Hypersensitive azobenzenes: facile synthesis of clickable and cleavable azo linkers with tunable and high reducibility

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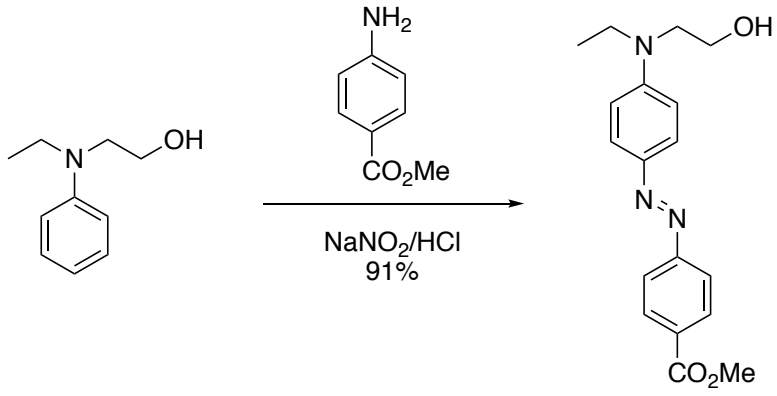
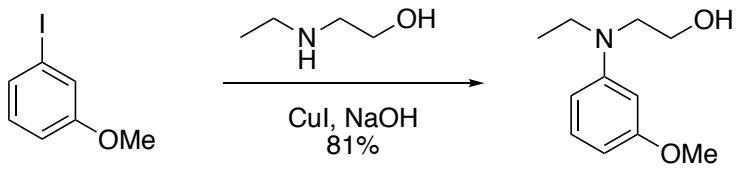
General Methods and Materials

Methyl 4-aminobenzoate, 2-(*N*-ethylanilino)ethanol, 3-iodoanisole, 2-(ethylamino)ethanol, CuI, sodium hydroxide (NaOH), 1-iodo-3,5-dimethoxybenzene (AK scientific, Inc.), *p*-toluenesulfonyl chloride, sodium azide (NaN_3), 4-aminobenzoic acid, tetraethylene glycol *p*-toluenesulfonate, potassium carbonate (K_2CO_3), hydrochloric acid 37% (HCl), sodium nitrite ($NaNO_2$), biotin, cyanine5.5 DBCO, N,N'-dicyclohexylcarbodiimide (DCC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl), 1-hydroxybenzotriazole hydrate (HOBt), 4-dimethylaminopyridine (DMAP), sodium bicarbonate ($NaHCO_3$) and sodium dithionite ($Na_2S_2O_4$) were purchased from commercial sources. NMR spectra were recorded on a Varian NMR system 500 MHz spectrometer, using DMSO-d_6 as the solvent. The UV/Vis measurements were carried out on a Lambda 265 UV/Vis-spectrometer from Perkin-Elmer. The fluorescence emission was carried out on a FP-6500 from JASCO. HRMS was carried out on a JMS-700 from JEOL in FAB mode. LC-MS/MS was carried out on a HP 1000 coupled with an Agilent 6130 single quadruple mass detector from Agilent technologies. Mobile phase A was H_2O, and mobile phase B was 5 mM ammonium formate in MeOH.

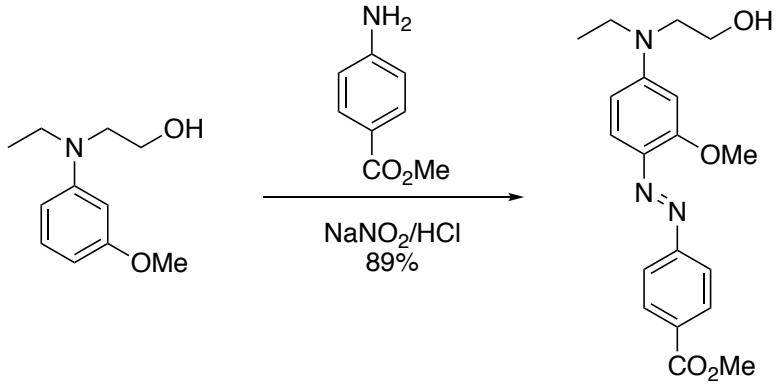
Synthesis



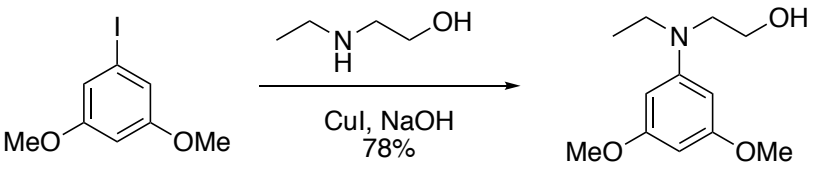
Donor/Acceptor hydroxy azobenzene (**1**): Methyl 4-aminobenzoate (250 mg, 1.65 mmol), was dissolved in 4 mL of 18% HCl and cooled using an ice bath. Then, a solution of sodium nitrite (171 mg, 2.48 mmol) in 0.5 mL of water was added dropwise into the reaction mixture. After 10 min of stirring, this solution was then transferred to a different reaction flask also ice-cooled containing solution of 2-(*N*-ethylanilino)ethanol (328 mg, 1.98 mmol) dissolved in 1.5 mL of 37% HCl. After the addition was complete, the cooling was removed and the resulting reaction mixture was stirred at room temperature for 3 hrs. After the reaction, saturated $NaHCO_3$ was added slowly. The product precipitated and was separated by centrifugation and the collected solid was washed with water to give 492 mg of the product as an orange solid (yield = 91%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.08 (d, J = 8.3 Hz, 2H), 7.82 (dd, J = 19.9, 8.6 Hz, 4H), 6.85 (d, J = 9.0 Hz, 2H), 4.85 (t, J = 5.4 Hz, 1H), 3.88 (s, 3H), 3.68 – 3.42 (m, 6H), 1.15 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.29, 155.79, 151.76, 142.79, 130.83, 129.85, 126.08, 122.24, 111.74, 58.80, 52.68, 52.58, 45.63, 12.46. HRMS (FAB+) m/z calcd for $C_{18}H_{22}N_3O_3$ $[M+H]^+$: 328.1661, observed: 328.1657.



2-(ethyl(3-methoxyphenyl)amino)ethanol (**I**): 3-iodoanisole (234 mg, 1.0 mmol), 2-(ethylamino)ethanol (1 g, 11.22 mmol), CuI (5 mg, 0.025 mmol), and NaOH (80.0 mg, 2.0 mmol) were added to a schlenk tube and was purged by bubbling Ar for 20 min. The reaction mixture was then stirred under inert atmosphere at 90 ºC for 24 hrs. After the reaction, the reaction mixture was cooled to room temperature and diluted with water and extracted with CH_2Cl_2 thrice. The combined organic layers were wash with 0.1 M NaOH, brine, and dried over Na_2SO_4. The organic solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent = EtOAc/DCM 20:80) which gave 158 mg of the product as a pale yellow oil (yield = 81%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.02 (dd, J = 9.2, 7.3 Hz, 1H), 6.32 – 6.22 (m, 1H), 6.20 – 6.08 (m, 2H), 4.69 (s, 1H), 3.68 (s, 3H), 3.51 (t, J = 6.7 Hz, 2H), 3.33 – 3.26 (m, 4H), 1.05 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.96, 149.44, 130.18, 104.88, 100.62, 97.99, 58.77, 55.12, 52.59, 45.09, 12.43. HRMS (FAB+) m/z calcd for $C_{11}H_{17}NO_2$ $[M]^+$: 195.1259, observed: 195.1259.

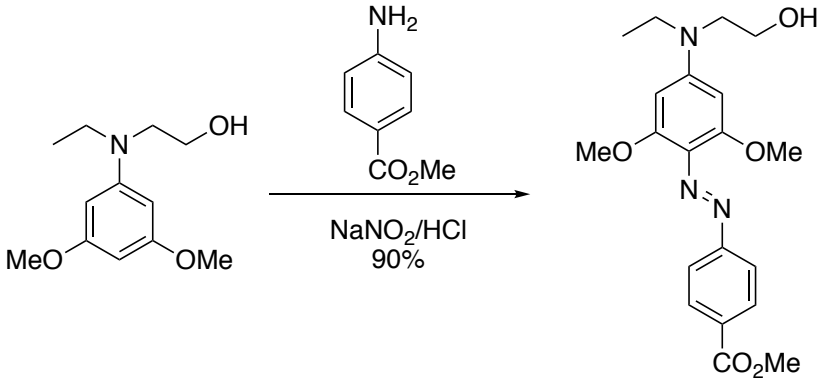


Donor-Donor/Acceptor hydroxy azobenzene (**2**): Methyl 4-aminobenzoate (250 mg, 1.65 mmol), was dissolved in 4 mL of 18% HCl and cooled using an ice bath. Then, a solution of sodium nitrite (171 mg, 2.48 mmol) in 0.5 mL of water was added dropwise into the reaction mixture. After 10 min of stirring, this solution was then transferred to another reaction flask containing compound **I** (387 mg, 1.98 mmol) dissolved in 1.5 mL of 37% HCl and ice-cooled. After the addition was complete, the cooling was removed and the resulting reaction mixture was stirred at room temperature for 3 hrs. After the reaction, saturated $NaHCO_3$ was added in the solution slowly precipitate the product. The precipitate was separated by centrifugation and the collected solid was washed with water to give 545 mg of the product as an orange solid (yield = 89 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.17 – 7.97 (m, 2H), 7.87 – 7.72 (m, 2H), 7.67 (d, J = 9.3 Hz, 1H), 6.43 (dd, J = 9.4, 2.5 Hz, 1H), 6.34 (d, J = 2.5 Hz, 1H), 4.85 (t, J = 5.4 Hz, 1H), 3.95 (s, 3H), 3.87 (s, 3H), 3.67 – 3.43 (m, 6H), 1.16 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.35, 160.60, 156.39, 153.74, 132.98, 130.78, 129.16, 122.13, 118.15, 105.29, 94.73, 58.94, 56.20, 52.67, 52.61, 45.74, 12.59. HRMS (FAB+) m/z calcd for $C_{19}H_{24}N_3O_4$ $[M+H]^+$: 358.1767, observed: 358.1765.

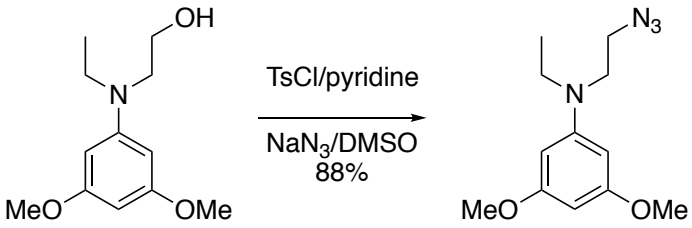


2-((3,5-dimethoxyphenyl)(ethyl)amino)ethanol (**II**): 1-iodo-3,5-dimethoxybenzene (264 mg, 1.0 mmol), 2-(ethylamino)ethanol (1 g, 11.22 mmol), CuI (5 mg, 0.025 mmol), and NaOH (80.0 mg, 2.0 mmol) were added to a schlenk tube and was purged by bubbling Ar for 20 min. The reaction mixture was then stirred under inert atmosphere at 90 ºC for 24 hrs. After the

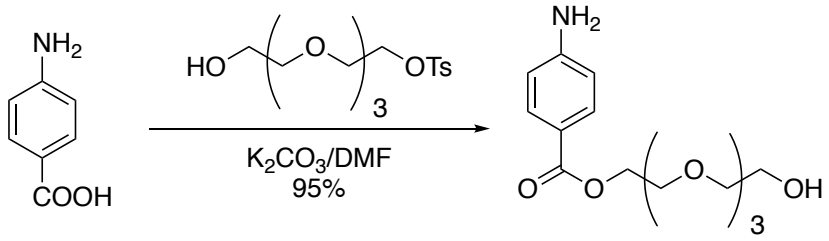
reaction, the reaction mixture was cooled to room temperature and diluted with water and extracted with CH_2Cl_2 thrice. The combined organic layers were washed with 0.1 M NaOH, brine, and dried over Na_2SO_4. The organic solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent = EtOAc/DCM 20:80) which gave about 176 mg of the product as a pale yellow oil (yield = 78 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.83 – 5.71 (m, 3H), 4.68 (t, J = 5.5 Hz, 1H), 3.67 (s, 6H), 3.50 (td, J = 6.6, 5.5 Hz, 2H), 3.33 – 3.25 (m, 4H), 1.05 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.79, 149.85, 91.01, 87.99, 58.82, 55.21, 52.65, 45.19, 12.51. HRMS (FAB+) m/z calcd for $C_{12}H_{19}NO_3$ $[M]^+$: 225.1365, observed: 225.1367.



