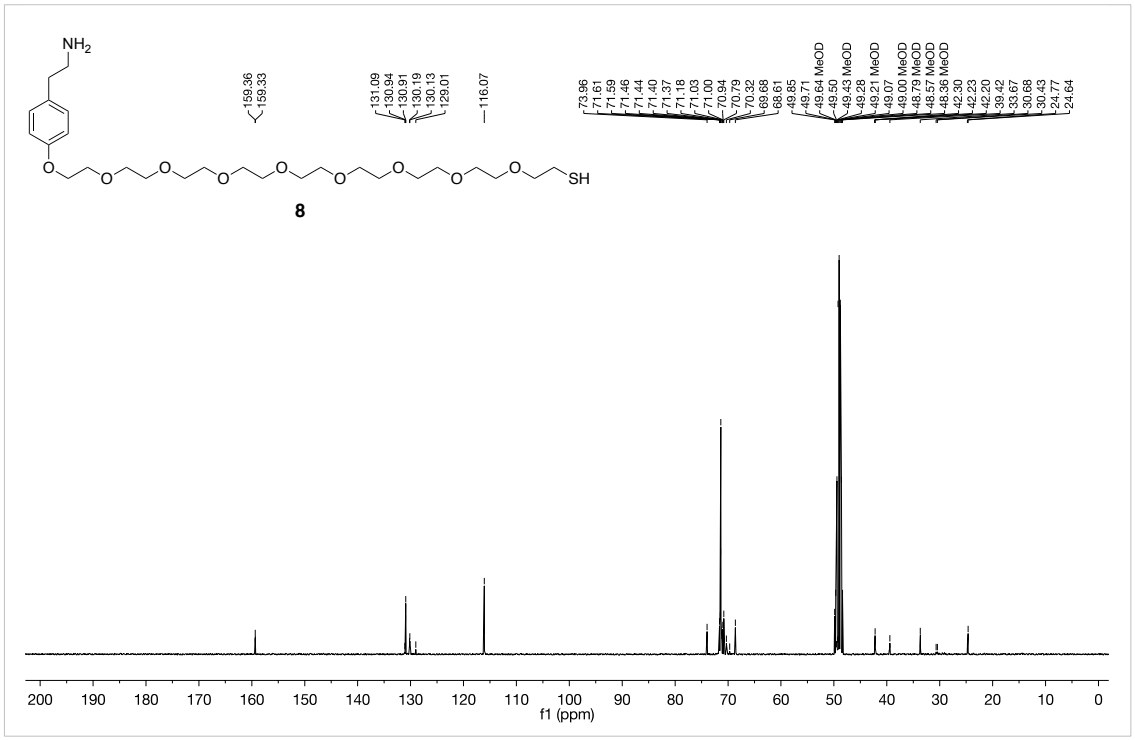
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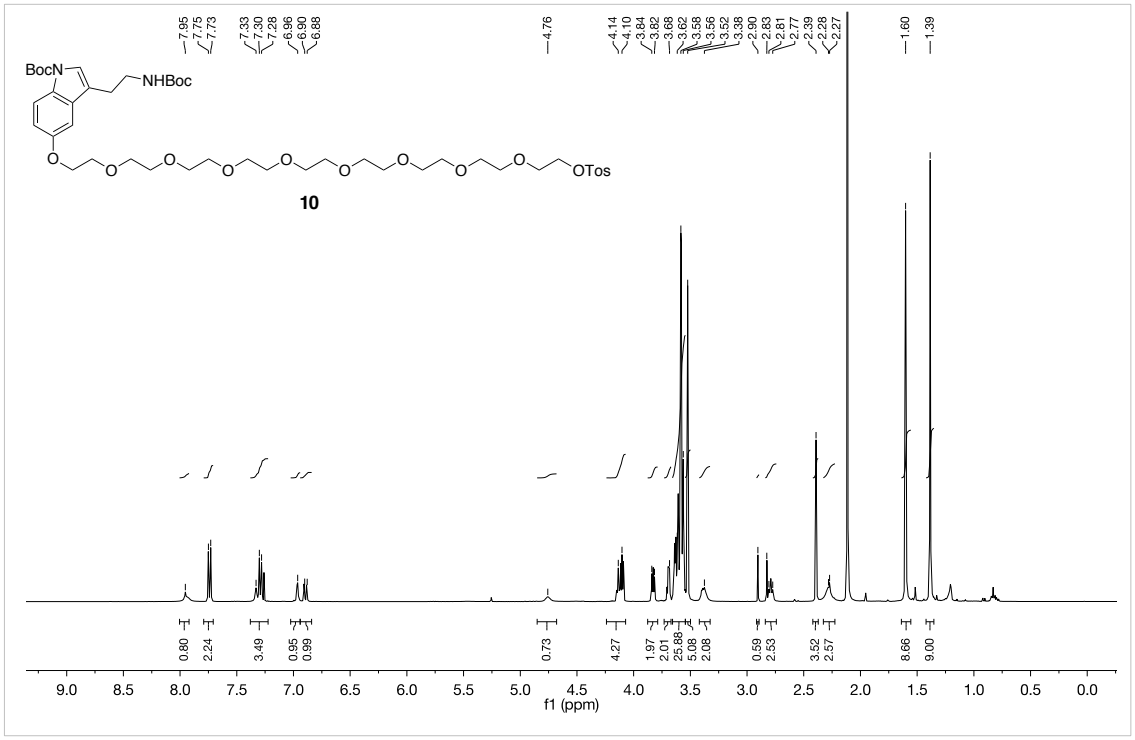
