*Electronic Supplementary Information (ESI) for:*

# Water Soluble, Deep-Red Fluorescent Squaraine Rotaxanes

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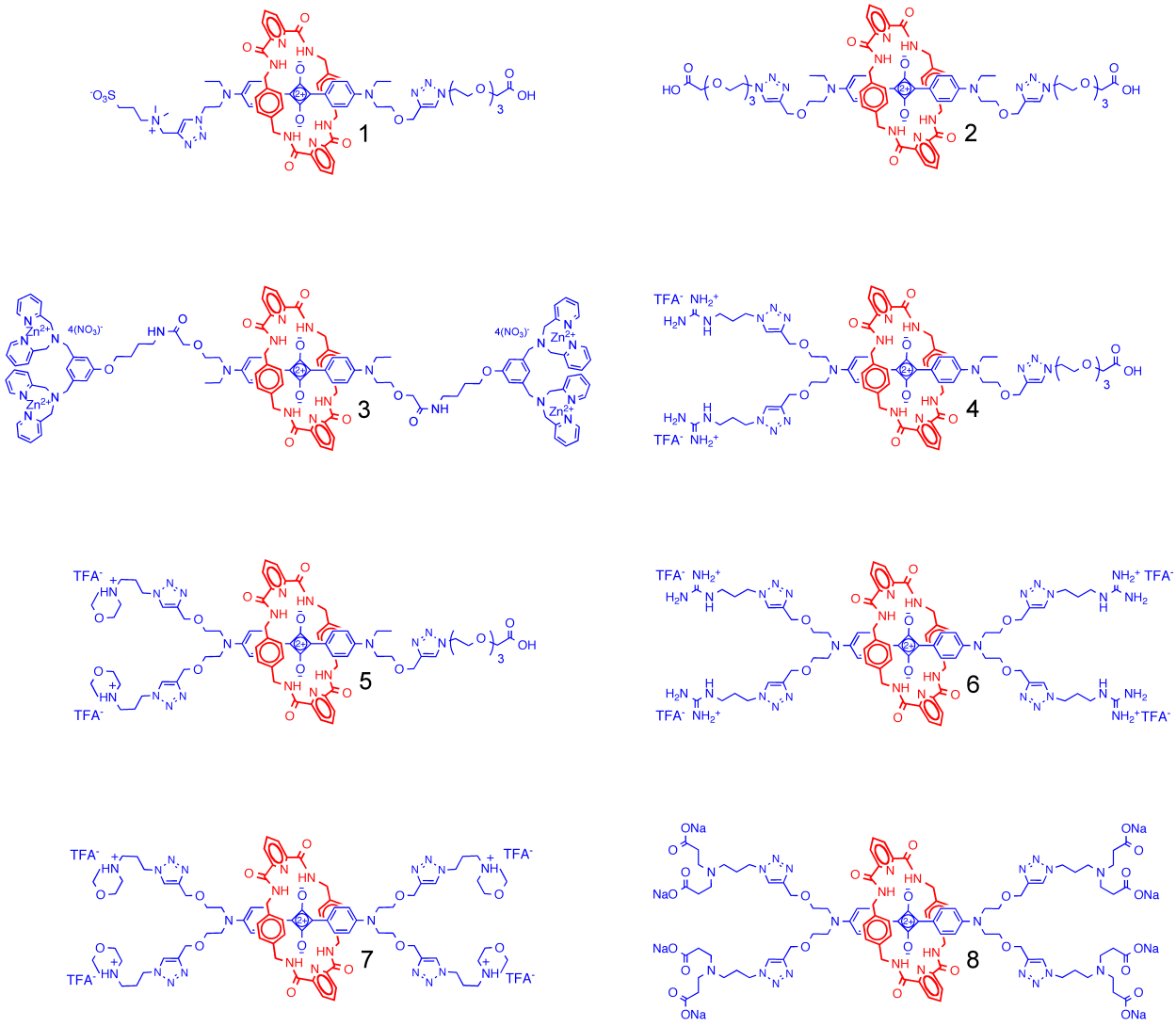
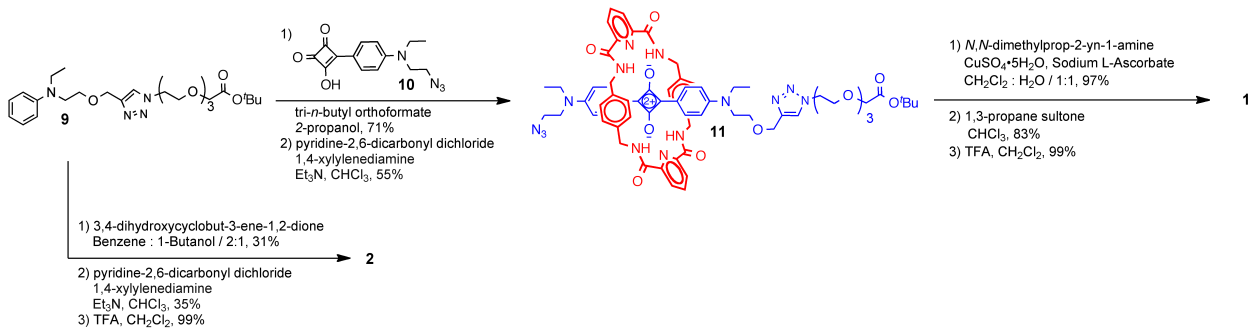
**1. Syntheses in General**

The structures of squaraine rotaxanes **1**-**8** are provided in Scheme S1.

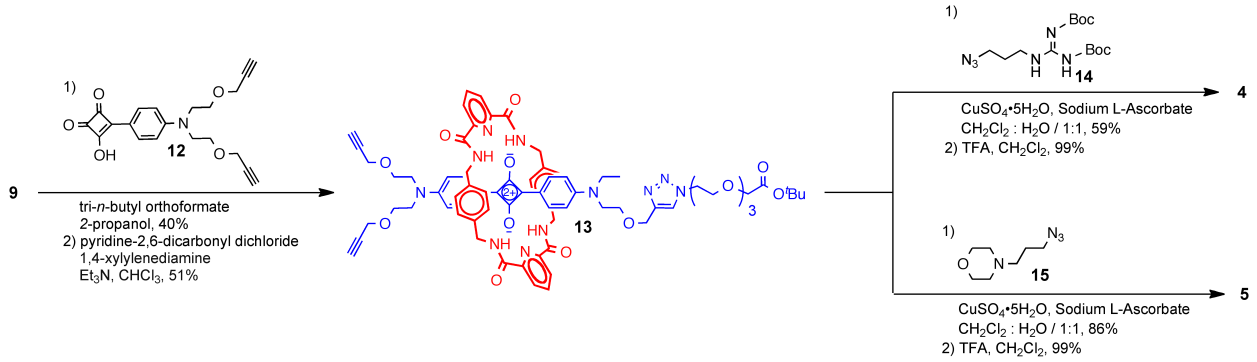



**Scheme S1**

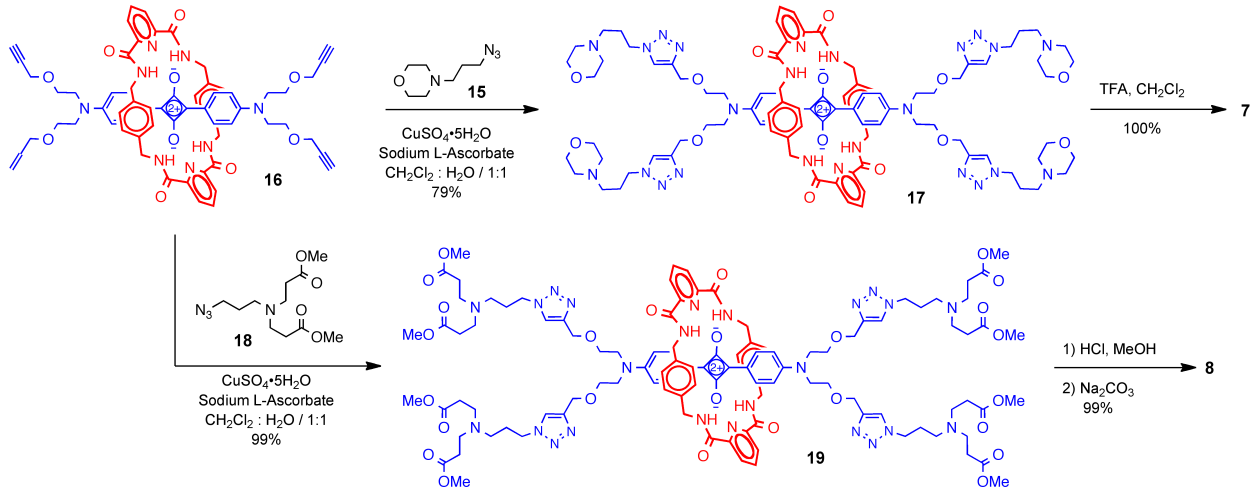
Squaraine rotaxanes **1**, **2**, **4**, **5**, **7**, and **8** are new compounds they were prepared by the pathways in Schemes S1-S3. As shown in Scheme S2, aniline **9** was reacted with semisquaraine **10** to create an unsymmetrical squaraine that was converted by standard clipping methods to the uncharged squaraine rotaxane **11**. This compound was converted in three steps to the zwitterionic squaraine rotaxane **1**. Condensation of two molar equivalents of aniline **9** with squaric acid, followed by rotaxane formation and deprotection produced the bis-carboxylic acid **2**. Similarly, aniline **9** was used to make the precursor squaraine rotaxane **13,** which was converted into cationic squaraine rotaxanes **4** and **5** (Scheme S3). Finally, the known squaraine rotaxane **16** was a common precursor for the squaraine rotaxanes **7** and **8**, each with four large groups appended to the ends of the squaraine dye. In each, the four groups were appended by conducting copper catalyzed azide/alkyne cycloaddition reactions (Scheme S4).




**Scheme S2**



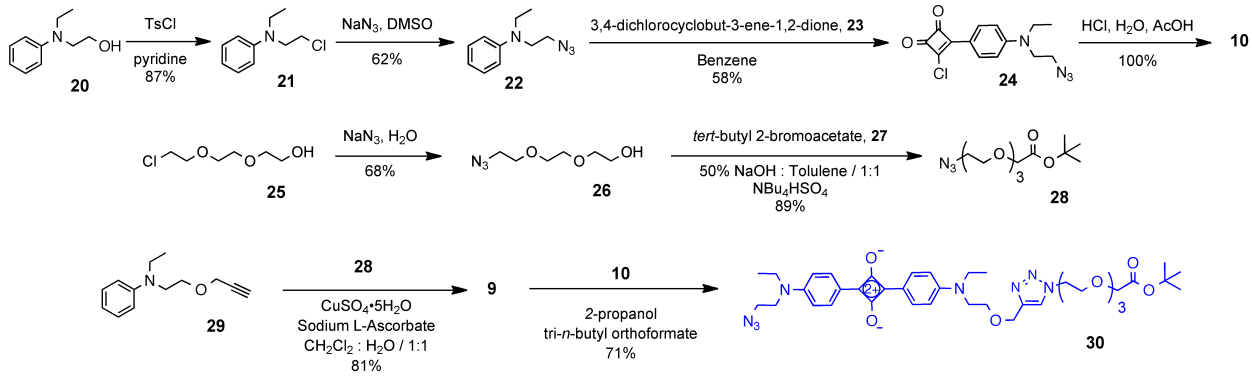

**Scheme S3**




**Scheme S4**

## 2. Syntheses in Detail

Reagents and starting materials were purchased from commercial supplies and used without further purification. Thin layer chromatography was performed on silica gel 60 F254. Column chromatography was performed on silica gel (230-400 mesh). NMR coupling constants (*J* values) are reported to the nearest 0.5 Hz. The known compounds **3**[S1], **6**[S2], **14**[S2], **16**[S2], **23**[S3], **29**[S4], **40**[S2], and **47**[S2] were synthesized and characterized by previously published methods.




**21.** The commercially available compound **20** (501 mg, 3.03 mmol, Aldrich) was dissolved in dry pyridine (10 mL) and the solution cooled to 0 °C. 4-toluenesulfonyl chloride (1.02 g, 5.35 mmol, Aldrich) was added slowly, and the stirring reaction was allowed to warm to room temp over 16 h. The reaction was extracted with chloroform and water, and after drying, the organic layer was evaporated to yield **21** a yellow oil (485 mg, 87%): $^{1}$H-NMR (300 MHz, $CDCl_3$): δ 1.25 (t, *J* = 7.0 Hz, 3H), 3.49 (q, *J* = 7.0 Hz, 2H), 3.65-3.72 (m, 4H), 6.75-6.82 (m, 3H), 7.32 (dd, *J* = 9.0 Hz, *J* = 7.5 Hz, 2H); $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 12.4, 40.4, 45.3, 52.3, 111.7, 116.4, 129.4, 146.8; HRMS (ESI-TOF): calculated for $C_{10}H_{15}ClN$ $[M+H]^+$ 184.0888, found 184.0876; $\lambda_{max}$ (abs, MeCN) = 257 nm.

**22.** Compound **21** (485 mg, 2.64 mmol) and sodium azide (1.04 g, 16.0 mmol, Sigma-Aldrich) were dissolved in DMSO (10 mL), and the mixture was heated for 16 h at 120 °C. The reaction was extracted with ethyl acetate and water, and after drying the organic layer was condensed and subjected to column chromatography using a silica gel column with $CHCl_3$ eluent to yield **22** a yellow oil. (312 mg, 62%): $^{1}$H-NMR (300 MHz, $CDCl_3$): δ 1.24 (t, *J* = 7.0 Hz, 3H), 3.46-4.58 (m, 6H), 6.77-6.82 (m, 3H), 7.29-7.34 (m, 2H); $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 12.1, 45.4, 48.8, 49.5, 112.1, 116.5, 129.3, 147.1; HRMS (ESI-TOF): calculated for $C_{10}H_{15}N_4$ $[M+H]^+$ 191.1291, found 191.1270; $\lambda_{max}$ (abs, MeCN) = 257 nm.

**24.** Compound **22** (1.97 g, 10.4 mmol) and the previously known compound **23**[S3] (2.04 g, 1.35 mmol) were refluxed in benzene (40 mL) for 16 h. The solvent was removed and the crude material was subjected to column chromatography using a silica gel column with $CH_2Cl_2$ eluent to yield **24** yellow oil (1.83 g, 58%): $^{1}$H-NMR (300 MHz, $CDCl_3$): δ 1.26 (t, *J* = 7.0 Hz, 3H), 3.53-3.65 (m, 6H), 6.78 (d, *J* = 9.5 Hz, 2H), 8.09 (d, *J* = 9.5 Hz, 2H); $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 12.0, 45.8, 48.7, 49.2, 111.7, 114.6, 131.6, 152.5, 172.3, 186.1, 190.0, 195.6; HRMS (ESI-TOF): calculated for $C_{14}H_{14}ClN_4O_2$ $[M+H]^+$ 305.0800, found 305.0793; $\lambda_{max}$ (abs, DMSO) = 382 nm.

**10.** Compound **24** (1.83 g, 6.01 mmol) was refluxed in water (60 mL), acetic acid (20 mL), and 5M HCl (20 mL) for 18 h. The hot reaction was poured over ice and allowed to warm to room temp then filtered. The brown solid was dried to give **10** (1.72 g, quantitative yield): $^{1}$H-NMR (500 MHz, DMSO-$d_6$): δ 1.12 (t, *J* = 7.0 Hz, 3H), 3.47 (q, *J* = 7.0 Hz, 2H), 3.52 (t, *J* = 5.0 Hz, 2H), 3.59 (t, *J* = 6.0 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 7.84 (d, *J* = 9.0 Hz, 2H); $^{13}$C-NMR (125 MHz, DMSO-$d_6$): δ 11.9, 44.5, 48.3, 48.5, 111.7, 116.9, 128.2, 149.6, 173.3, 194.5; HRMS (ESI-TOF): calculated for $C_{14}H_{15}N_4O_3$ $[M+H]^+$ 287.1139, found 287.1157; $\lambda_{max}$ (abs, DMSO) = 380 nm.

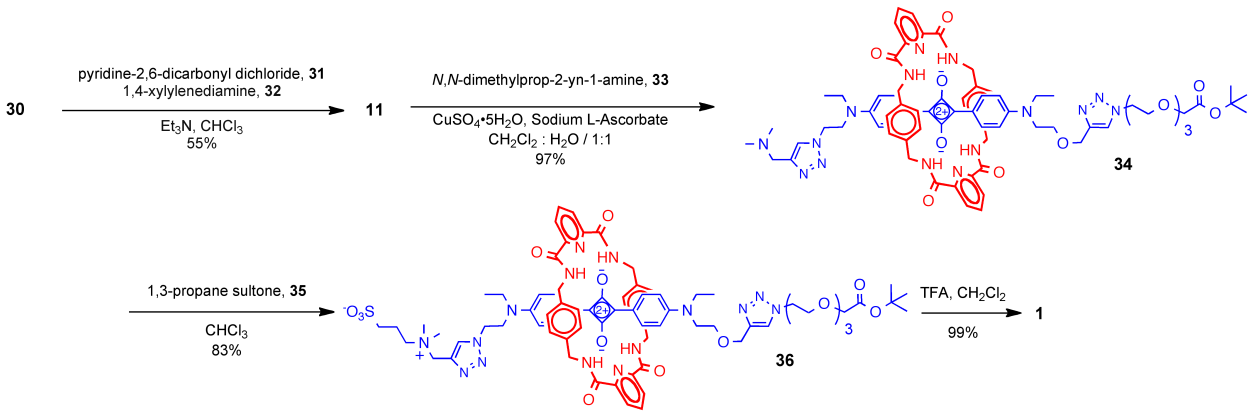
**26.** The commercially available compound **25** (2.01 g, 11.9 mmol, Aldrich), sodium azide (2.00 g, 30.8 mmol, Sigma-Aldrich), and sodium iodide (100 mg) were dissolved in water (60 mL). The reaction was refluxed for 13 h then cooled before extraction with diethyl ether. The later was washed with brine, condensed then subjected to column chromatography using a silica gel column with $CHCl_3$ eluent to yield **26** a yellow oil (1.42 g, 68%): $^{1}$H-NMR (300 MHz, $CDCl_3$): δ 2.61 (t, *J* = 5.5 Hz, 1H), 3.38 (t, *J* = 5.0 Hz, 2H), 3.59 (t, *J* = 4.5, 2H), 3.64-3.73 (m,

8H); $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 50.4, 61.5, 69.8, 70.2, 70.4, 72.4; HRMS (ESI-TOF): calculated for $C_6H_{14}N_4O_2$ $[M+H]^+$ 174.1111, found 174.1099.

**28.** A 50% solution of sodium hydroxide was allowed to cool to room temperature (10 g in 10 mL water). To this **26** (1.06 g, 6.05 mmol), **27** (2.02 g, 10.4 mmol, Aldrich), and $NBu_4HSO_4$ (500 mg, Sigma-Aldrich) in toluene (10 mL) were added. The biphasic reaction was stirred for 20 h. The organic layer was condensed and extracted with $CHCl_3$ and water. The organic layer was condensed then subjected to column chromatography using a silica gel column with $CHCl_3$ eluent to yield **28** a yellow oil (1.56 g, 89%): $^1$H-NMR (300 MHz, $CDCl_3$): δ 1.47 (s, 9H), 3.39 (t, $J$ = 5.5 Hz, 2H), 3.66-3.75 (m, 10H), 4.02 (s, 2H); $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 27.6, 27.9, 50.5, 68.3, 68.8, 69.8, 70.48, 70.51, 81.3, 169.4; HRMS (ESI-TOF): calculated for $C_{12}H_{23}N_3NaO_5$ $[M+Na]^+$ 312.1530, found 312.1509.

**9.** A mixture of the previously known compound **29**[S4] (352 mg, 1.73 mmol) and **28** (497 mg, 1.72 mmol) was dissolved in $CH_2Cl_2$ (5mL). To this a solution of $CuSO_4$•$5H_2O$ (11.63 mg, Aldrich) and sodium L-ascorbate (17.05 mg, Sigma) in water (5 mL) was added. The reaction was stirred at room temperature over 16 h, then extraction with $CHCl_3$ and water. The organic layer was washed with EDTA, dried with $MgSO_4$, and condensed. The crude product was purified by column chromatography using a silica gel column with 0 to 5% MeOH in $CHCl_3$ eluent to give **9** a yellow oil (685 mg, 81%): $^1$H-NMR (300 MHz, $CDCl_3$): δ 1.13 (t, $J$ = 7.0 Hz, 3H), 1.46 (s, 9H), 3.39 (q, $J$ = 7.0 Hz, 2H), 3.50 (t, $J$ = 6.0 Hz, 2H), 3.61 (s, 4H), 3.67 (q, $J$ = 6.0 Hz, 6H), 3.85 (t, $J$ = 5.0 Hz, 2H), 4.00 (s, 2H), 4.52 (t, $J$ = 5.0 Hz, 2H), 4.65 (s, 2H), 6.77-6.82 (m, 3H), 7.19 (dd, $J$ = 8.5 Hz, $J$ = 7.5 Hz, 2H), 7.68 (s, 1H); $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 12.0, 27.7, 28.0, 45.3, 49.8, 50.1, 58.4, 64.6, 67.6, 68.0, 69.4, 70.41, 70.44, 70.48, 70.62, 81.5, 111.7, 115.6, 115.7, 123.6, 129.2, 169.5, 188.4; HRMS (ESI-TOF): calculated for $C_{25}H_{41}N_4O_6$ $[M+H]^+$ 493.3021, found 493.3036; $\lambda_{max}$ (abs, MeCN) = 257 nm.

**Squaraine Dye 30.** A solution of **10** (300 mg, 1.05 mmol), **9** (500 mg, 1.02 mmol), and tri-*n*-butyl orthoformate (1 mL, Alfa Aesar) in 2-propanol (30 mL) was allowed to reflux for 12 h. The crude product was condensed and purified by column chromatography using a silica gel column with 0 to 3% MeOH in $CHCl_3$ eluent to yield **30** a green-blue solid (549 mg, 71%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 1.25 (tt, $J$ = 7.0 Hz, $J$ = 4.5 Hz, 6H), 1.46 (s, 9H), 3.57-3.61 (m, 10H), 3.63-3.65 (m, 4H), 3.68-3.69 (m, 4H), 3.77 (t, $J$ = 6.0 Hz, 2H), 3.85 (t, $J$ = 5.0 Hz, 2H), 4.00 (s, 2H), 4.52 (t, $J$ = 5.0 Hz, 2H), 4.64 (s, 2H), 6.77 (dd, $J$ = 9.5 Hz, $J$ = 3.5 Hz, 4H), 7.70 (s, 1H), 8.38 (dd, $J$ = 9.0 Hz, $J$ = 4.5 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 12.2, 12.4, 28.1, 29.7, 46.2, 46.4, 48.9, 49.5, 50.2, 50.3, 64.5, 67.6, 68.9, 69.3, 70.4, 70.6, 70.93, 81.6, 112.2, 112.5, 119.7, 120.3, 123.9, 133.1, 133.7, 144.2, 152.6, 154.0, 169.5, 183.2, 188.0, 188.9; HRMS (ESI-TOF): calculated for $C_{39}H_{53}N_8O_8$ $[M+H]^+$ 761.3981, found 761.3991; $\lambda_{max}$ (abs, $CHCl_3$) = 629 nm, log ε = 5.42, $\lambda_{max}$ (em, $CHCl_3$) = 649 nm, $\Phi_f$ = 0.36.