Donor-Donor-Donor/Acceptor hydroxy azobenzene (**3**): Methyl 4-aminobenzoate (250 mg, 1.65 mmol) was dissolved in 4 mL of 18% HCl and cooled using an ice bath. Then, a solution of sodium nitrite (171 mg, 2.48 mmol) in 0.5 mL of water was added dropwise into the reaction mixture. After 10 min of stirring, this solution was then transferred to another reaction flask containing compound **II** (446 mg, 1.98 mmol) dissolved in 1.5 mL of 37% HCl cooled with ice-bath. After the addition was complete, the cooling was removed and the resulting reaction mixture was stirred at room temperature for 3 hrs. After the reaction, saturated $NaHCO_3$ was added into the solution slowly to precipitate out the product. The precipitate was separated by centrifugation and the collected solid was washed with water to give about 575 mg of the product as an orange solid (yield = 90 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.03 (d, J = 8.3 Hz, 2H), 7.65 (d, J = 8.3 Hz, 2H), 6.00 (s, 2H), 4.88 (t, J = 5.5 Hz, 1H), 3.86 (s, 3H), 3.82 (s, 6H), 3.67 – 3.49 (m, 6H), 1.18 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.48, 157.32, 130.62 (likely to represent multiple carbon atoms), 128.56, 123.85, 121.55, 88.78, 59.02, 52.65, 52.55, 45.68, 12.64. HRMS (FAB+) m/z calcd for $C_{20}H_{26}N_3O_5$ $[M+H]^+$: 388.1872, observed: 388.1868.

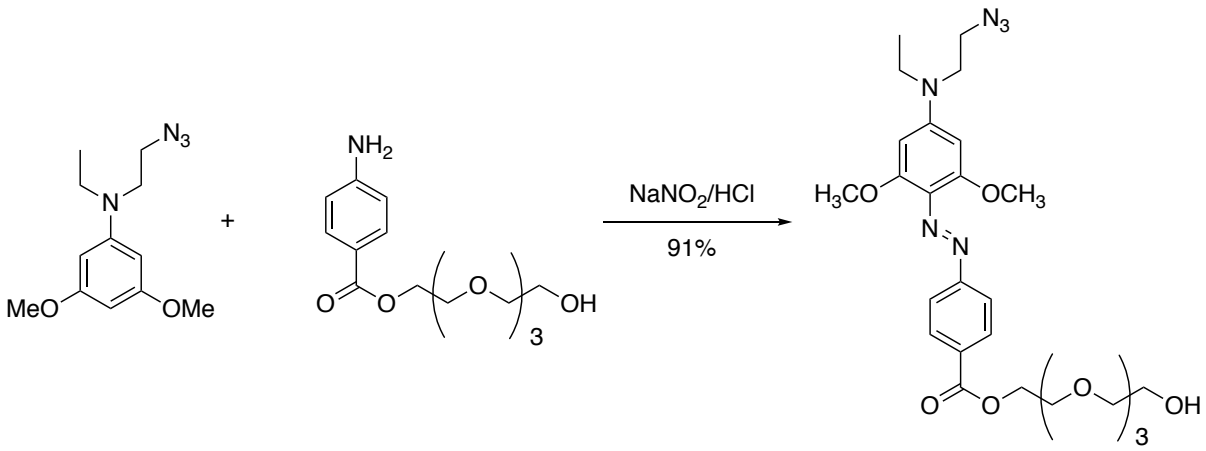


Azide-N-ethylaniline (**4**): To a solution of compound **II** (200 mg, 0.89mmol) in 4 mL pyridine cooled to 0 °C, *p*-toluenesulfonyl chloride (339 mg, 1.78 mmol) was added and the reaction mixture was stirred overnight at room temperature. The resulting solution was extracted with $CHCl_3$, water, and dried over Na_2SO_4. The organic solvent was removed under reduced pressure. The crude product and sodium azide (347 mg, 5.34 mmol) was dissolved in 5 mL DMSO and stirred overnight at 120 °C. The resulting solution was extracted with EtOAc and dried over Na_2SO_4. The organic solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent = $CHCl_3$) which gave about 220 mg of the product as a yellow oil (yield = 88 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.84 (s, 3H), 3.69 (s, 6H), 3.45 (d, J = 1.4 Hz, 4H), 3.38 – 3.34 (m, 2H), 1.07 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.82, 149.39, 91.52, 88.76, 55.25, 49.24, 49.02, 44.97, 12.39. HRMS (FAB+) m/z calcd for $C_{12}H_{19}N_4O_2$ $[M+H]^+$: 251.1508, observed: 251.1501.

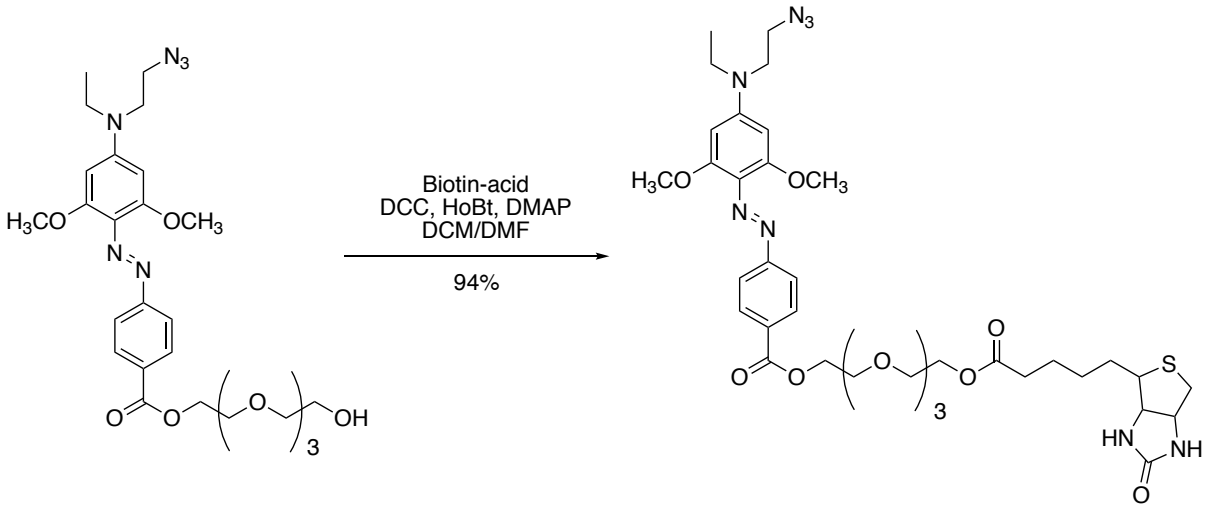


Hydroxy-TEG-aniline (**5**): To a solution of 4-amionbenzoic acid (428 mg, 3.12 mmol) and tetraethylene glycol *p*-toluenesulfonate (400 mg, 1.04 mmol) in 15 mL DMF, K_2CO_3 (431 mg, 3.12 mmol) was added and the reaction mixture was stirred overnight at 65 °C. After the reaction, the solution was extracted with DCM and washed with ice water. The organic layer was dried over Na_2SO_4 and the organic solvent was removed under reduced pressure and then precipitated into hexane thrice. The obtained colorless oil was then dried under high vacuum conditions to give 308 mg of the product as a yellow oil (yield = 95 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.67 – 7.61 (m, 2H), 6.61 – 6.53 (m, 2H), 5.97 (s, 2H), 4.57 (s, 1H), 4.30 – 4.22 (m, 2H), 3.72 – 3.65 (m, 2H), 3.59 – 3.29 (m, 12H). ^{13}C NMR (126 MHz, DMSO-d_6) δ

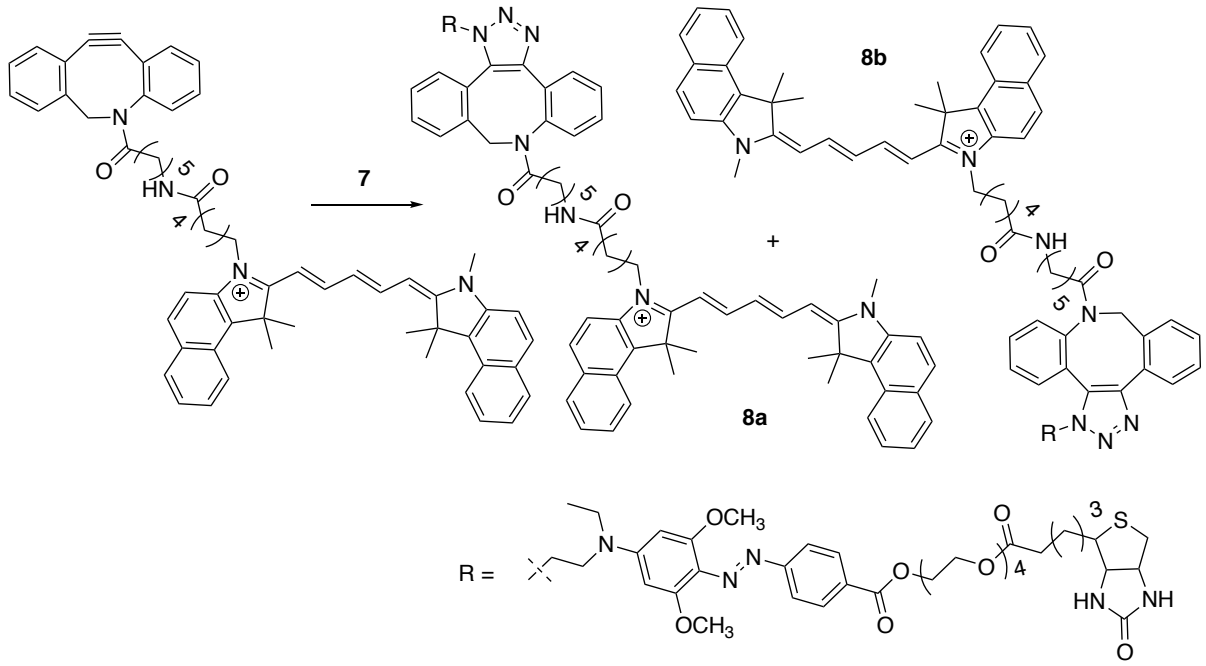
166.27, 153.97, 131.58, 116.16, 113.06, 72.78, 70.31, 70.27, 70.23, 70.19, 69.03, 63.43, 60.65. HRMS (FAB+) m/z calcd for $C_{15}H_{23}NO_6$ $[M]^+$: 313.1525, observed: 313.1524.



Azide-DDDA-AZO-OH (**6**): Compound **5** (100 mg, 0.32 mmol) was dissolved in water (1 mL) and cooled using an ice bath. Then, 0.2 mL of 37 % HCl was added, followed by the addition of sodium nitrite (33 mg) solution in 1 mL of water. After 30 min of stirring, this solution was then transferred ice cooled solution of compound **4** (120 mg, 0.48 mmol) dissolved in 3 mL of DMF. The reaction mixture was stirred at room temperature for 24 hrs under inert atmosphere. After the reaction, the reaction mixture was dissolved in chloroform and extracted with water thrice. The combined organic layer was dried over $MgSO_4$ and the organic solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent = DCM/MeOH 95:5) which gave about 167 mg of the product as a red oil (yield = 91 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.09 – 7.98 (m, 2H), 7.79 – 7.59 (m, 2H), 6.05 (s, 2H), 4.56 (s, 1H), 4.47 – 4.32 (m, 2H), 3.85 (s, 6H), 3.80 – 3.35 (m, 20H), 1.20 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.91, 157.42, 130.71 (likely to represent multiple carbon atoms), 128.79, 124.12, 121.42, 89.25, 72.79, 70.33, 70.28, 70.22, 70.20, 68.80, 64.52, 60.65, 56.49, 49.15, 45.40, 12.68. HRMS (FAB+) m/z calcd for $C_{27}H_{39}N_6O_8$ $[M+H]^+$: 575.2829, observed: 575.2826.