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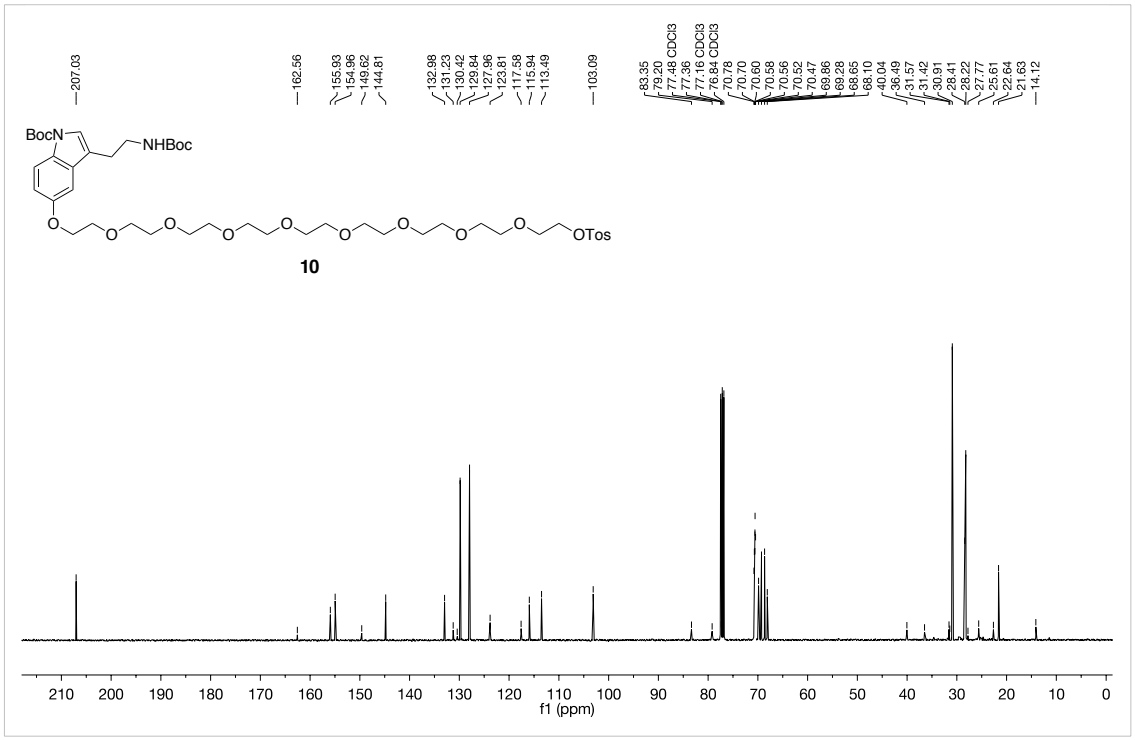
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