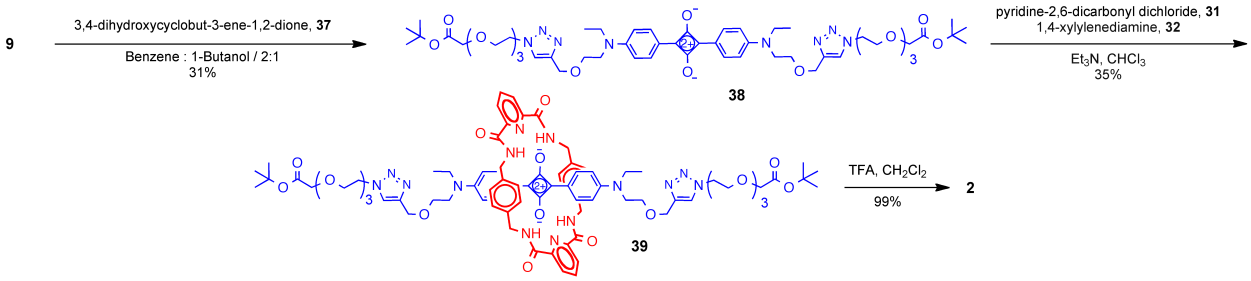

**Squaraine Rotaxane 11.** In a 50 mL syringe pyridine-2,6-dicarbonyl dichloride **31** (804 mg, 3.92 mmol, Aldrich) was dissolved in anhydrous $CHCl_3$ (25 mL). In a second 50 mL syringe 1,4-xylylenediamine **32** (557 mg, 4.09 mmol, Alfa Aesar) and triethylamine (0.50 mL, 3.6 mmol, Sigma-Aldrich) were dissolved in anhydrous $CHCl_3$ (24.5 mL). Each syringe was added dropwise over 8 hours, using a mechanical syringe pump to a solution of squaraine dye **30** (385 mg, 0.506 mmol) in anhydrous $CHCl_3$ (25 mL). The reaction mixture was filtered through celite® 545 to remove any polymeric material and condensed. The crude product was purified by column chromatography using

a column of silica gel with 0 to 5% MeOH in $CHCl_3$ as the eluent to give pure **11** (361 mg, 55%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 1.18 (tt, *J* = 7.0 Hz, *J* = 4.5 Hz 6H), 1.47 (s, 9H), 3.41-3.52 (m, 6H), 3.58-3.61 (m, 4H), 3.64 (s, 2H), 3.65-3.67 (m, 4H), 3.69-3.73 (m, 4H), 3.90 (t, *J* = 5.0 Hz, 2H), 4.01 (s, 2H), 4.44 (dd, *J* = 14.5 Hz, *J* = 5.5 Hz, 4H), 4.57 (t, *J* = 5.0 Hz, 2H), 4.62 (d, *J* = 6.5 Hz, 2H), 4.64-4.65 (m, 4H), 6.11 (d, *J* = 9.5 Hz, 2H), 6.29 (d, *J* = 9.5 Hz, 2H), 6.61 (d, *J* = 14.0 Hz, 8H), 7.79 (s, 1H), 8.09 (d, *J* = 9.0 Hz, 2H), 8.13-8.17 (m, 4H), 8.52 (d, *J* = 8.0 Hz, 4H), 10.02 (t, *J* = 6.0 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 12.2, 12.3, 28.1, 29.7, 43.3, 46.0, 46.4, 48.7, 49.3, 50.2, 50.3, 64.5, 67.6, 68.9, 69.4, 70.4, 70.5, 70.7, 81.6, 111.2, 111.9, 119.0, 119.4, 124.0, 125.2, 128.9, 133.4, 133.9, 136.6, 138.8, 144.1, 149.5, 152.7, 154.0, 163.6, 169.5, 184.2, 185.0, 185.9; HRMS (ESI-TOF): calculated for $C_{69}H_{79}N_{14}O_{12}$ $[M+H]^+$ 1295.5996, found 1295.5984; $\lambda_{max}$ (abs, $CHCl_3$) = 640 nm, log ε = 5.32, $\lambda_{max}$ (em, $CHCl_3$) = 656 nm, $\Phi_f$ = 0.47.

**Squaraine Rotaxane 34.** A mixture of **11** (30.4 mg, 23.4 μmol) and the commercially available compound **33** (54.9 mg, 660 μmol, Aldrich) was dissolved in $CH_2Cl_2$ (5mL). To this a solution of $CuSO_4$•$5H_2O$ (17.63 mg, Aldrich) and sodium L-ascorbate (28.45 mg, Sigma) in water (5 mL) was added. The reaction was stirred at room temperature over 16 h, then extraction with $CHCl_3$ and water. The organic layer was washed with EDTA, dried with $MgSO_4$, and condensed. The crude product was purified by column chromatography using a silica gel column with 0 to 15% MeOH in $CHCl_3$ eluent to give **34** a blue solid (31.4 mg, 97%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 1.07 (t, *J* = 7.0 Hz, 3H), 1.17 (t, *J* = 7.0 Hz, 3H), 1.47 (s, 9H), 2.30 (s, 6H), 3.18 (q, *J* = 7.0 Hz, 2H), 3.47-3.51 (m, 2H), 3.58 (t, *J* = 5.5 Hz, 2H), 3.63 (s, 2H), 3.64-3.66 (m, 4H), 3.69-3.73 (m, 4H), 3.85-3.90 (m, 4H), 4.00 (s, 2H), 4.46-4.52 (m, 8H), 4.55-4.59 (m, 6H), 4.63 (s, 2H), 6.13 (d, *J* = 9.0 Hz, 2H), 6.23 (d, *J* = 9.5 Hz, 2H), 6.60 (br. s., 8H), 7.48 (s, 1H), 7.79 (s, 1H), 8.10-8.18 (m, 6H), 8.52 (d, *J* = 8.0 Hz, 4H), 10.00 (t, *J* = 6.0 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 12.4, 12.5, 18.7, 28.4, 43.6, 50.6, 64.7, 67.9, 69.2, 69.7, 70.73, 70.75, 70.78, 81.9, 111.4, 112.2, 119.3, 120.0, 124.3, 125.5, 129.2, 133.5, 134.5, 136.9, 139.2, 144.3, 149.8, 152.3, 154.6, 163.8, 169.8, 184.2, 185.2, 187.1; HRMS (ESI-TOF): calculated for $C_{74}H_{88}N_{15}O_{12}$ $[M+H]^+$ 1378.6731, found 1378.6722; $\lambda_{max}$ (abs, $CHCl_3$) = 637 nm, log ε = 5.36, $\lambda_{max}$ (em, $CHCl_3$) = 654 nm, $\Phi_f$ = 0.44.

**Squaraine Rotaxane 36.** The commercially available compound **35** (23.5 mg, 191 μmol, Aldrich) and **34** (21.2 mg, 15.4 μmol) were dissolved in $CHCl_3$ (3 mL) and stirred for 16 h. The reaction was condensed then purified by column chromatography using a silica gel column with 0 to 10% $NH_4OH$ in MeCN eluent to give **36** a blue solid (19.2 mg, 83%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 1.10 (t, *J* = 7.0 Hz, 3H), 1.20 (t, *J* = 7.0 Hz, 3H), 1.47 (s, 9H), 2.35 (br. s., 2H), 2.86 (br. s., 2H), 3.15 (br. s., 6H), 3.37 (d, *J* = 8.0 Hz, 2H), 3.48-3.55 (m, 2H), 3.61 (br. s., 2H), 3.64 (s, 2H), 3.65-3.67 (m, 4H), 3.70-3.74 (m, 6H), 3.81 (br. s., 2H), 3.89 (t, *J* = 5.0 Hz, 2H), 4.01 (s, 2H), 4.36 (dd, *J* = 14.5 Hz, *J* = 4.29 Hz, 4H), 4.57 (t, *J* = 5.0 Hz, 4H), 4.65-4.70 (m, 6H), 4.74 (br. s., 2H), 5.90 (d, *J* = 8.50 Hz, 2H), 6.33 (d, *J* = 9.0 Hz, 2H), 6.57 (m, 8H), 7.79 (s, 1H), 7.98 (d, *J* = 9.0 Hz, 2H), 8.13 (d, *J* = 9.0 Hz, 2H), 8.19 (t, *J* = 8.0 Hz, 2H), 8.52 (d, *J* = 8.0 Hz, 4H), 8.83 (br. s., 1H), 10.02 (t, *J* = 5.5 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 12.0, 12.2, 28.1, 29.7, 43.3, 50.3, 61.6 62.0 67.6, 68.9, 70.45, 70.47, 70.5, 70.7, 81.6, 111.0, 112.0, 119.5, 124.0 125.4, 128.9, 132.4, 134.4, 136.6, 139.0, 144.1, 149.3, 154.0, 163.6, 184.1, 185.0, 187.1; HRMS (ESI-TOF): calculated for $C_{77}H_{94}N_{15}O_{15}S$ $[M+H]^+$ 1500.6769, found 1500.6725; $\lambda_{max}$ (abs, $CHCl_3$) = 640 nm, log ε = 5.51, $\lambda_{max}$ (em, $CHCl_3$) = 657 nm, $\Phi_f$ = 0.31.

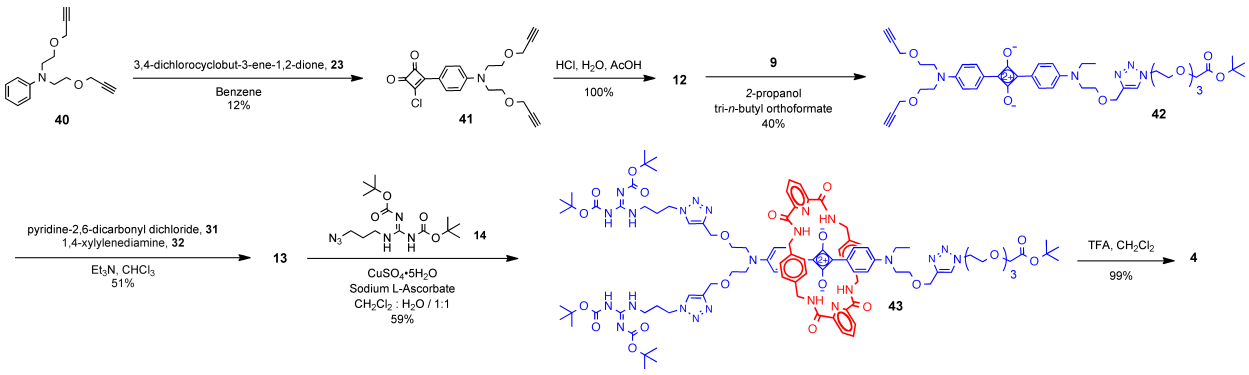
**Squaraine Rotaxane 1.** Squaraine Rotaxane **36** (19.2 mg, 12.8 μmol) was dissolved in $CH_2Cl_2$ (5 mL) and TFA (1.0 mL, 13 mmol, Sigma-Aldrich), and then stirred for 8 h. The reaction was condensed then washed with diethyl ether to produce **1** a blue solid (19.7 mg, 99%): $^1$H-NMR (500 MHz, $CD_3OD$): δ 1.08 (t, *J* = 7.0, 3H), 1.19 (t, *J* = 7.0 Hz, 3H), 2.30-2.36 (m, 2H), 2.88 (t, *J* = 6.5 Hz, 6.5 Hz, 2H), 3.14 (s, 6H), 3.56-3.62 (m, 10H), 3.65-3.67 (m, 4H), 3.69-3.73 (m, 4H), 3.86-3.89 (m, 4H), 4.10 (s, 2H), 4.31 (dd, *J* = 14.5, 5.0 Hz, 4H), 4.58 (t, *J* = 5.0 Hz, 2H), 4.62 (s, 2H), 4.64-3.65 (m, 4H), 4.71 (dd, *J* = 14.5 Hz, *J* = 7.0 Hz, 4H), 5.95 (d, *J* = 9.0 Hz, 2H), 6.55 (d, *J* = 9.0 Hz, 10H), 7.94 (d, *J* = 9.0 Hz, 2H), 8.06 (s, 1H), 8.10 (d, *J* = 9.0 Hz, 2H), 8.35 (s, 1H), 8.36 (t, *J* = 8.0 Hz, 3H), 8.52 (d, *J* = 8.0 Hz, 4H), 10.15 (tt, *J* = 7.0, 5.0 Hz, 4H); $^{13}$C-NMR: unable to obtain due to poor solubility; HRMS (ESI-TOF): calculated for $C_{73}H_{86}N_{15}O_{15}S$ $[M+H]^+$ 1444.6158, found 1444.6143.; $\lambda_{max}$ (abs, $CHCl_3$) = 640 nm, log ε = 5.14, $\lambda_{max}$ (em, $CHCl_3$) = 654 nm, $\Phi_f$ = 0.28, $\lambda_{max}$ (abs, $H_2O$) = 651 nm, log ε = 5.56, $\lambda_{max}$ (em, $H_2O$) = 672 nm, $\Phi_f$ = 0.22, $\lambda_{max}$ (abs, PBS) = 651 nm, log ε = 5.57, $\lambda_{max}$ (em, PBS) = 672 nm, $\Phi_f$ = 0.22, $\lambda_{max}$ (abs, 10% FBS) = 651 nm, log ε = 5.57, $\lambda_{max}$ (em, 10% FBS) = 671 nm.




**Squaraine Dye 38.** Compound **9** (1.79 g, 3.64 mmol) was added to a solution of 3,4-dihydroxy-3-cyclobutene-1,2-dione **37** (0.216 g, 1.90 mmol, Aldrich) in a mixture of benzene : 1-butanol / 2:1 (30 mL). The flask was equipped with a Dean-Stark apparatus and refluxed for 16 h. The reaction was condensed and the crude product purified by column chromatography using a column of silica gel with 0 to 1% MeOH in $CHCl_3$ eluent to give **38** a green-blue solid (599 mg, 31%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 1.24 (t, $J$ = 7.0 Hz, 6H), 1.47 (s, 18H), 3.55-3.70 (m, 24H), 3.77 (t, $J$ = 4.0, 4H), 3.86 (t, $J$ = 5.0 Hz, 4H), 4.00 (s, 4H), 4.53 (t, $J$ = 5.0 Hz, 4H), 4.65 (s, 4H), 6.76 (d, $J$ = 9.0 Hz, 4H), 7.71 (s, 2H), 8.36 (d, $J$ = 9.0 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 12.2, 28.1, 46.3, 50.2, 64.6, 67.3, 67.7, 68.9, 69.4, 70.45, 70.50, 70.64, 75.0, 81.6, 112.4, 119.8, 123.9, 133.3, 144.2, 153.5, 169.5, 183.3, 188.4, 189.3; HRMS (ESI-TOF): calculated for $C_{54}H_{78}N_8NaO_{14}$ $[M+Na]^+$ 1085.5530, found 1085.5558; $\lambda_{max}$ (abs, $CHCl_3$) = 633 nm, log ε = 5.45, $\lambda_{max}$ (em, $CHCl_3$) = 652 nm, $\Phi_f$ = 0.45.

**Squaraine Rotaxane 39.** In a 100 mL syringe pyridine-2,6-dicarbonyl dichloride **31** (1.47 g, 723 mmol, Aldrich) was dissolved in anhydrous $CHCl_3$ (70 mL). In a second 100 mL syringe 1,4-xylylenediamine **32** (952 mg, 6.99 mmol, Alfa Aesar) and triethylamine (3.0 mL, 22 mmol, Sigma-Aldrich) were dissolved in anhydrous $CHCl_3$ (67 mL). Each syringe was added dropwise over 8 hours, using a mechanical syringe pump to a solution of squaraine dye **38** (769 mg, 0.723 mmol) in anhydrous $CHCl_3$ (25 mL). The reaction mixture was filtered through celite® 545 to remove any polymeric material and condensed. The crude product was purified by column chromatography using a column of silica gel with 0 to 5% MeOH in $CHCl_3$ as the eluent to give pure **39** a blue solid (401 mg, 35%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 1.14 (t, $J$ = 10.0, 6H), 1.45 (s, 18H), 3.42-3.47 (m, 4H), 3.54 (t, $J$ = 5.5 Hz, 4H), 3.60-3.70 (m, 20H), 3.88 (t, $J$ = 5.0 Hz, 4H), 3.99 (s, 4H), 4.52 (d, $J$ = 5.5 Hz, 8H), 4.56 (t, $J$ = 5.0 Hz, 4H), 4.62 (s, 4H), 6.17 (d, $J$ = 9.0 Hz, 4H), 6.60 (s, 8H), 7.78 (s, 2H), 8.07 (d, $J$ = 9.0 Hz, 4H), 8.14 (t, $J$ = 8.0 Hz, 2H), 8.50 (d, $J$ = 8.0 Hz, 4H), 10.03 (t, $J$ = 6.0 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 12.1, 28.0, 43.3, 46.2, 50.0, 50.2, 64.4, 67.6, 68.9, 69.4, 70.39, 70.42, 70.45, 70.6, 81.6, 111.5, 118.9, 123.9, 125.1, 128.9, 133.5, 138.8, 144.1, 149.4, 153.4, 163.5, 169.5, 184.2, 185.1, 187.3; HRMS (ESI-TOF): calculated for $C_{84}H_{105}N_{14}O_{18}$ $[M+H]^+$ 1597.7726, found 1597.7689; $\lambda_{max}$ (abs, $CHCl_3$) = 640 nm, log ε = 5.19, $\lambda_{max}$ (em, $CHCl_3$) = 663 nm, $\Phi_f$ = 0.44.

**Squaraine Rotaxane 2.** Squaraine Rotaxane **39** (398 mg, 249 μmol) was dissolved in $CH_2Cl_2$ (5 mL) and TFA (1.0 mL, 13 mmol, Sigma-Aldrich), and then stirred for 8 h. The reaction was condensed then washed with diethyl ether to produce **2** a blue solid (414 mg, 97%): $^1$H-NMR (500 MHz, $CD_3OD$): δ 1.12 (t, $J$ = 7.0 Hz, 6H), 3.63 (m, 28H), 3.89 (s, 8H), 4.51 (d, $J$ = 5.5 Hz, 8H), 4.59 (m, 8H), 6.29 (d, $J$ = 9.0 Hz, 4H), 6.57 (s, 8H), 8.02 (d, $J$ = 9.0 Hz, 4H), 8.05 (s, 2H), 8.32 (m, 2H), 8.49 (d, $J$ = 8.0 Hz, 4H), 10.17 (t, $J$ = 6.0 Hz, 4H); $^{13}$C-NMR (125 MHz, $CD_3OD$): δ 12.5, 15.6, 44.3, 47.4, 51.7, 52.4, 65.1, 67.1, 69.0, 70.5, 71.5, 71.6, 71.7, 71.9, 72.0, 111.3, 120.1, 126.3, 126.7, 130.1, 134.7, 138.0, 141.1, 150.6, 155.4, 165.2, 172.8, 187.1; HRMS (ESI-TOF): calculated for $C_{76}H_{88}N_{14}NaO_{18}$ $[M+Na]^+$ 1507.6293, found 1507.6324; $\lambda_{max}$ (abs, $H_2O$) = 655 nm, log ε = 5.06, $\lambda_{max}$ (em, $H_2O$) = 677 nm, $\Phi_f$ = 0.16, $\lambda_{max}$ (abs, PBS) = 655 nm, log ε = 5.05, $\lambda_{max}$ (em, PBS) = 676 nm, $\Phi_f$ = 0.16, $\lambda_{max}$ (abs, 10% FBS) = 655 nm, log ε = 5.02, $\lambda_{max}$ (em, 10% FBS) = 675 nm.




**41.** The previously known compounds **40**[S2] (2.21 g, 8.59 mmol) and **23**[S3] (1.25 g, 8.23 mmol) were refluxed in benzene (40 mL) for 16 h. The solvent was removed and the crude material was subjected to column chromatography using a silica gel column with $CH_2Cl_2$ eluent to yield **41** a yellow oil (369 mg, 12%): $^1$H-NMR (300 MHz, $CDCl_3$): δ 2.45 (t, $J$ = 2.5, 2H), 3.71 (s, 8H), 4.13 (d, $J$ = 2.0 Hz, 4H), 6.75 (d, $J$ = 9.5 Hz, 2H), 7.96 (d, $J$ = 9.0 Hz, 2H); $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 50.6, 58.1, 66.6, 74.6, 79.0, 111.6, 114.0, 131.0, 152.9, 171.4, 185.5, 189.5, 195.3; HRMS (ESI-TOF): calculated for $C_{20}H_{19}ClNO_4$ $[M+H]^+$ 372.1003, found 372.0997.

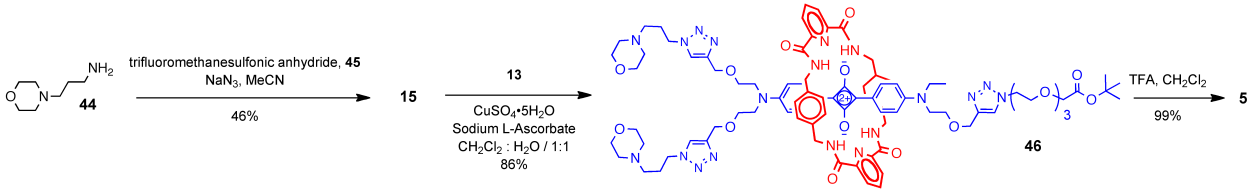
**12.** Compound **41** (364 mg, 979 µmol) was refluxed in water (60 mL), acetic acid (20 mL), and 5M HCl (20 mL) for 18 h. The hot reaction was poured over ice and allowed to warm to room temp then filtered. The filter paper was allowed to dry, then rinsed with acetone to remove the brown oil that was condensed to yield **12** (346 mg, quantitative yield): $^1$H-NMR (300 MHz, DMSO-$d_6$): δ 3.45 (t, $J$ = 2.5, 2H), 3.61 (s, 8H), 4.16 (d, $J$ = 2.5 Hz, 4H), 6.82 (d, $J$ = 9.0 Hz, 2H), 7.82 (d, $J$ = 9.0 Hz, 2H); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): δ 49.9, 57.7, 66.7, 77.3, 80.2, 111.5, 127.2, 144.4, 162.7, 185.5, 195.3; HRMS (ESI-TOF): calculated for $C_{20}H_{20}NO_5$ $[M+H]^+$ 354.1336, found 354.1315.

**Squaraine Dye 42.** A solution of **12** (350 mg, 990 µmol), **9** (800 mg, 1.63 mmol), and tri-*n*-butyl orthoformate (1 mL, Alfa Aesar) in 2-propanol (50 mL) was allowed to reflux for 12 h. The crude product was condensed and purified by column chromatography using a silica gel column with 0 to 2% MeOH in $CHCl_3$ eluent to yield **42** a green-blue solid (328 mg, 40%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 1.23 (t, $J$ = 10.0 Hz, 3H), 1.46 (s, 9H), 2.44 (t, $J$ = 2.5, 2H), 3.56-3.69 (m, 12H), 3.76-3.79 (m, 10H), 3.85 (t, $J$ = 5.0 Hz, 2H), 4.00 (s, 2H), 4.16 (d, $J$ = 2.5 Hz, 4H), 4.53 (t, $J$ = 5.0 Hz, 2H), 4.64 (s, 2H), 6.79 (dd, $J$ = 15.0, 9.0 Hz, 4H), 7.70 (s, 1H), 8.36 (d, $J$ = 9.0 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 12.5, 28.3, 46.6, 50.5, 51.6, 58.8, 64.8, 67.4, 67.9, 69.1, 69.6, 70.68, 70.72, 70.87, 75.2, 79.4, 112.7, 120.0, 124.1, 133.3, 133.8, 144.4, 153.6, 154.0, 183.5, 188.4, 189.7; HRMS (ESI-TOF): calculated for $C_{45}H_{58}N_5O_{10}$ $[M+H]^+$ 828.4178, found 828.4163; $\lambda_{max}$ (abs, $CHCl_3$) = 631 nm, log ε = 5.65, $\lambda_{max}$ (em, $CHCl_3$) = 651 nm, $\Phi_f$ = 0.43.