Azide-DDDA-AZO-biotin (**7**): Biotin (20 mg, 0.08 mmol), HOBt (3 mg, 0.02 mmol) and 3 Å molecular sieves were dissolved in 1 mL dry DMF. Then a solution of DCC (24 mg, 0.12 mmol) in 0.5 mL DCM was added at 0 °C and the reaction mixture was stirred at room temperature for 2 hrs. Compound **6** (35 mg, 0.06 mmol) and DMAP (1 mg, 0.01 mmol) were added, and the reaction mixture was stirred at 60 °C for 3 hrs and then at room temperature for 24 hrs. After the reaction, the reaction mixture was filtered and extracted with DCM and water. The organic layer was dried over Na_2SO_4 and the organic solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent = DCM/MeOH 97:3) to give 46 mg of the product as a red oil (yield = 94 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.09 – 7.96 (m, 2H), 7.71 – 7.60 (m, 2H), 6.37 (d, J = 32.3 Hz, 2H), 6.04 (s, 2H), 4.45 – 4.33 (m, 2H), 4.31 – 4.24 (m, 1H), 4.15 – 4.04 (m, 3H), 3.84 (s, 6H), 3.80 – 3.72 (m, 2H), 3.68 (t, J = 6.0 Hz, 2H), 3.64 – 3.46 (m, 14H), 3.06 (ddd, J = 8.7, 6.3, 4.5 Hz, 1H), 2.80 (dd, J = 12.4, 5.1 Hz, 1H), 2.56 (d, J = 12.4 Hz, 1H), 2.28 (t, J = 7.5 Hz, 2H), 1.63 – 1.26 (m, 6H), 1.20 (d, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.26, 165.94, 163.13, 157.74, 157.13, 152.07, 130.67, 128.90, 124.24, 121.67, 89.03, 70.33, 70.23, 70.21, 70.20, 68.80, 68.75, 64.51, 63.50, 61.46, 59.62, 56.38, 55.78, 49.16, 49.07, 45.24, 33.67, 28.40, 28.42, 24.91, 12.64. HRMS (FAB+) m/z calcd for $C_{38}H_{57}N_8O_9S$ $[M+H]^+$: 801.3969, observed: 801.3960.



Compound **8**: **7** (2 mg, 0.002 mmol) and cyanine 5.5-DBCO (2.6 mg, 0.002 mmol) were dissolved in 0.5 mL DMSO. The reaction mixture was stirred in the dark overnight at 40 °C. LC-MS indicated full conversion. In absence of any other reagents and full conversion of the starting materials, no purification was carried out (For UV-Vis see Figure 4. For IR see Figure S24. For LC-MS see Figure S25). **8a** and **8b** are positional isomers.

Cleavage of the azo bond

50 µM of azo-compound solution was prepared in 2/8 DMSO/Tris-HCl buffer mixture. Then, 50 µL of freshly prepared solution of sodium dithionite with individual concentrations of 40 mM, 20 mM, 15 mM and 10 mM, respectively were added to 950 µL of azo-compound solution. Final dithionite concentrations were 2 mM, 1 mM, 0.75 mM and 0.5 mM, respectively. The cleavage of the azobenzene group was followed by UV-Vis spectroscopy. The time-absorbance graph fitting was performed using OriginPro 9.0.

$1/y = 1/y_o + kt$ (k = rate constant, t = time, y_0 = initial absorbance)

Half-life is given by: $t_{1/2} = 1/ky_o$

$[Na_2S_2O_4]$		y_0 (initial absorbance)	k ($M^{-1}s^{-1}$) (rate constant)	$t_{1/2}$ (s) (half-life)	R^2
2 mM	**1** (DA)	1.13±0.002	0.05±0.91	15	0.96
	2 (DDA)	1.24±0.01	1.02±0.08	0.7	0.93
	3 (DDDA)	1.20±0.001	5.08±0.79	0.1	0.95
1 mM	**1** (DA)	1.12±0.002	0.02±0.31	35	0.97
	2 (DDA)	1.14±0.002	0.59±0.05	1.4	0.95
	3 (DDDA)	1.21±0.002	4.71±0.92	0.1	0.99
0.75 mM	**1** (DA)	-	-	-	-
	2 (DDA)	1.16±0.002	0.38±0.02	2.2	0.96
	3 (DDDA)	1.21±0.004	2.52±0.25	0.3	0.96
0.5 mM	**1** (DA)	-	-	-	-
	2 (DDA)	-	-	-	-
	3 (DDDA)	1.18±0.006	1.28±0.12	0.6	0.93

- = a full azo scission reaction could not be observed.

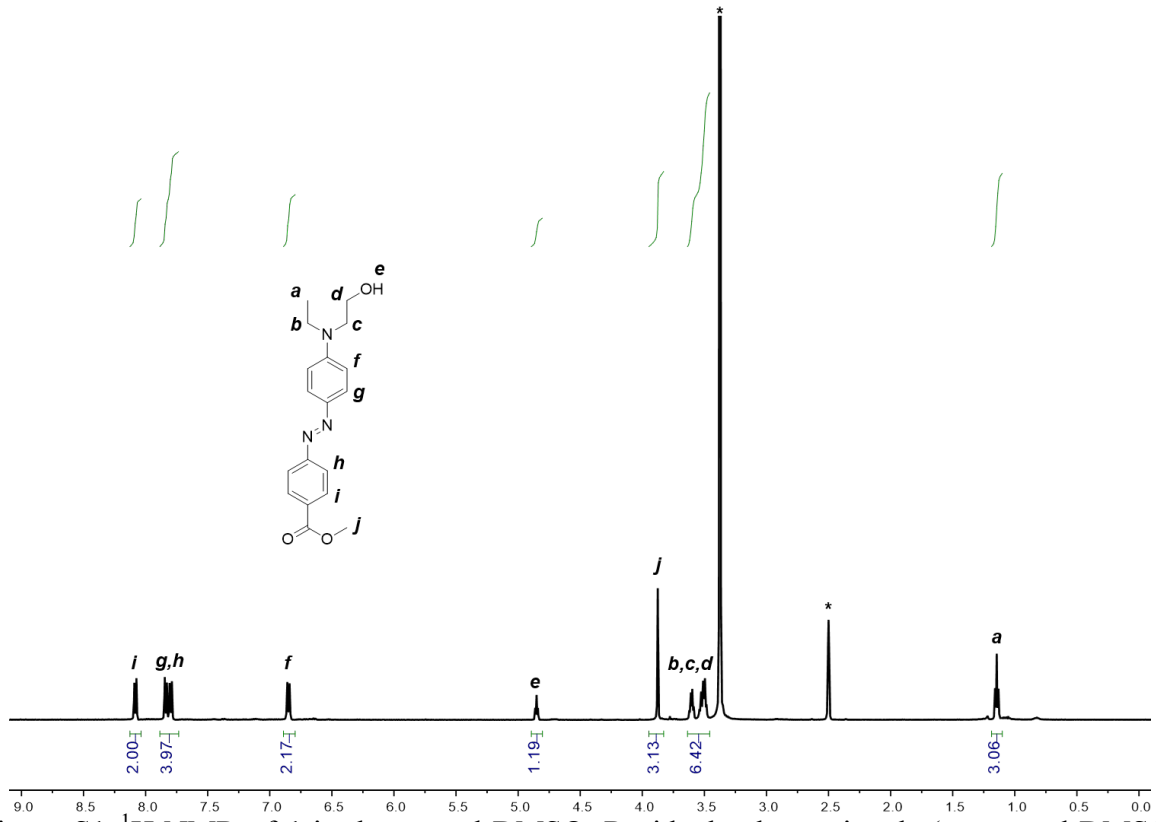


Figure S1. ^{1}H-NMR of **1** in deuterated DMSO. Residual solvent signals (water and DMSO) are shown with an asterisk.

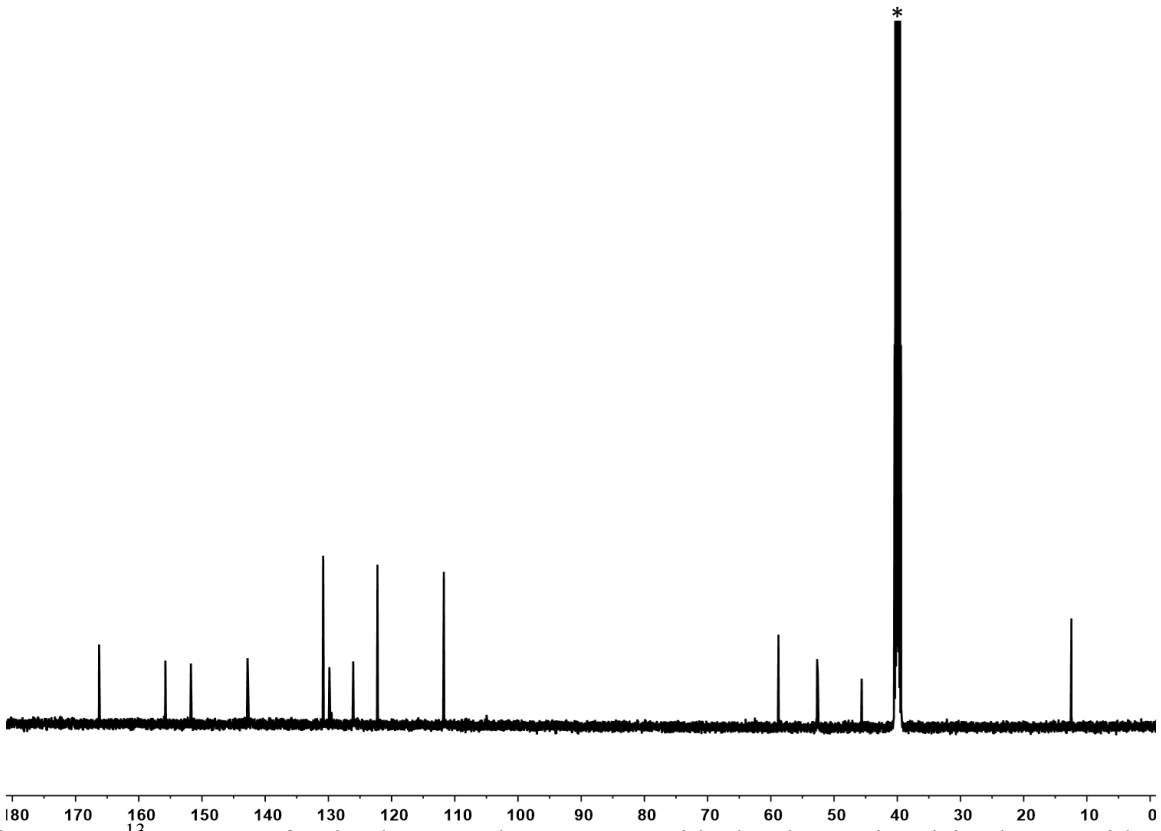


Figure S2. ^{13}C-NMR of **1** in deuterated DMSO. Residual solvent signal is shown with an asterisk.

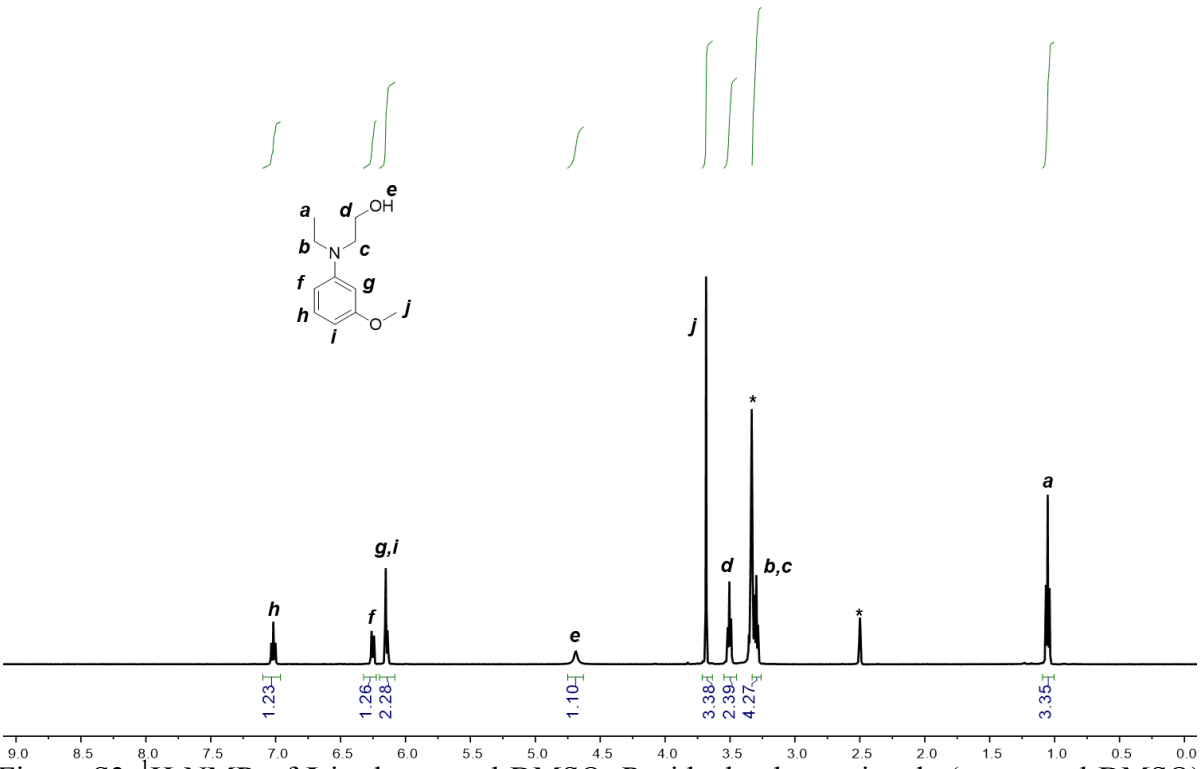


Figure S3. ^{1}H-NMR of **I** in deuterated DMSO. Residual solvent signals (water and DMSO) are shown with an asterisk.