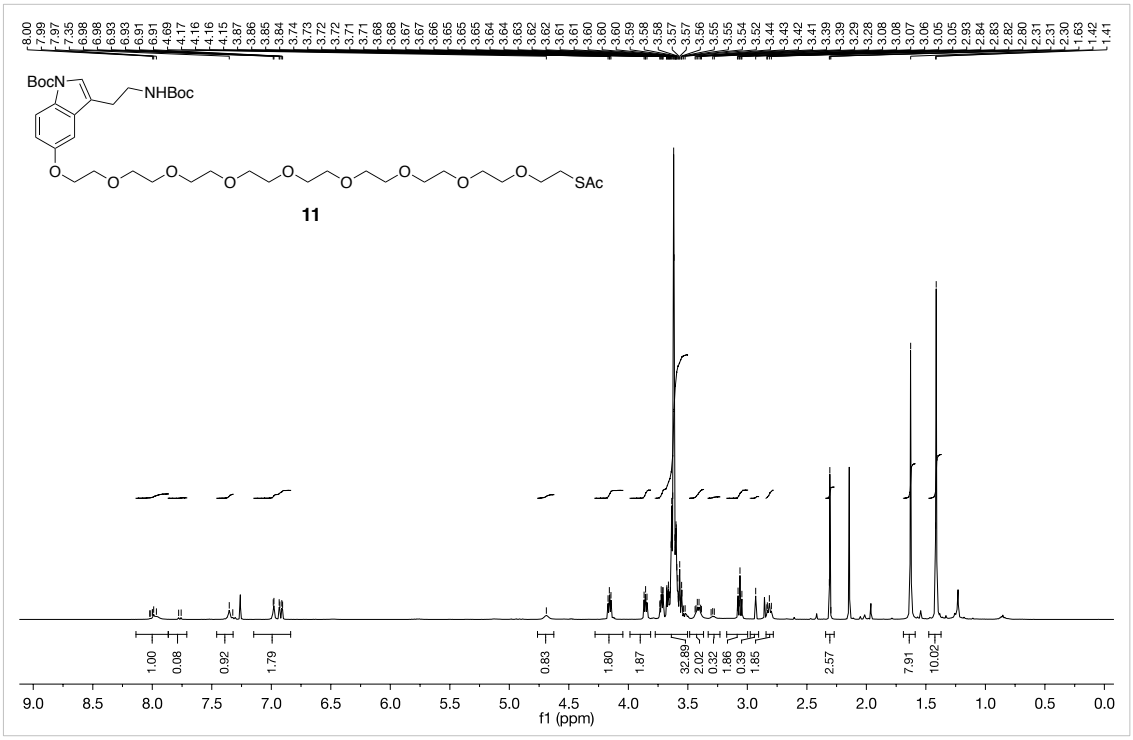
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