**Squaraine Rotaxane 13.** In a 100 mL syringe pyridine-2,6-dicarbonyl dichloride **31** (1.13 mg, 5.54 mmol, Aldrich) was dissolved in anhydrous $CHCl_3$ (50 mL). In a second 100 mL syringe 1,4-xylylenediamine **32** (810 mg, 5.95 mmol, Alfa Aesar) and triethylamine (1.0 mL, 7.16 mmol, Sigma-Aldrich) were dissolved in anhydrous $CHCl_3$ (49 mL). Each syringe was added dropwise over 8 hours, using a mechanical syringe pump to a solution of squaraine dye **42** (525 mg, 0.634 mmol) in anhydrous $CHCl_3$ (50 mL). The reaction mixture was filtered through celite to remove any polymeric material and condensed. The crude product was purified by column chromatography using a column of silica gel with 0 to 1% MeOH in $CHCl_3$ as the eluent to give pure **13** (440 mg, 51%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 1.21 (t, $J$ = 7.0 Hz, 3H), 1.47 (s, 9H), 2.51 (t, $J$ = 2.5 Hz, 2H), 3.52 (q, $J$ = 7.0 Hz, 2H), 3.61-3.64 (m, 14H), 3.65-3.72 (m, 4H), 3.74 (t, $J$ = 6.0 Hz, 2H), 3.90 (t, $J$ = 5.0 Hz, 2H), 4.01 (s, 2H), 4.15 (d, $J$ = 2.5 Hz, 4H), 4.35 (dd, $J$ = 14.5, 4.5 Hz, 4H), 4.58 (t, $J$ = 5.0 Hz, 2H), 4.66 (s, 2H), 4.72 (dd, $J$ = 14.5, 7.0 Hz, 4H), 6.09 (d, $J$ = 9.5 Hz, 2H), 6.36 (d, $J$ = 9.5 Hz, 2H), 6.61 (d, $J$ = 28.0 Hz, 8H), 7.80 (s, 1H), 8.05 (d, $J$ = 9.0 Hz, 2H), 8.14-8.17 (m, 4H), 8.52 (d, $J$ = 8.0 Hz, 4H), 10.02 (t, $J$ = 6.5 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 12.2, 28.1, 43.3, 50.3, 51.2, 58.5, 64.5, 66.9, 67.6, 68.9, 69.4, 70.45, 70.51, 70.7, 75.1, 79.1, 111.6, 111.9, 119.0, 124.0, 125.2, 128.9, 133.4, 133.7, 136.6, 138.8, 144.1, 149.5, 152.5, 163.6, 169.5, 184.3, 185.1; HRMS (ESI-TOF): calculated for $C_{75}H_{84}N_{11}O_{14}$ $[M+H]^+$ 1362.6194, found 1362.6203; $\lambda_{max}$ (abs, $CHCl_3$) = 641 nm, log ε = 5.46, $\lambda_{max}$ (em, $CHCl_3$) = 661 nm, $\Phi_f$ = 0.45.

**Squaraine Rotaxane 43.** A mixture of **13** (15.33 mg, 11.25 µmol) and the previously known compound **14**[S2] (22.34 mg, 65.2 µmol) was dissolved in $CH_2Cl_2$ (3mL). To this a solution of $CuSO_4$•$5H_2O$ (19.51 mg, Aldrich) and sodium L-ascorbate (23.34 mg, Sigma) in water (3 mL) was added. The reaction was stirred at room temperature over 16 h, then extraction with $CHCl_3$ and water. The organic layer was washed with EDTA, dried with $MgSO_4$, and condensed. The crude product was purified by column chromatography using a silica gel column with 0 to 2% MeOH in $CHCl_3$ eluent to give **43** a blue solid (13.6 mg, 59%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 1.15 (t, *J* = 7.0 Hz, 3H), 1.47 (s, 9H), 1.50 (d, *J* = 2.5 Hz, 36H), 2.21 (quin, *J* = 6.5 Hz, 4H), 3.44-3.50 (m, 6H), 3.55 (br. s., 2H), 3.62-3.71 (m, 18H), 3.90 (t, *J* = 5.0 Hz, 2H), 4.01 (s, 2H), 4.46-4.50 (m, 8H), 4.57-4.63 (m, 12H), 6.13 (d, *J* = 9.0 Hz, 2H), 6.26 (d, *J* = 9.0 Hz, 2H), 6.61 (s, 8H), 7.80 (br. s., 1H), 8.00 (br. s., 2H), 8.08 (dd, *J* = 9.0 Hz, *J* = 3.5 Hz, 4H), 8.16 (t, *J* = 8.0 Hz, 2H), 8.48-8.52 (m, 6H), 10.03 (t, *J* = 6.0 Hz, 4H), 11.47 (s, 2H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 12.2, 28.0, 28.3, 29.7, 30.5, 37.4, 43.3, 47.6, 51.2, 64.5, 67.6, 68.9, 69.4, 70.45, 70.47, 70.51, 70.7, 79.4, 81.6, 83.4, 111.5, 111.8, 118.9, 119.2, 125.2, 128.9, 133.3, 133.7, 136.6, 138.9, 149.5, 153.2, 153.6, 156.6, 163.6, 169.5, 184.2, 184.9, 185.1; HRMS (ESI-TOF): calculated for $C_{103}H_{135}N_{23}Na_2O_{22}$ [*M*+2Na]$^+$ 2091.9942, found 2091.9945; $\lambda_{max}$ (abs, $CHCl_3$) = 643 nm, log ε = 5.49, $\lambda_{max}$ (em, $CHCl_3$) = 662 nm, $\Phi_f$ = 0.45.

**Squaraine Rotaxane 4.** Squaraine Rotaxane **43** (12.2 mg, 5.96 µmol) was dissolved in $CH_2Cl_2$ (5 mL) and TFA (1.0 mL 13 µmol, Sigma-Aldrich), and then stirred for 8 h. The reaction was condensed then washed with diethyl ether to produce **4** a blue solid (11.4 mg, 99%): $^1$H-NMR (500 MHz, $CD_3OD$): δ 1.18 (t, *J* = 7.0 Hz, 3H), 2.17 (quin, *J* = 7.0 Hz, 4H), 3.21 (t, *J* = 7.0 Hz, 6H), 3.35 (s, 2H), 3.51-3.73 (m, 18H), 3.89 (t, *J* = 5.0 Hz, 2H), 4.12 (s, 2H), 4.31 (dd, *J* = 14.5 Hz, *J* = 5.0 Hz, 4H), 4.49 (t, *J* = 7.0 Hz, 4H), 4.54 (s, 4H), 4.59 (t, *J* = 4.5 Hz, 2H), 4.62 (s, 2H), 4.70 (dd, *J* = 14.5 Hz, *J* = 7.0 Hz, 4H), 6.08 (d, *J* = 9.0 Hz, 2H), 6.54 (d, *J* = 9.0 Hz, 10H), 7.93 (d, *J* = 9.0 Hz, 2H), 7.99 (s, 3H), 8.08 (t, *J* = 9.0 Hz, 2H), 8.31 (t, *J* = 8.0 Hz, 2H), 8.48 (d, *J* = 8.0 Hz, 4H), 10.15 (t, *J* = 7.0 Hz, 4H); $^{13}$C-NMR: 30.5, 30.9, 39.7, 44.4, 65.1, 66.7, 69.0, 70.47, 70.53, 71.6, 80.5, 82.3, 91.2, 91.8, 111.3, 113.8, 119.4, 120.1, 126.7, 130.1, 133.6, 135.1, 138.0, 149.0, 154.4, 157.7, 164.8, 168.2, 185.6, 186.4; HRMS (ESI-TOF): calculated for $C_{79}H_{96}N_{23}O_{14}$ [*M*+H]$^+$ 1590.7502, found 1590.7536; $\lambda_{max}$ (abs, $H_2O$) = 654 nm, log ε = 5.09, $\lambda_{max}$ (em, $H_2O$) = 675 nm, $\Phi_f$ = 0.22, $\lambda_{max}$ (abs, PBS) = 654 nm, log ε = 5.07, $\lambda_{max}$ (em, PBS) = 673 nm, $\Phi_f$ = 0.23, $\lambda_{max}$ (abs, 10% FBS) = 654 nm, log ε = 5.05, $\lambda_{max}$ (em, 10% FBS) = 672 nm.

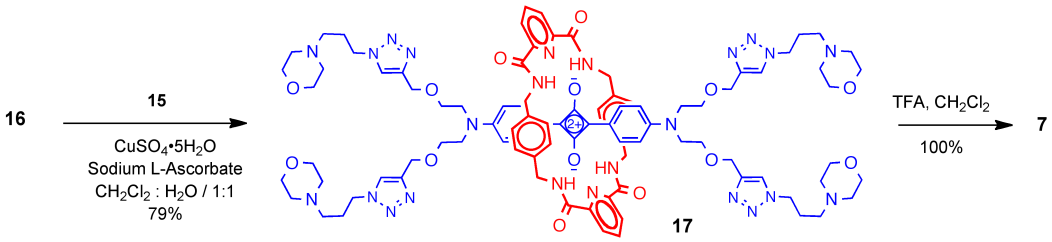



**15.** Compound **45** (2.00 mL, 11.9 mmol) was added dropwise to a mixture of sodium azide (1.07 g, 16.5 mmol, Sigma-Aldrich) in acetonitrile (35 mL) at 0 ºC. The reaction was allowed to warm to room temperature and after 2 h filtered to remove salts. The solution was again cooled to 0 ºC and the commercially available compound **44** (1.04 g, 7.21 mmol, Aldrich) was added dropwise and stirred for 16 h at room temperature. The reaction was extracted with $CHCl_3$ and water, the organic layer was condensed to yield **15** a yellow oil (566 mg, 46%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 1.74 (quin, *J* = 7.0 Hz, 2H), 2.40-2.44 (m, 6H), 3.32 (t, *J* = 7.0 Hz, 2H), 3.68 (t, *J* = 4.5 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 25.6, 49.2, 53.4, 55.5 66.6; HRMS (ESI-TOF): calculated for $C_7H_{15}N_4O$ [*M*+H]$^+$ 171.1240, found 171.1253.

**Squaraine Rotaxane 46.** A mixture of **13** (33.4 mg, 24.5 µmol) and **15** (29.84 mg, 175.3 µmol) was dissolved in $CH_2Cl_2$ (3mL). To this a solution of $CuSO_4$•$5H_2O$ (27.90 mg, Aldrich) and sodium L-ascorbate (31.55 mg, Sigma) in water (3 mL) was added. The reaction was stirred at room temperature over 16 h, then extraction with $CHCl_3$ and water. The organic layer was washed with EDTA, dried with $MgSO_4$, and condensed. The crude product was purified by column chromatography using a silica gel column with 0 to 15% MeOH in $CHCl_3$ eluent to give **46** a blue solid (35.8 mg, 86%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 1.19 (t, *J* = 7.0 Hz, 3H), 1.46 (s, 9H), 2.12 (t, *J* = 6.5 Hz, 4H), 2.40-2.44 (m, 12H), 3.50 (t, *J* = 6.0 Hz, 2H), 3.59-3.73 (m, 28H), 3.90 (t, *J* = 5.0 Hz, 2H), 4.01 (s, 2H), 4.41 (dd, *J* = 14.0, 5.0 Hz, 4H), 4.47 (t, *J* = 6.5 Hz, 4H), 4.57-4.68 (m, 12H), 6.08 (d, *J* = 9.0 Hz, 2H), 6.30 (d, *J* = 9.0 Hz, 2H), 6.59 (d, *J* = Hz, 8H), 7.68 (br. s., 2H), 7.81 (br. s., 1H), 8.04 (d, *J* = 9.0 Hz, 2H), 8.11-8.17 (m, 4H), 8.51 (d, *J* = 8.0 Hz, 4H), 10.02 (t, *J* = 5.5 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 12.2, 26.9 28.1, 43.3, 48.3, 50.2, 51.1, 53.5,

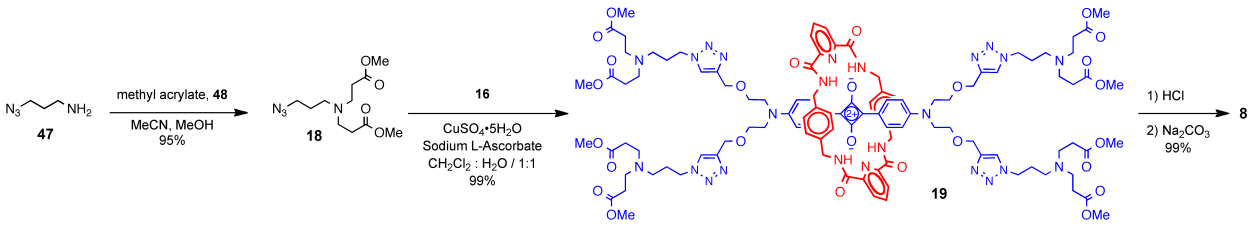
55.1, 66.7, 67.6, 67.7, 68.9, 69.4, 70.43, 70.45, 70.48, 70.6, 81.6, 111.5, 111.8, 119.0, 119.2, 125.2, 128.9, 133.3, 133.7, 136.6, 138.9, 149.5, 153.8, 163.5, 184.1, 185.1; HRMS (ESI-TOF): calculated for $C_{89}H_{112}N_{19}O_{16}$ $[M+H]^+$ 1702.8490, found 1702.8557; $\lambda_{max}$ (abs, $CHCl_3$) = 644 nm, log ε = 5.51, $\lambda_{max}$ (em, $CHCl_3$) = 662 nm, $\Phi_f$ = 0.47.

**Squaraine Rotaxane 5.** Squaraine Rotaxane **46** (20.7 mg, 12.2 µmol) was dissolved in $CH_2Cl_2$ (5 mL) and TFA (1.0 mL, 13 mmol, Sigma-Aldrich), and then stirred for 8 h. The reaction was condensed then washed with diethyl ether to produce **5** a blue solid (23.9 mg, 99%): $^1$H-NMR (500 MHz, $CD_3OD$): δ 1.13 (t, *J* = 7.0 Hz, 3H), 2.40 (br. s., 4H), 3.22-3.25 (m, 12H), 3.35 (s, 2H), 3.59-3.70 (m, 28 H), 3.91 (br. s., 2H), 4.13 (s, 2H), 4.30 (dd, *J* = 14.5 Hz, *J* = 4.0 Hz, 4H), 4.56-4.60 (m, 12H), 4.71 (dd, *J* = 14.0, 7.0 Hz, 4H), 6.07 (d, *J* = 8.0 Hz, 2H), 6.55 (br. s., 10H), 7.41 (br. s., 2H), 7.54 (s, 1H), 7.93 (d, *J* = 8.5 Hz, 2H), 8.09 (d, *J* = 8.5 Hz, 2H), 8.19 (br. s., 2H), 8.32 (t, 2H), 8.47 (d, *J* = 8.0 Hz, 4H), 10.14 (t, *J* = 5.5 Hz, 4H); $^{13}$C-NMR: 25.5, 44.2, 53.4, 55.8, 65.2, 69.1, 70.4, 71.5, 71.6, 71.7, 71.9, 112.3, 112.9, 120.1, 125.2, 126.7, 129.3, 130.1, 134.5, 134.9, 138.1, 141.1, 150.6, 157.4, 157.6, 165.3, 187.1; HRMS (ESI-TOF): calculated for $C_{85}H_{104}N_{19}O_{16}$ $[M+H]^+$ 1646.7864, found 1646.8011; $\lambda_{max}$ (abs, $H_2O$) = 654 nm, log ε = 5.25, $\lambda_{max}$ (em, $H_2O$) = 675 nm, $\Phi_f$ = 0.21, $\lambda_{max}$ (abs, PBS) = 653 nm, log ε = 5.23, $\lambda_{max}$ (em, PBS) = 674 nm, $\Phi_f$ = 0.26, $\lambda_{max}$ (abs, 10% FBS) = 654 nm, log ε = 5.24, $\lambda_{max}$ (em, 10% FBS) = 673 nm.




**Squaraine Rotaxane 17.** A mixture of the previously published squaraine rotaxane **16**[S2] (9.54 mg, 8.46 µmol) and **15** (12.78 mg, 75.08 µmol) was dissolved in $CH_2Cl_2$ (5mL). To this a solution of $CuSO_4$•$5H_2O$ (31.87 mg, Aldrich) and sodium L-ascorbate (36.08 mg, Sigma) in water (5 mL) was added. The reaction was stirred at room temperature over 16 h, then extraction with $CHCl_3$ and water. The organic layer was washed with EDTA, dried with $MgSO_4$, and condensed. The crude product was purified by column chromatography using a silica gel column with 0 to 15% $NH_4OH$ in MeCN eluent to give **17** a blue solid (12.1 mg, 79%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 2.08 (quin, *J* = 7.0 Hz, 8H), 2.34 (t, *J* = 7.0 Hz, 12H), 2.39 (br. s., 12H), 3.60 - 3.74 (m, 32H), 4.44 (t, *J* = 7.0 Hz, 8H), 4.52 (d, *J* = 6.0 Hz, 8H), 4.58 (s, 8H), 6.20 (d, *J* = 9.0 Hz, 4H), 6.58 (s, 8H), 7.63 (s, 4H), 8.07 (d, *J* = 9.0 Hz, 4H), 8.14 (t, *J* = 8.0 Hz, 2H), 8.49 (d, *J* = 8.0 Hz, 4H), 10.00 (t, *J* = 5.5 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 26.4, 29.7, 43.3, 48.2, 51.3, 53.5, 55.1, 64.5, 66.7, 67.7, 111.8, 119.3, 125.0, 128.9, 133.5, 136.6, 138.9, 149.5, 153.9, 163.6, 185.1; HRMS (ESI-TOF): calculated for $C_{94}H_{119}N_{24}O_{14}$ $[M+H]^+$ 1807.9293, found 1807.9522; $\lambda_{max}$ (abs, $CHCl_3$) = 643 nm, log ε = 5.32, $\lambda_{max}$ (em, $CHCl_3$) = 661 nm, $\Phi_f$ = 0.44.

**Squaraine Rotaxane 7.** Squaraine Rotaxane **17** (9.40 mg, 5.21 µmol) was dissolved in $CH_2Cl_2$ (5 mL) and TFA (1.0 mL, 13 mmol, Sigma-Aldrich), and then stirred for 8 h. The reaction was condensed then washed with diethyl ether to produce **7** a blue solid (11.65 mg, 99%): $^1$H-NMR (500 MHz, $CD_3OD$): δ 2.39 (br. s., 8H), 3.19 - 3.25 (m, 24H), 3.65 (br. s., 32H), 4.50 (d, *J* = 5.5 Hz, 8H), 4.55 (d, *J* = 7.5 Hz, 16H), 6.32 (d, *J* = 9.0 Hz, 4H), 6.53 (s, 8H), 7.99 - 8.05 (m, 8H), 8.31 (t, *J* = 8.0 Hz, 2H), 8.46 (d, *J* = 8.0 Hz, 4H), 10.11 (t, *J* = 6.0 Hz, 4H); $^{13}$C-NMR (125 MHz, $CD_3OD$): δ 25.6, 27.9, 29.4, 29.8, 30.1, 31.2, 35.5, 44.3, 48.5, 53.4, 55.8, 59.7, 65.2, 69.1, 72.3, 109.2, 117.1, 124.5, 127.6, 131.4, 138.1, 142.5, 145.4, 150.1, 161.2, 175.4, 179.2; HRMS (ESI-TOF): calculated for $C_{94}H_{119}N_{24}O_{14}$ $[M+H]^+$ 1807.9293, found 1807.9322; $\lambda_{max}$ (abs, $H_2O$) = 652 nm, log ε = 5.25, $\lambda_{max}$ (em, $H_2O$) = 673 nm, $\Phi_f$ = 0.23, $\lambda_{max}$ (abs, PBS) = 652 nm, log ε = 5.20, $\lambda_{max}$ (em, PBS) = 672 nm, $\Phi_f$ = 0.24, $\lambda_{max}$ (abs, 10% FBS) = 652 nm, log ε = 5.24, $\lambda_{max}$ (em, 10% FBS) = 672 nm.




**18.** The commercially available compound **48** (2.00 mL, 22.0 mmol, Aldrich) in MeOH (20 mL) was cooled to 0 ºC. To this reaction a solution of the previously known compound **47**[S2] (1.03g, 10.3 mmol) in MeOH (8 mL) was added dropwise and the reaction allowed to warm to room temperature and stir for 48 h. The crude product was purified by column chromatography using a silica gel column with 0 to 50% EtOAc in hexane eluent to give **18** a yellow oil (2.66 g, 95%): $^1$H-NMR (300 MHz, $CDCl_3$): δ 1.64 (quin, $J$ = 6.5 Hz, 2H), 2.36-2.45 (m, 6H), 2.68 (t, $J$ = 7.0 Hz, 4H), 3.26 (t, $J$ = 6.5 Hz, 2H), 3.61 (s, 6H); $^{13}$C-NMR (75 MHz, $CDCl_3$): δ 26.5, 32.4, 49.0, 49.1, 50.3, 51.3, 172.7; HRMS (ESI-TOF): calculated for $C_{11}H_{21}N_4O_4$ $[M+H]^+$ 273.1557, found 273.1545.

**Squaraine Rotaxane 19.** A mixture of the previously published squaraine rotaxane **16**[S2] (46.20 mg, 41.0 µmol) and **18** (141.78 mg, 521 µmol) was dissolved in $CH_2Cl_2$ (6mL). To this a solution of $CuSO_4$•$5H_2O$ (47.48 mg, Aldrich) and sodium L-ascorbate (60.23 mg, Sigma) in water (6 mL) was added. The reaction was stirred at room temperature over 16 h, then extraction with $CHCl_3$ and water. The organic layer was washed with EDTA, dried with $MgSO_4$, and condensed. The crude product was purified by column chromatography using a silica gel column with 0 to 2% MeOH in $CHCl_3$ eluent to give **19** a blue solid (89.9 mg, 99%): $^1$H-NMR (500 MHz, $CDCl_3$): δ 2.07 (quin, $J$ = 6.5 Hz, 8H), 2.43 (q, $J$ = 7.0 Hz, 24H), 2.74 (t, $J$ = 7.0 Hz, 16H), 3.67 (s, 40H), 4.38 (t, $J$ = 7.0 Hz, 8H), 4.54 (d, $J$ = 5.5 Hz, 8H), 4.61 (s, 8H), 6.22 (d, $J$ = 9.0 Hz, 4H), 6.61 (s, 8H), 7.76 (s, 4H), 8.09 (d, $J$ = 9.0 Hz, 4H), 8.16 (t, $J$ = 8.0 Hz, 2H), 8.51 (d, $J$ = 8.0 Hz, 4H), 10.02 (t, $J$ = 5.5 Hz, 4H); $^{13}$C-NMR (125 MHz, $CDCl_3$): δ 28.2, 29.7, 32.4, 43.3, 48.0, 49.1, 50.4, 51.2, 51.6, 64.5, 67.5, 111.8, 119.2, 123.2, 125.2, 128.9, 130.8, 133.5, 136.6, 138.9, 144.1, 149.5, 153.9, 163.5, 172.9, 184.9, 185.1; HRMS (ESI-TOF): calculated for $C_{110}H_{143}N_{24}O_{26}$ $[M+H]^+$ 2216.0600, found 2216.0623; $\lambda_{max}$ (abs, $CHCl_3$) = 643 nm, log ε = 5.24, $\lambda_{max}$ (em, $CHCl_3$) = 660 nm, $\Phi_f$ = 0.35.

**Squaraine Rotaxane 8.** Squaraine Rotaxane **19** (15.74 mg, 7.10 µmol) was dissolved in MeOH (3 mL) and 10M HCl (0.05 mL) then stirred for 8 h. The reaction was condensed to remove MeOH then dissolved in 10M HCl (2.00 mL) allowing it to stir for an additional 24 h. The reaction was neutralized with aqueous $Na_2CO_3$ then condensed, redissolved in MeOH, and then filtered and condensed again to yield **8** a blue solid (16.02 mg, 99%): $^1$H-NMR (300 MHz, $CD_3OD$:$D_2O$/1:1): δ 2.45 (br. s., 8H), 2.55 - 2.77 (m, 16H), 3.16 - 3.30 (m, 16H), 3.59 - 3.80 (m, 16H), 4.43 - 4.66 (m, 24H), 6.34 (br. s., 4H), 6.55 (s, 8H), 8.00 (d, $J$ = 7.5 Hz, 4H), 8.07 (s, 4H), 8.36 (t, $J$ = 7.5 Hz, 2H), 8.49 (d, $J$ = 7.5 Hz, 4H), 10.09 (t, $J$ = 6.0 Hz, 4H); $^{13}$C-NMR (125 MHz, $CD_3OD$): δ 25.7, 29.6, 29.7, 44.4, 50.9, 51.1, 53.3, 64.7, 69.7, 114.0, 120.6, 124.4, 126.9, 127.6, 130.2, 132.4, 134.7, 138.1, 144.8, 150.5, 155.8, 165.2, 172.5, 173.7, 173.8, 184.3, 186.9; HRMS (ESI-TOF): calculated for $C_{102}H_{127}N_{24}O_{26}$ $[M+H]^+$ 2103.9309, found 2103.9348; $\lambda_{max}$ (abs, $H_2O$) = 653 nm, log ε = 5.20, $\lambda_{max}$ (em, $H_2O$) = 674 nm, $\Phi_f$ = 0.23, $\lambda_{max}$ (abs, PBS) = 653 nm, log ε = 5.22, $\lambda_{max}$ (em, PBS) = 673 nm, $\Phi_f$ = 0.26, $\lambda_{max}$ (abs, 10% FBS) = 652 nm, log ε = 5.21, $\lambda_{max}$ (em, 10% FBS) = 673 nm.