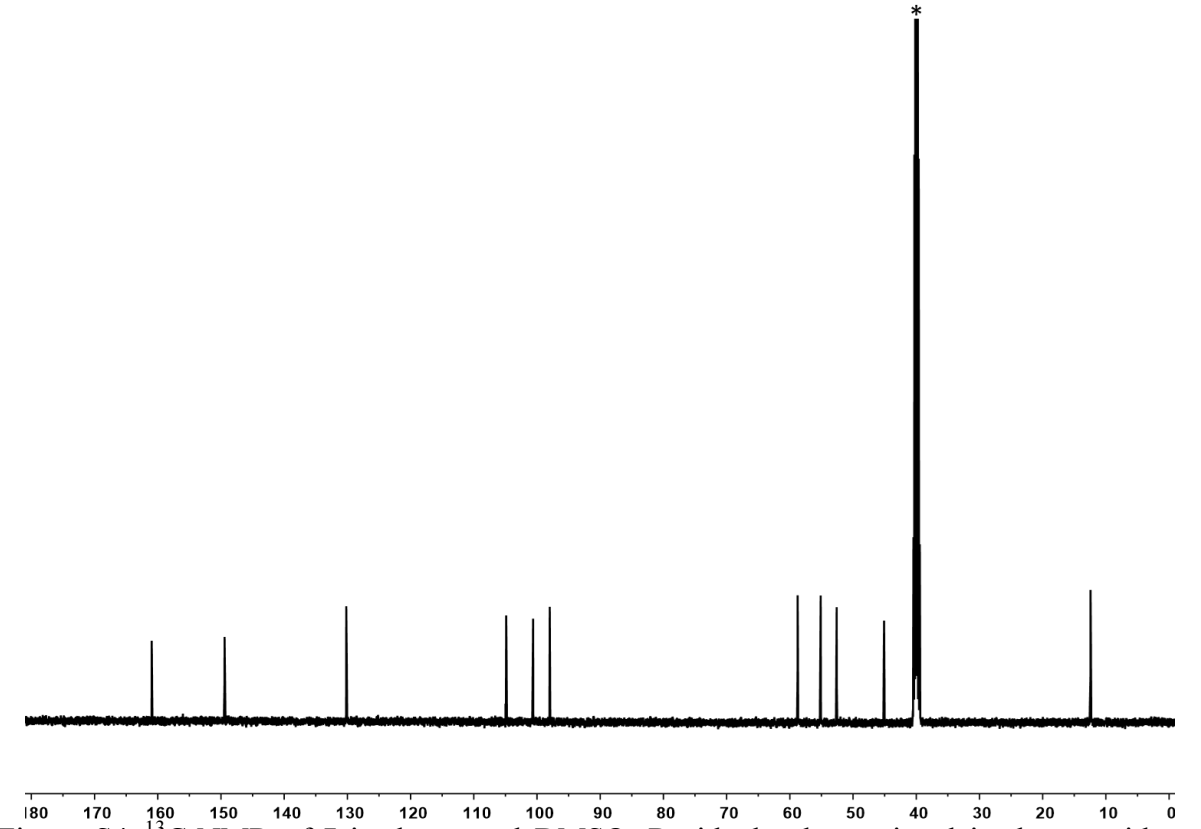


Figure S4. ^{13}C-NMR of **I** in deuterated DMSO. Residual solvent signal is shown with an asterisk.

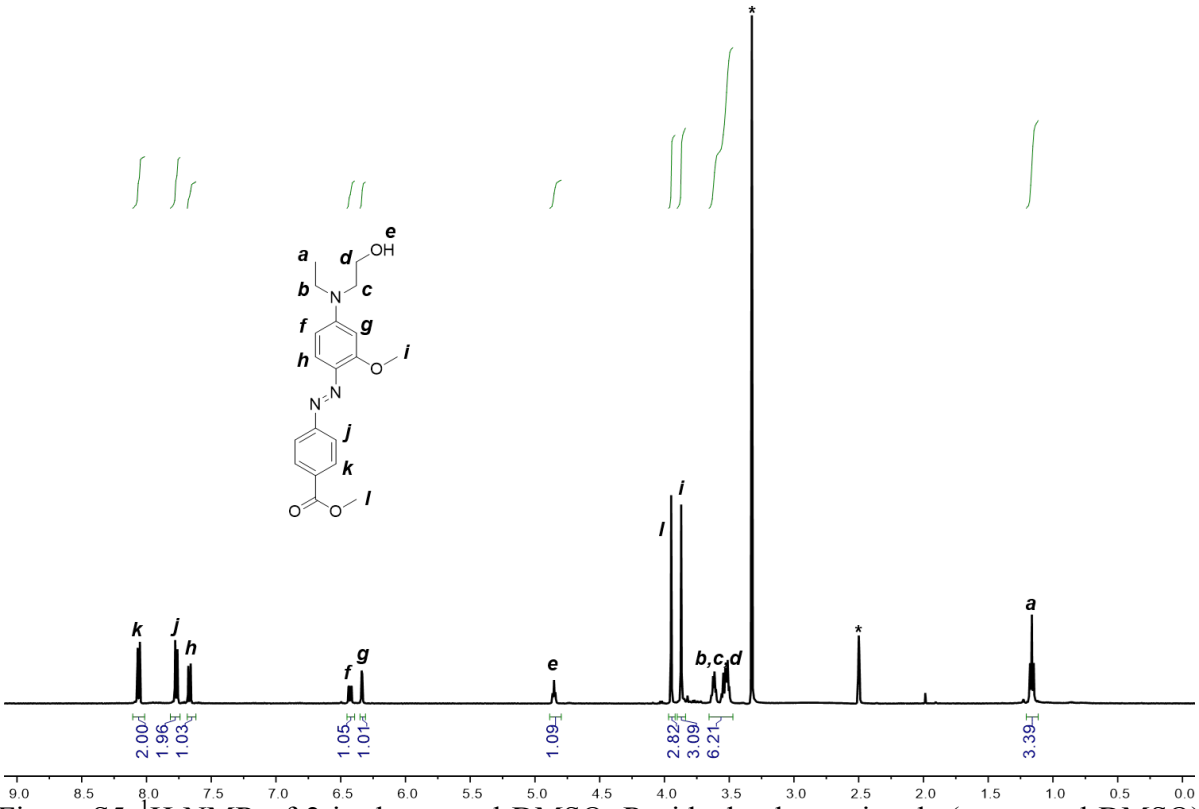


Figure S5. ^{1}H-NMR of **2** in deuterated DMSO. Residual solvent signals (water and DMSO) are shown with an asterisk.

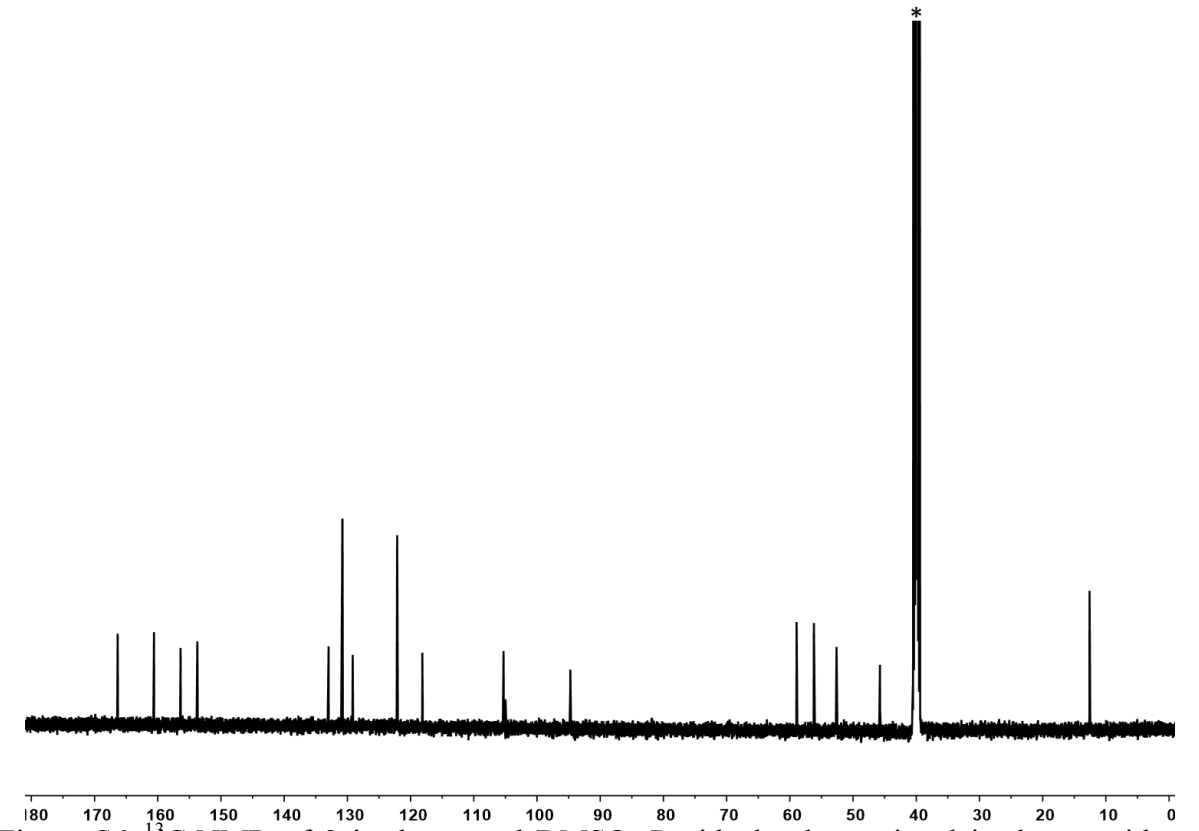


Figure S6. ^{13}C-NMR of **2** in deuterated DMSO. Residual solvent signal is shown with an asterisk.

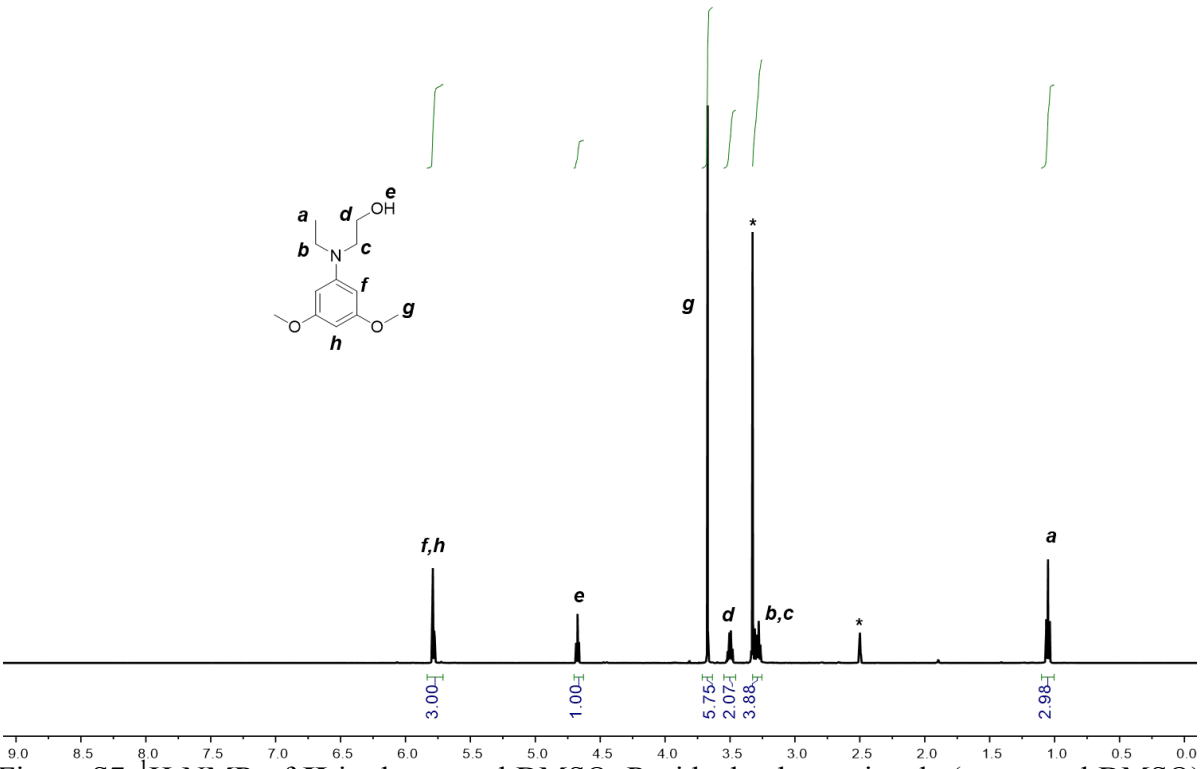


Figure S7. ^{1}H-NMR of **II** in deuterated DMSO. Residual solvent signals (water and DMSO) are shown with an asterisk.