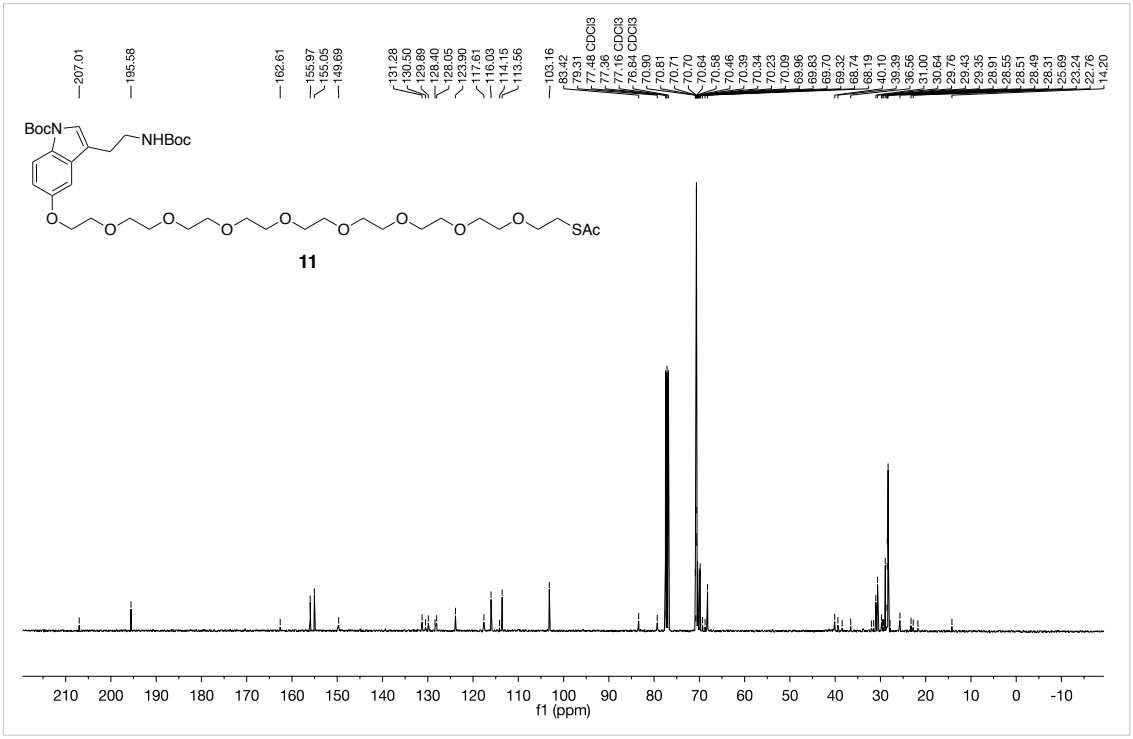
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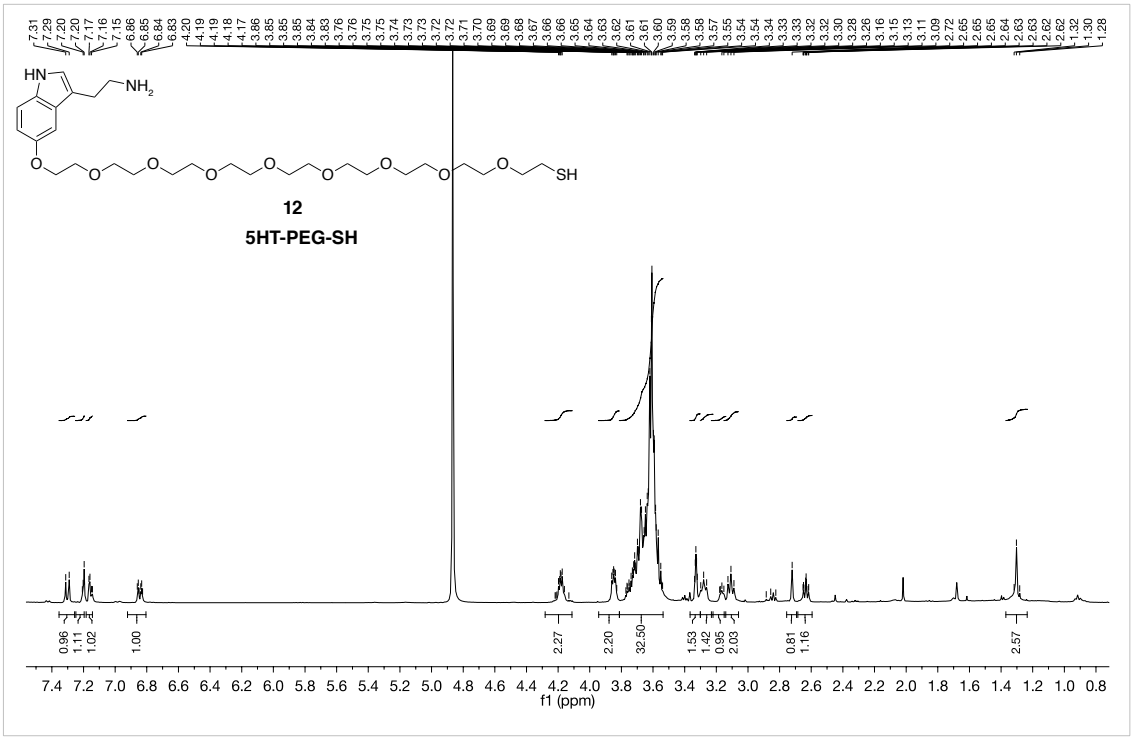