**3. Photophysical Properties**

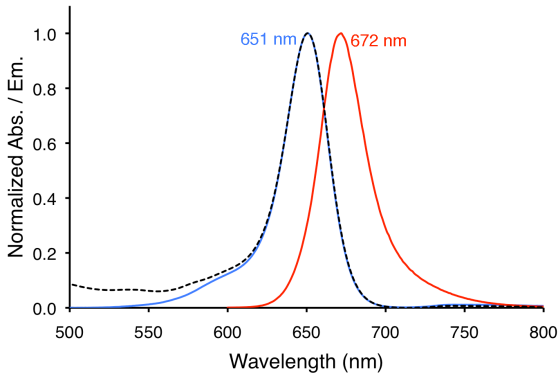
The absorption/emission values for samples in phosphate buffered saline (PBS, sodium phosphate 10 mM, NaCl 150 mM, pH 7.4) are very similar to the values in water (compare Table S1 and Table 1). The absorption maxima bands for **7** and **8** are coincident with their excitation maxima bands indicating that the compounds do not form non-fluorescent aggregates.

**Table S1**. Absorption/Emission Maxima in Phosphate Buffered Saline (PBS)

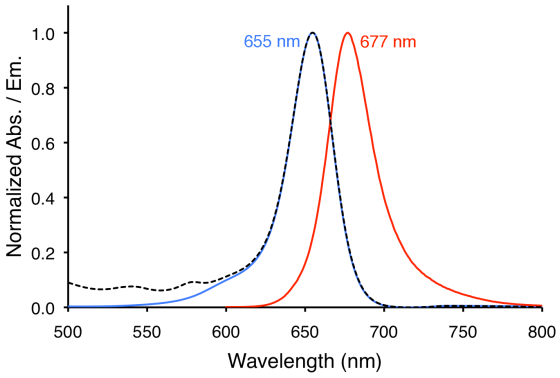
| Compound | $\lambda_{abs}$ (nm) | log ε | $\lambda_{em}$ [a] (nm) | $\Phi_f$ [b] |
|---|---|---|---|---|
| **1** | 651 | 5.57 | 672 | 0.22 |
| **2** | 655 | 5.05 | 676 | 0.16 |
| **3** | | Aggregation | | |
| **4** | 654 | 5.07 | 673 | 0.23 |
| **5** | 653 | 5.23 | 674 | 0.26 |
| **6** | 653 | 5.21 | 670 | 0.27 |
| **7** | 652 | 5.20 | 672 | 0.24 |
| **8** | 653 | 5.22 | 673 | 0.26 |

[a] Spectra were obtained in Phosphate Buffered Saline (PBS) 0.01M phosphate buffer, 0.154M sodium chloride, and pH 7.4. Solutions were excited at optically matching wavelengths and emission monitored in the region 600–900 nm. [b]Fluorescence quantum yields (error limit ± 5%) were determined using 4,4-[bis(N,N-dimethylamino)phenyl] squaraine dye as the standard ($\Phi_f = 0.70$ in $CHCl_3$).

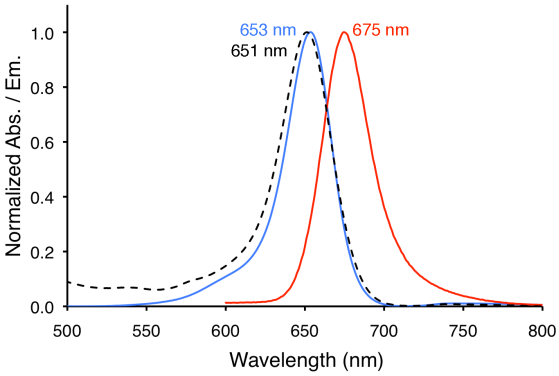
Absorption spectra were also recorded for the eight compounds in 10% FBS (fetal bovine serum) and they are shown below. In this environment, the absorption bands for **4**, **5**, and **6** showed the presence of a minor, blue shifted band at ~575 nm , which is highly characteristic of a non fluorescent H-aggregate (Chen, H.; Farahat, M. S.; Law, K.-Y.; Whitten, D. G. *J. Am. Chem. Soc.* **1996**, *118*, 2584). Thus, serum proteins promote self-aggregation of these specific dyes leading to intermolecular coupling of transition dipoles and diminished fluorescence brightness.



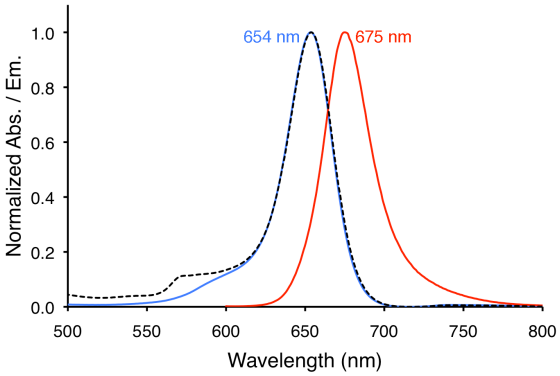

Squaraine Rotaxane **1** abs (—), em (—) in water, abs (- -) in 10% FBS



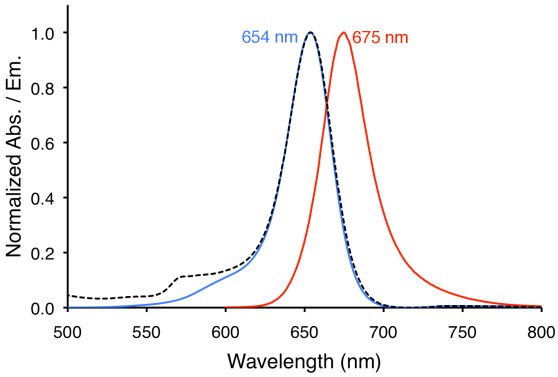

Squaraine Rotaxane **2** abs (—), em (—) in water, abs (- -) in 10% FBS



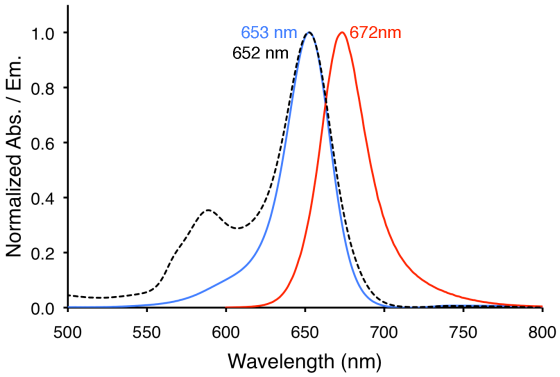

Squaraine Rotaxane **3** abs (—), em (—) in water, abs (- -) in 10% FBS



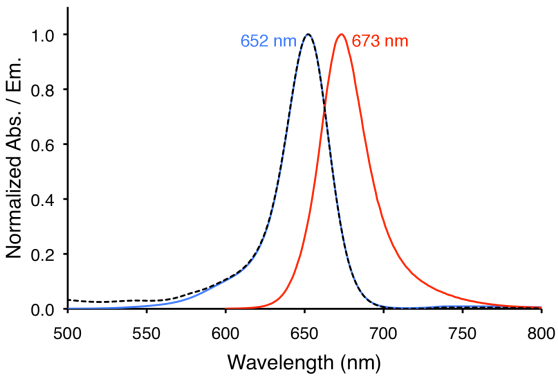

Squaraine Rotaxane **4** abs (—), em (—) in water, abs (- -) in 10% FBS



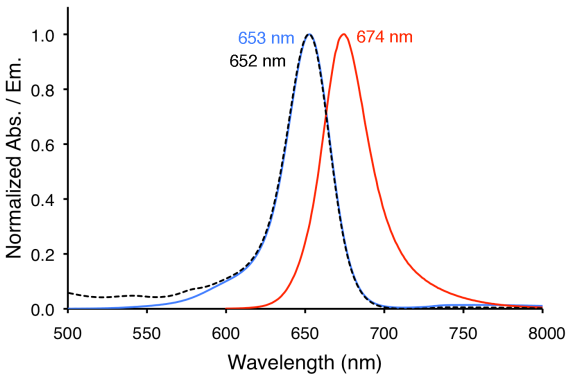

Squaraine Rotaxane **5** abs (—), em (—) in water, abs (- -) in 10% FBS



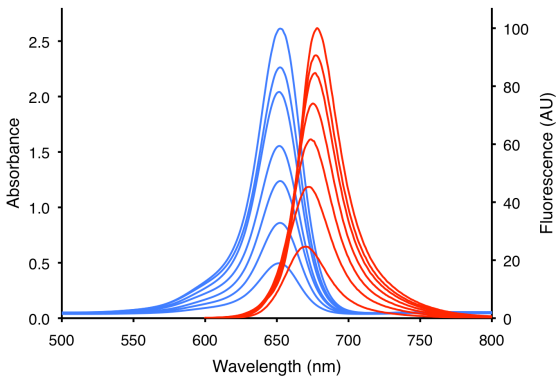

Squaraine Rotaxane **6** abs (—), em (—) in water, abs (- -) in 10% FBS



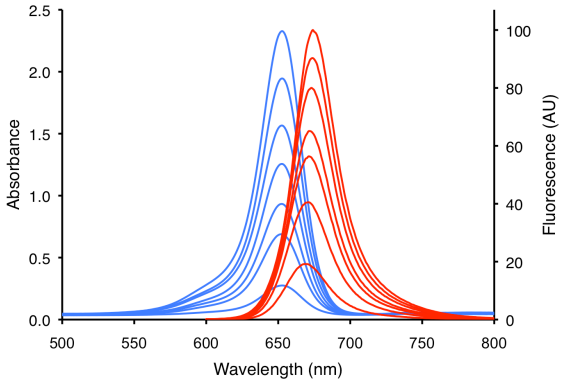

Squaraine Rotaxane **7** abs (—), em (—) in water, abs (- -) in 10% FBS




Squaraine Rotaxane **8** abs (—), em (—) in water, abs (- -) in 10% FBS




Squaraine Rotaxane **7** abs (—), em (—) in PBS, 2 to 14 μM




Squaraine Rotaxane **8** abs (—), em (—) in PBS, 2 to 14 μM

**4. Equilibrium Dialysis**

A single-use Rapid Equilibrium Dialysis (RED) device (Thermo) was used to measure the relative affinity of bovine serum albumin (BSA) for squaraine rotaxanes **7** and **8**, and control dye Indocyanine Green (**ICG**). The RED device comprised a plate that held a series of disposable dialysis cells. Each cell contained a source chamber that was separated by a porous membrane (10,000 MW cutoff) from a receiving chamber. Separate solutions of **7**, **8**, or **ICG** (10 μM), with or without BSA (10 μM), in 500 μL of phosphate buffered saline (PBS, sodium phosphate 10 mM, NaCl 150 mM, pH 7.4) were placed in the source chamber of separate dialysis cells and 750 μL of PBS was placed in the corresponding receiving chamber of each cell. The plate holding the cells was incubated at 37 °C and 100 rpm using a thermoregulated shaker. After 24 h, a 300 μL aliquot was removed from each chamber, mixed with 700 μL of PBS, and scanned at the absorption maxima of the respective dye. The various absorption spectra are shown in Fig. S1. The control dye, **ICG**, is known to decompose in water with a half-life of ~12 h at 37 °C,[S5] but the dye is protected when it associates with BSA. Thus, the spectra in Fig. S1A show that most of ICG is sequestered by the BSA in source chamber of the dialysis cell, in agreement with its known very high affinity for BSA (log $K_a$ ~ 5.5).[S6] The data in Fig. S1B and Fig. S1C show that the presence of BSA has a small sequestering effect on the equilibrium distribution of dye **7** and no measurable effect on the equilibrium distribution of dye **8** across the dialysis cell. This is strong evidence that squaraine rotaxanes **7** and **8** have low or no affinity for BSA. Furthermore, fluorescence experiments confirm that the presence of 5% BSA has essentially no effect on the quantum yield of **8** in PBS.

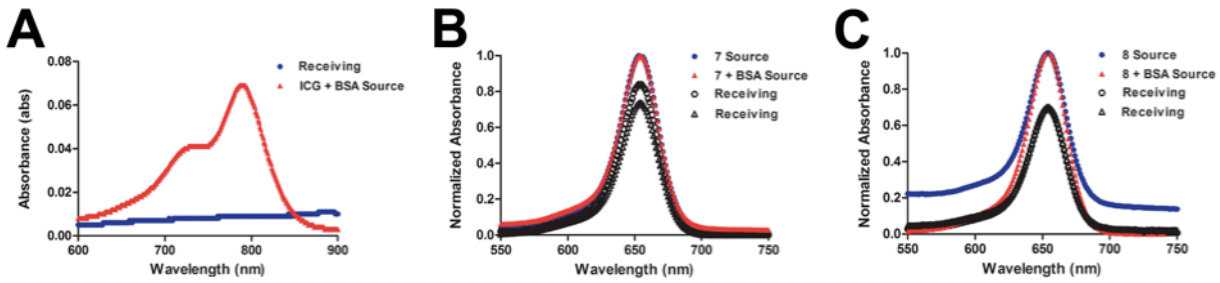
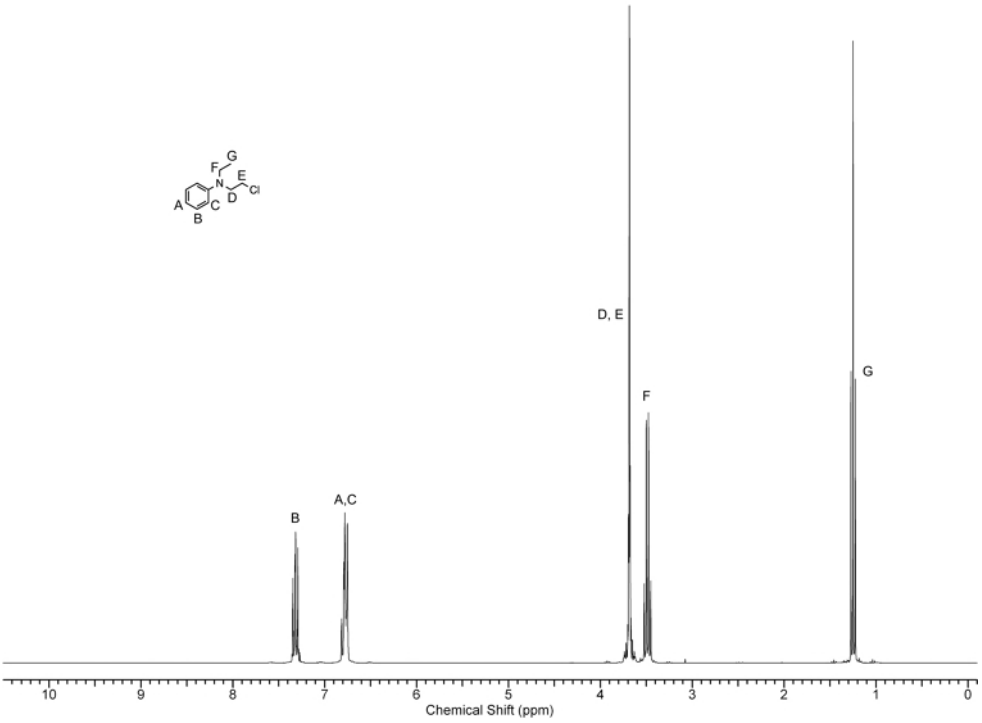


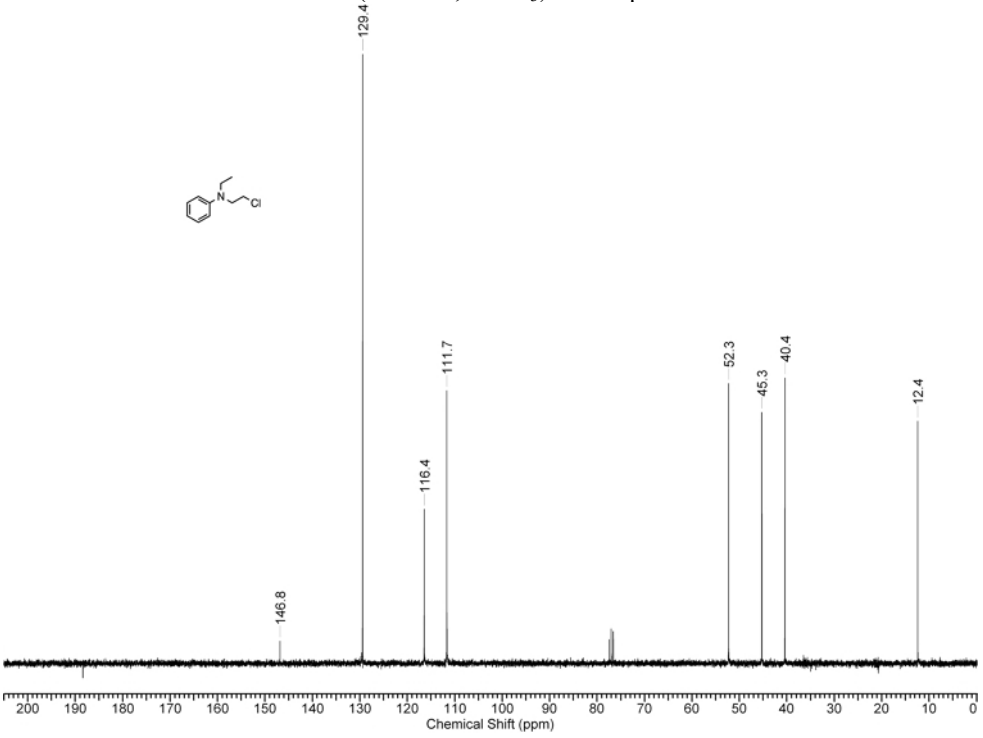

**Fig. S1.** Absorbance spectra of dye in the source and receiving chambers of the RED device after 24 h of incubation at 37 °C. (**A**) Incubation started with a mixture of **ICG** and BSA (10 μM each) in the source chamber. (**B**) Incubation started with only **7** (10 μM) in the source chamber (circles), or a mixture of **7** and BSA (10 μM each) in the source chamber (triangles). (**C**) Incubation started with only **8** (10 μM) in the source chamber (circles), or a mixture of **8** and BSA (10 μM each) in the source chamber (triangles).

## 5. $^{1}$H NMR and $^{13}$C NMR spectra

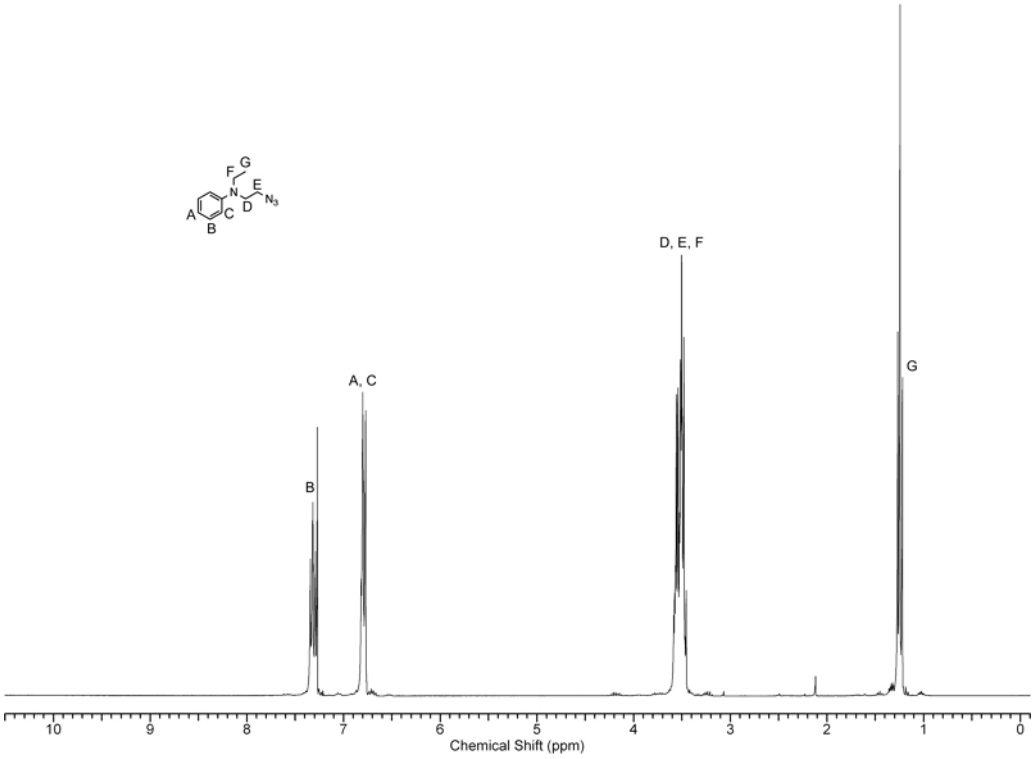
$^{1}$H NMR (300 MHz, $CDCl_3$) of compound **21**