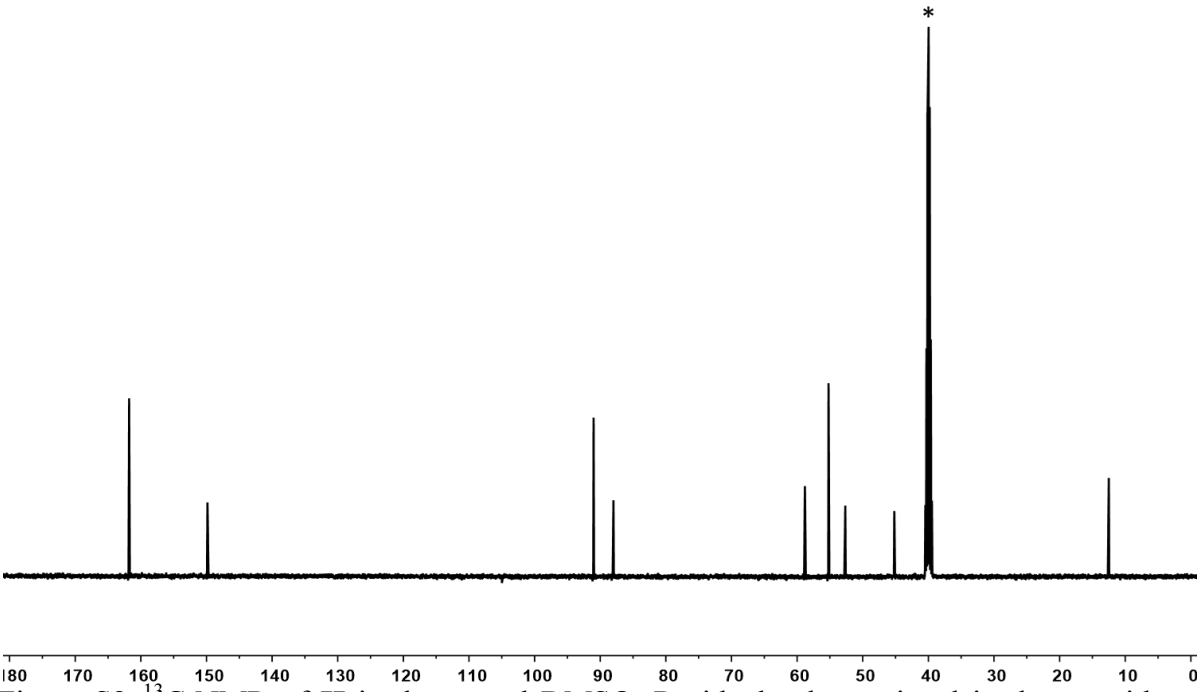


Figure S8. ^{13}C-NMR of **II** in deuterated DMSO. Residual solvent signal is shown with an asterisk.

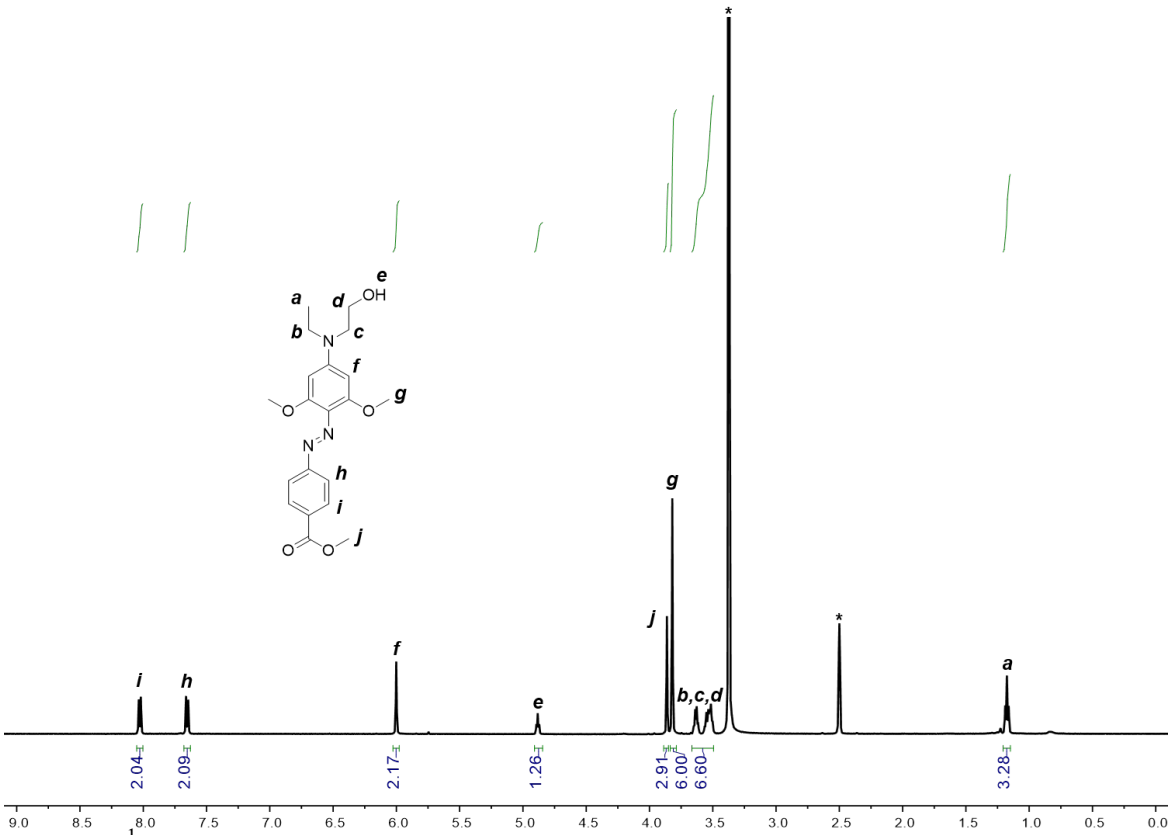


Figure S9. ^{1}H-NMR of **3** in deuterated DMSO. Residual solvent signals (water and DMSO) are shown with an asterisk.

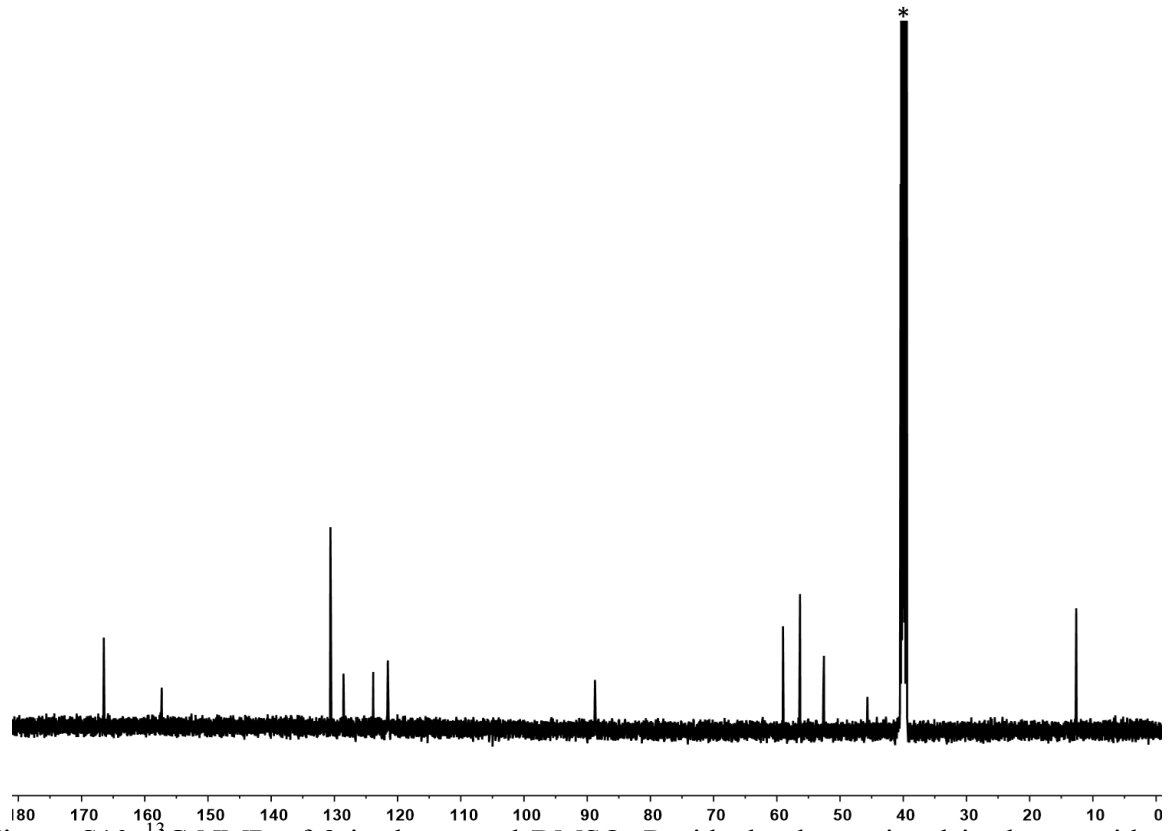


Figure S10. ^{13}C-NMR of **3** in deuterated DMSO. Residual solvent signal is shown with an asterisk.

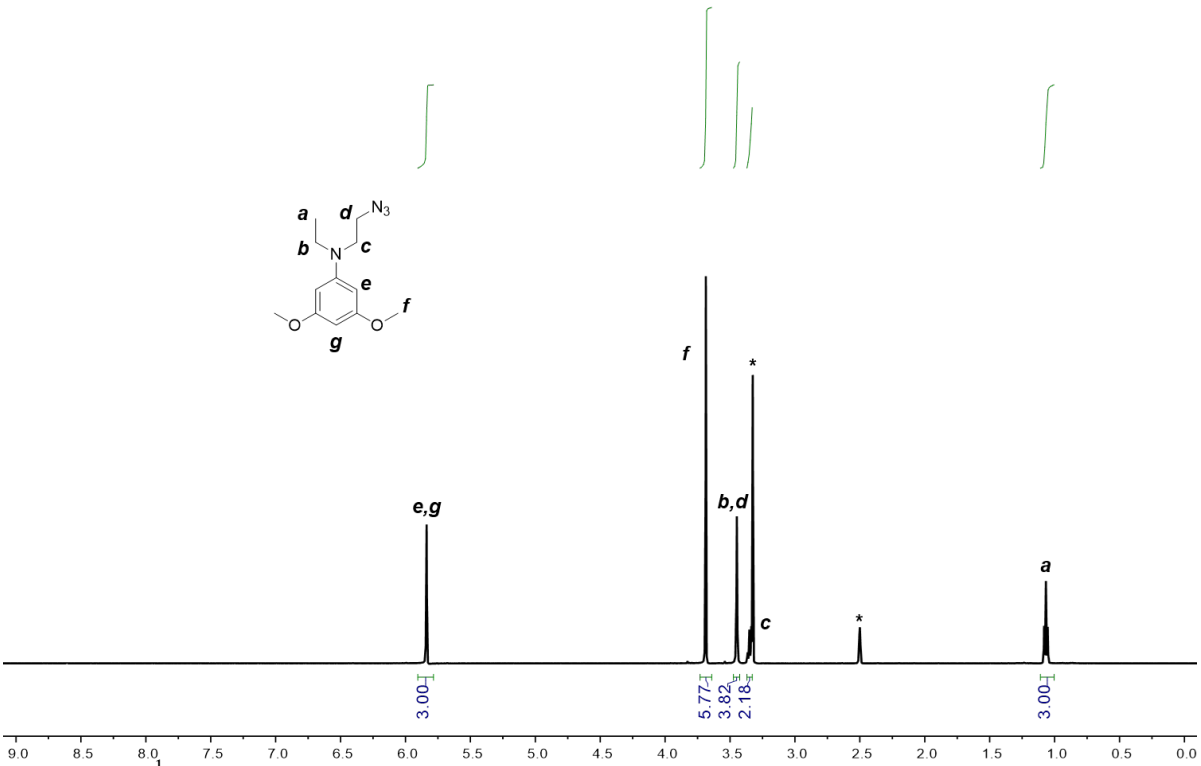


Figure S11. ^{1}H-NMR of **4** in deuterated DMSO. Residual solvent signals (water and DMSO) are shown with an asterisk.

