Electronic Supplementary Information (ESI) for:

Time-Lapse Imaging of Cell Death in Cell Culture and Whole Living Organisms Using Turn-On Deep-Red Fluorescent Probes

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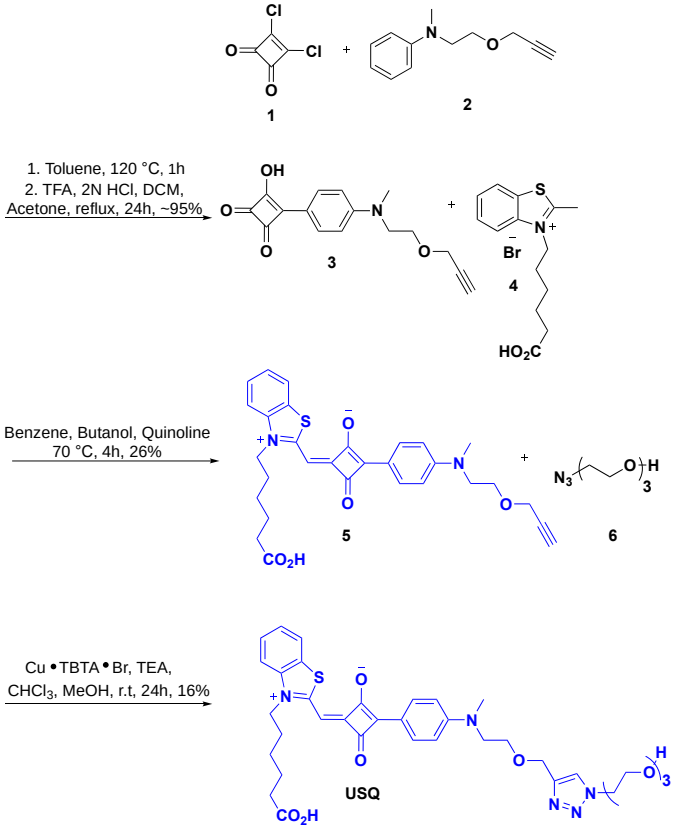
A. Synthesis and Characterization

B. Photophysical Studies

C. In Vitro Studies

D. Xenopus Studies

A. Synthesis and Characterization



Semisquaraine **3**. The known compounds **1** (615 mg, 4.1 mmol) and **2** (1.02 g, 4.5 mmol)[S1] were dissolved in 20 mL of toluene and heated to reflux for one hour. The solvent was removed under pressure, and the residue was redissolved in 2.0 mL 2N HCl, 3.0 mL dichloromethane, 8.0 mL acetone, and 2.0 mL trifluoroacetic acid (TFA) then refluxed for 24 hours. The hot solution was poured over ice, upon cooling to room temperature, the precipitate was vacuum filtered to yield **3** as a golden yellow solid (708 mg, 2.34 mmol, 60%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.84 (d, *J* = 10 Hz, 2H), 6.86 (d, *J* = 10 Hz, 2H), 4.15 (d, *J* = 3 Hz, 2H), 3.63 (s, 4H), 3.42 (t, *J* = 3 Hz, 1H), 3.01 (s, 3H), ^{13}C NMR (400 MHz, DMSO-d_6) δ 195.1, 174.1, 128.3, 112.3, 80.7, 77.7, 67.1, 58.15, 51.4

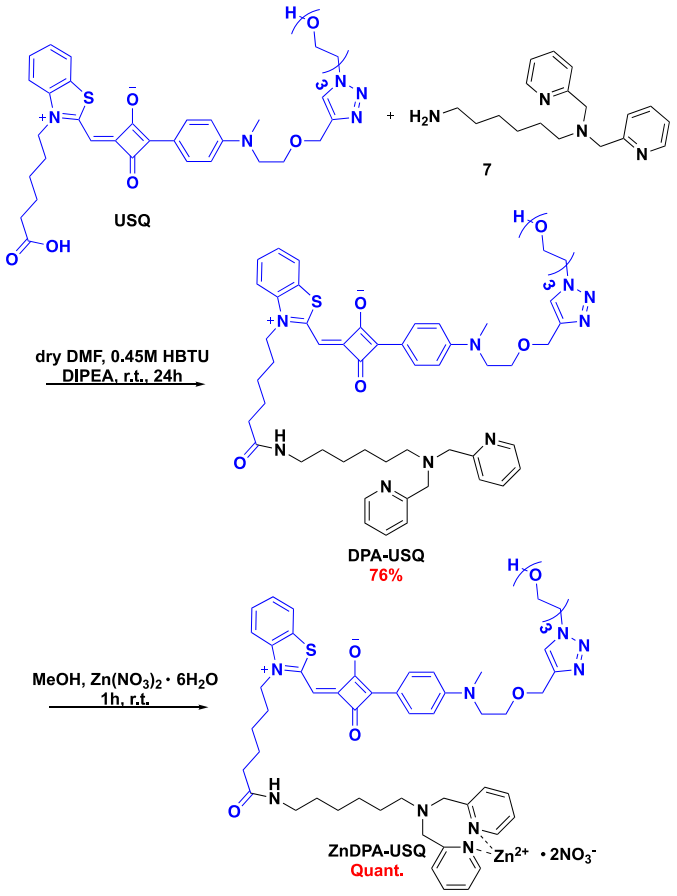
Benzothiazole **4***.* 2-Methylbenzothiazole (803 µL, 6.4 mmol) and 6-bromohexanoic acid were placed in a sealed pressure-tube and heated to reflux for 24 hours. The pink residue was

dissolved with a minimal amount of methanol then precipitated with excess ether. The precipitate was vacuum filtered then washed with ether to yield **4** as a light pink solid (1.63 g, 4.74 mmol, 74%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.31 (d, *J* = 8 Hz, 1H), 8.27 (d, *J* = 8 Hz, 1H), 7.92 (t, *J* = 8 Hz, 1H), 7.82 (t, *J* = 8 Hz, 1H), 4.77 (t, *J* = 8 Hz, 2H), 3.24 (s, *J* = Hz, 3H), 2.34 (t, *J* = 7 Hz, 2H), 2.00 (m, 2H), 1.71 (m, 2H), 1.57 (m, 2H); ^{13}C NMR NMR (400 MHz, CD_3OD) δ 176.7, 174.2, 141.2, 129.6, 129.3, 128.4, 124.0, 116.5, 49.3 33.0, 27.7, 25.6, 24.0, 15.7; HRMS (ESI-TOF) found m/z 264.1071, calculated $C_{14}H_{18}NO_2S$ $[M+H]^+$ 264.1053.

*Squaraine **5**.* Compound **3** (237 mg, 0.689 mmol) and compound **4** (197 mg, 0.689 mmol) were dissolved in 50 mL benzene, 50 mL butanol, and 1.0 mL quinolone and heated at 75 °C for 4 hours. The solvent was removed and excess hexane was added. The precipitate was allowed to settle, and the filtrate was decanted. The solid was washed with hexane until the filtrate was colorless. The solid was dissolved in chloroform and purified via column chromatography (0 – 35% methanol in chloroform) to yield **5** as dark blue solid (93.8 mg, 0.177 mmol, 26%). mp 106 – 110 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.03 (s, 1H), 8.17 (d, *J* = 8 Hz, 1H), 7.92 (d, *J* = 8 Hz, 1H), 7.88 (d, *J* = 9 Hz, 2H), 7.63 (t, *J* = 8 Hz, 1H), 7.50 (t, *J* = 8 Hz, 1H), 6.78 (d, *J* = 9 Hz, 2H), 6.32 (s, 1H), 4.56 (t, *J* = 7 Hz, 2H), 4.14 (s, 2H), 3.62 (s, 4H), 3.42 (s, 1H), 3.00 (s, 3H), 2.20 (t, *J* = 7 Hz, 2H), 1.76 (m, 2H), 1.54 (m, 2H), 1.42 (m, 2H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 186.7, 181.9, 180.7, 174.8, 166.1, 164.5, 150.5, 140.9, 129.4, 128.8, 128.3, 126.7, 124.0, 120.2, 115.4, 112.3, 90.7, 80.8, 77.7, 67.3, 58.2, 51.4, 47.5, 34.0, 28.1, 26.0, 24.6. HRMS (ESI-TOF) found m/z 531.1932, calculated $C_{30}H_{30}N_2O_5S$ $[M+H]^+$ 531.1948.

USQ. Compound **5** (48.2 mg, 0.091 mmol), **6** (43.3 mg, 0.247 mmol), and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine copper(I)bromide (12.3 mg, 0.0123 mmol) were dissolved in 4 mL chloroform and 1.0 mL methanol, along with triethylamine (41 µL, 0.273 mmol), and shaken at room temperature for 24 hours. The solvent was evaporated under reduced pressure. The

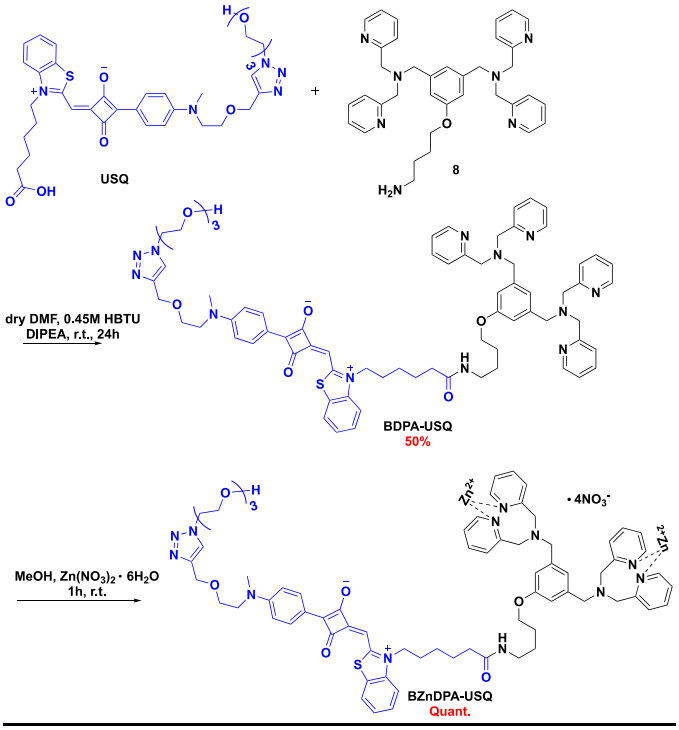
residue was dissolved in water and purified via reverse phase column chromatography (0 – 50% acetonitrile in water) to yield **USQ** as a purple film (10.2 mg, 14.5 µmol, 16%). mp > 260 °C ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.15 (d, *J* = 8.0 Hz, 1H), 8.00 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 9 Hz, 2H), 7.64 (t, *J* = 8 Hz, 1H), 7.50 (t, *J* = 8 Hz, 1H), 6.76 (d, *J* = 9 Hz, 2H), 6.32 (s, 1H), 4.56 (t, *J* = 7 Hz, 2H), 4.52 (s, 2H), 4.49 (t, *J* = 5 Hz, 2H), 3.77 (t, *J* = 5 Hz, 2H), 3.60 – 3.35 (m, 12H), 3.00 (s, 3H), 2.19 (t, *J* = 7 Hz, 2H), 1.76 (m, 2H), 1.54 (m, 2H), 1.42 (m, 2H). ^{13}C NMR (400 MHz, DMSO-d_6) δ 186.7, 181.9, 180.7, 174.8, 166.1, 164.5, 150.5, 140.9, 129.4, 128.8, 128.3, 126.74, 124.7, 124.0, 120.2, 115.4, 112.3, 90.7, 72.8, 70.1, 70.0, 69.1, 67.4, 64.1, 60.7, 51.5, 49.8, 47.4, 46.1, 34.0, 28.1, 26.0, 24.6, 11.4. HRMS (ESI-TOF) found m/z 706.2921, calculated $C_{36}H_{43}N_5O_8S$ $[M+H]^+$ 706.2905.