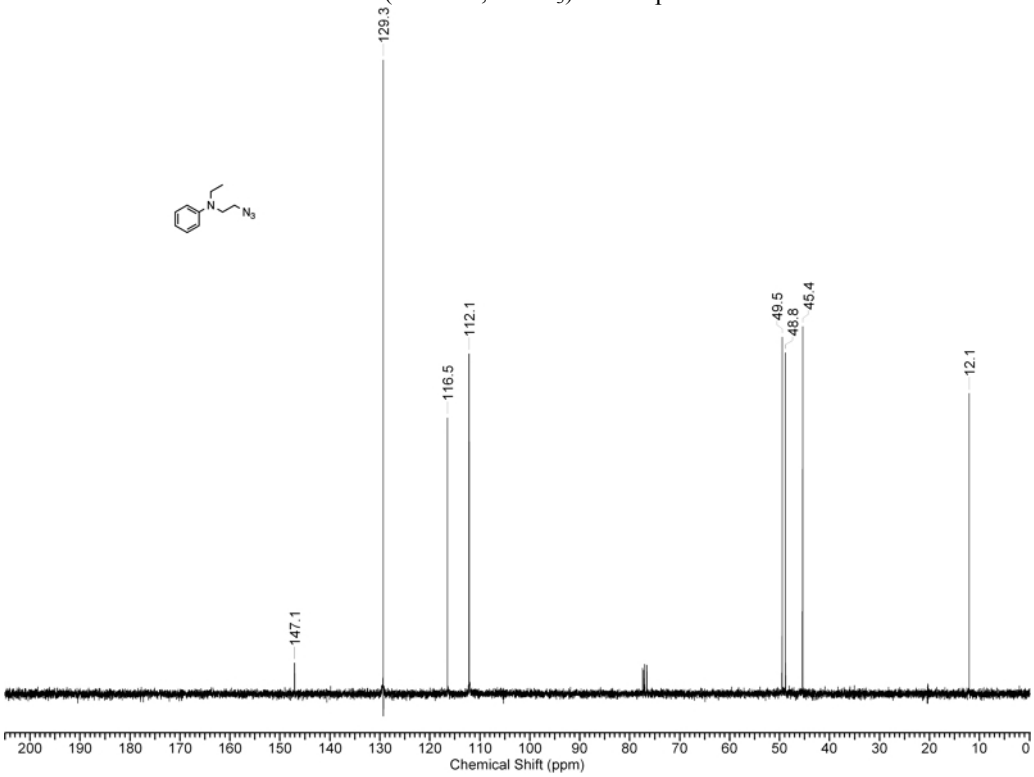

$^{13}$C NMR (75 MHz, $CDCl_3$) of compound **21**



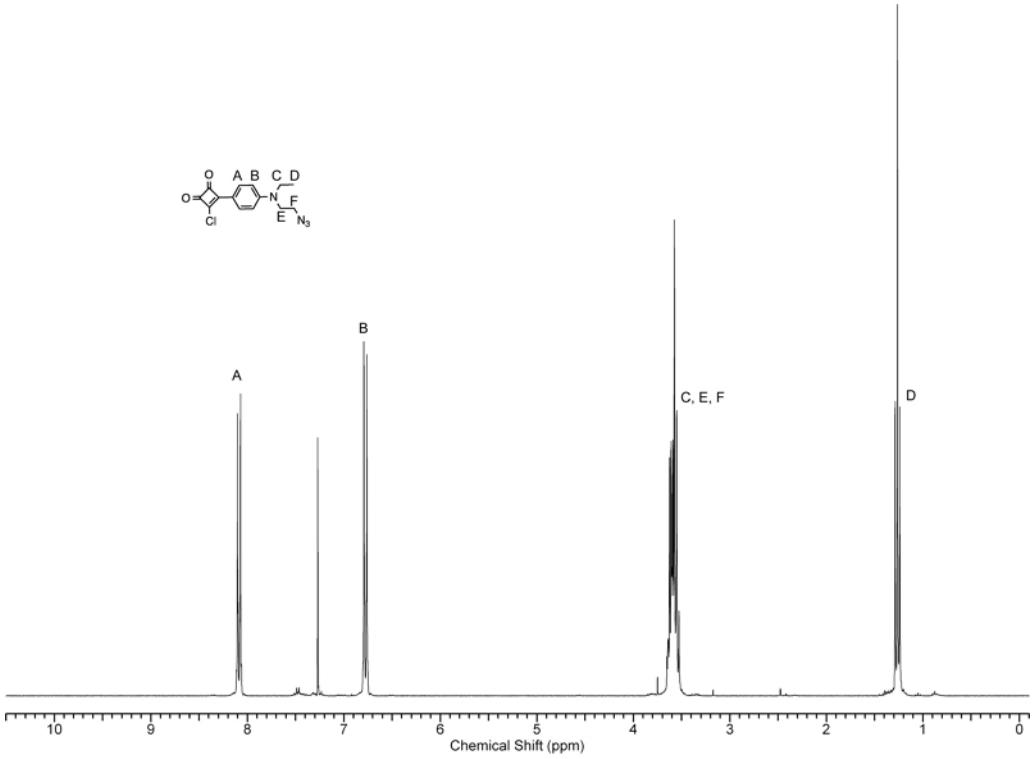
$^{1}$H NMR (300 MHz, $CDCl_3$) of compound **22**



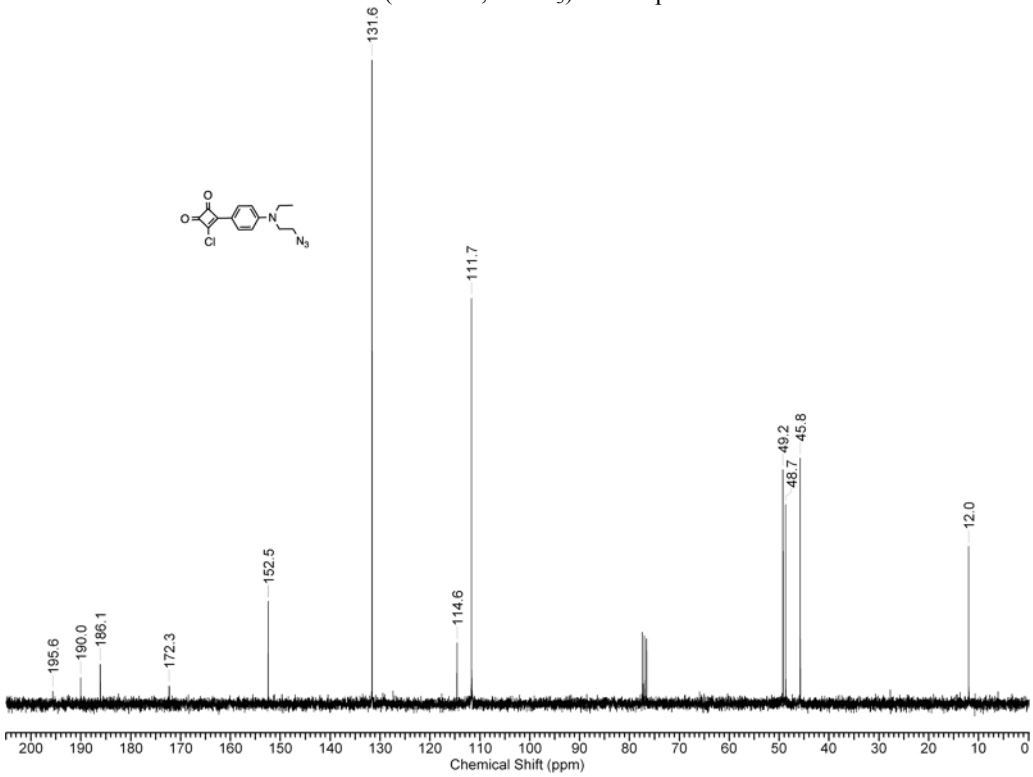

$^{13}$C NMR (75 MHz, $CDCl_3$) of compound **22**



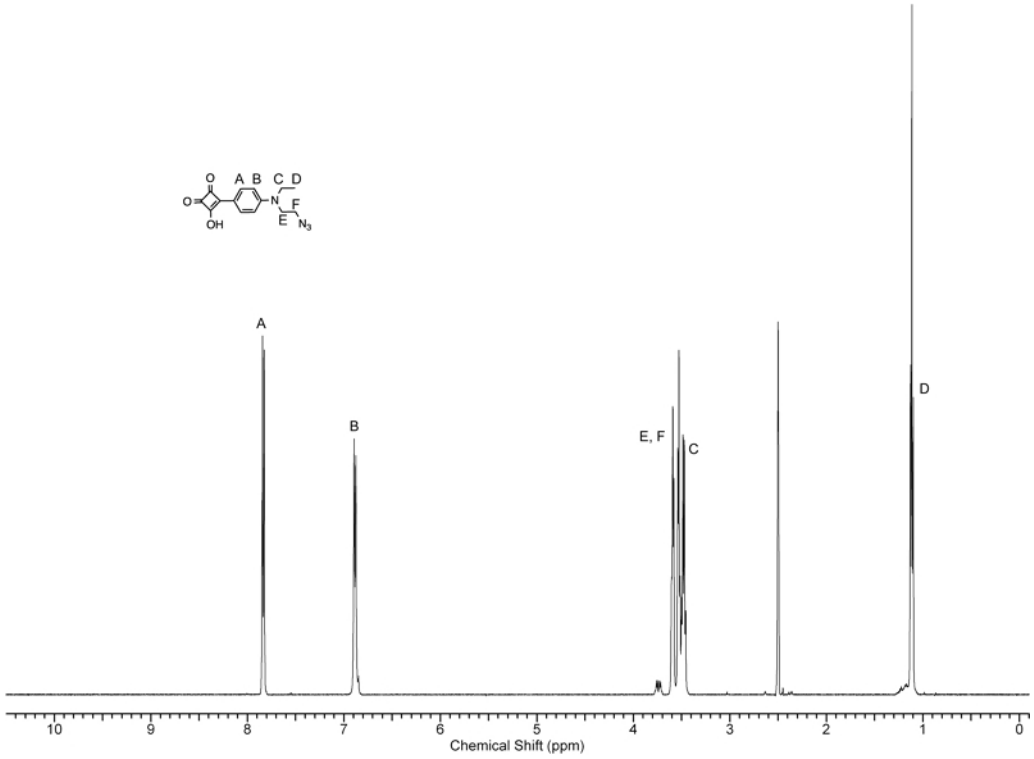
$^{1}$H NMR (300 MHz, $CDCl_3$) of compound **24**



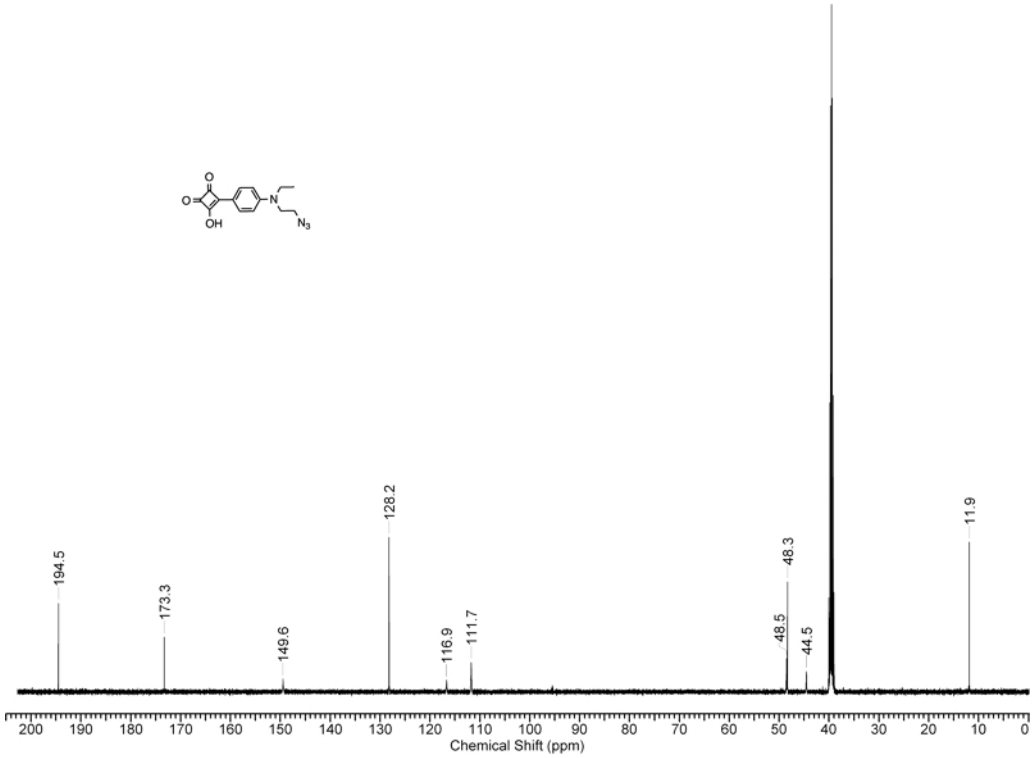

$^{13}$C NMR (75 MHz, $CDCl_3$) of compound **24**



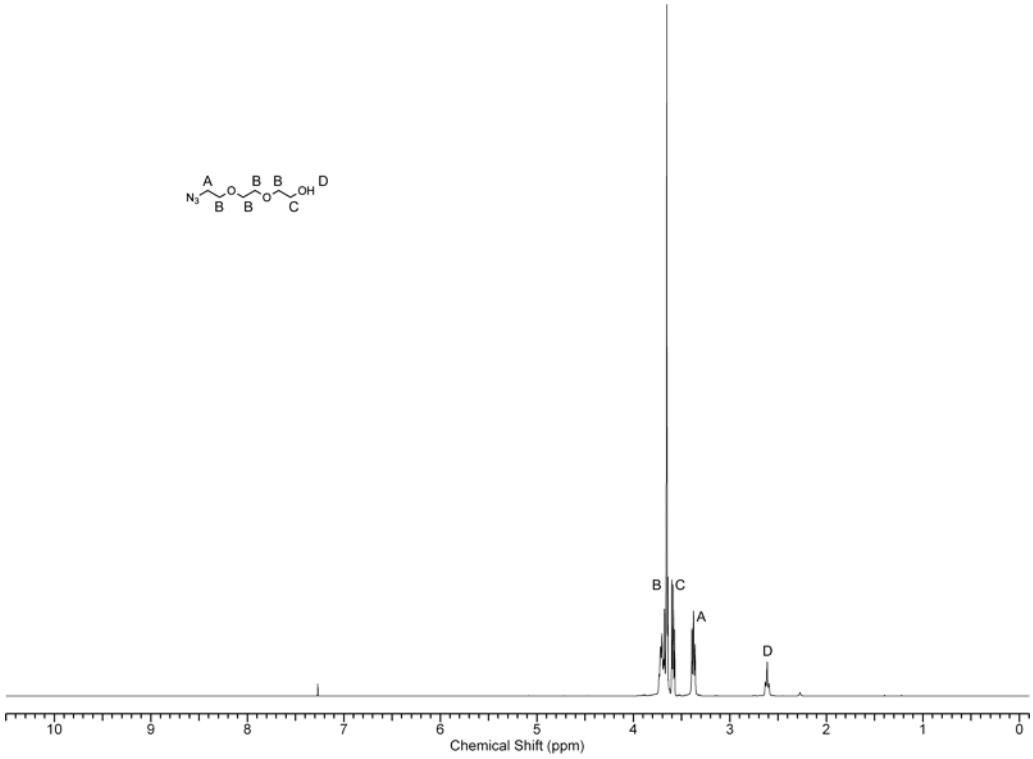
$^{1}$H NMR (500 MHz, DMSO-$d_6$) of compound **10**



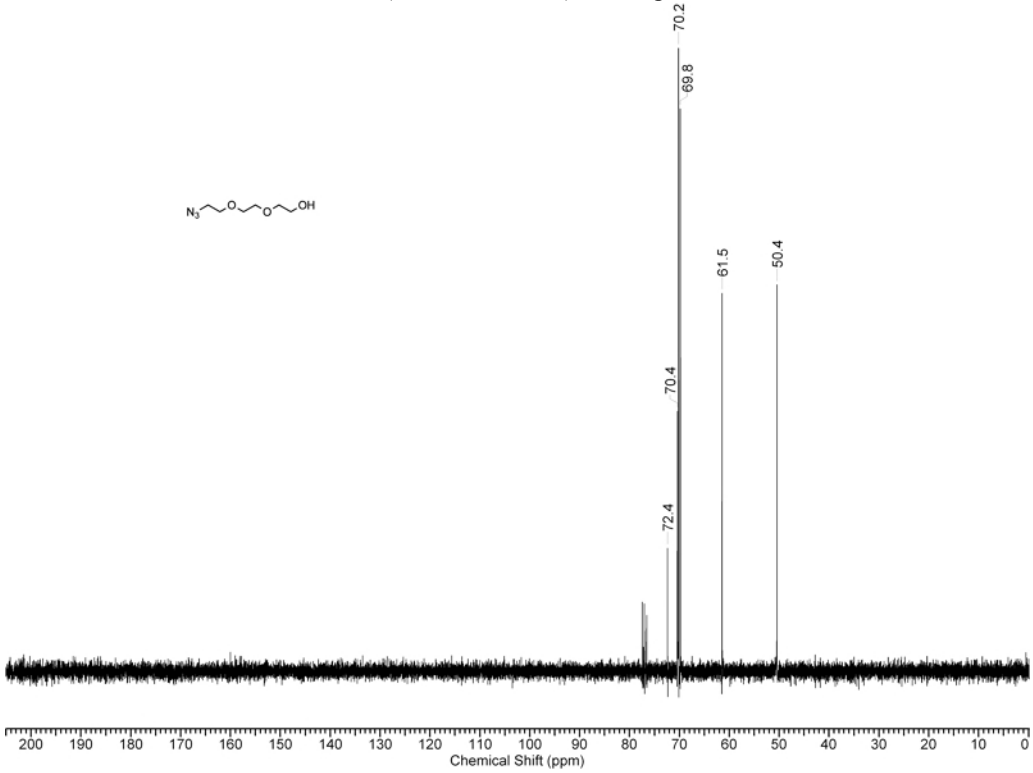

$^{13}$C NMR (125 MHz, DMSO-$d_6$) of compound **10**



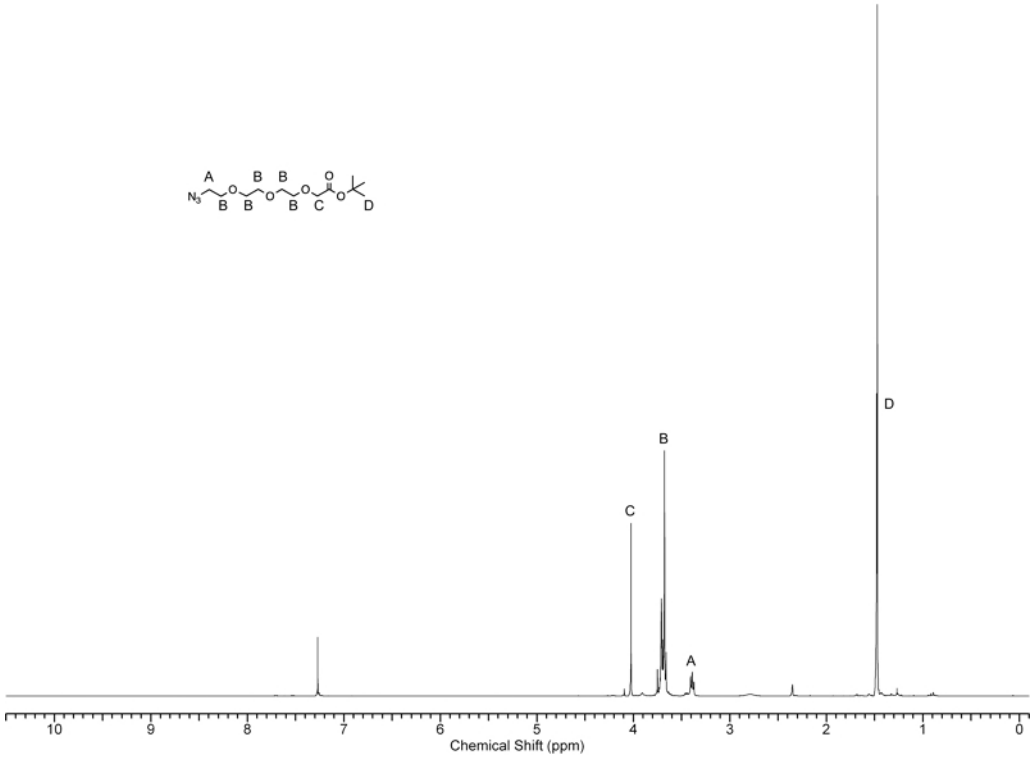
$^{1}$H NMR (300 MHz, $CDCl_3$) of compound **26**



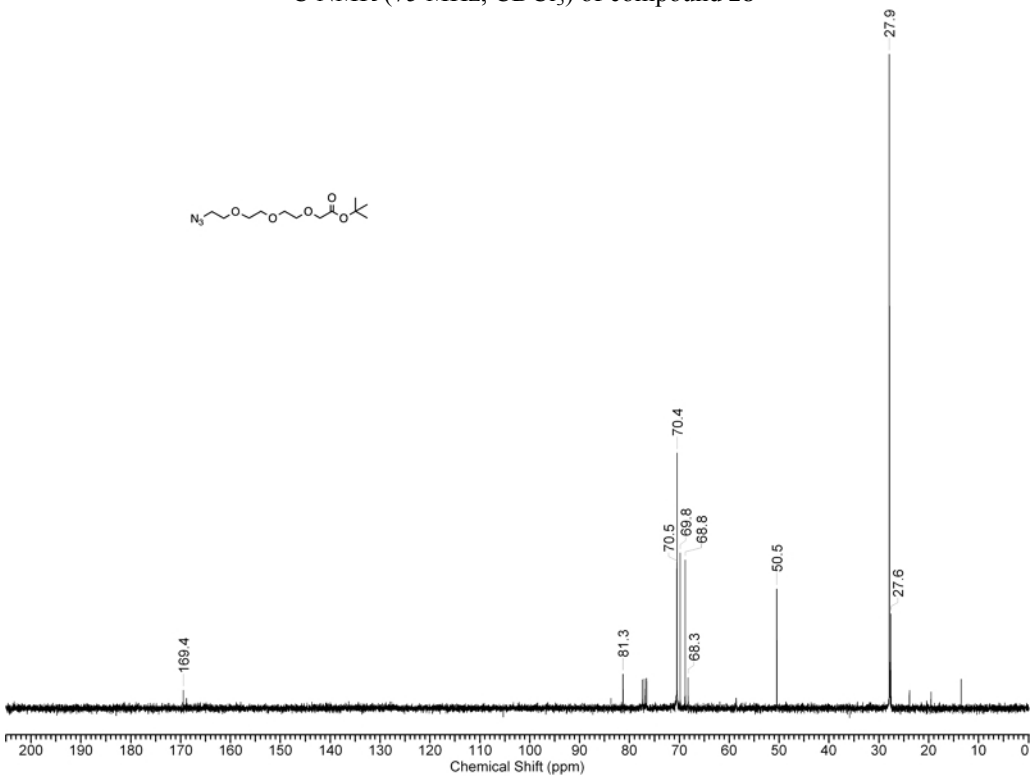

$^{13}$C NMR (75 MHz, $CDCl_3$) of compound **26**



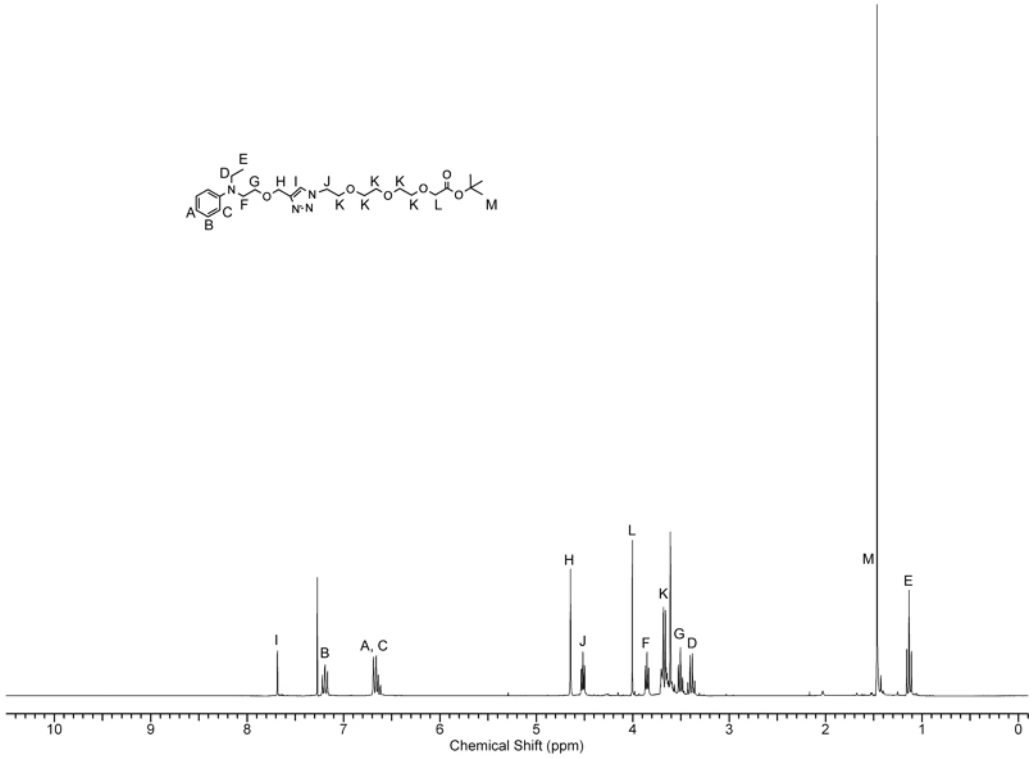
$^{1}$H NMR (300 MHz, $CDCl_3$) of compound **28**