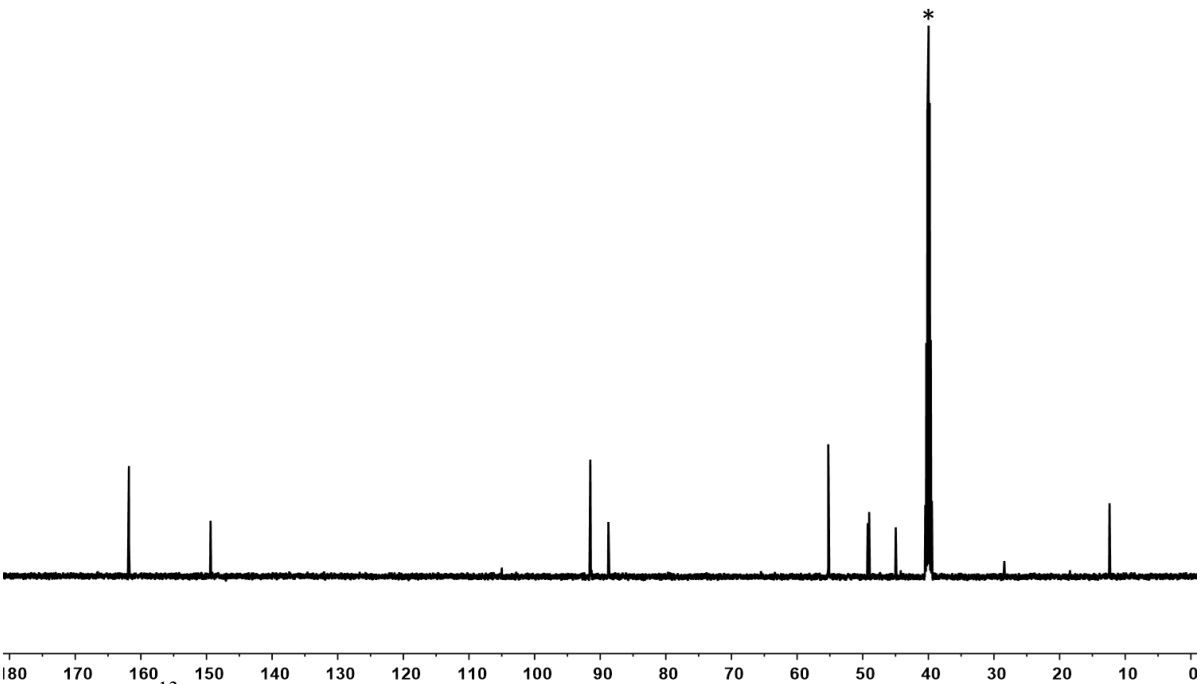


Figure S12. ^{13}C-NMR of **4** in deuterated DMSO. Residual solvent signal is shown with an asterisk.

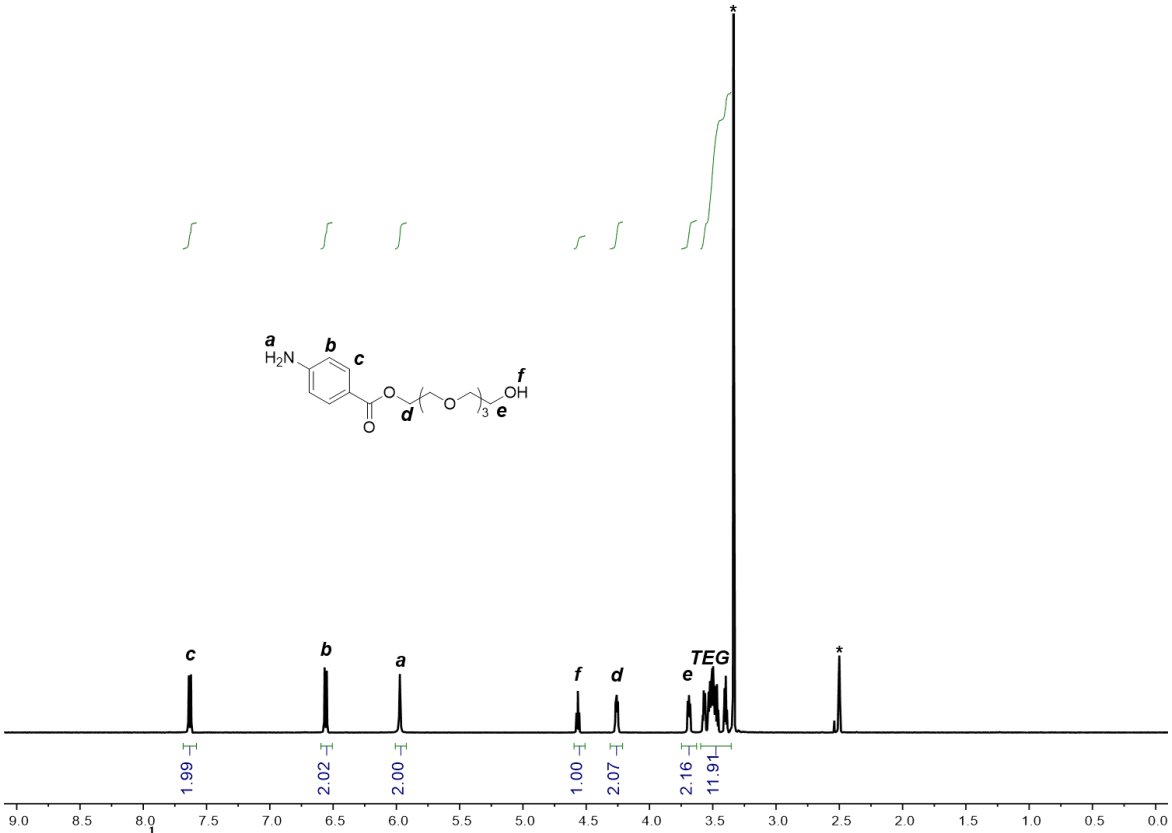


Figure S13. ^{1}H-NMR of **5** in deuterated DMSO (TEG = triethylene glycol unit). Residual solvent signals (water and DMSO) are shown with an asterisk.

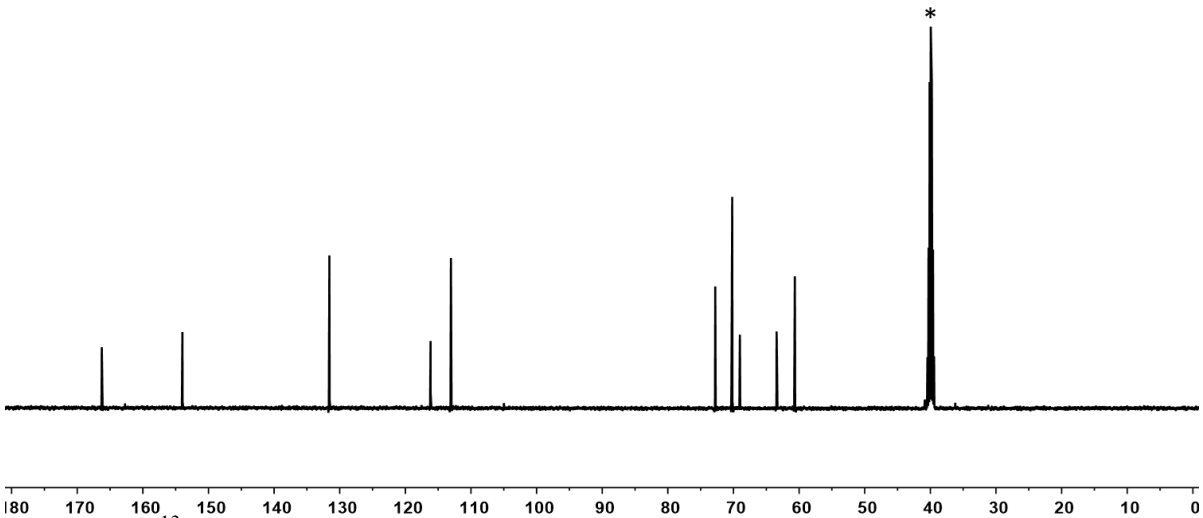


Figure S14. ^{13}C-NMR of **5** in deuterated DMSO. Residual solvent signal is shown with an asterisk.

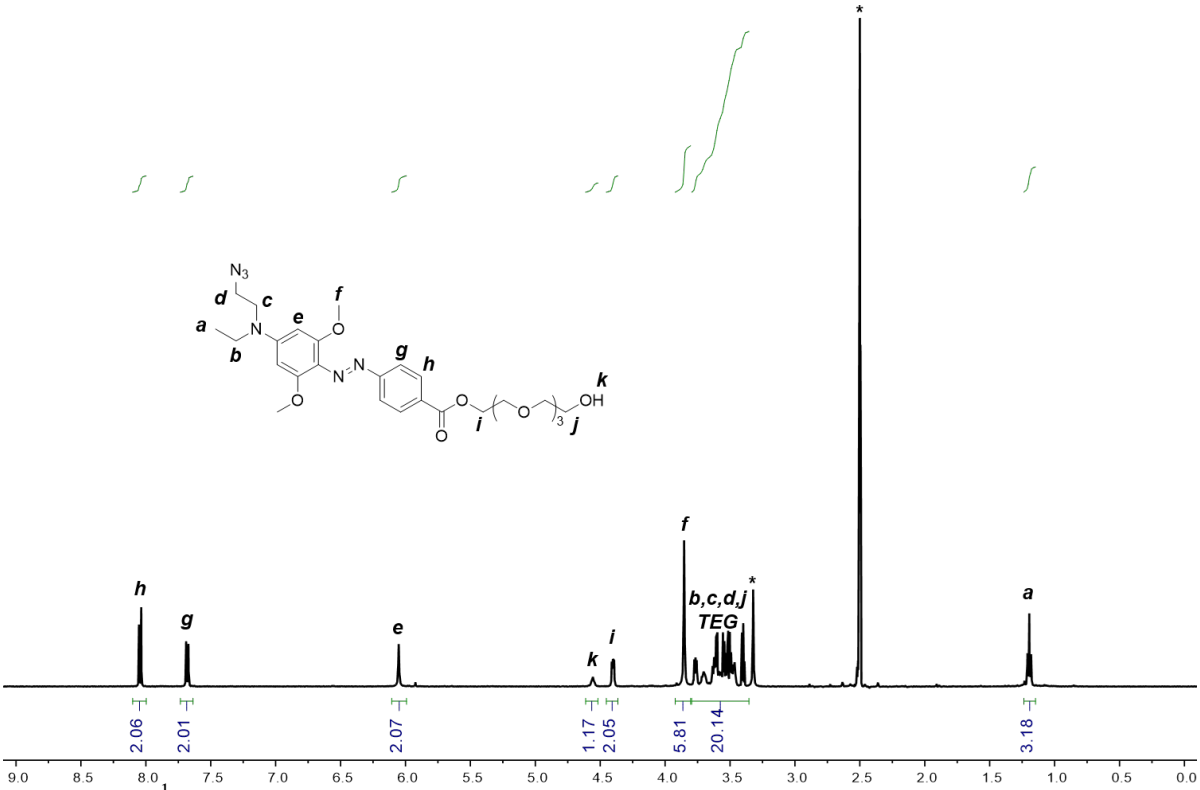


Figure S15. ^{1}H-NMR of **6** in deuterated DMSO (TEG = triethylene glycol unit). Residual solvent signals (water and DMSO) are shown with an asterisk.