DPA-USQ. Compounds **USQ** (54.1 mg, 76.6 µmol) and **7** (58.3 mg, 0.194 mmol) were dissolved in 900 µL of dimethylformamide then chilled to 0 °C. 1.5 molar equivalence of 0.45 M HBTU

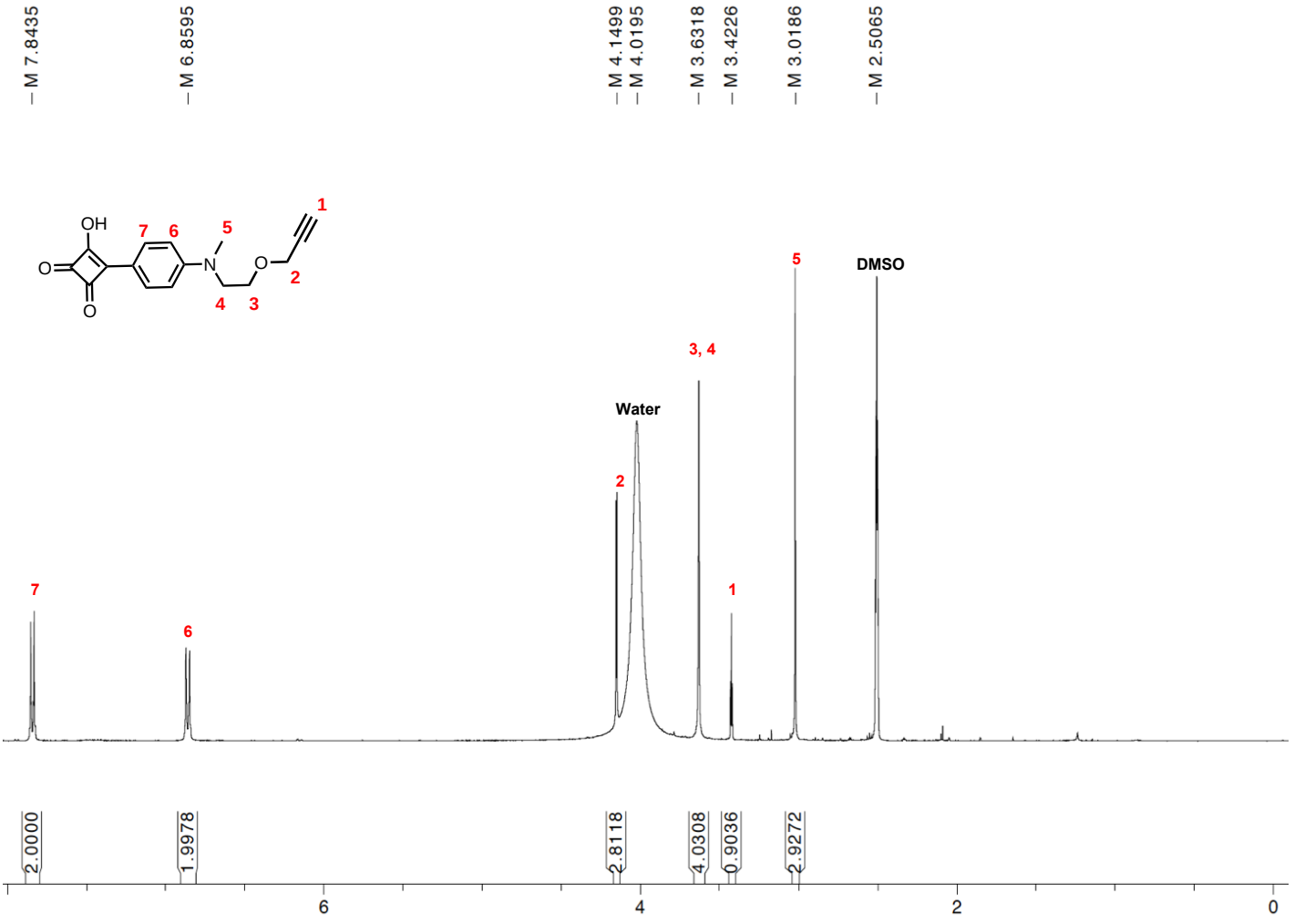
solution in dimethylformamide was added to the reaction mixture followed by N,N-diisopropylethylamine (67 µL, 0.383 mmol), all under argon gas. The reaction mixture was allowed to reach room temperature gradually overnight. The solvent was removed under reduced pressure, and the crude product was purified via reverse column chromatography (0 – 100% acetonitrile in water with 0.1% TFA). 1M HCl (22 uL) was added to the collected fractions to convert the TFA salt. The solvent was reduced to ~1 mL then diluted with 50% acetonitrile in water and was repeated twice. After the final reduction, the 1 mL was transferred to a tarred vial and lyophilized to yield **DPA-USQ** (70.3 mg, 71.3 µmol, 93%) as a purple film. ^{1}H NMR (500 MHz, 1:1 – CD_3CN:D_2O) δ 9.08 (s, 2H), 8.56 (m, 2H), 8.36 (bs, 2H), 8.10 (m, 8H), 8.00 (bs 1H), 7.92 (bs, 1H), 7.15 (bs, 2H), 6.80 (s, 1H), 5.05 (s, 2H), 4.99 (bs, 2H), 4.82 (m, 6H), 4.31 (bs, 2H), 4.07 – 3.94 (m, 12H), 3.49 (bs, 3H), 3.41 (bs, 4H), 2.63 (bs, 2H), 2.29 (bs, 2H), 2.06 (m, 4H), 1.87 (bs, 2H), 1.79 (bs, 2H), 1.64 (bs, 4H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 186.6, 181.9, 180.7, 174.5, 166.1, 164.5, 150.5, 144.3, 140.9, 129.4, 128.8, 128.3, 126.7, 124.7, 124.0, 120.1, 115.4, 112.3, 112.1, 90.7, 72,8, 70.1, 70.0, 69.2, 67.4, 66.0, 64.1, 60.7, 51.5, 49.8, 47.4, 46.2, 33.9, 28.1, 26.0, 24.6 HRMS (ESI-TOF) found 986.4959, calculated m/z $C_{54}H_{68}N_9O_7S$ $[M+H]^+$ 986.4959.

ZnDPA-USQ. Compound **DPA-USQ** (0.51 mg, 0.52 µmol) was dissolved in 256 µL of methanol to make a 2.0 mM solution. Two molar equivalents of a 200 mM zinc nitrate solution was added and the solution shaken at room temperature for 1 hour. The solvent was removed to yield **ZnDPA-USQ** (0.618 mg, 0.52 µmol, 100%).

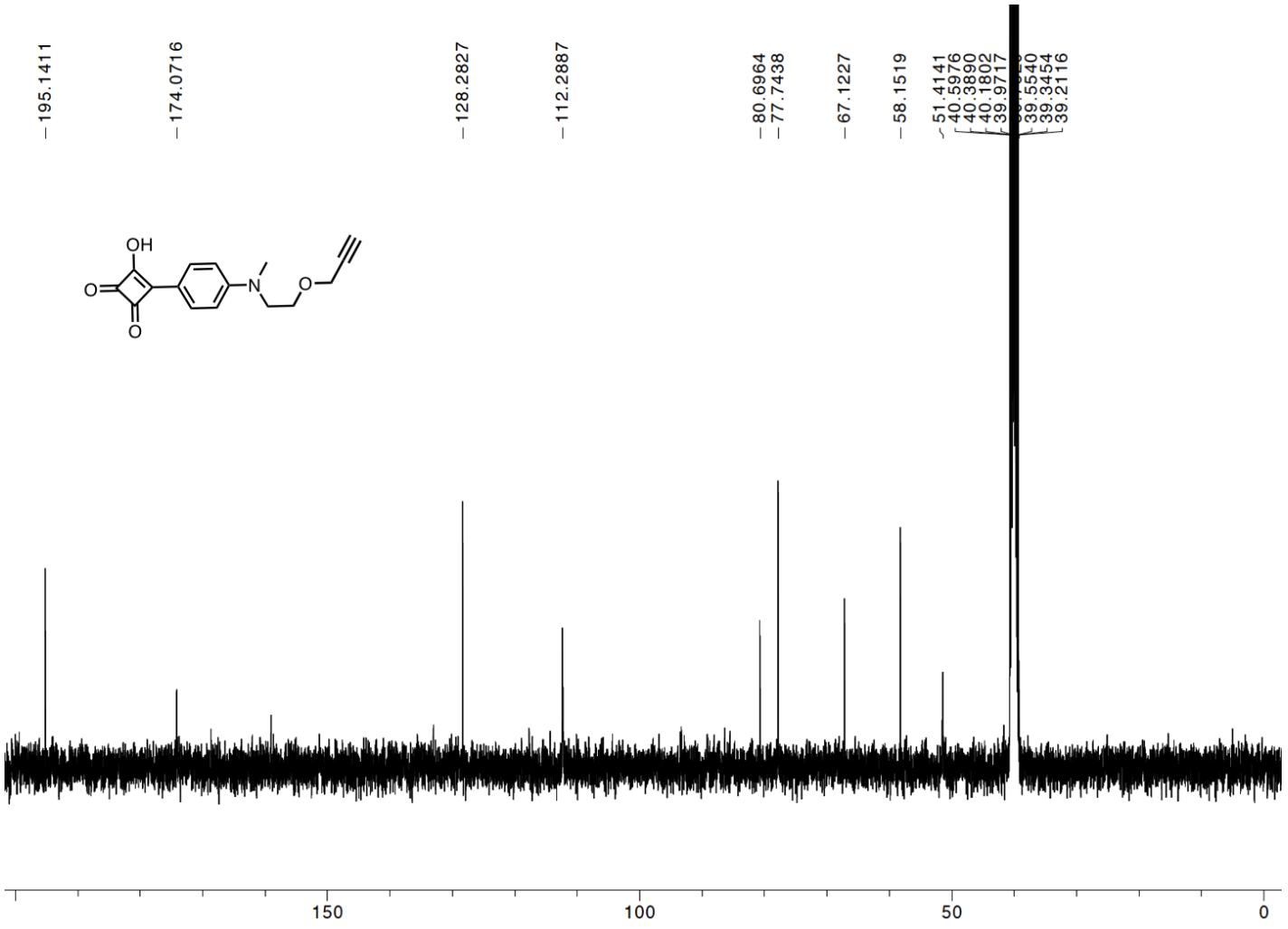


BDPA-USQ. Using the same procedure for **DPA-SQ**, the dye **USQ** was coupled with the known compound **8**.[S2] ^{1}H NMR (500 MHz, 1:1 – CD_3CN:D_2O) δ 8.59 (s, 4H), 8.20 (t, *J* = 8 Hz, 4H), 7.97 (d, *J* = 8 Hz, 1H), 7.86 (s, 1H), 7.81 (d, *J* = 8 Hz, 1H), 7.71 (m, 11H), 7.56 (t, 1H), 6.83 (d, *J* = 8 Hz, 2H), 6.75 (s, 1H), 6.67 (bs, 1H), 6.62 (s, 2H), 6.43 (s, 1H), 4.59 (s, 2H), 4.49 (m, 4H), 4.23 (m, 10H), 4.17 (s, 4H), 3.84 (m, 2H), 3.72 (s, 3H), 3.80 – 3.46 (m, 12H), 3.17 (t, *J* = 7 Hz, 2H), 2.20 (t, *J* = 7 Hz, 2H), 1.89 (m, 2H), 1.66 (m, 4H), 1.56 (m, 2H), 1.46 (m, 2H); ^{13}C NMR (400 MHz, 1:1 – CH_3CN:D_2O) δ 185.2, 183.0, 175.7, 175.7, 162.0, 161.7, 159.1, 153.4, 152.7, 146.8, 145.7, 143.6, 142.2, 138.0, 129.6, 128.1, 127.3, 127.0, 126.4, 125.6, 124.3, 124.0, 119.3, 115.9, 72.3, 70.3, 70.0, 69.2, 68.2, 63.6, 60.9, 59.8, 56.8, 56.2, 54.8, 50.5, 39.2, 38.9, 36.0, 28.3, 26.6, 26.4, 26.1, 25.9, 25.6, 25.5, 25.9, 25.6, 25.5, 22.9. HRMS (ESI-TOF) found m/z 1275.6205, calculated $C_{72}H_{82}N_{12}O_8S$ $[M+H]^+$ 1275.6172.