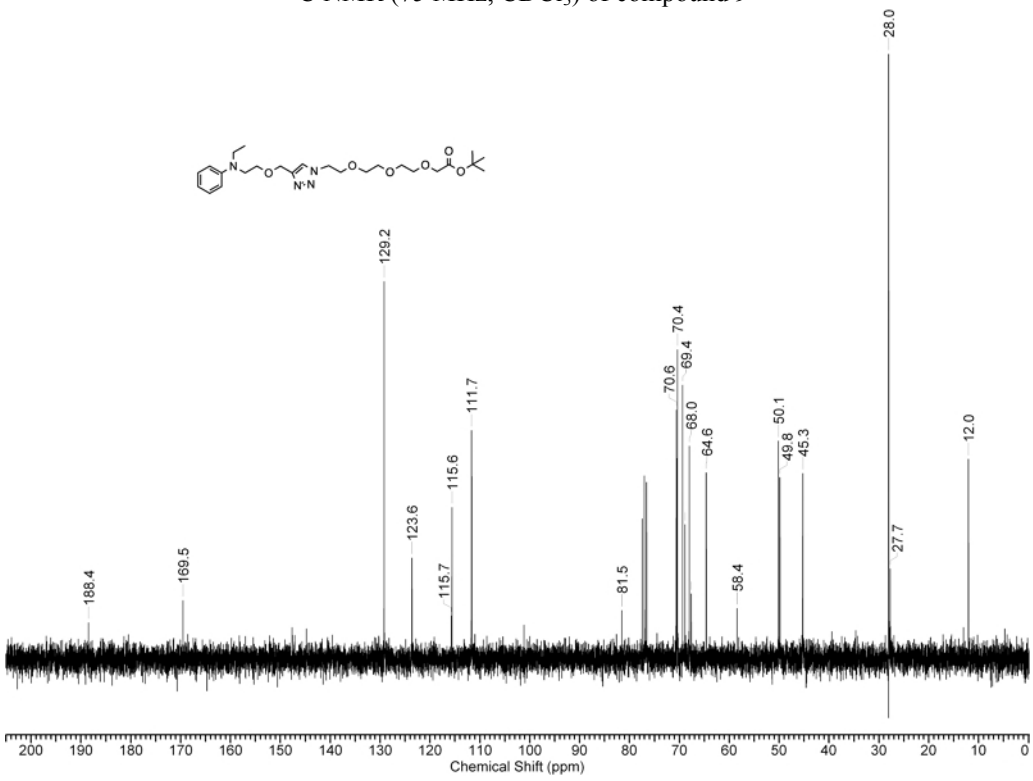

$^{13}$C NMR (75 MHz, $CDCl_3$) of compound **28**



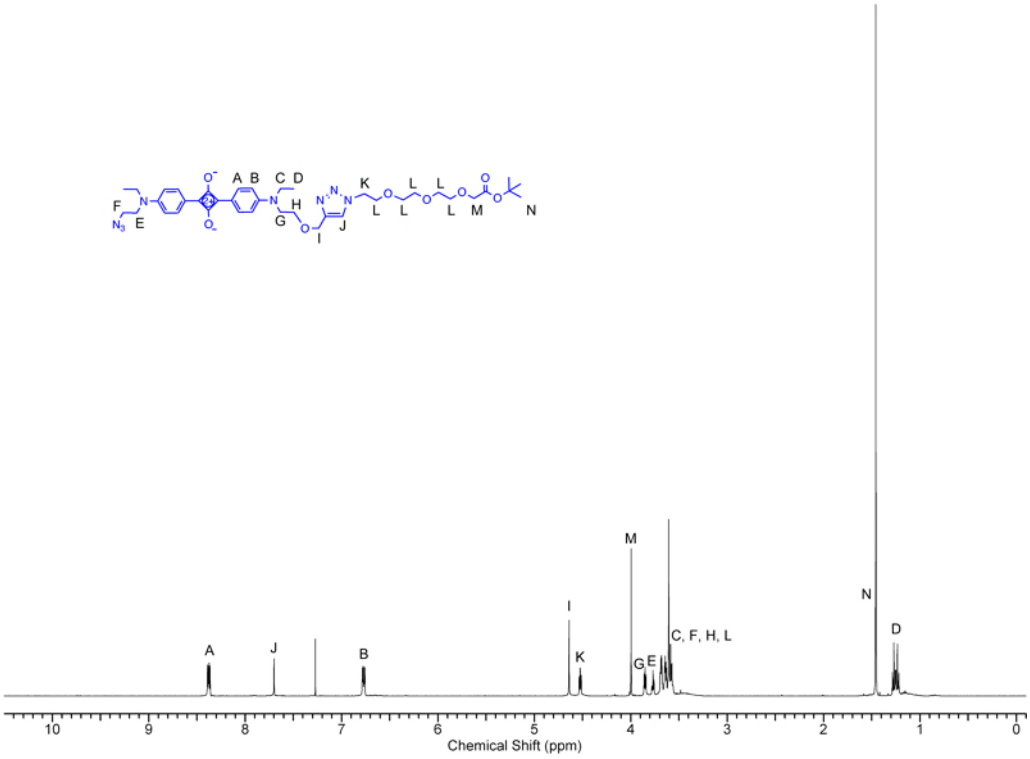
$^{1}$H NMR (300 MHz, $CDCl_3$) of compound **9**



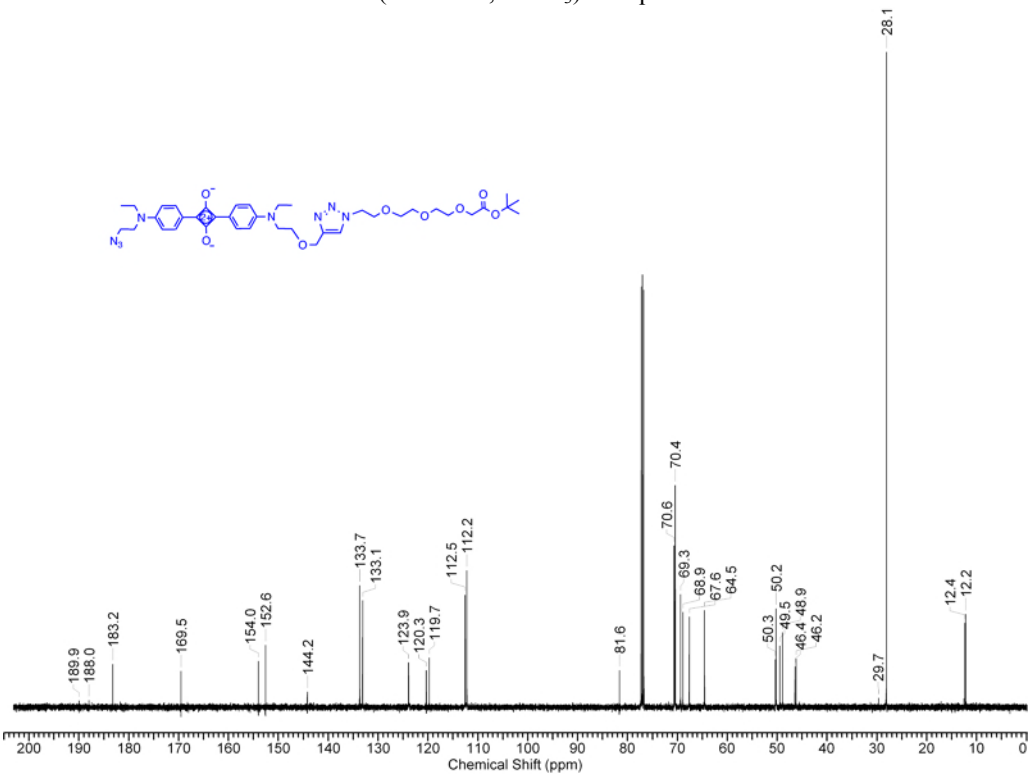

$^{13}$C NMR (75 MHz, $CDCl_3$) of compound **9**



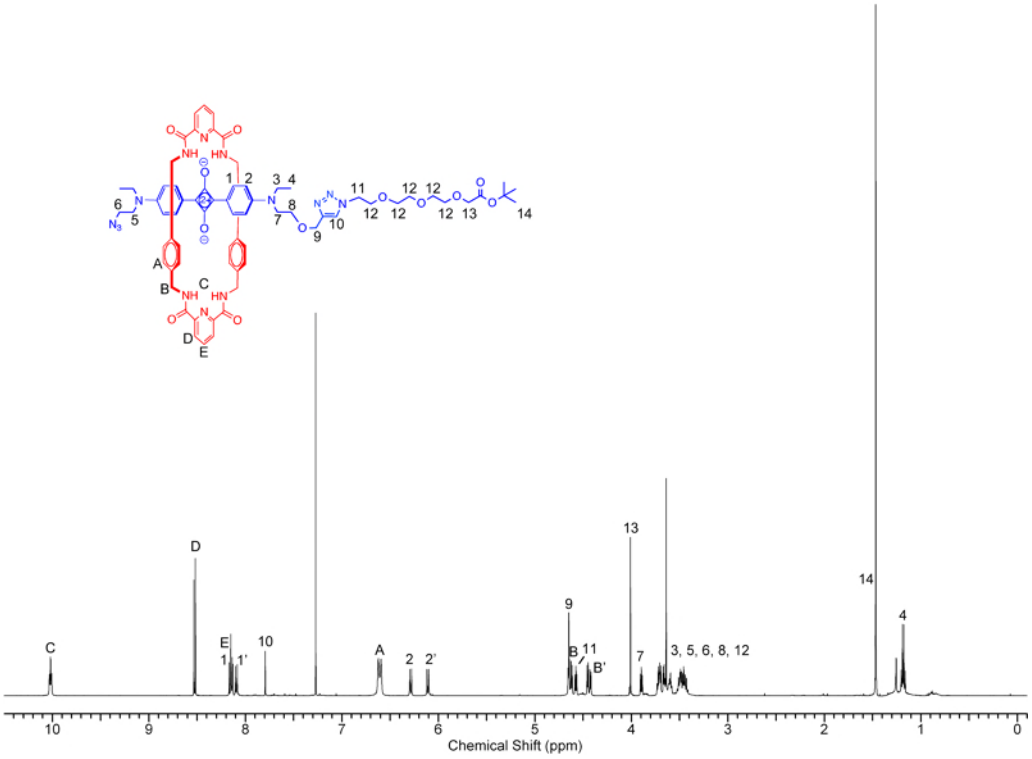
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine **30**



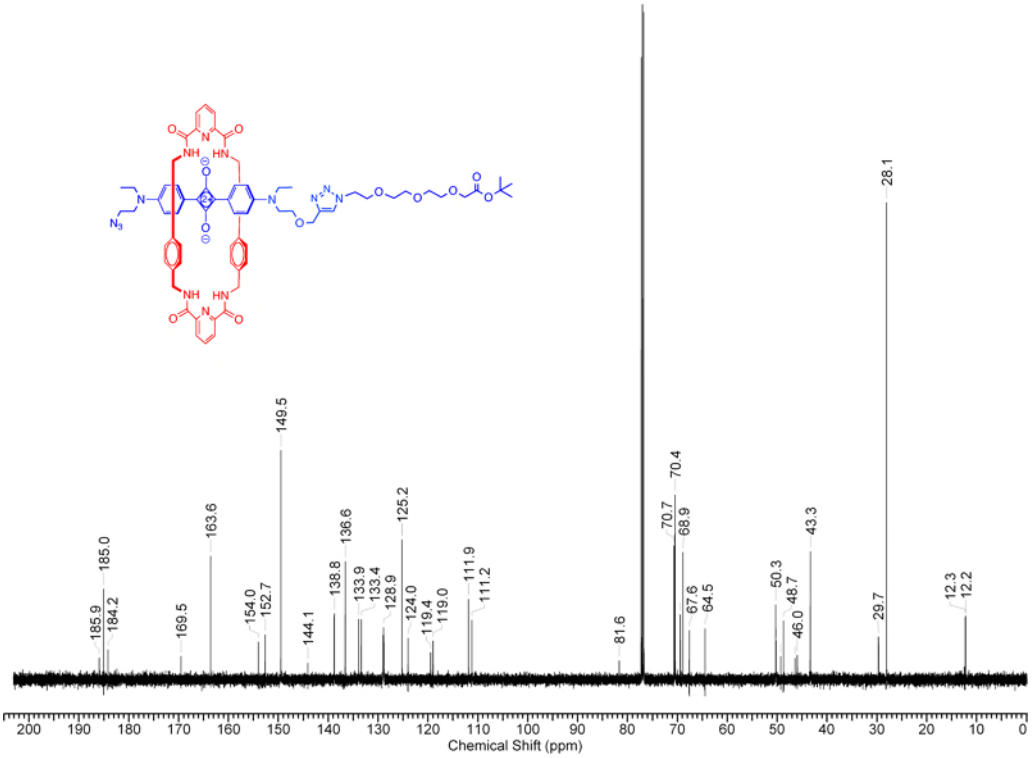

$^{13}$C NMR (125 MHz, $CDCl_3$) of Squaraine **30**



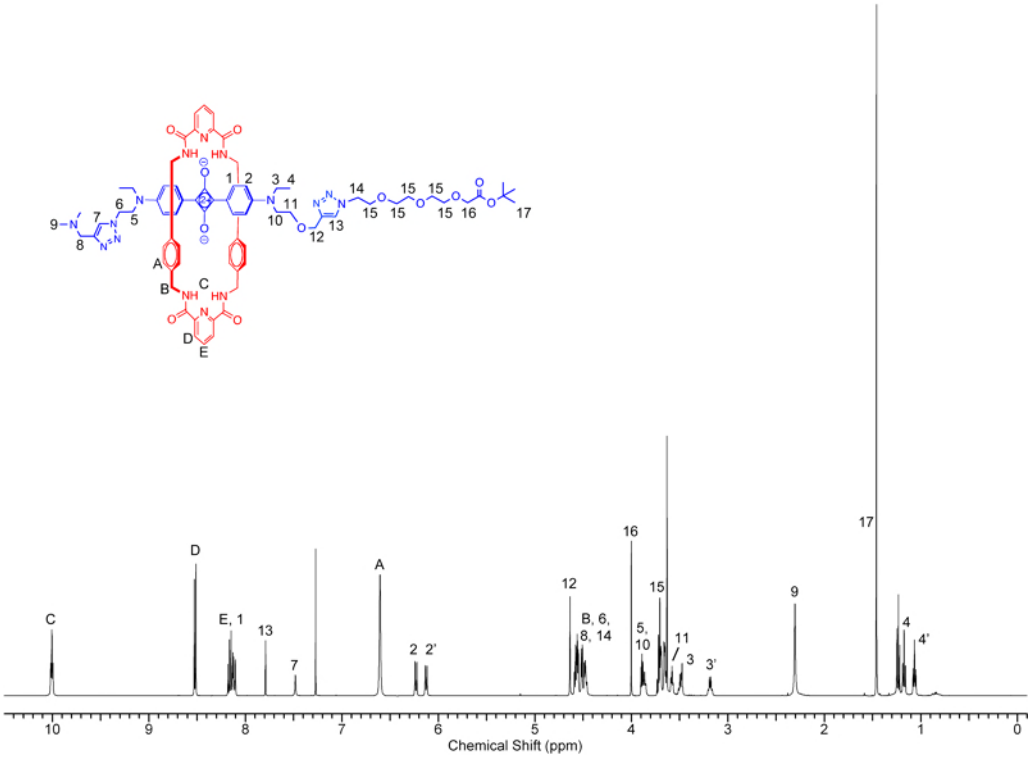
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine Rotaxane **11**



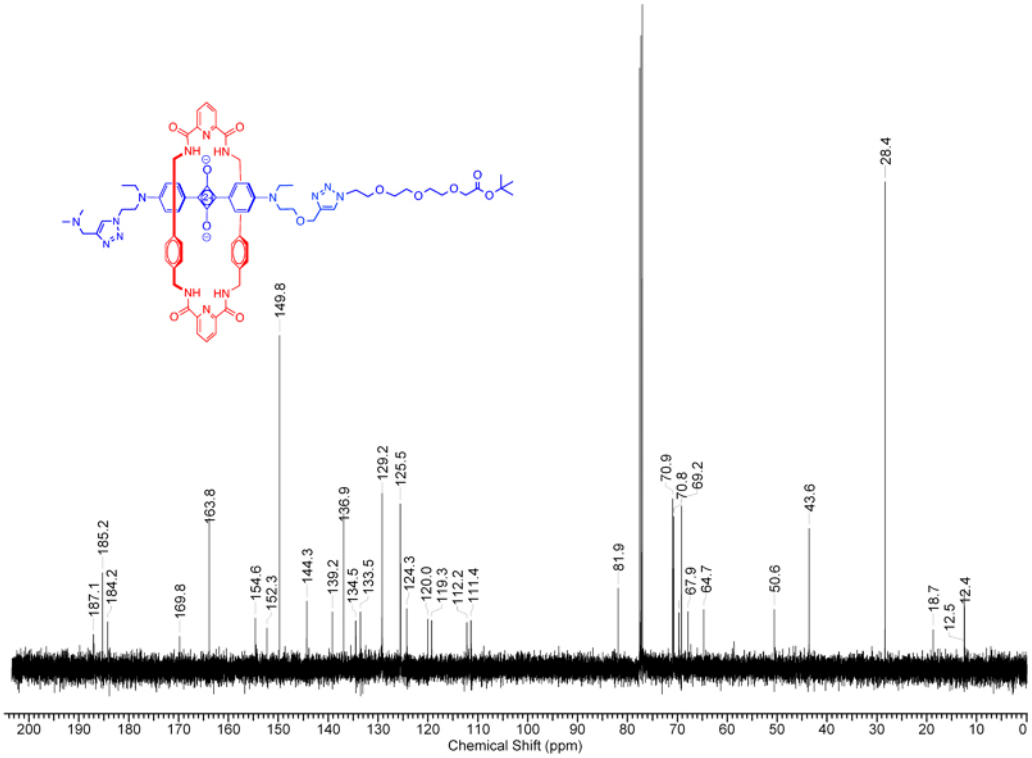

$^{13}$C NMR (125 MHz, $CDCl_3$) of Squaraine Rotaxane **11**



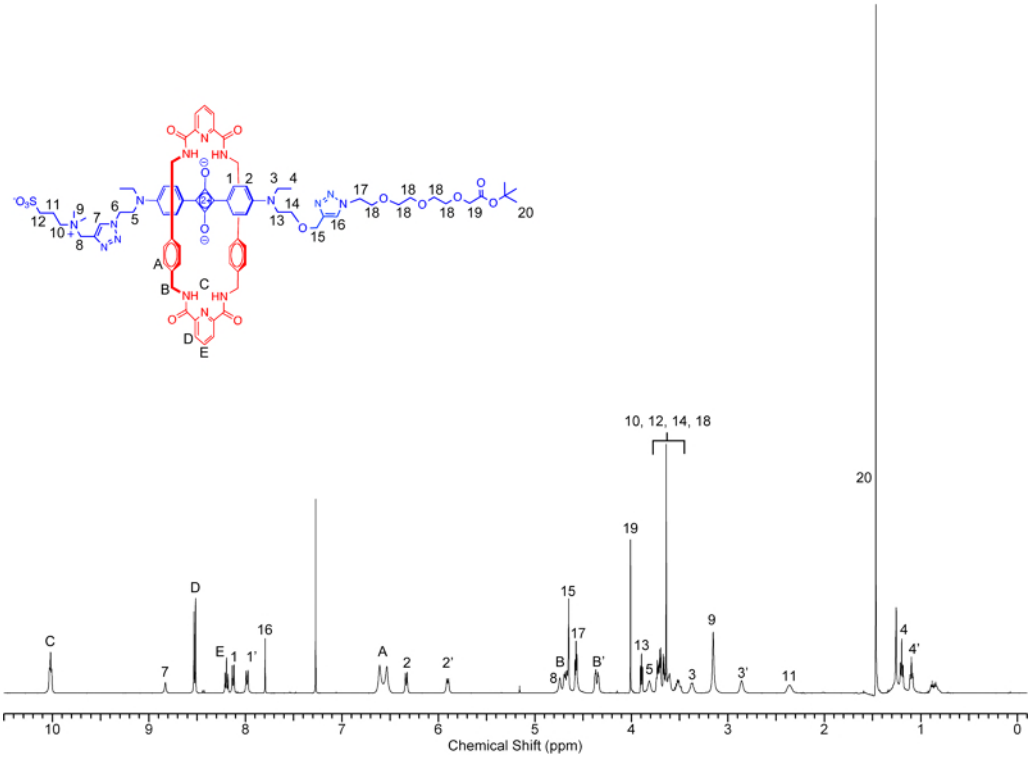
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine Rotaxane **34**



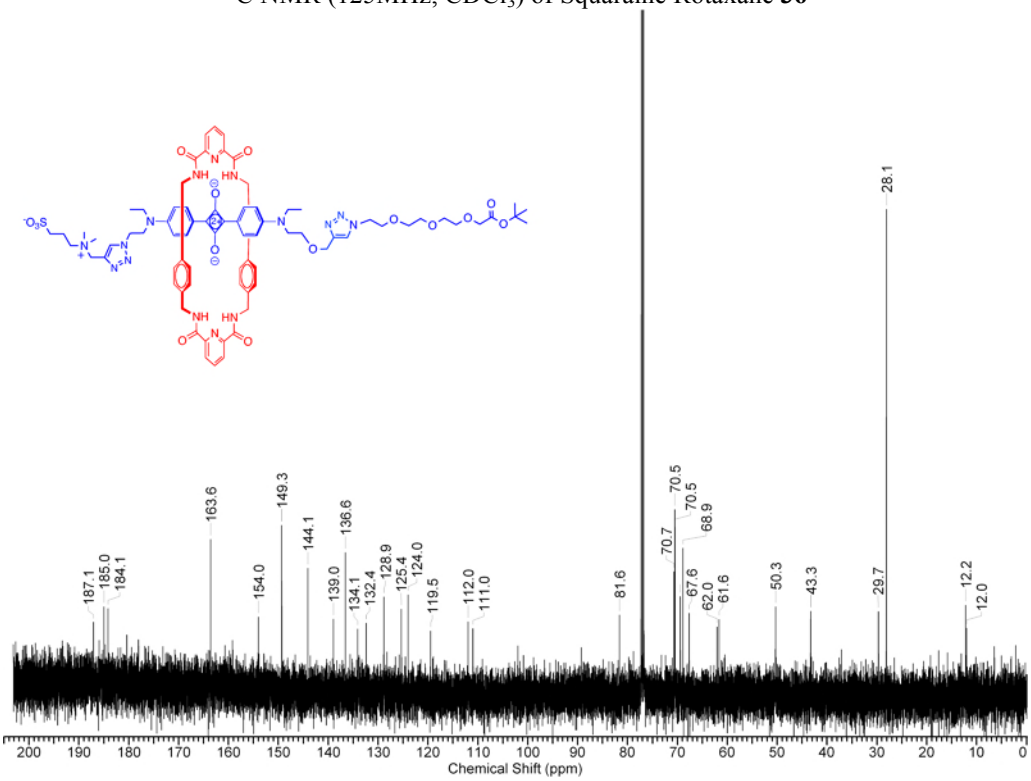

$^{13}$C NMR (125 MHz, $CDCl_3$) of Squaraine Rotaxane **34**



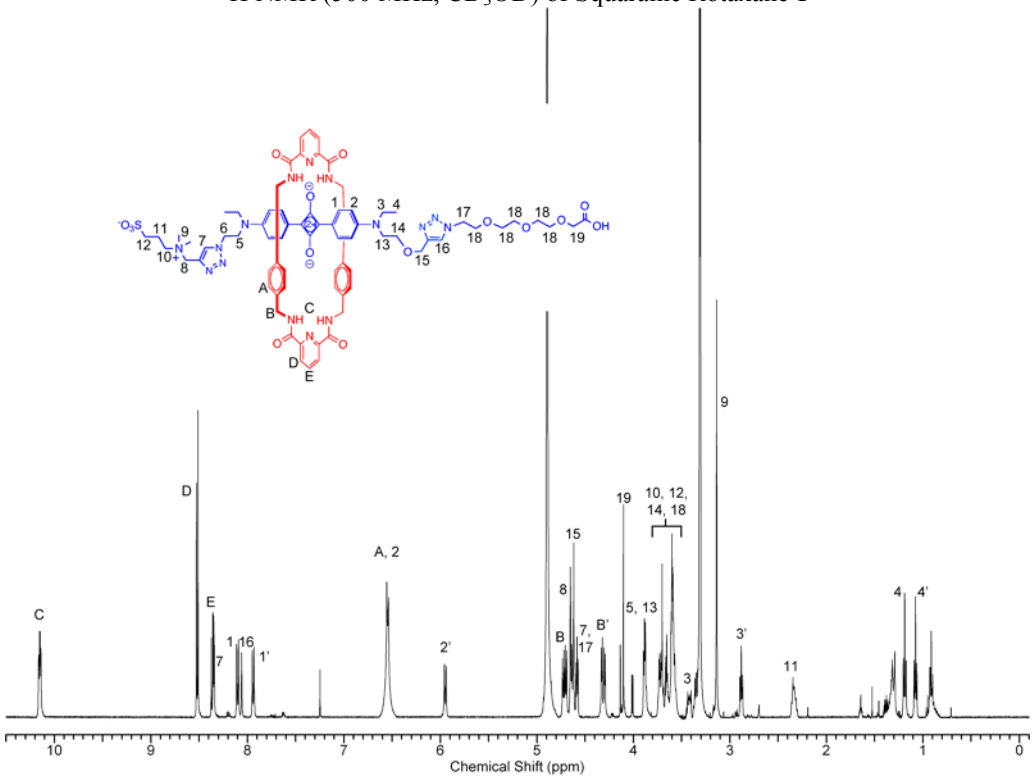
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine Rotaxane **36**