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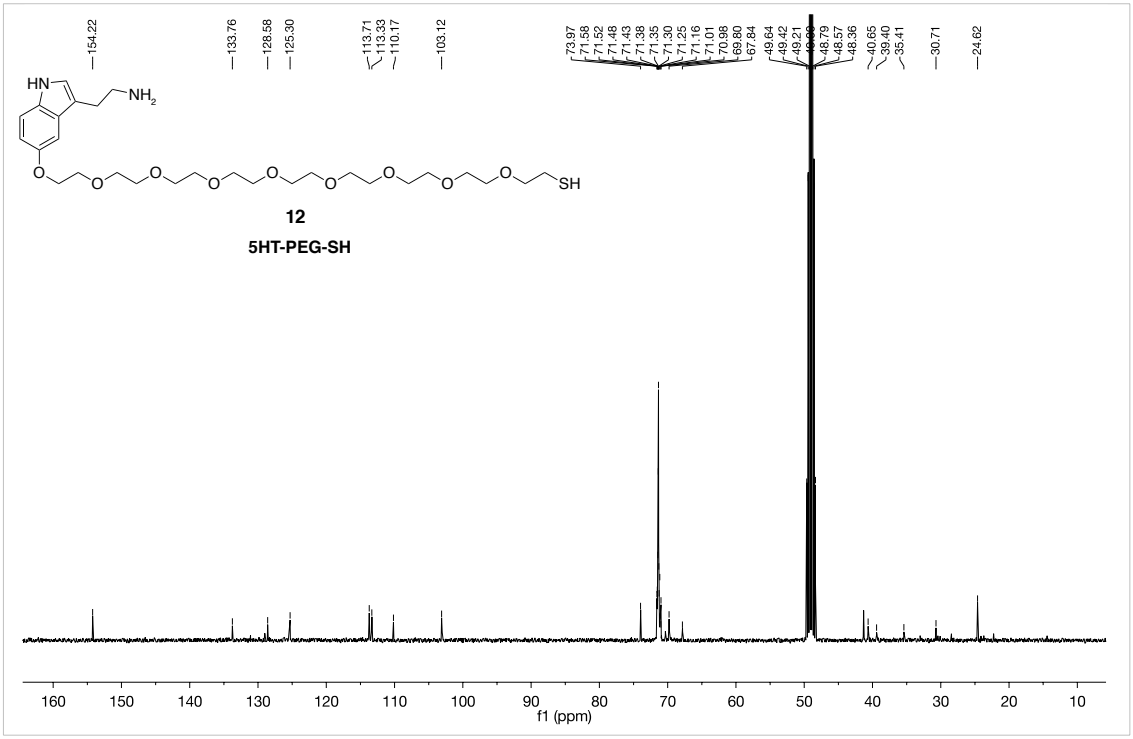
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