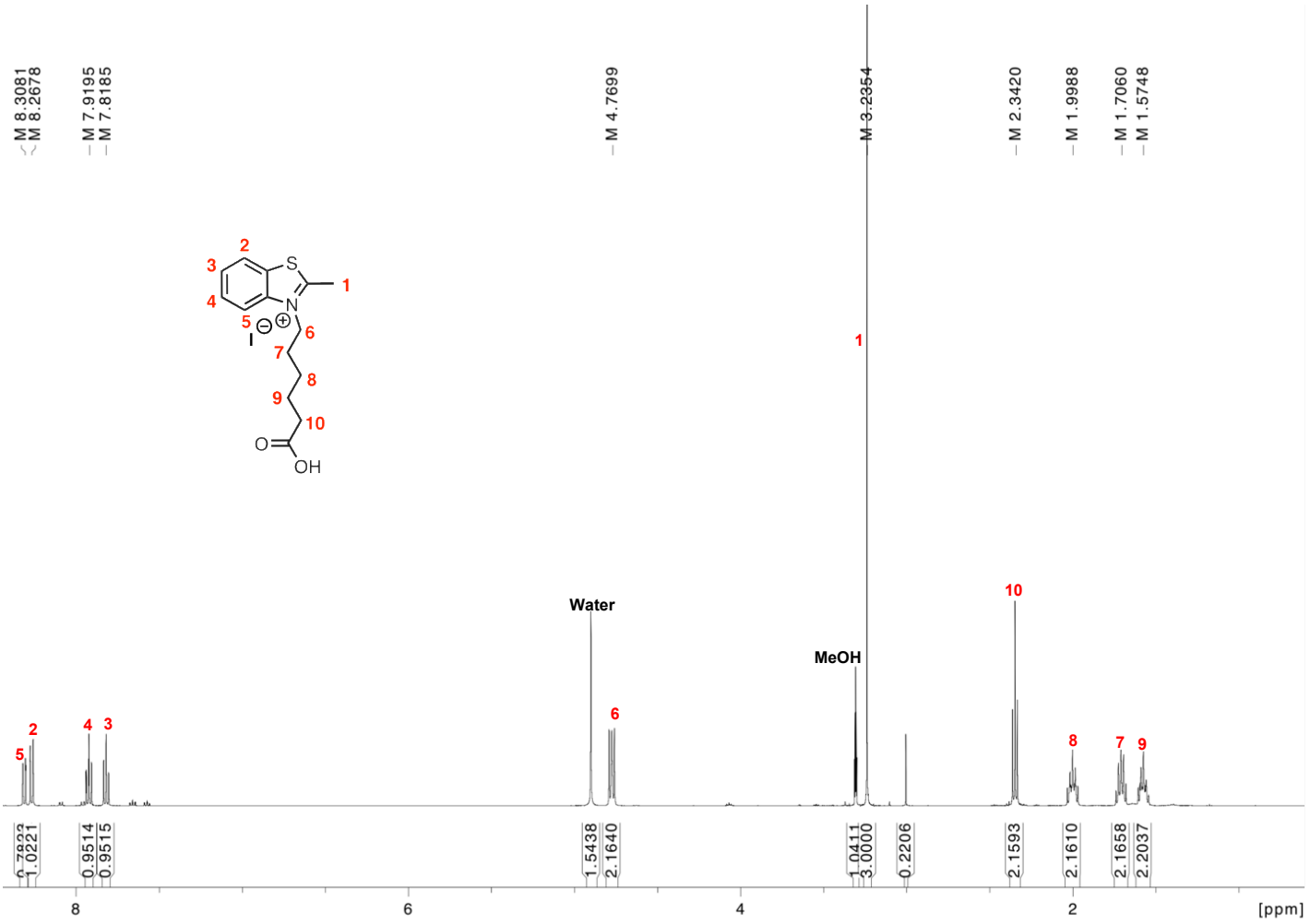
BZnDPA-USQ. The zinc chelation procedure was the same used to make ***ZnDPA-USQ.***



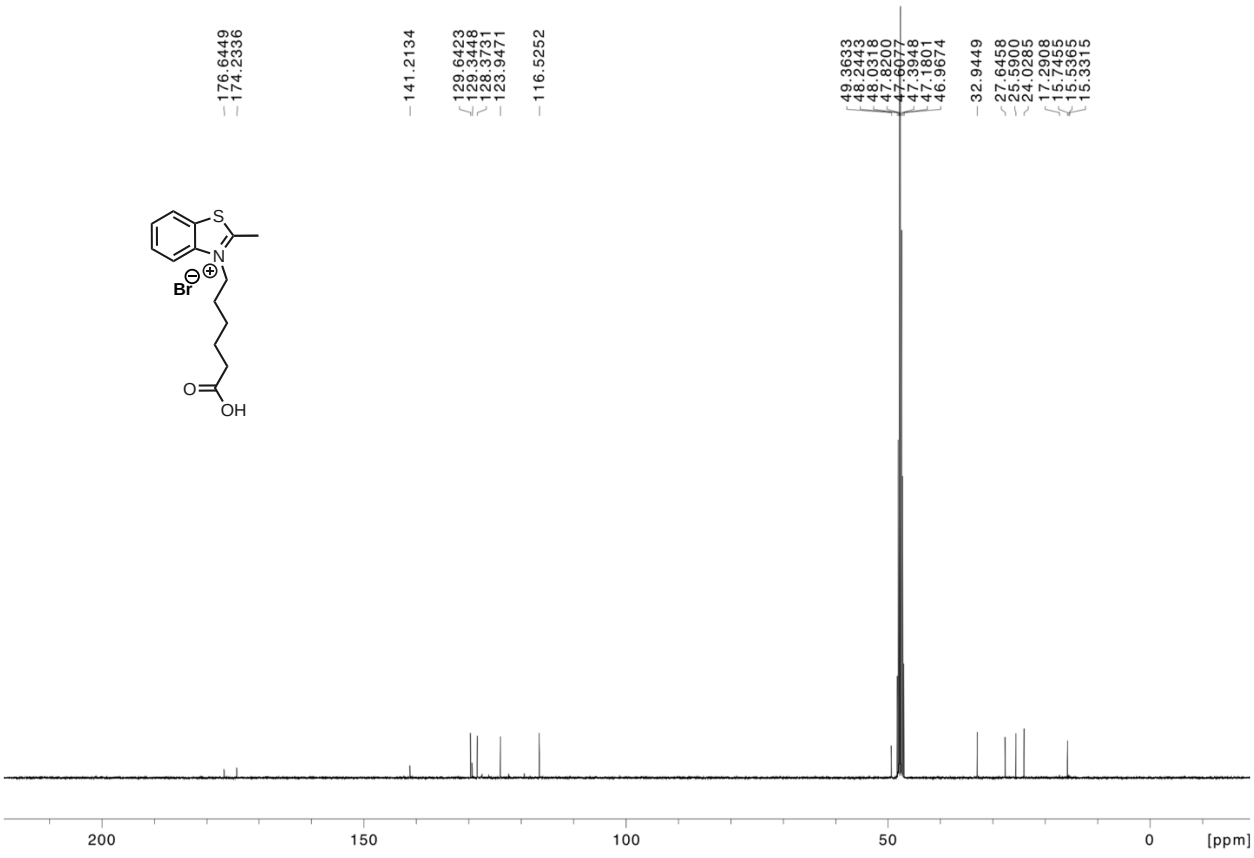
^{1}H NMR spectrum (500 MHz, DMSO-d_6) of **3**.



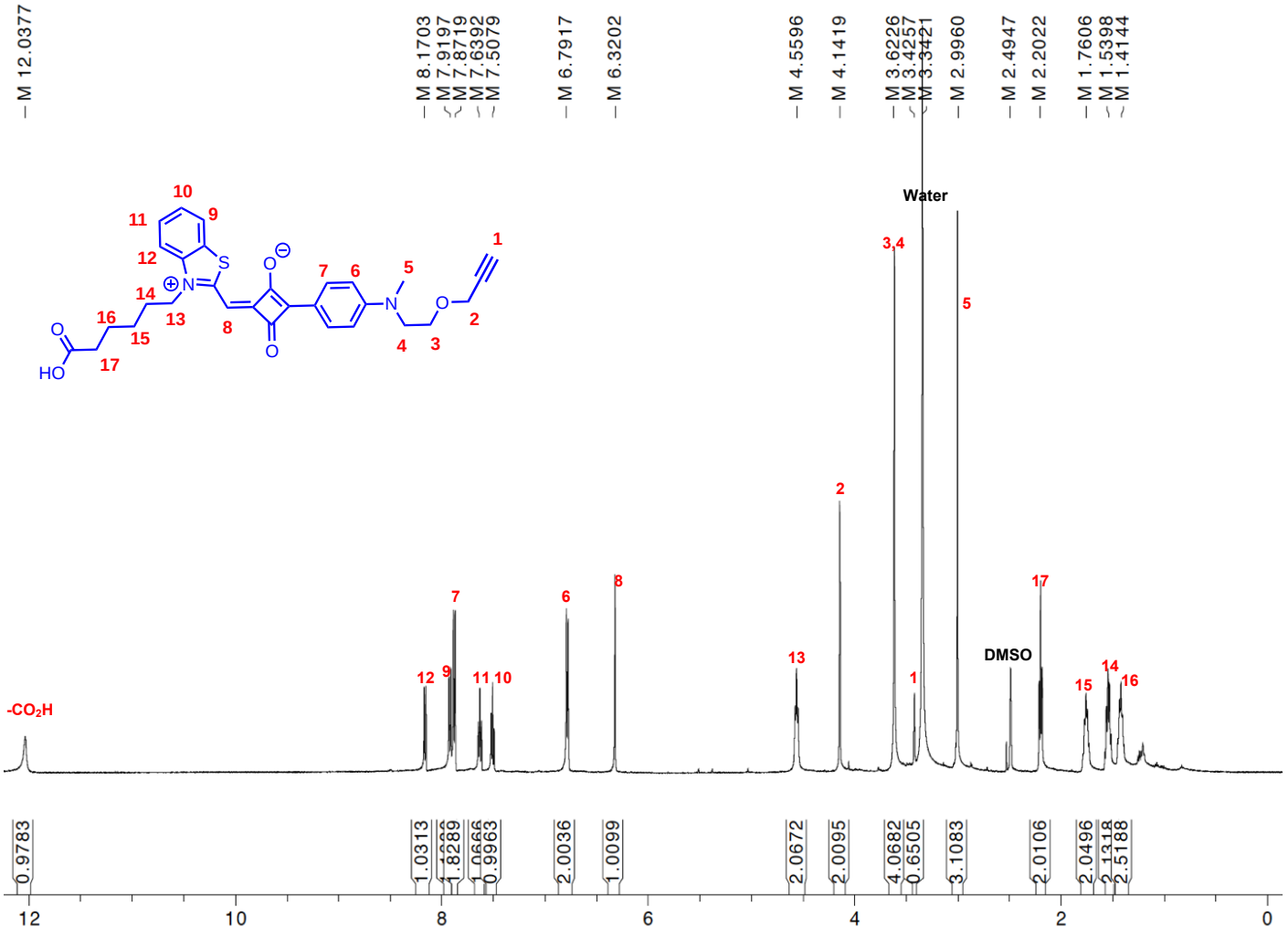
^{13}C NMR spectrum (400 MHz, DMSO-d_6) of **3**.