$^{13}$C NMR (125MHz, $CDCl_3$) of Squaraine Rotaxane **36**

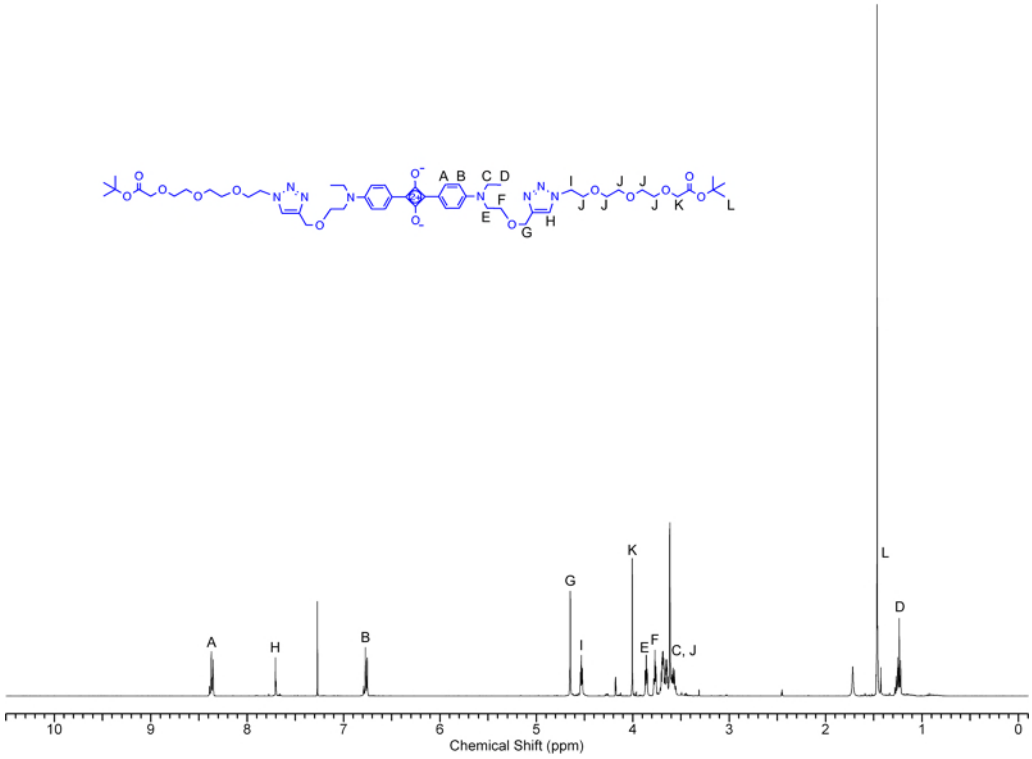


$^{1}$H NMR (500 MHz, $CD_3OD$) of Squaraine Rotaxane **1**

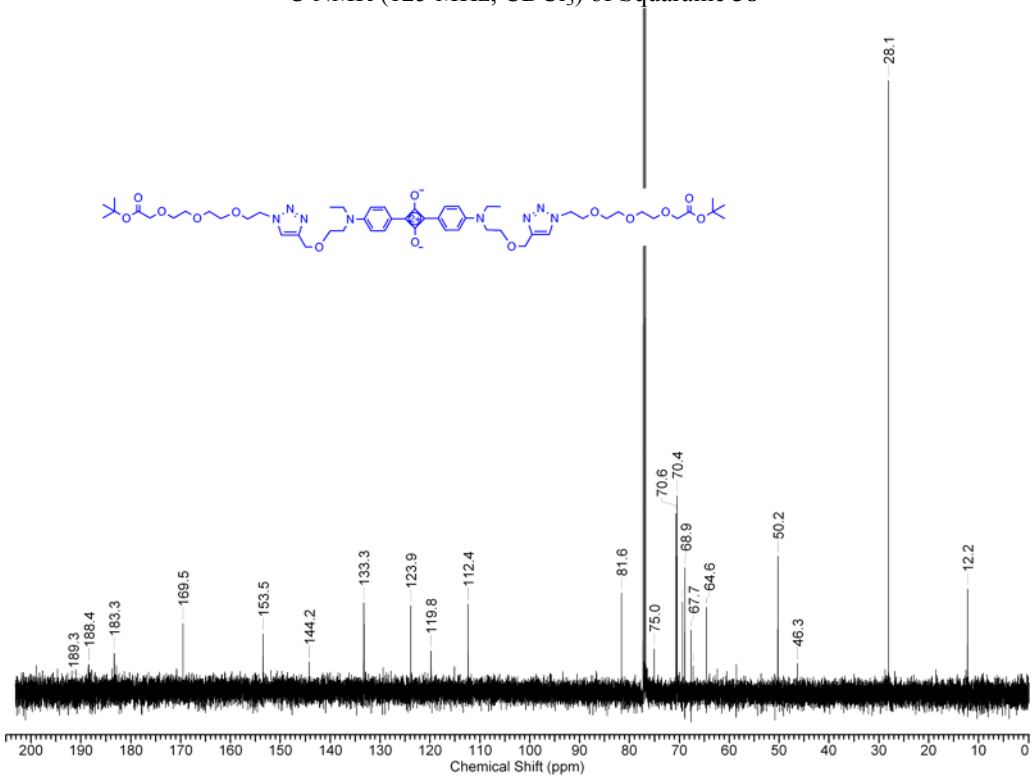



$^{13}$C NMR of Squaraine Rotaxane **1** was not obtained due to poor solubility

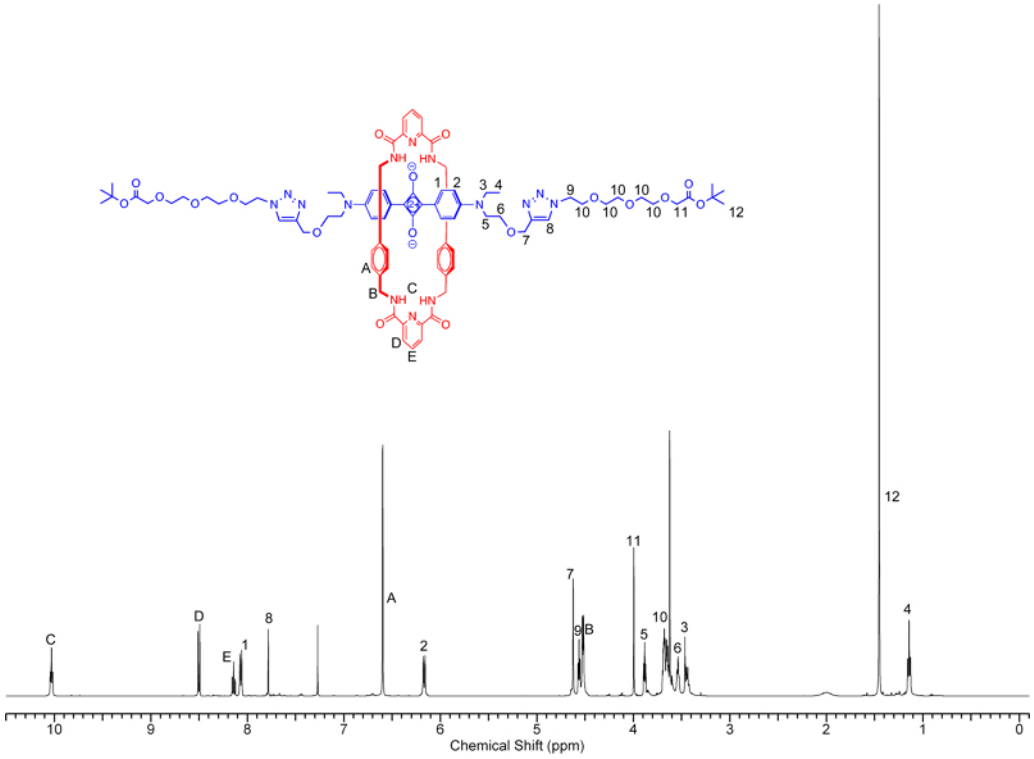
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine **38**



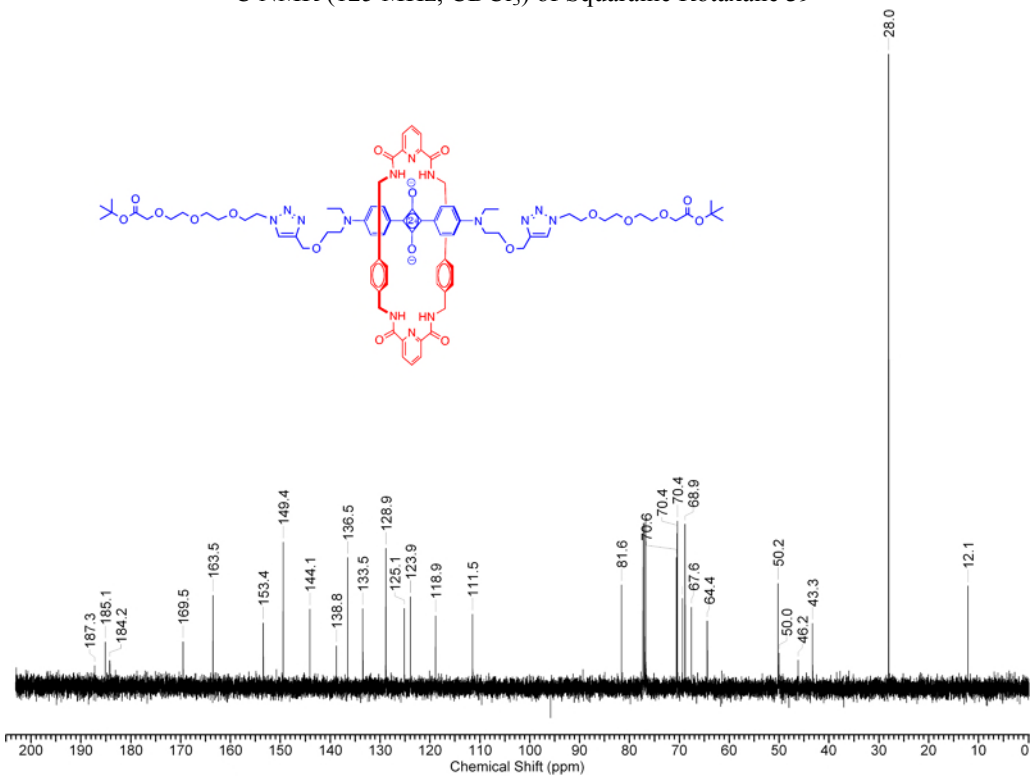

$^{13}$C NMR (125 MHz, $CDCl_3$) of Squaraine **38**



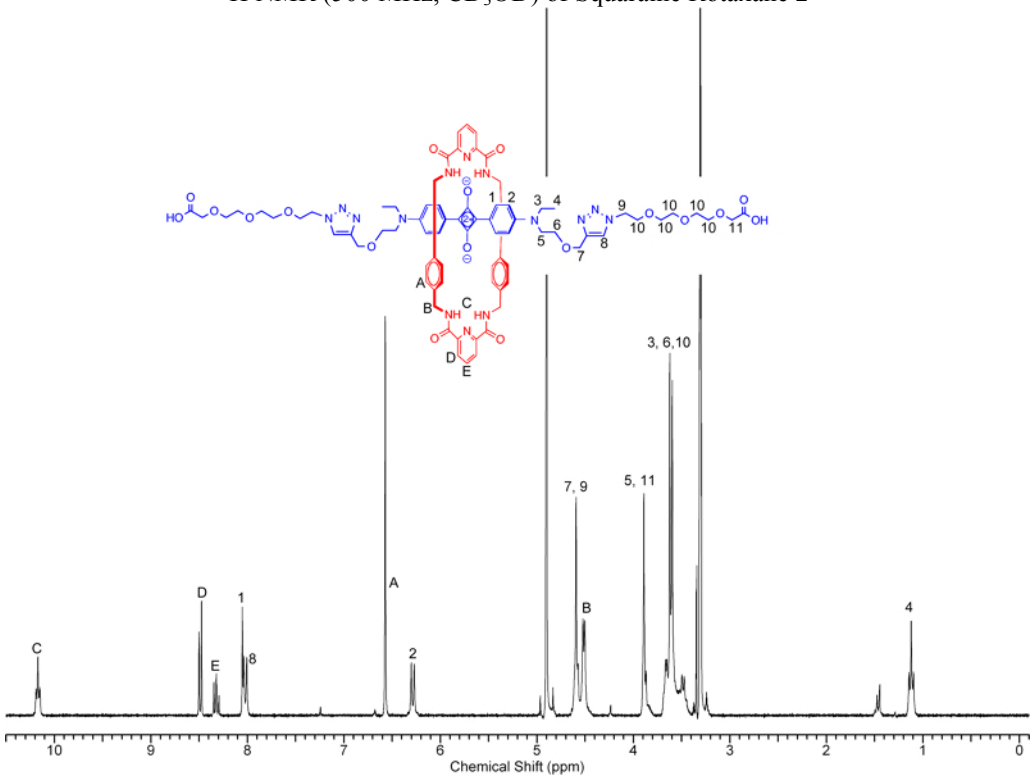
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine Rotaxane **39**



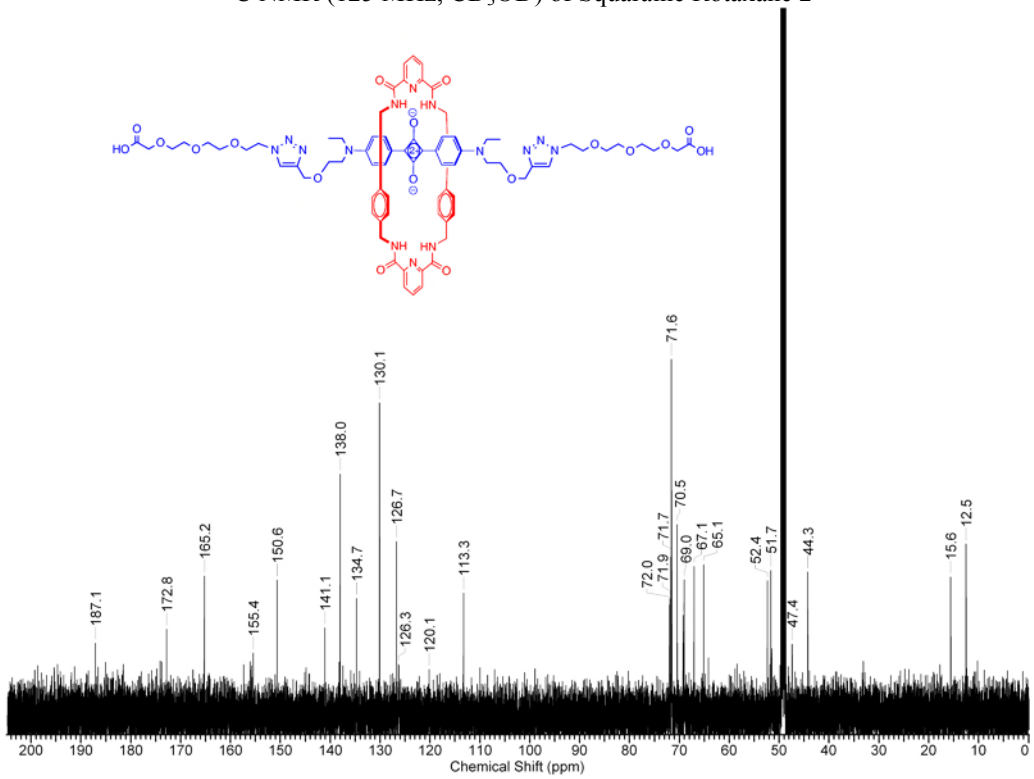

$^{13}$C NMR (125 MHz, $CDCl_3$) of Squaraine Rotaxane **39**



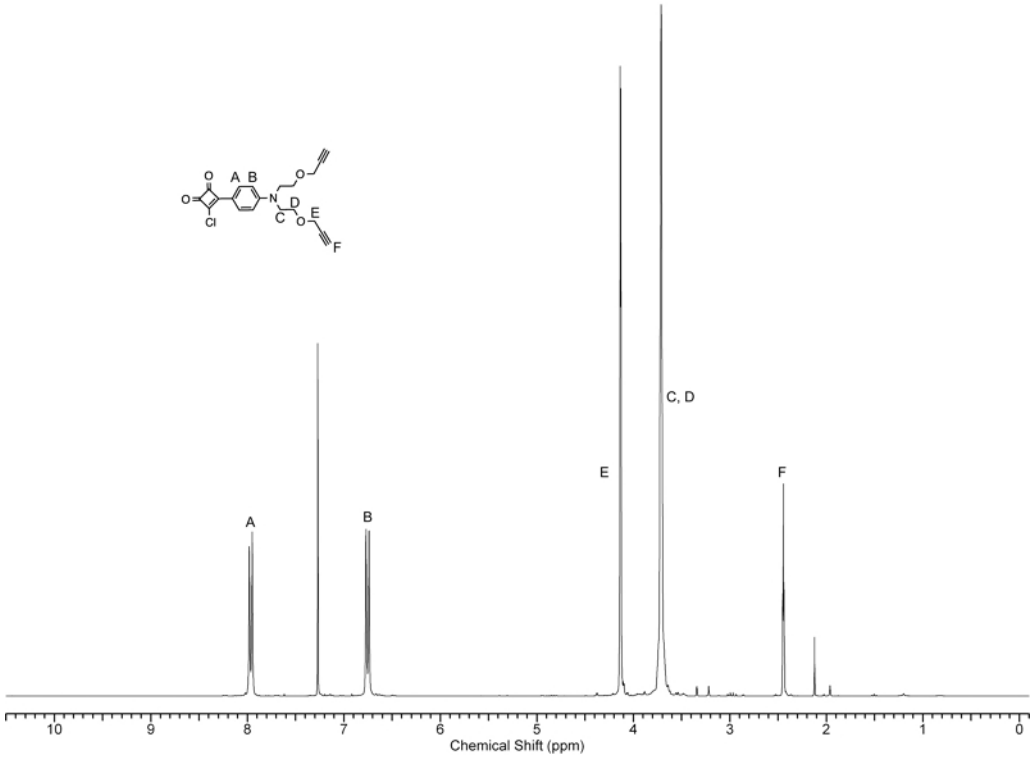
$^{1}$H NMR (500 MHz, $CD_3OD$) of Squaraine Rotaxane **2**



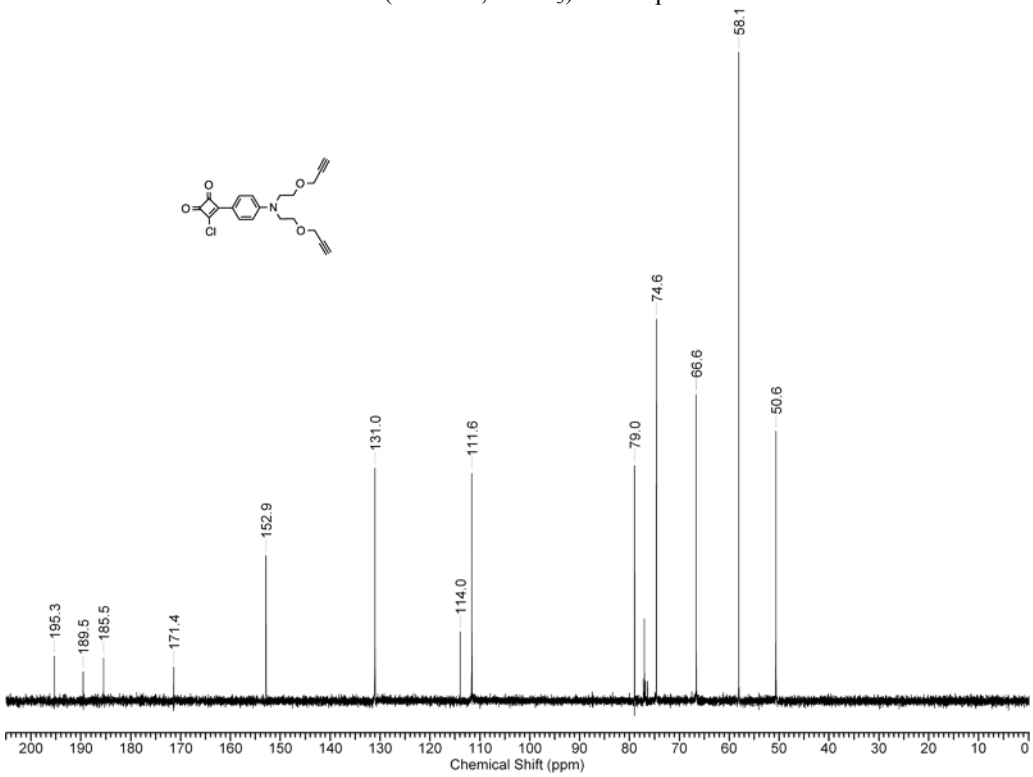

$^{13}$C NMR (125 MHz, $CD_3OD$) of Squaraine Rotaxane **2**



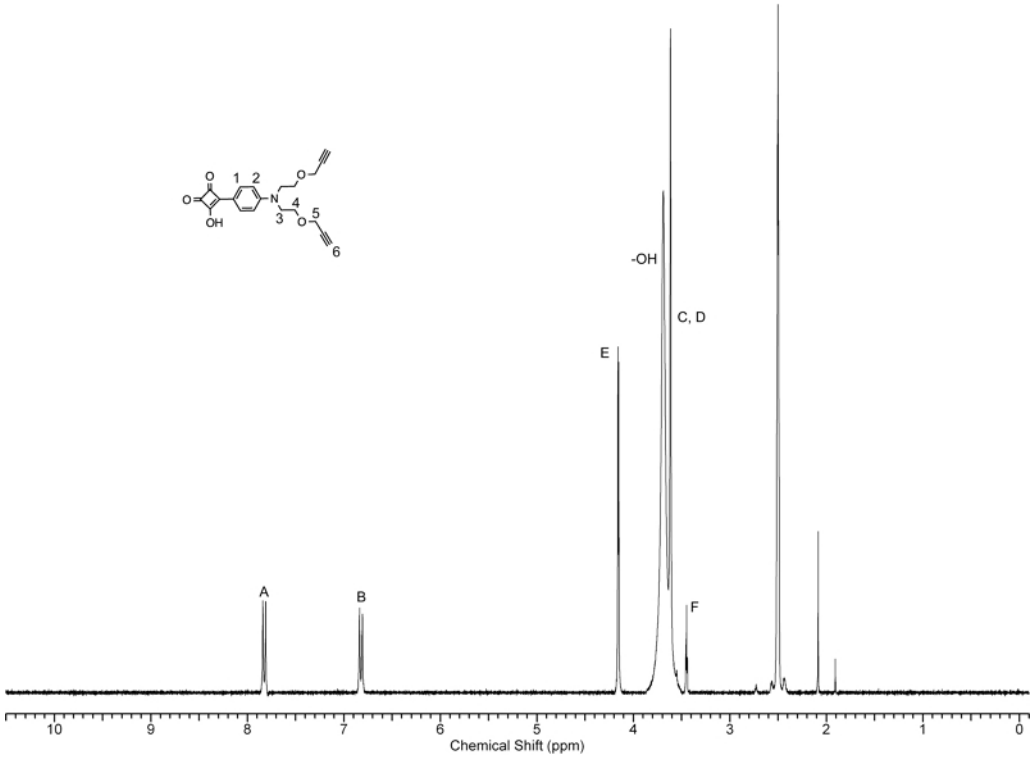
$^{1}$H NMR (300 MHz, $CDCl_3$) of compound **41**