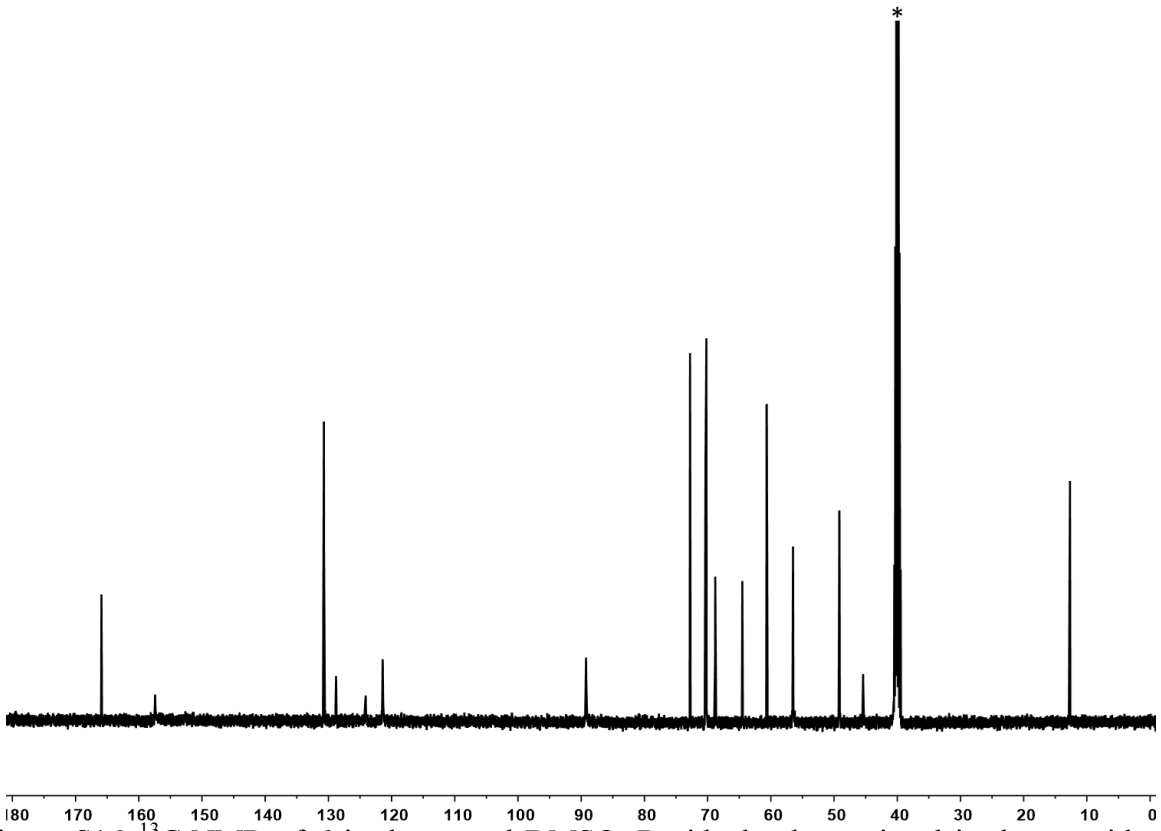


Figure S16. ^{13}C-NMR of **6** in deuterated DMSO. Residual solvent signal is shown with an asterisk.

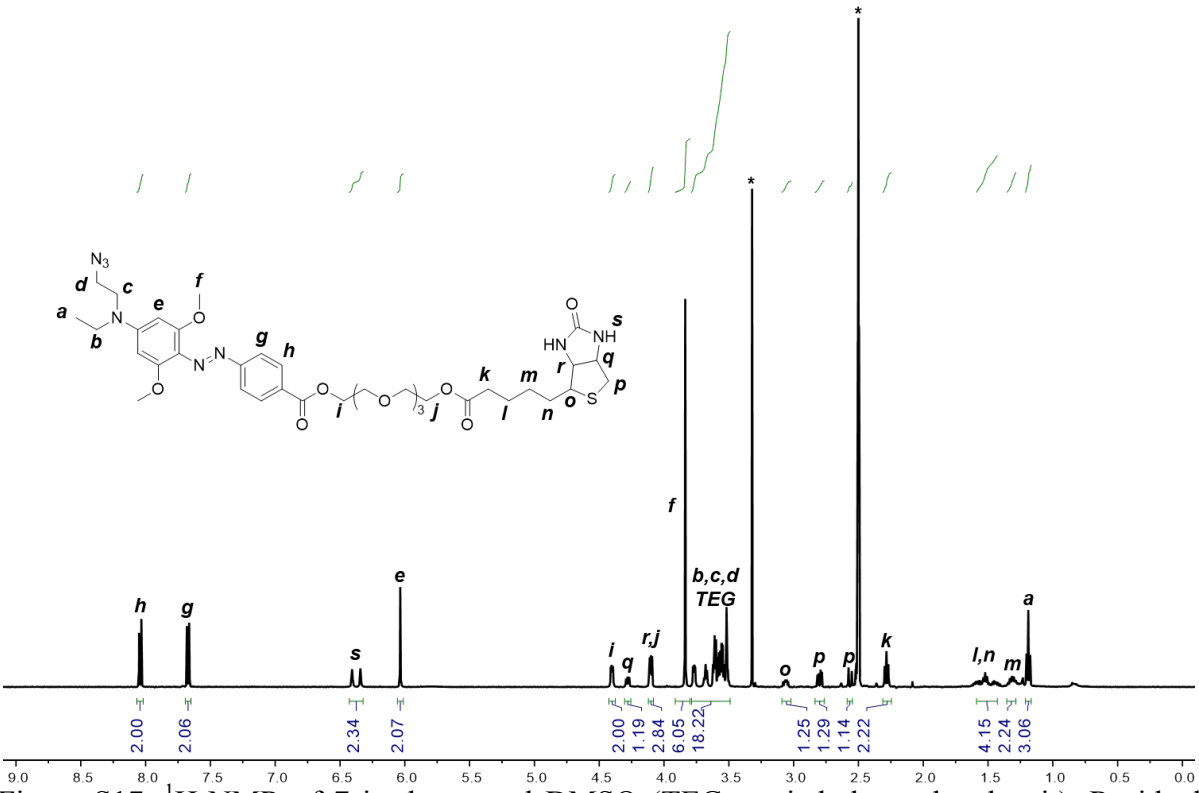


Figure S17. ^{1}H-NMR of **7** in deuterated DMSO (TEG = triethylene glycol unit). Residual solvent signals (water and DMSO) are shown with an asterisk.

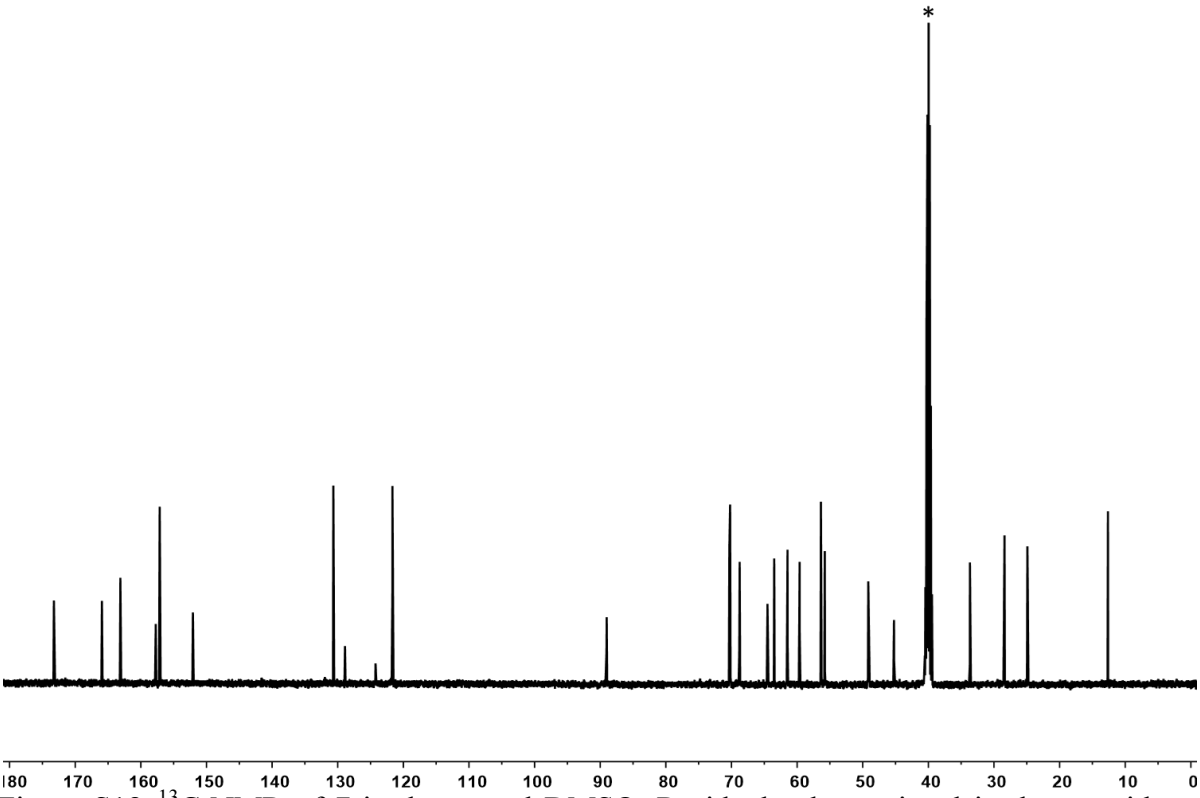


Figure S18. ^{13}C-NMR of **7** in deuterated DMSO. Residual solvent signal is shown with an asterisk.

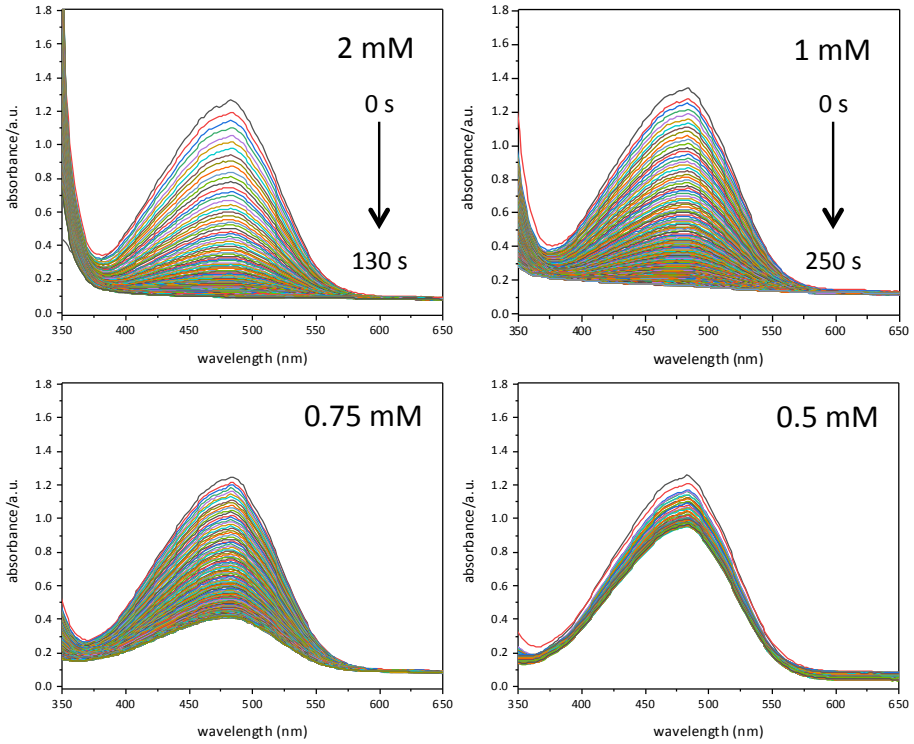


Figure S19. UV-Vis spectra of **1** upon exposure with varying sodium dithionite concentrations as indicated in each frame. The time difference between each trace is 1 second.