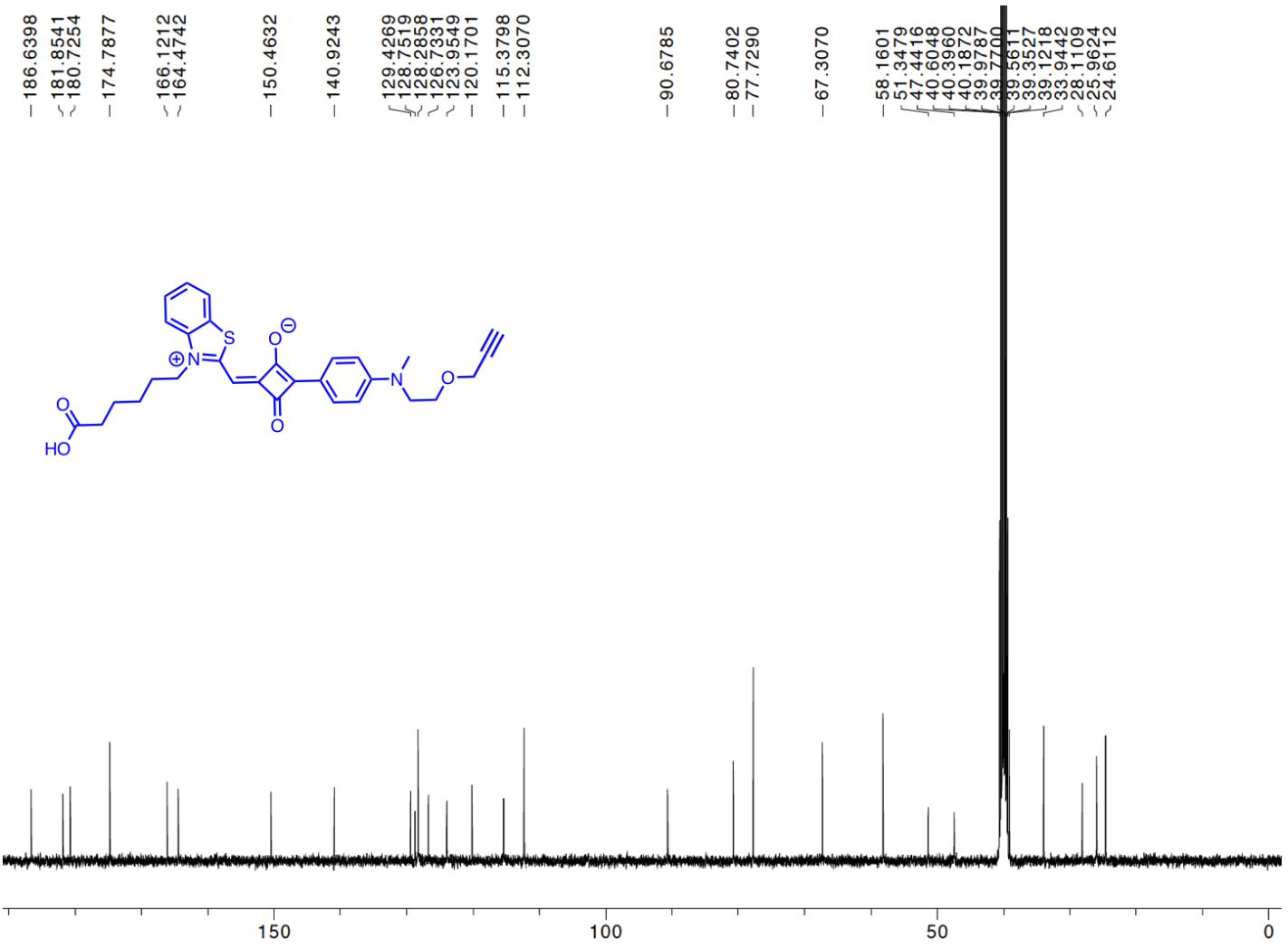
^{1}H NMR spectrum (500 MHz, CD_3OD) of **4**.



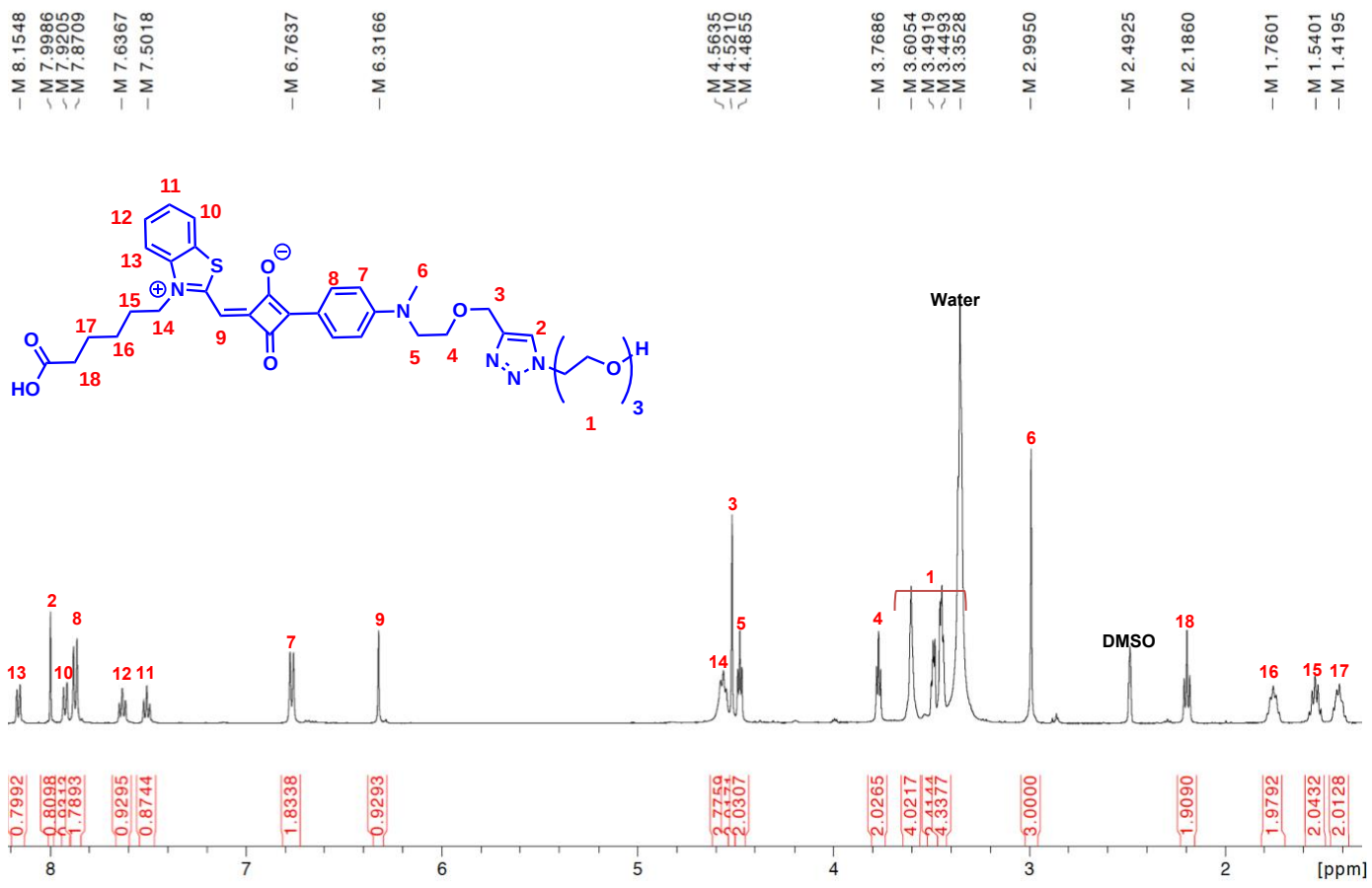
^{13}C NMR spectrum (400 MHz, CD_3OD) of **4**.



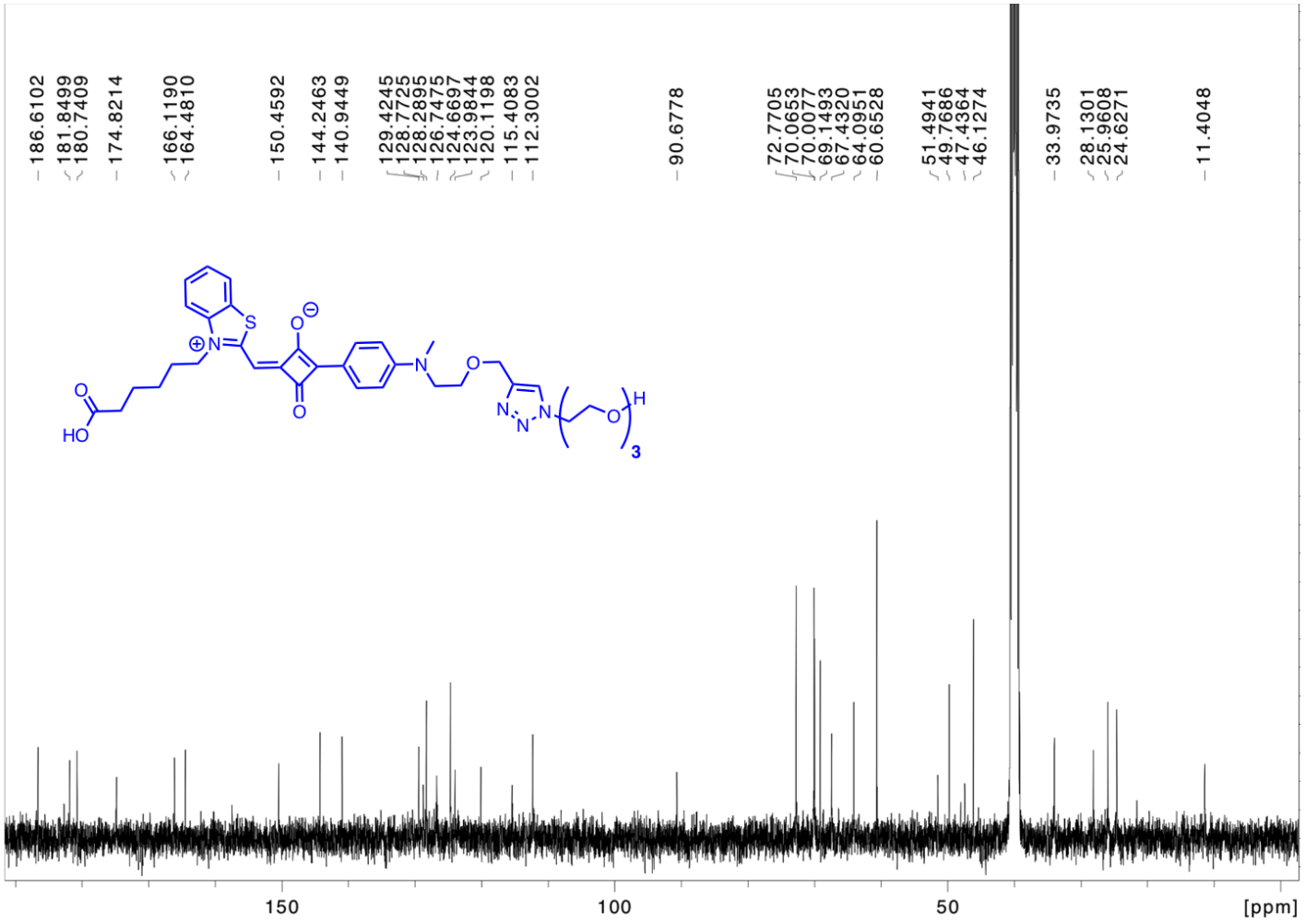
^{1}H NMR spectrum (400 MHz, DMSO-d_6) of **5**.



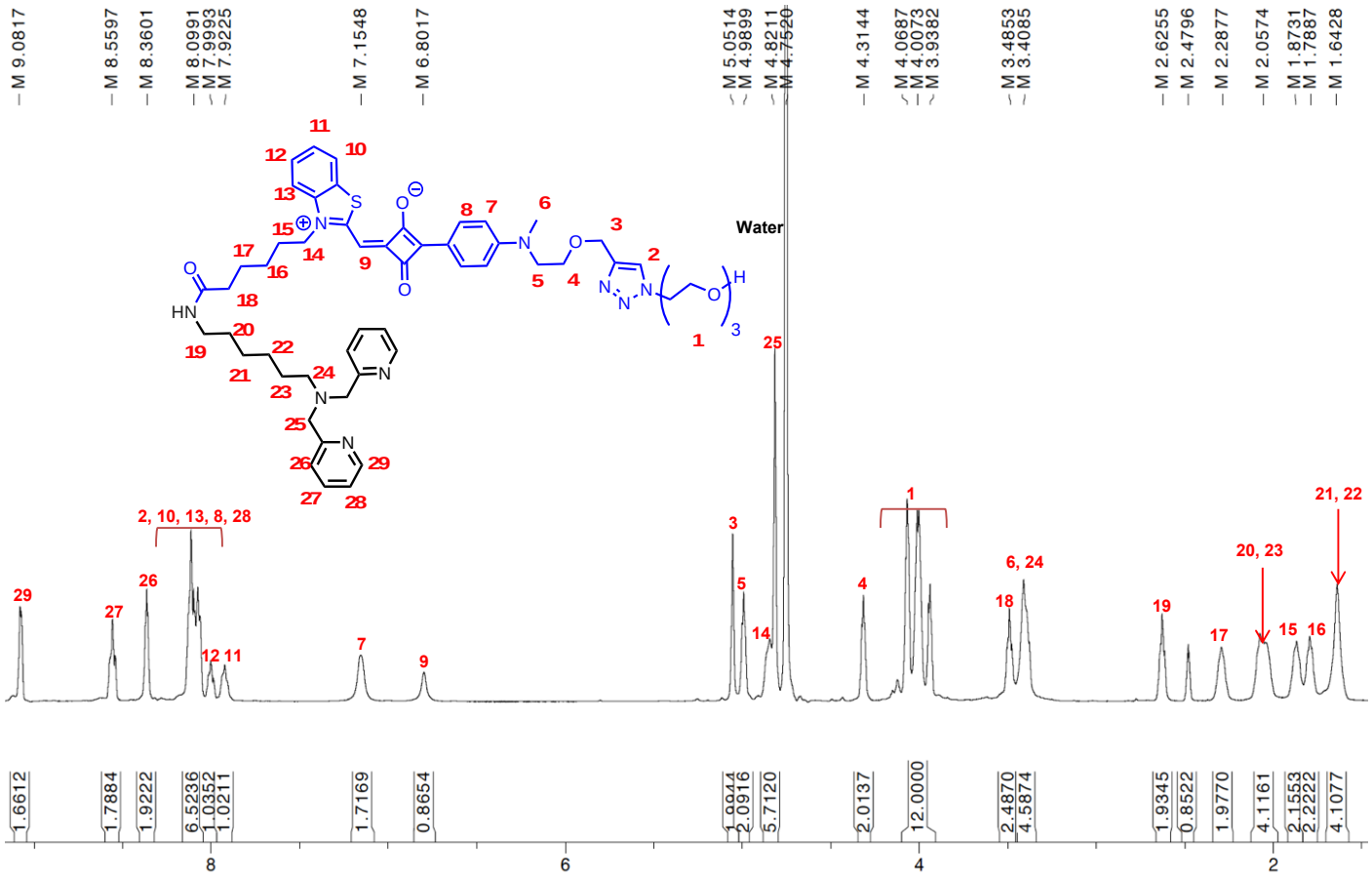
^{13}C NMR spectrum (400 MHz, DMSO-d_6) of **5**.



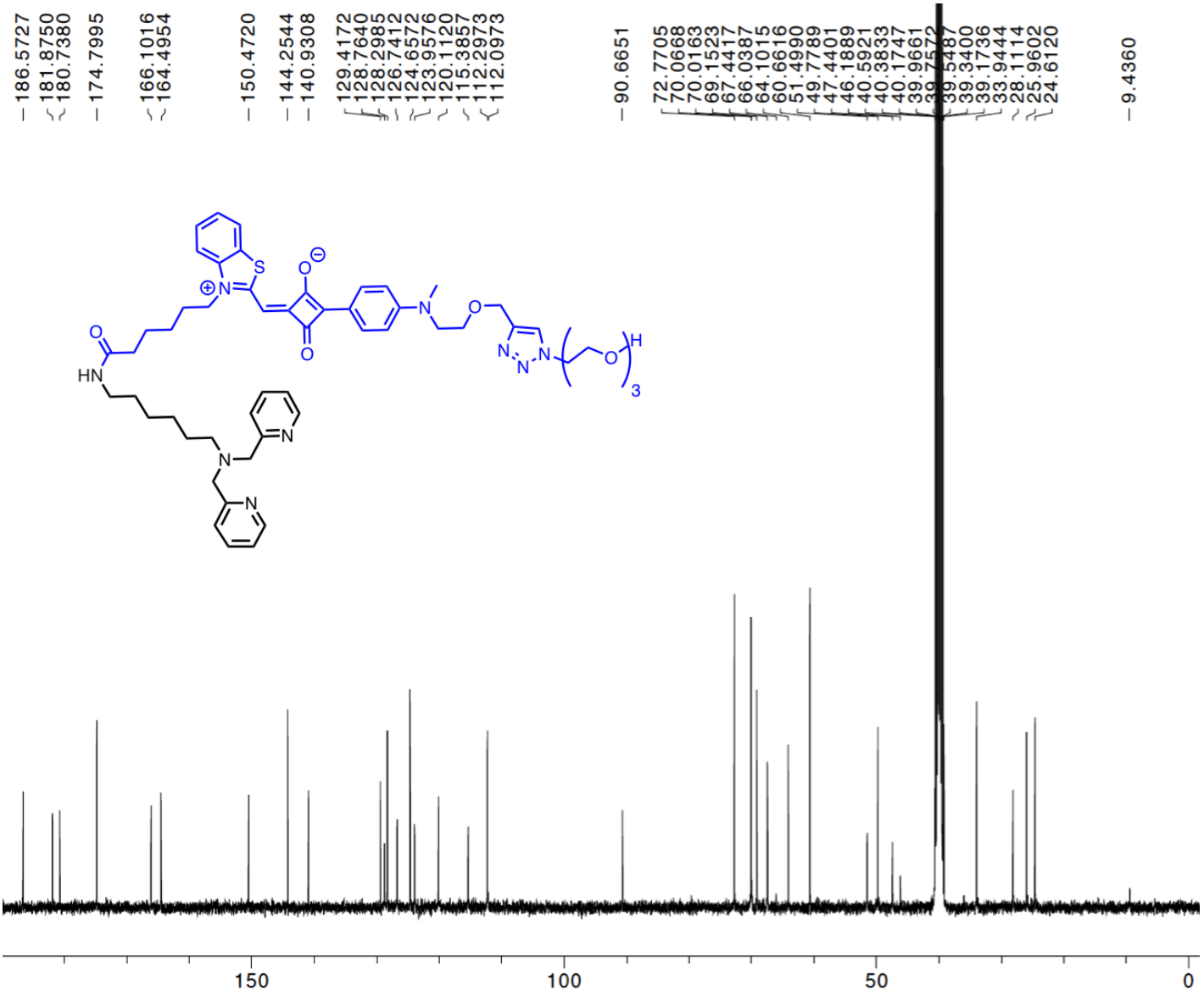
^{1}H NMR spectrum (500 MHz, DMSO-d_6) of **USQ**.



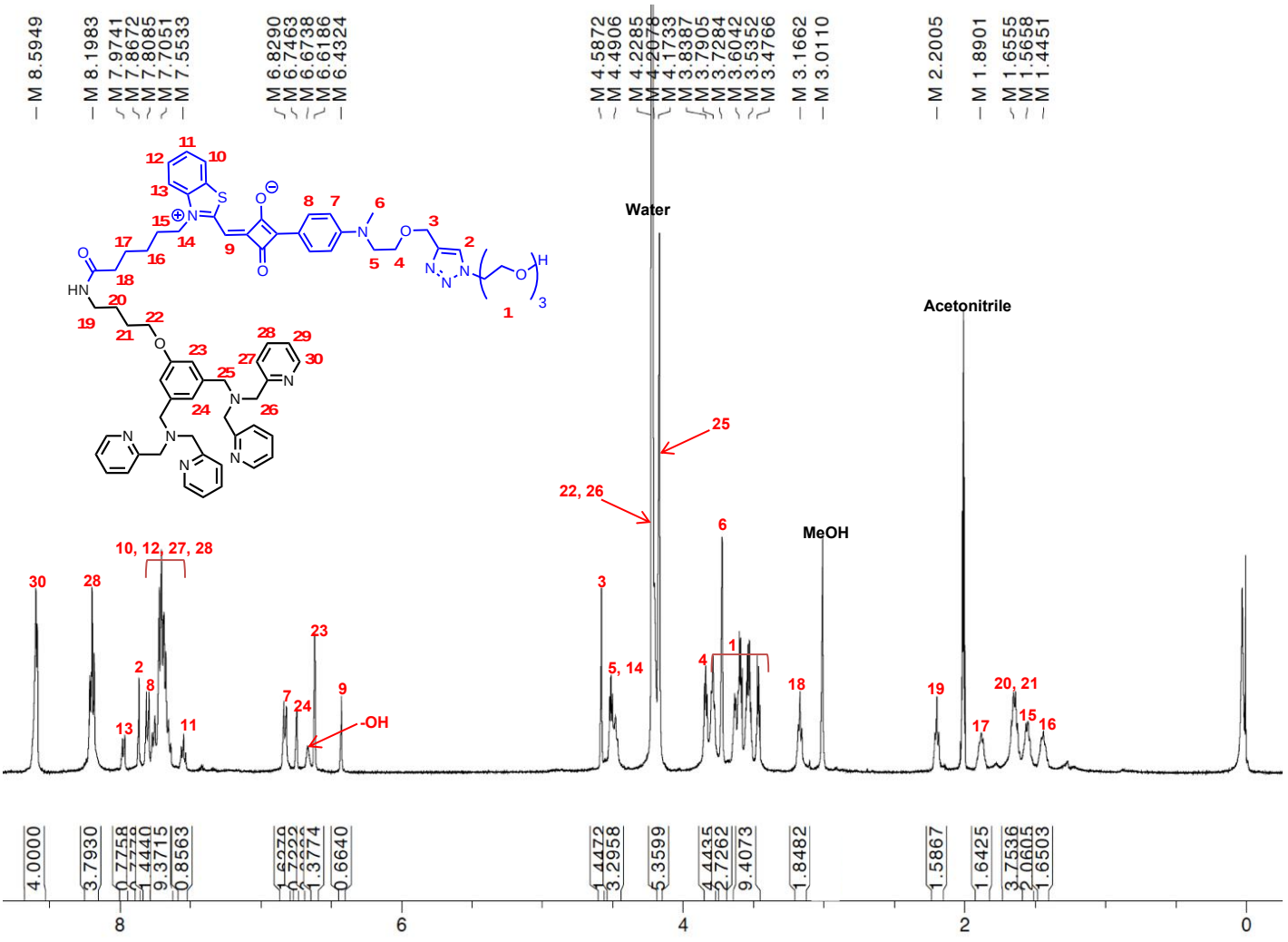
^{13}C NMR spectrum (400 MHz, DMSO-d_6) of **USQ**.



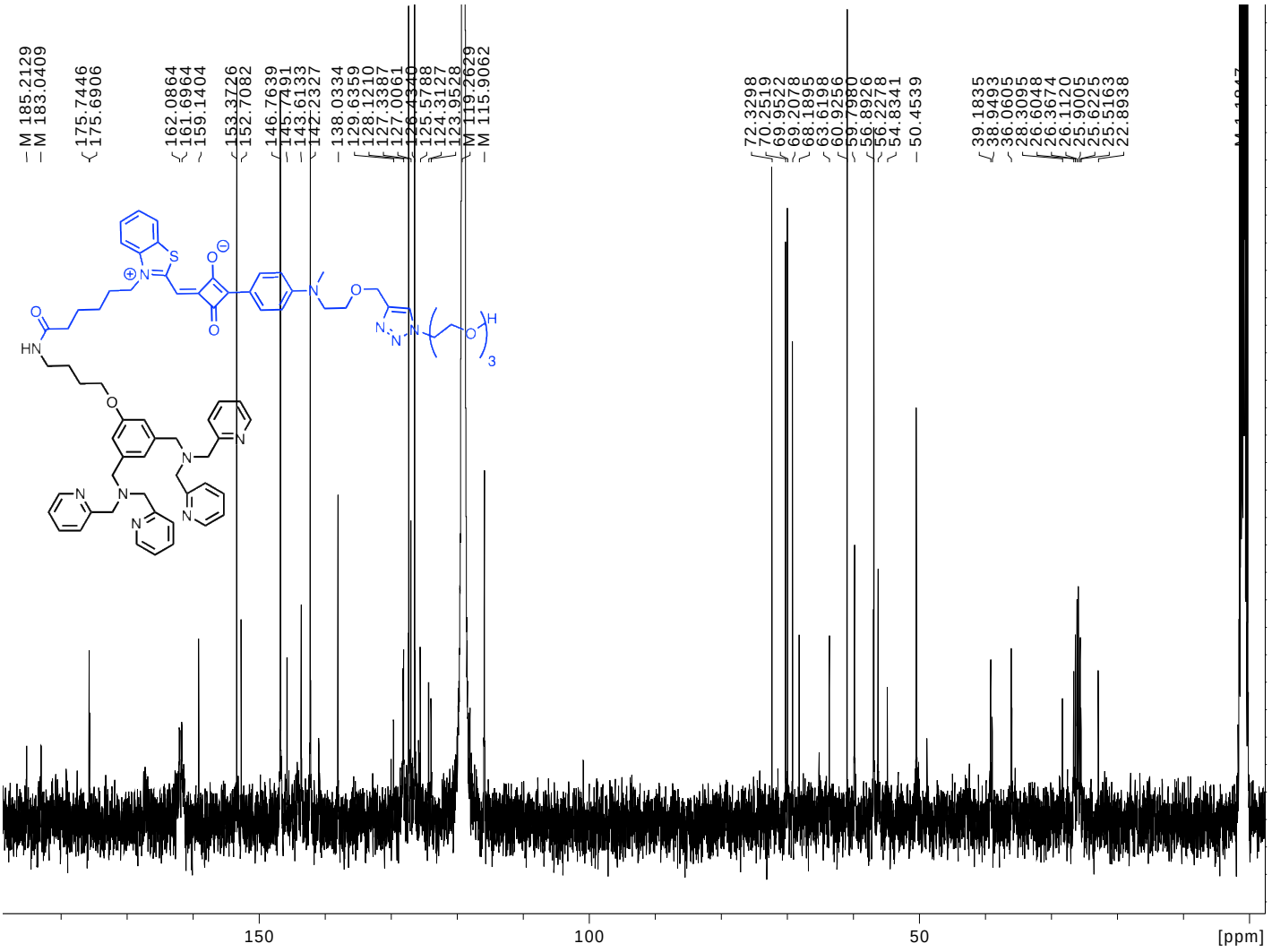
^{1}H NMR spectrum (500 MHz, 1:1 – CD_3CN:D_2O) of **DPA-USQ**.