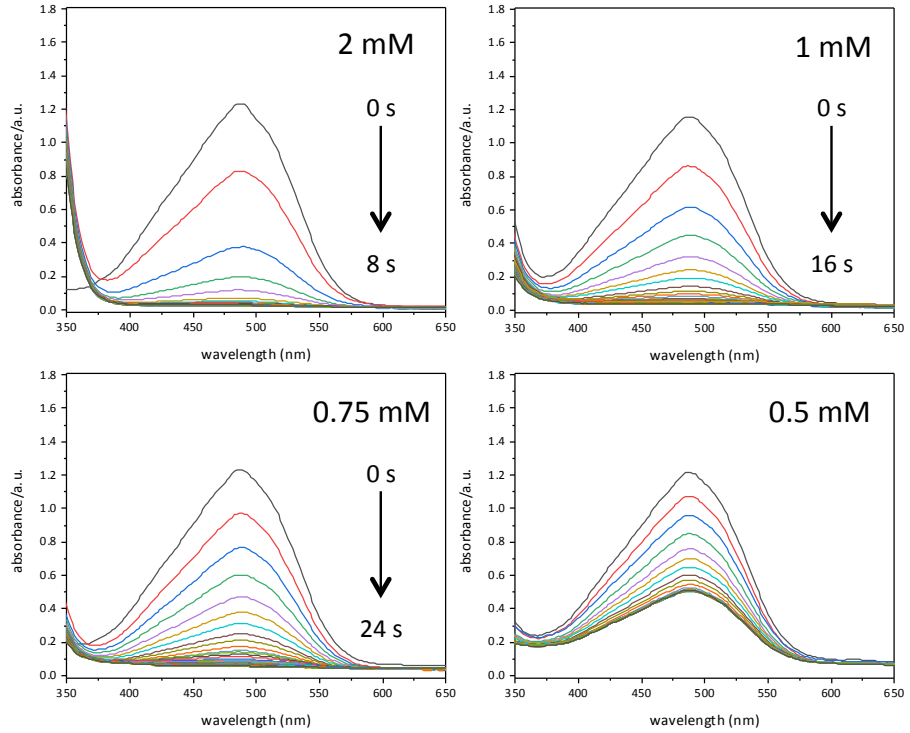


Figure S20. UV-Vis spectra of **2** upon exposure with varying sodium dithionite concentrations as indicated in each frame. The time difference between each trace is 1 second.

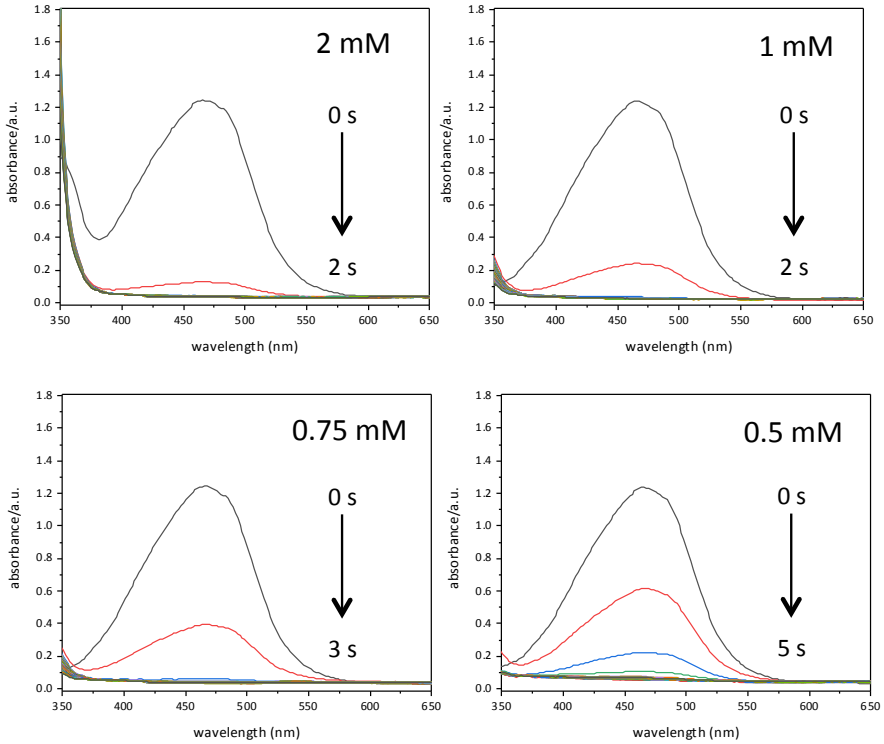


Figure S21. UV-Vis spectra of **3** upon exposure with varying sodium dithionite concentrations as indicated in each frame. The time difference between each trace is 1 second.

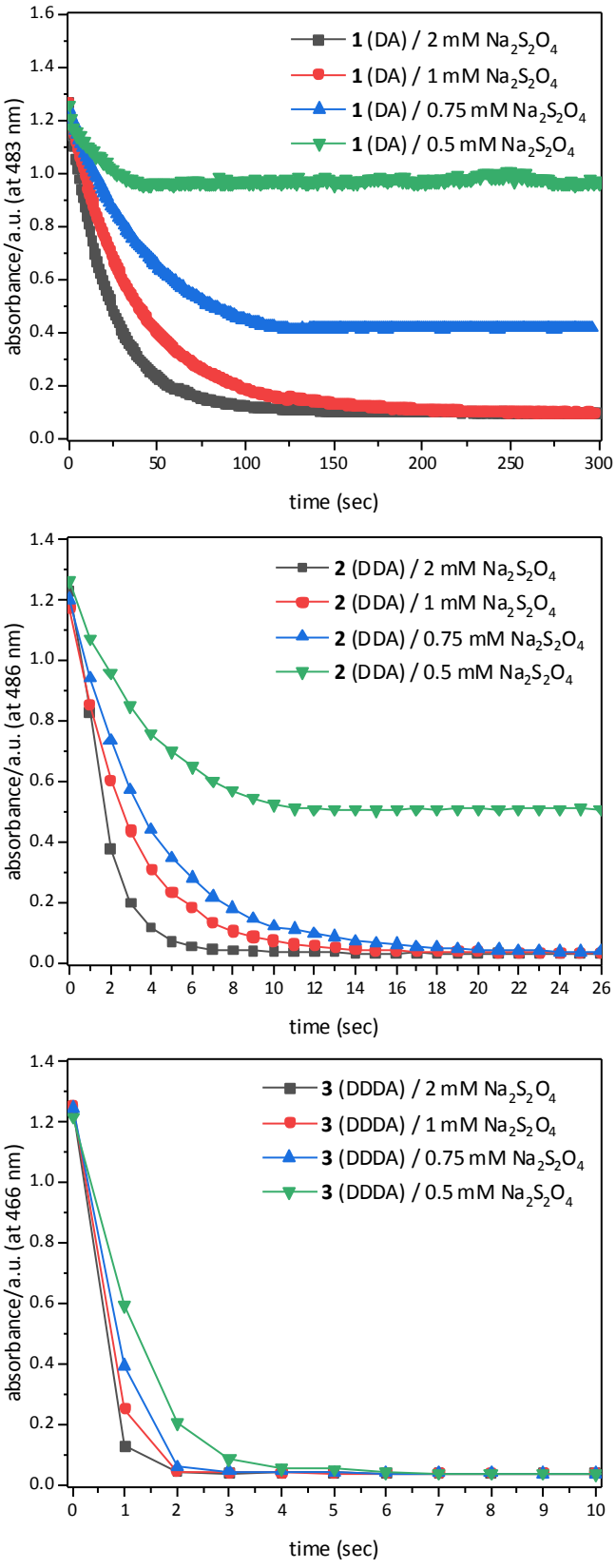


Figure S22. Absorption intensity as a function of time in **1**, **2**, and **3**.

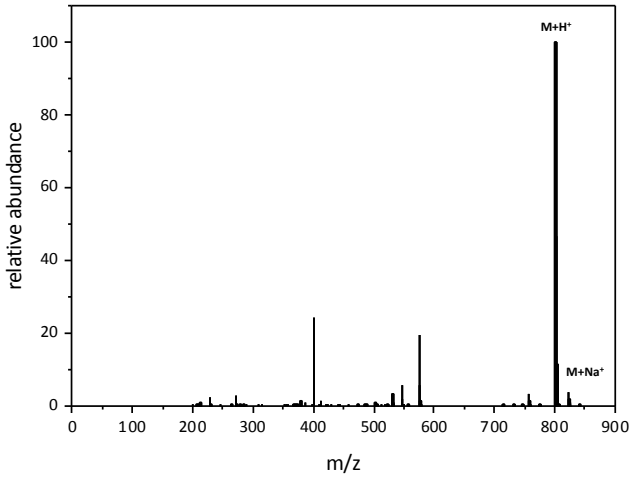


Figure S23. Mass spectrum of **7** in LC-MS analysis.

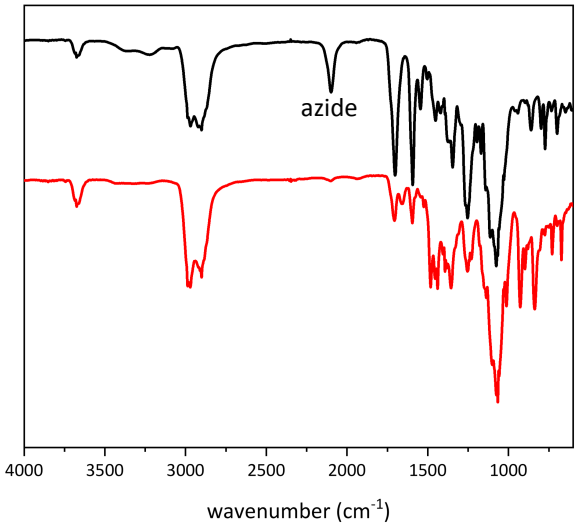


Figure S24. IR spectra of **7** (black) and **8** (red).

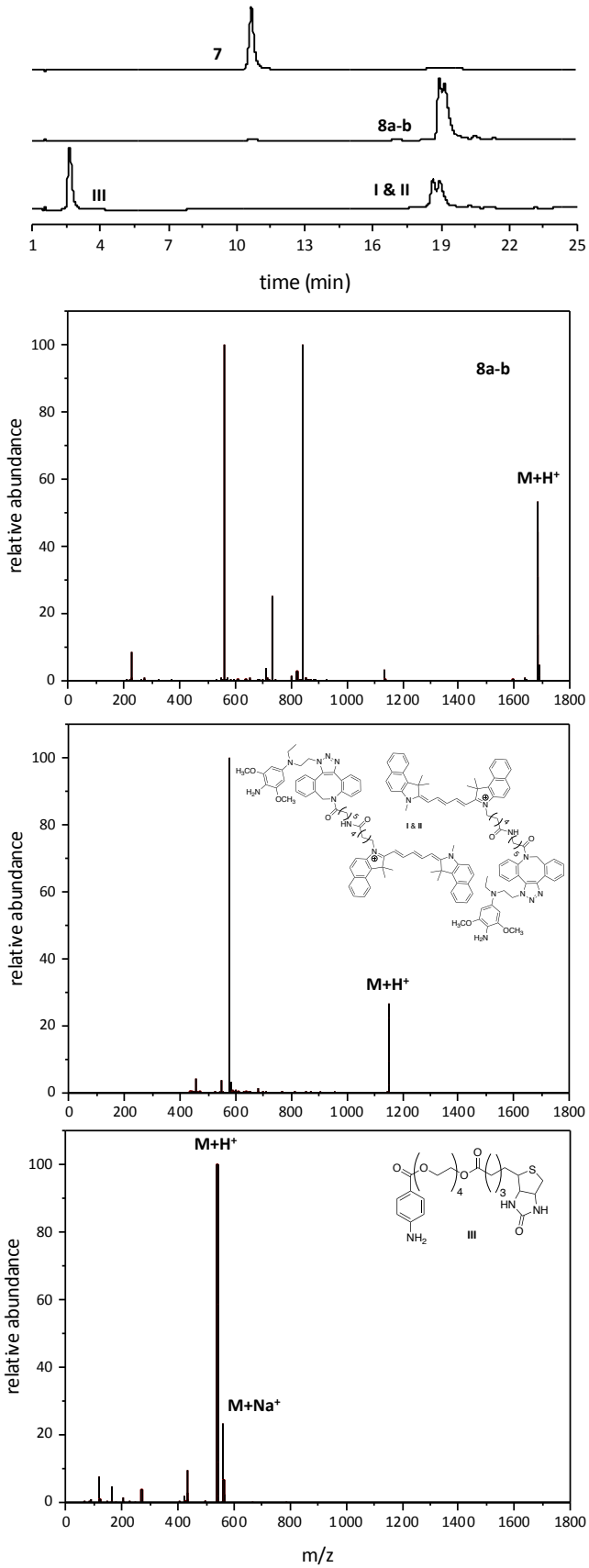


Figure S25. Liquid chromatography (top) and mass spectrometry (bottom) data for **8** before and after the azo cleavage.