^{13}C NMR spectrum (400 MHz, DMSO-d_6) of **DPA-USQ**.



^{1}H NMR spectrum (500 MHz, 1:1 – CD_3CN:D_2O) of **BDPA-USQ**.



^{13}C NMR spectrum (400 MHz, DMSO-d_6) of **BDPA-USQ.**

Acquisition Parameter

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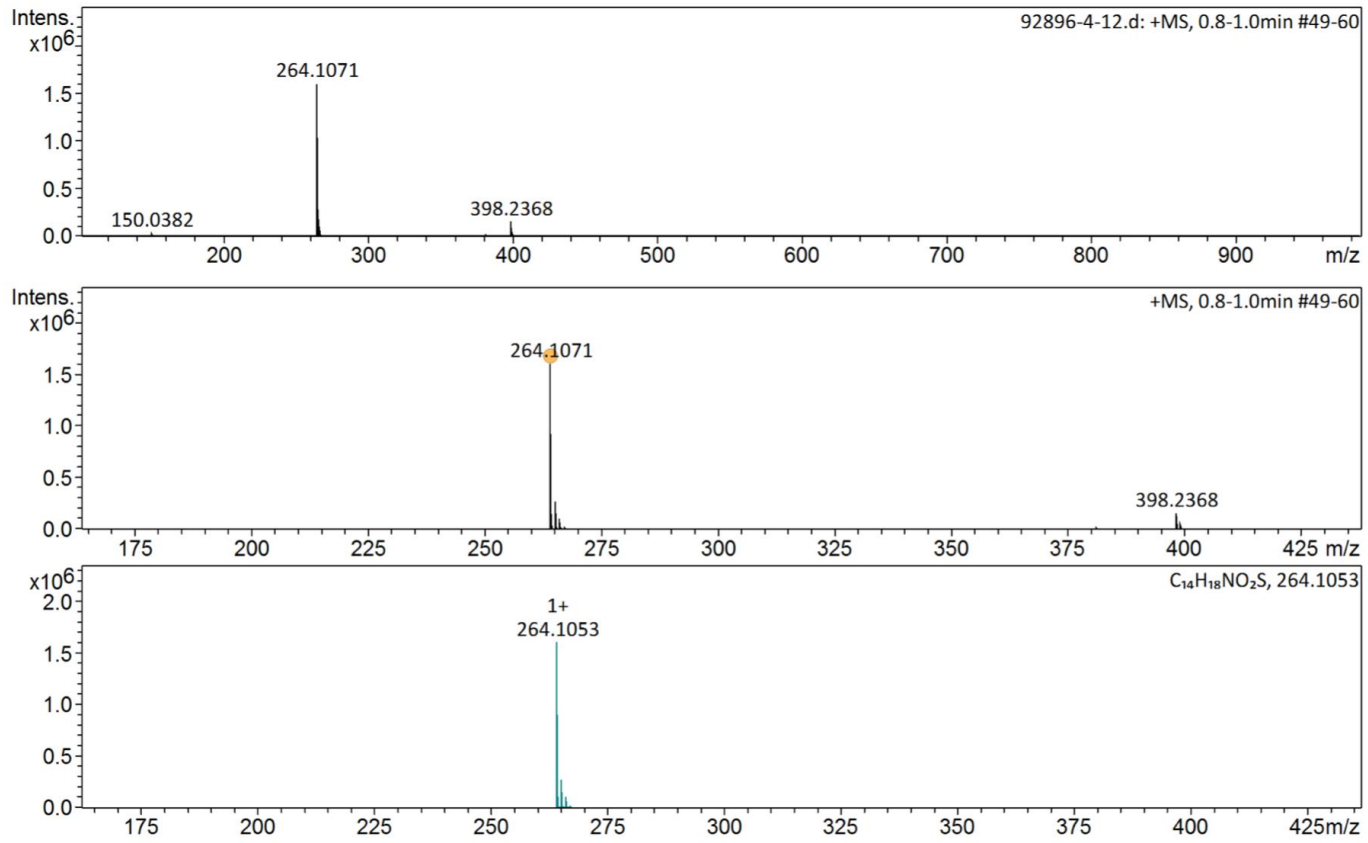
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source



Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
264.107123	1	C14H18NO2S	264.105276	-7.0	-6.8	6.5	ok	even

HR-MS spectrum of **4**

Acquisition Parameter

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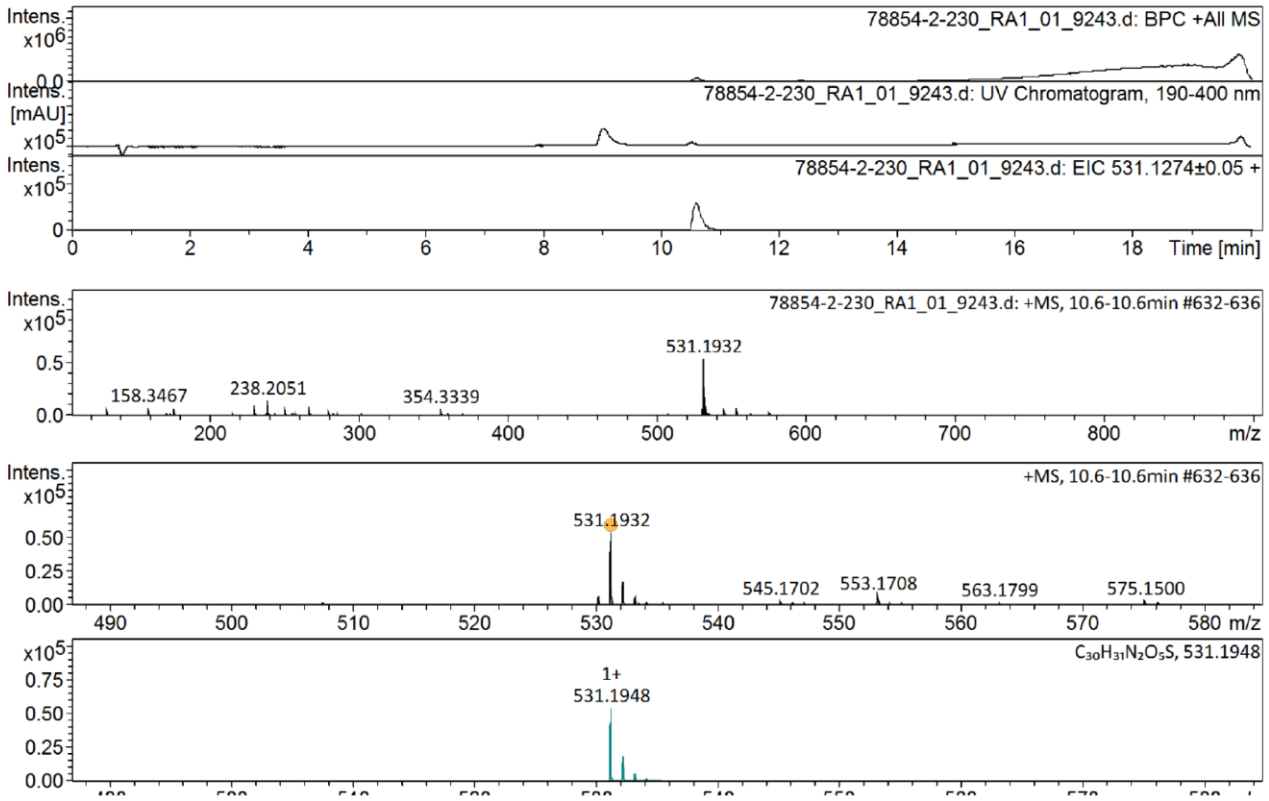
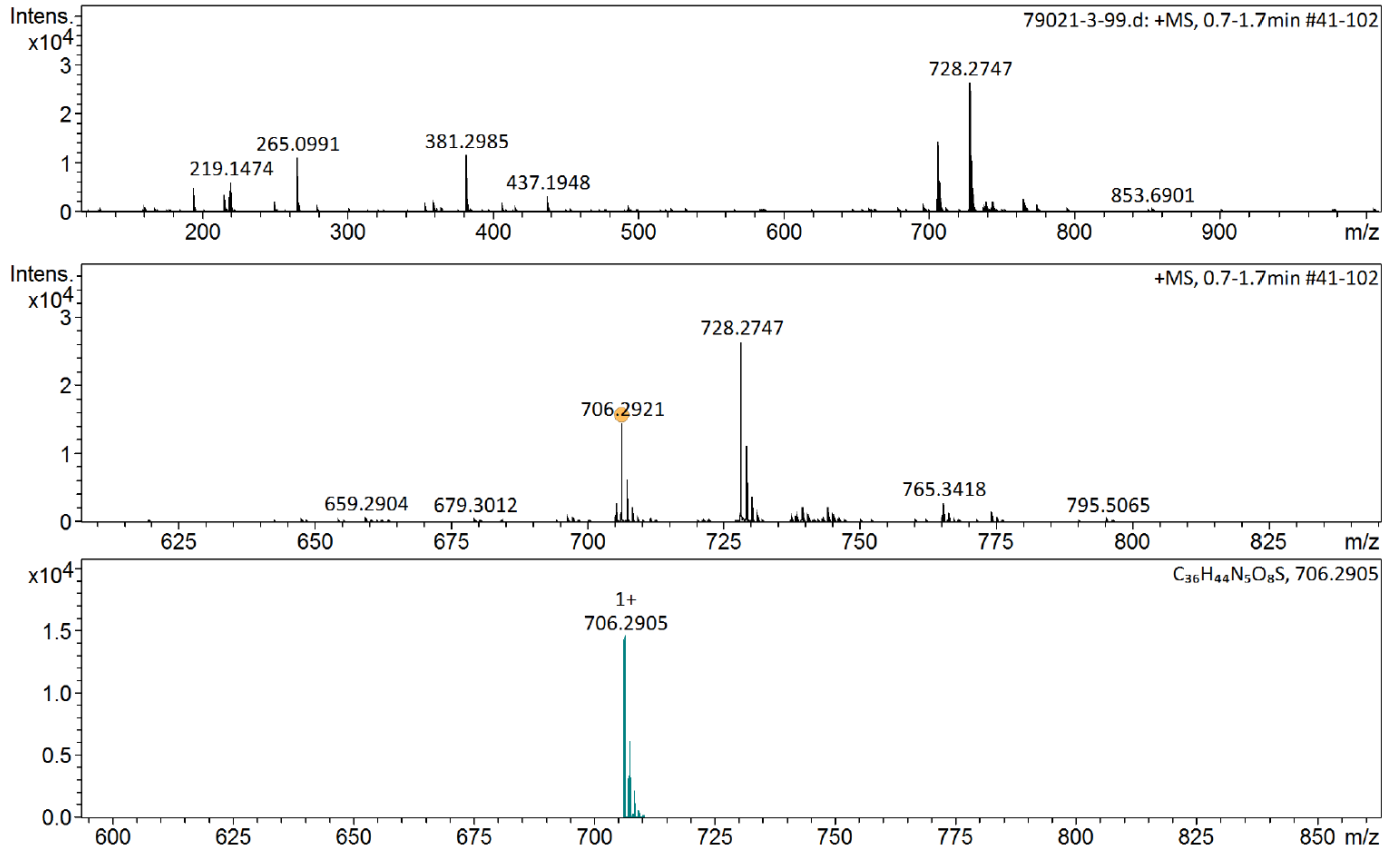


Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
531.1932	1	C30H31N2O5S	531.1948	3.1	7.7	1	100.00	16.5	even	ok

HR-MS spectrum of **5**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source

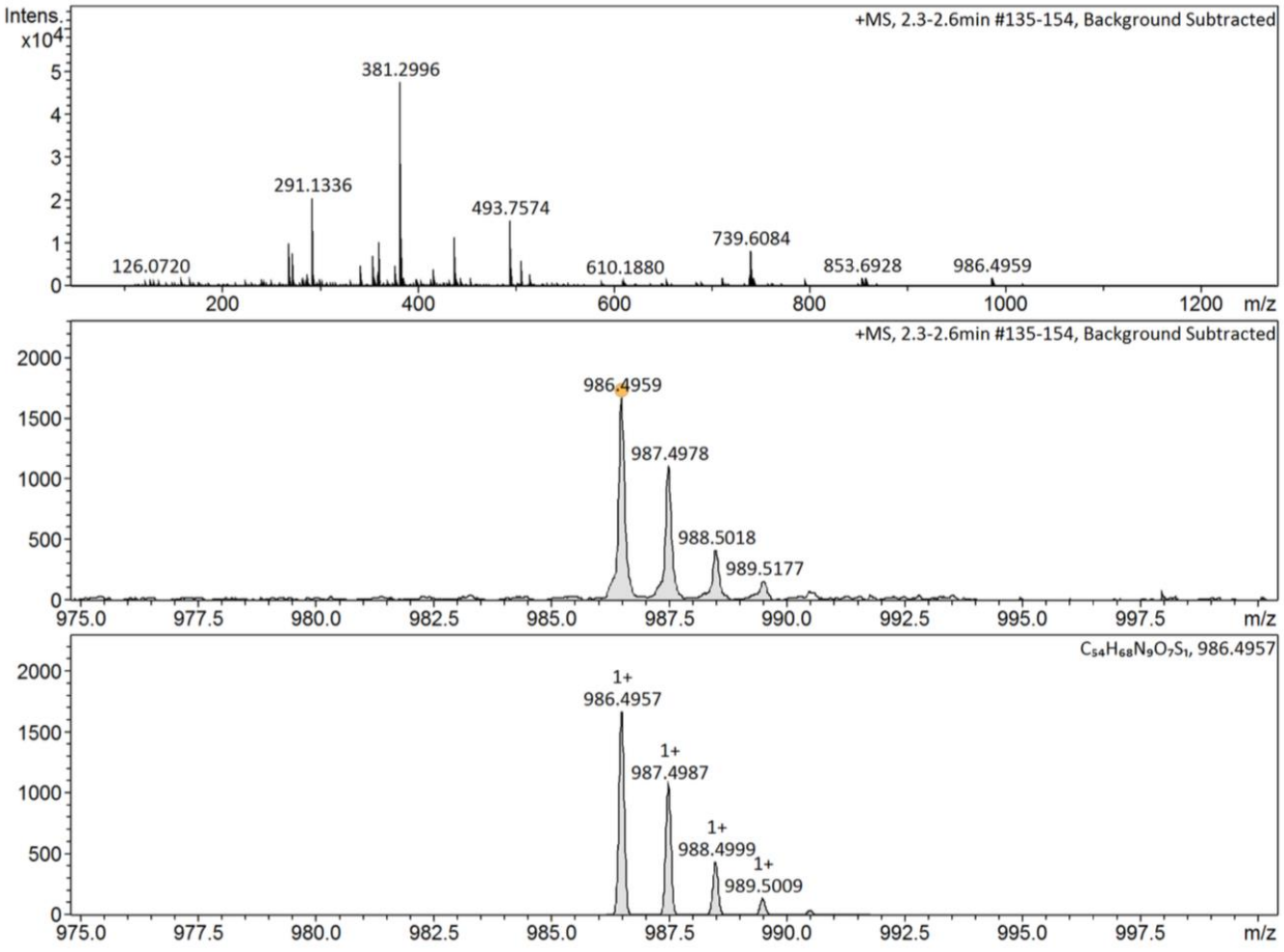


Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
706.292074	1	C36H44N5O8S	706.290511	-2.2	-2.3	17.5	ok	even

HR-MS spectrum of **USQ**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Waste



Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
986.495907	1	C54H68N9O7S	986.495693	-0.2	927.1	25.5	ok	even
	2	C59H68N7O5S	986.499716	3.9	919.9	29.5	ok	even
	3	C51H72N9O7S2	986.499064	3.2	939.7	20.5	ok	even

HR-MS spectrum of **DPA-USQ**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	4.0 Bar
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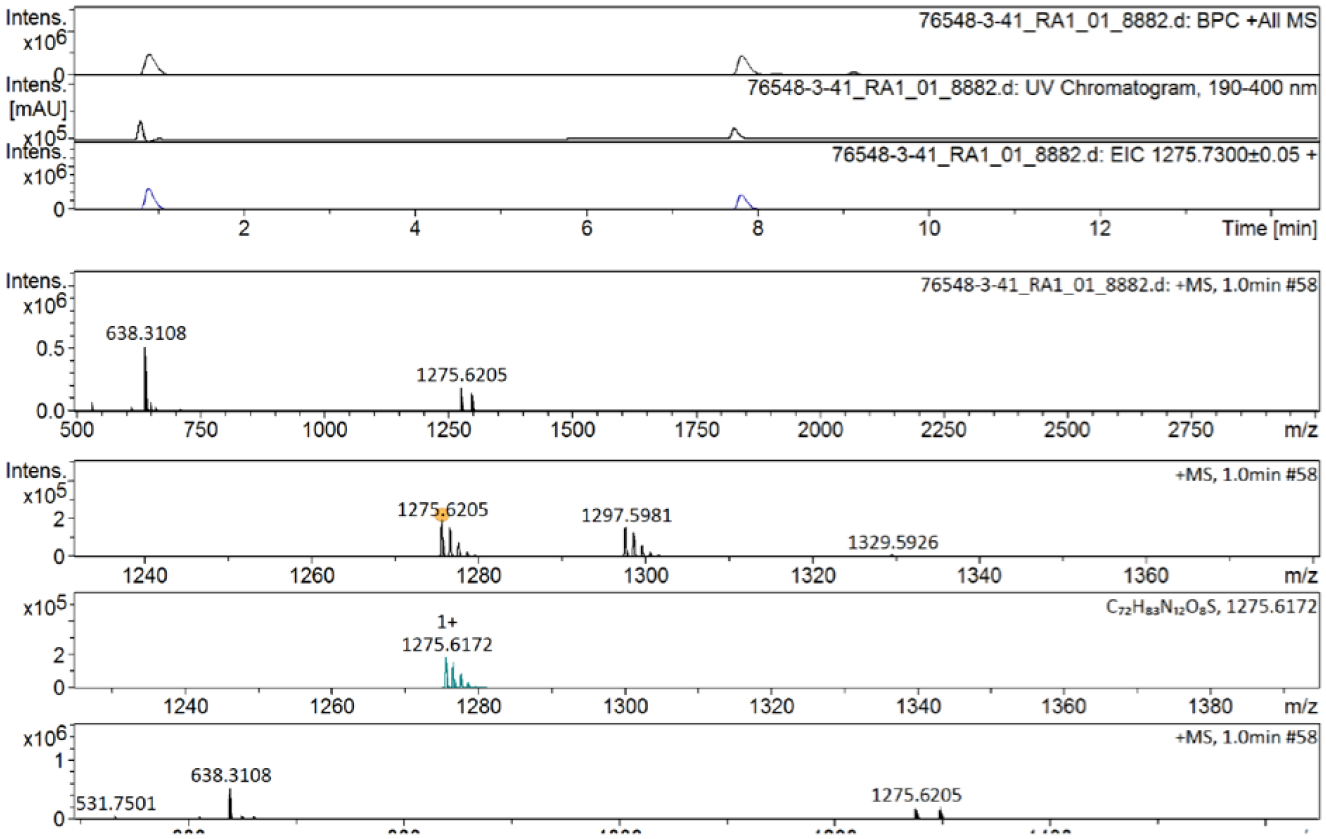
Scan End	3000 m/z	Set Collision Cell RF	800.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e¯ Conf	N-Rule
1275.6205	1	C72H83N12O8S	1275.6172	-2.6	4.1	1	100.00	37.5	even	ok

HR-MS spectrum of **BDPA-USQ**

B. Photophysical Studies

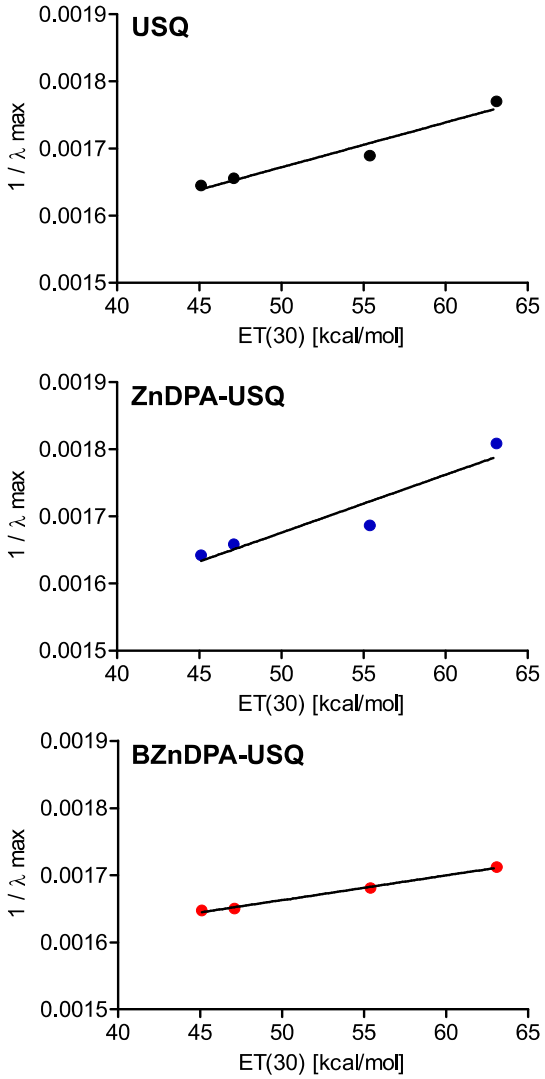


Figure S1. Linear correlation between $E_T(30)$ values and $1/\lambda_{max}$ for absorption in solvents of different polarity dimethylsulfoxide (DMSO), butanol (BuOH), methanol (MeOH), and water (H_2O).

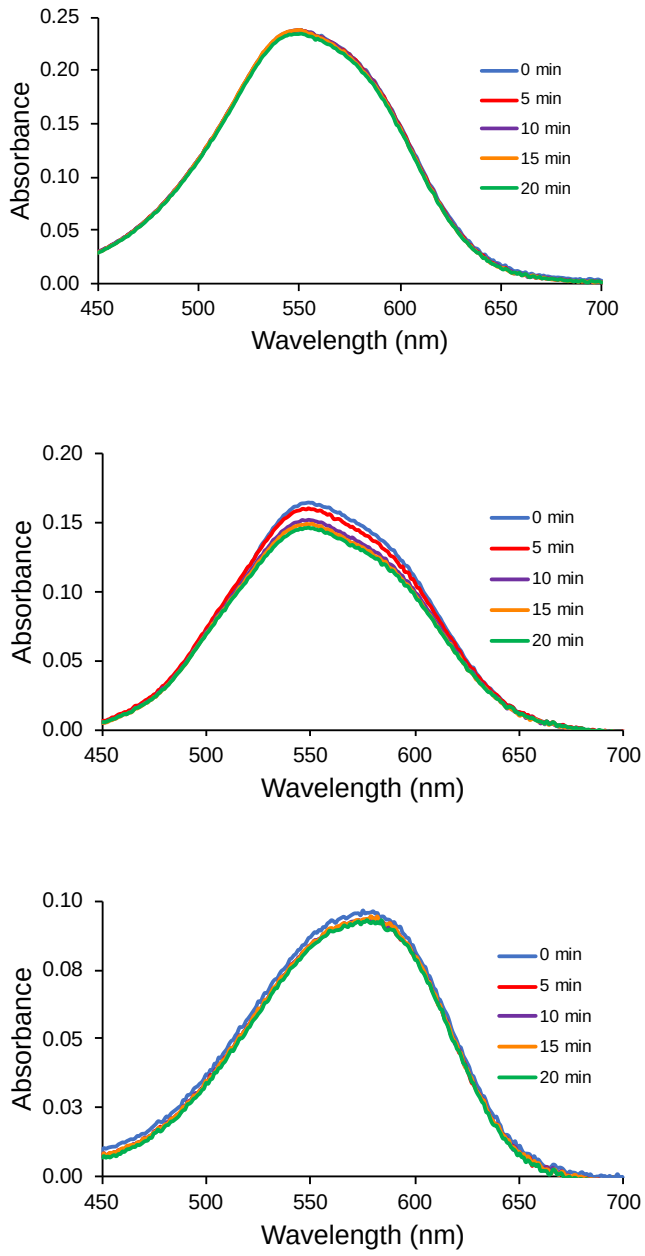


Figure S2. Photostability of: (*top*) **USQ**, (*middle*) **ZnDPA-USQ**, and (*bottom*) **ZnDPA-USQ**. In each case, a cuvette containing an aqueous solution (6 µM) at 20 °C was irradiated with intense light from a Xenon lamp, passed through a 495 nm longpass filter, for 20 minutes. There was little or no change in sample absorbance over time indicating high photostability.

C. In Vitro Studies

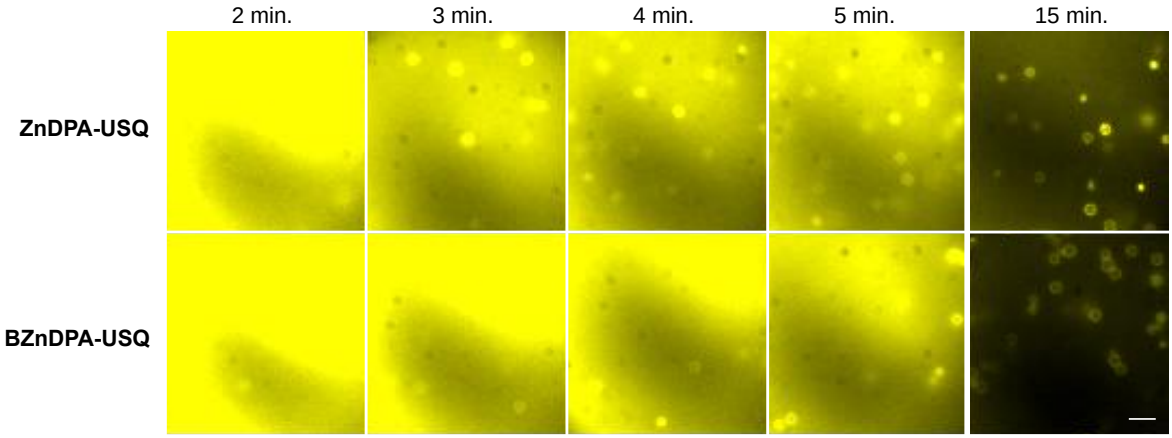


Figure S3. Fluorescent micrographs of RBC ghosts treated with 10 μM **ZnDPA-USQ** or **BZnDPA-USQ** and imaged at subsequent time points using a standard TxRed filter set. Scale bar = 25 μM

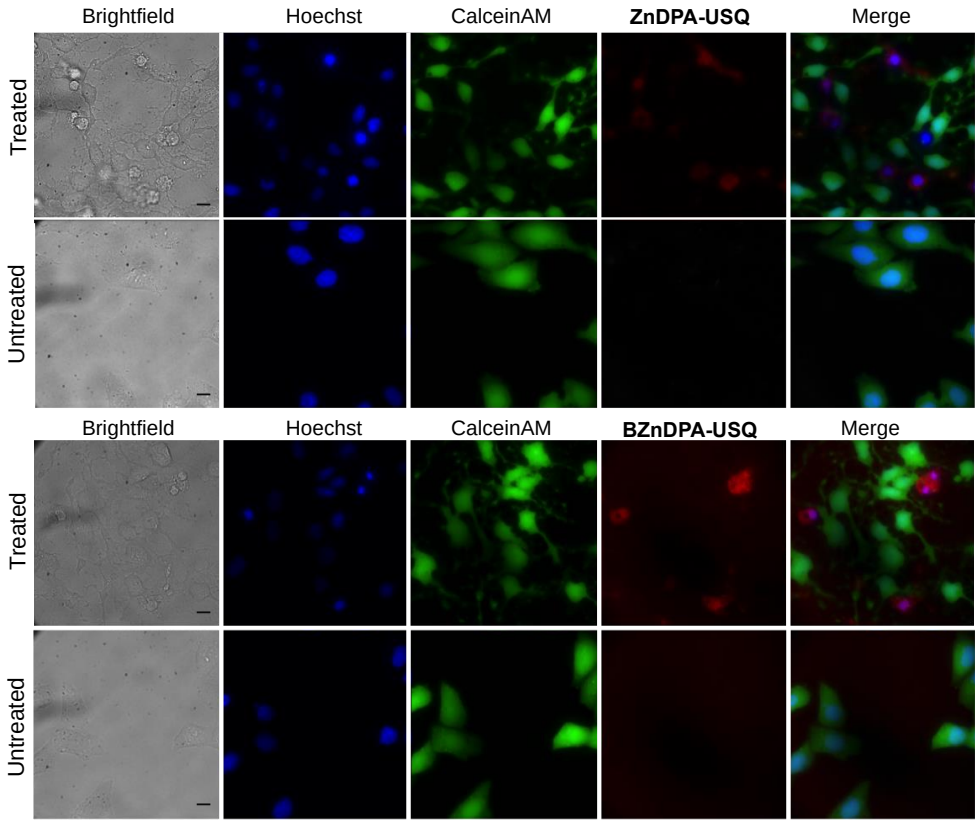


Figure S4. Fluorescence micrographs of CHO-K1 cells that had been treated with Staurosporine (500 nM) for 3 hours or were untreated. The cells were subsequently treated with **ZnDPA-USQ** or **BZnDPA-USQ** (red, 500 nM), nuclear stain Hoechst33342 (blue, 3 μM) and live cell indicator CalceinAM (green, 5 μM). Scale bar = 50 μM

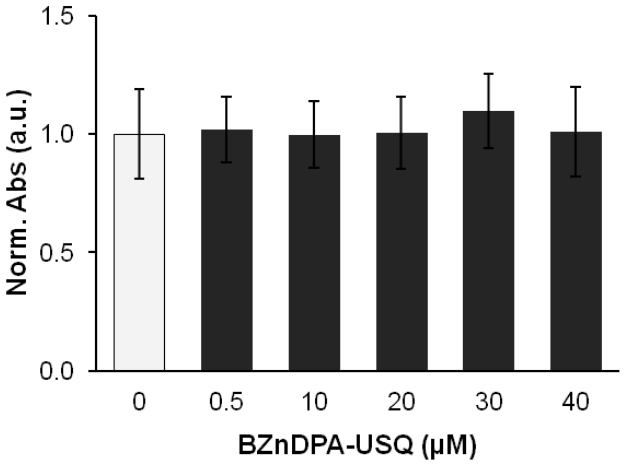


Figure S5. MTT assay measuring vitality of CHO-K1 cells after incubation with **BZnDPA-USQ** for 24 hours.

D. Xenopus Studies

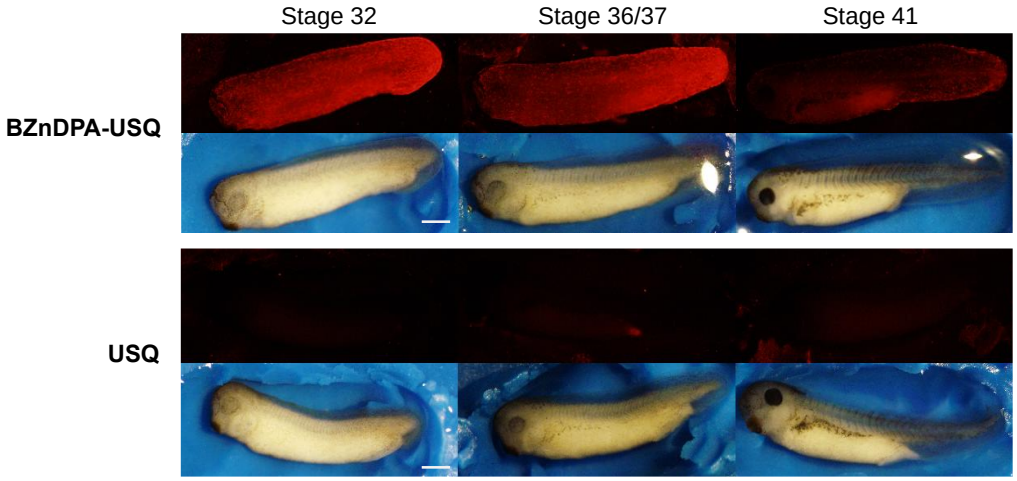


Figure S6. Fluorescent images of *Xenopus* embryos treated with **BZnDPA-USQ** (top) or **USQ** (bottom) (30 μM) for 16 hours (stage 32). At that point, the incubation medium was change to probe-free 1/3 MMR, and incubation continued, with imaging occurring at additional time periods of 8 hours (stage 36/37) and 30 hours (stage 41). Scale bar = 0.5 mm

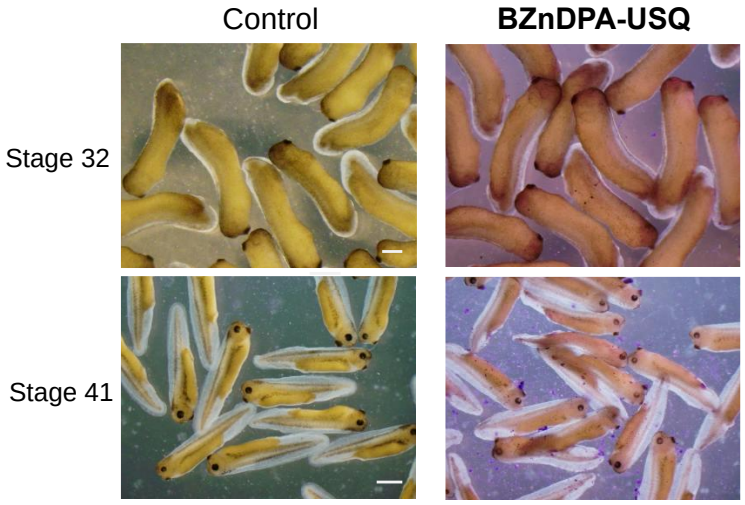


Figure S7. Brightfield images showing *Xenopus* embryos after incubation in probe-free 1/3 MMR solution (Control) or **BZnDPA-USQ** (30 μM) for 16 hours (stage 32) or 46 hours (stage 41). Scale bar = 1 mm

References

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(S2) Lakshmi, C.; Hanshaw, R. G.; Smith, B. D. Fluorophore-linked Zinc(II)dipicolylamine Coordination Complexes as Sensors for Phosphatidylserine-containing Membranes. *Tetrahedron* **2004**, *60*, 11307–11315.