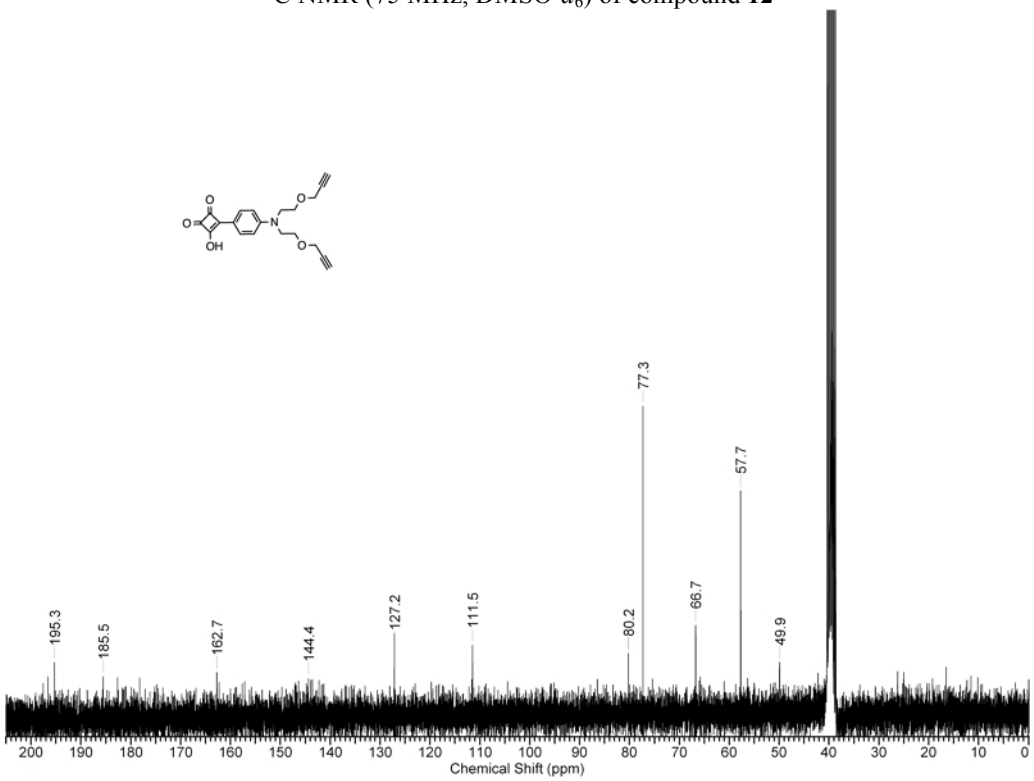

$^{13}$C NMR (75 MHz, $CDCl_3$) of compound **41**



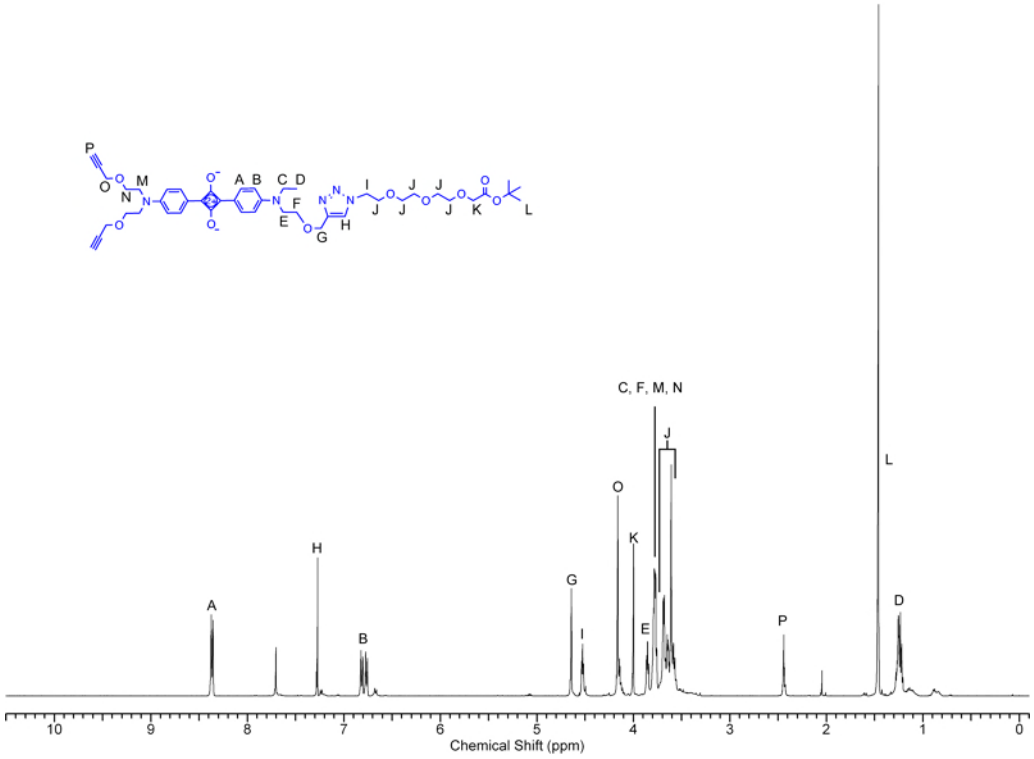
$^{1}$H NMR (300 MHz, DMSO-$d_6$) of compound **12**



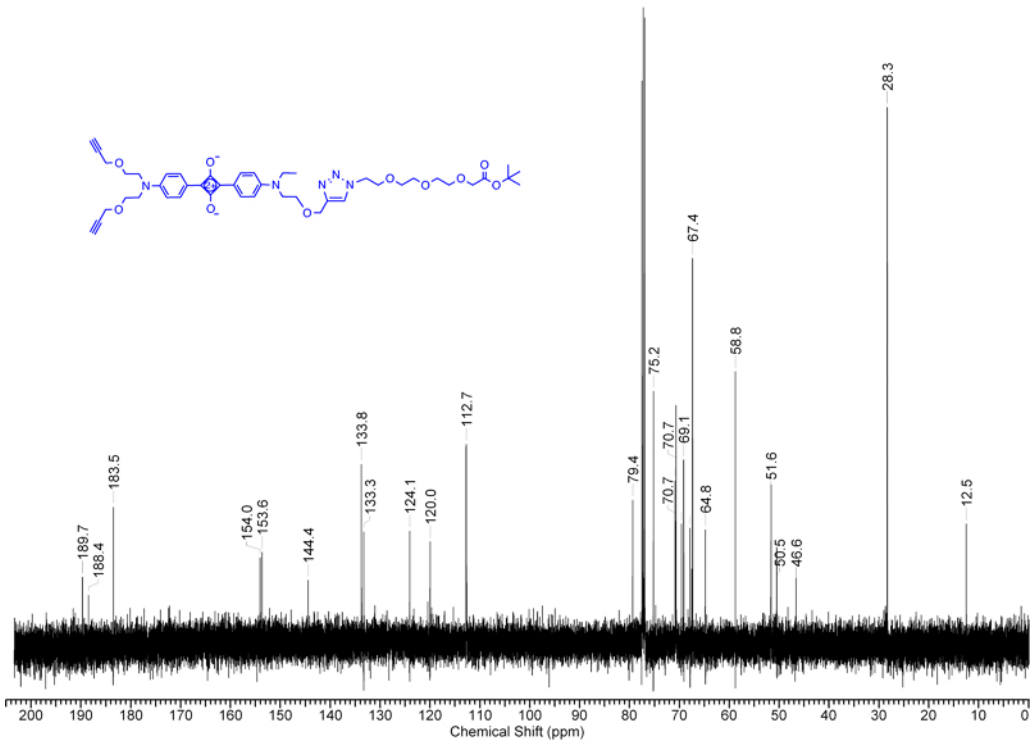

$^{13}$C NMR (75 MHz, DMSO-$d_6$) of compound **12**



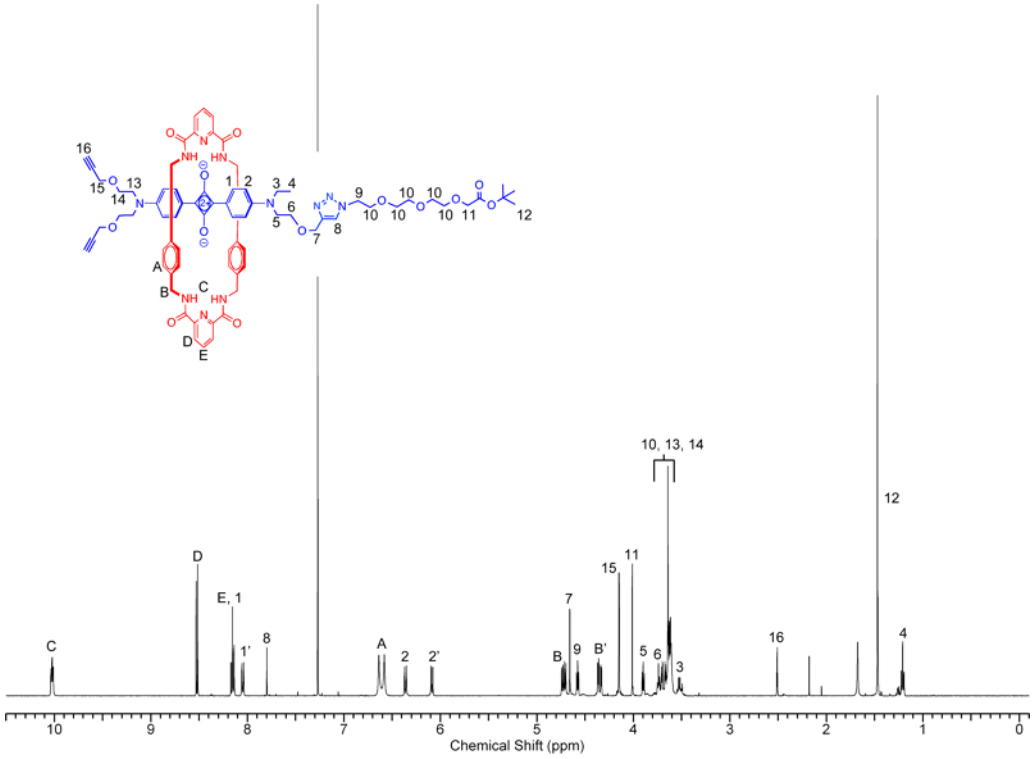
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine **42**



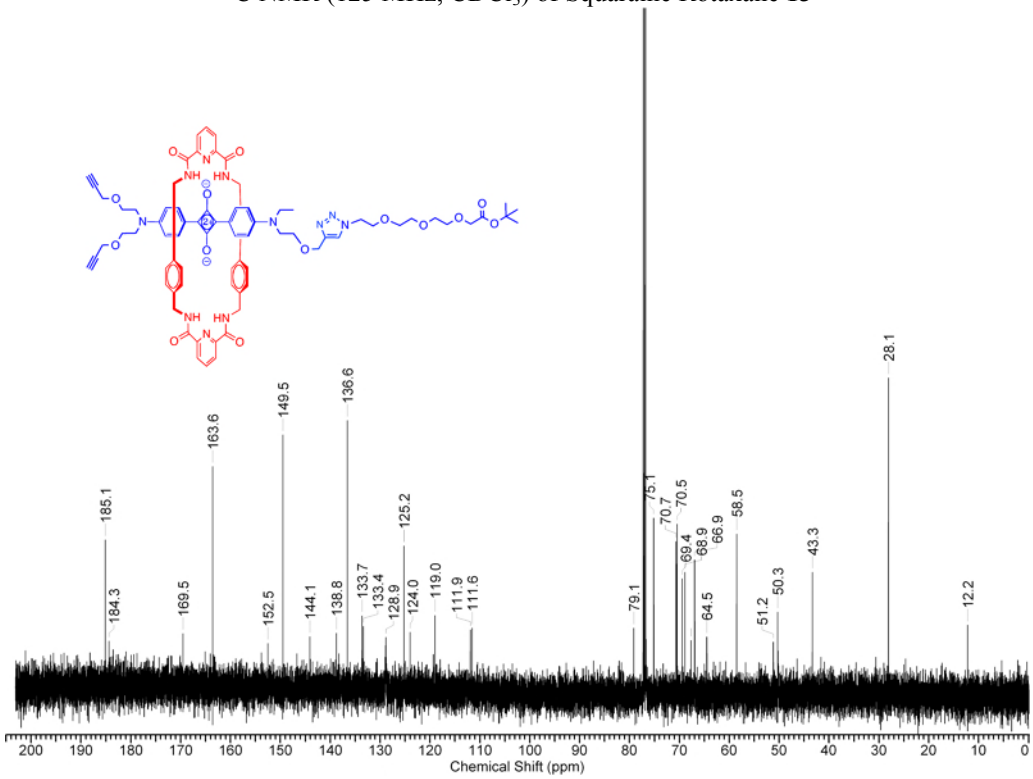

$^{13}$C NMR (125 MHz, $CDCl_3$) of Squaraine **42**



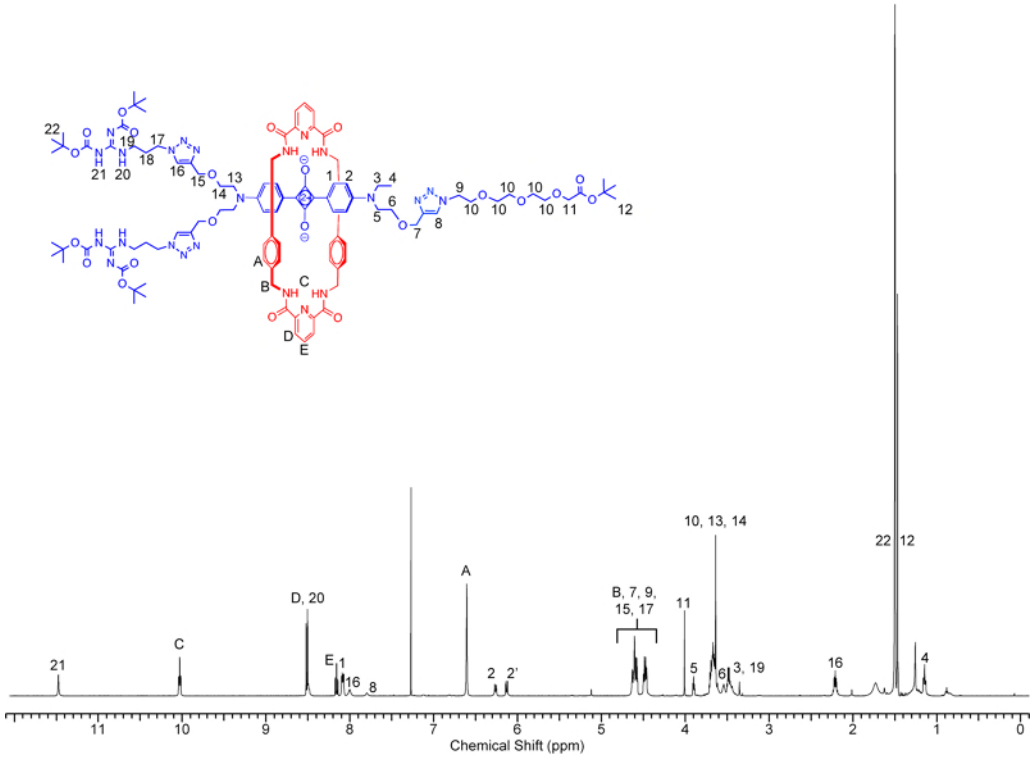
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine Rotaxane **13**



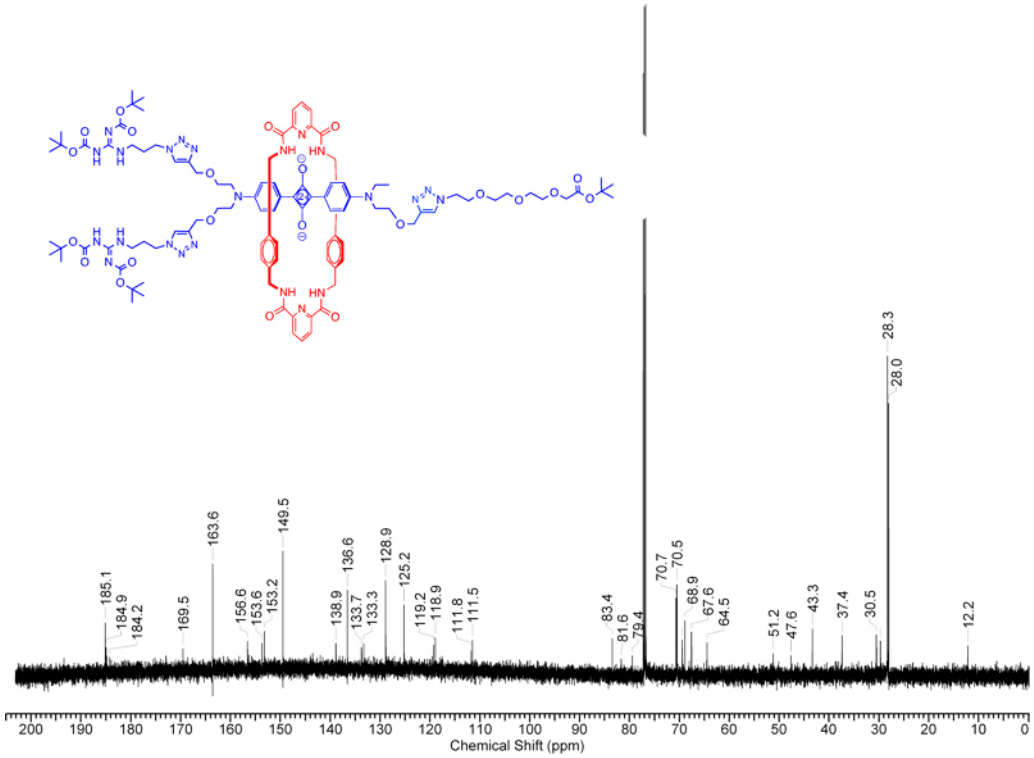

$^{13}$C NMR (125 MHz, $CDCl_3$) of Squaraine Rotaxane **13**



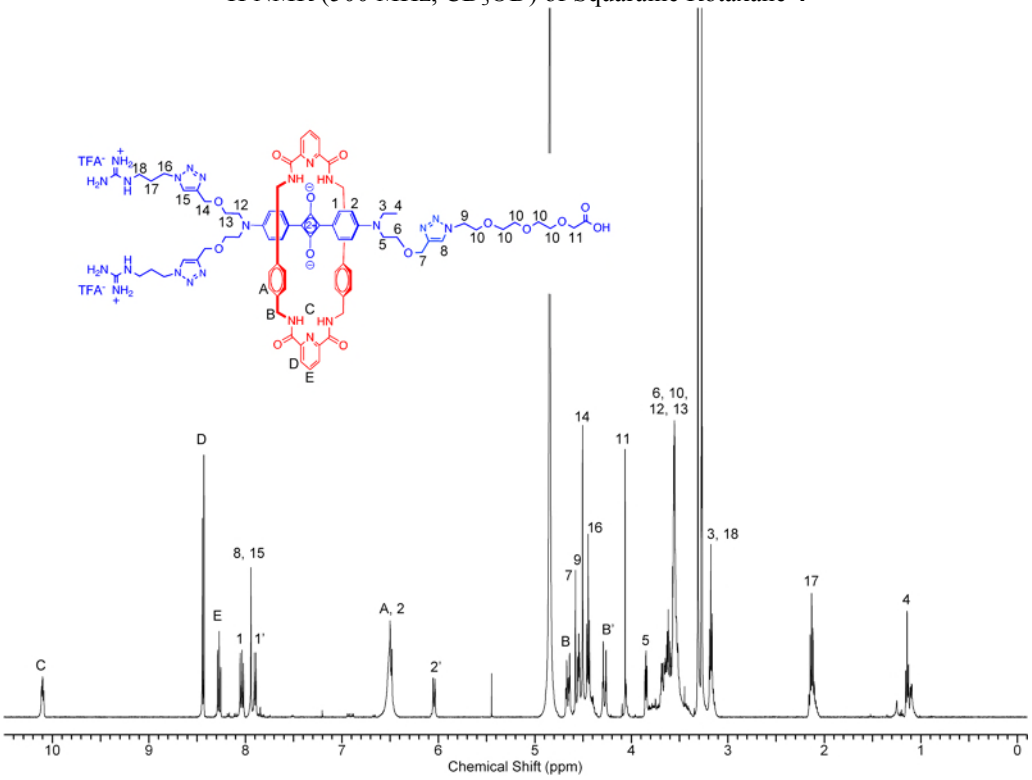
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine Rotaxane **43**



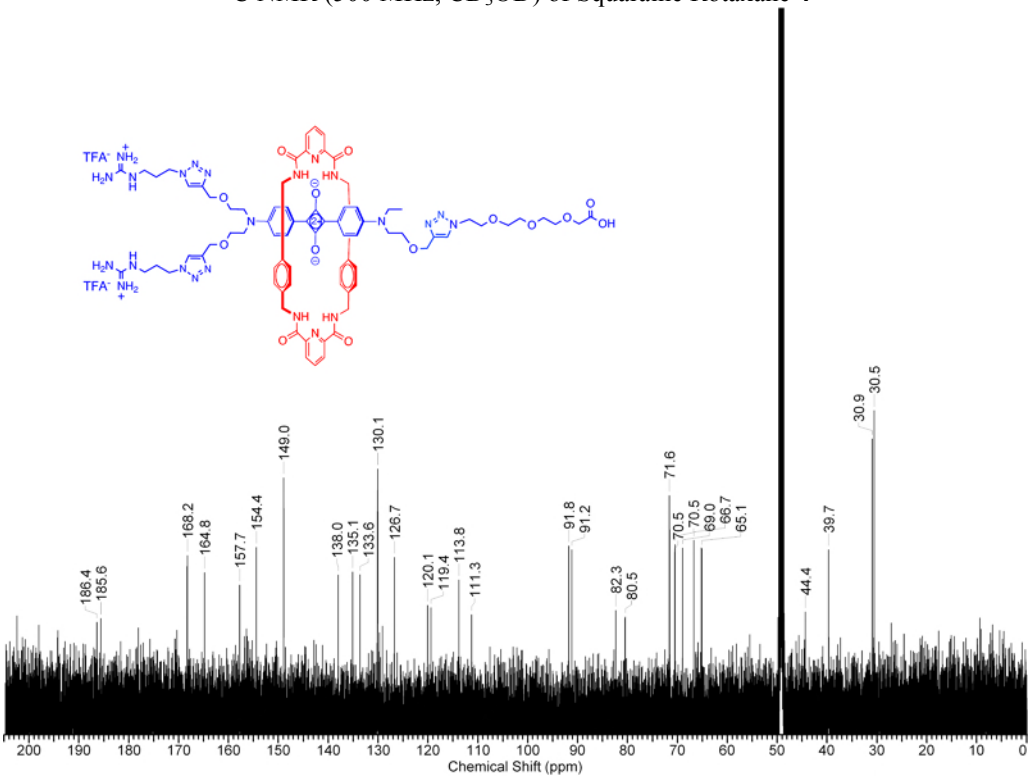

$^{13}$C NMR (125 MHz, $CDCl_3$) of Squaraine Rotaxane **43**



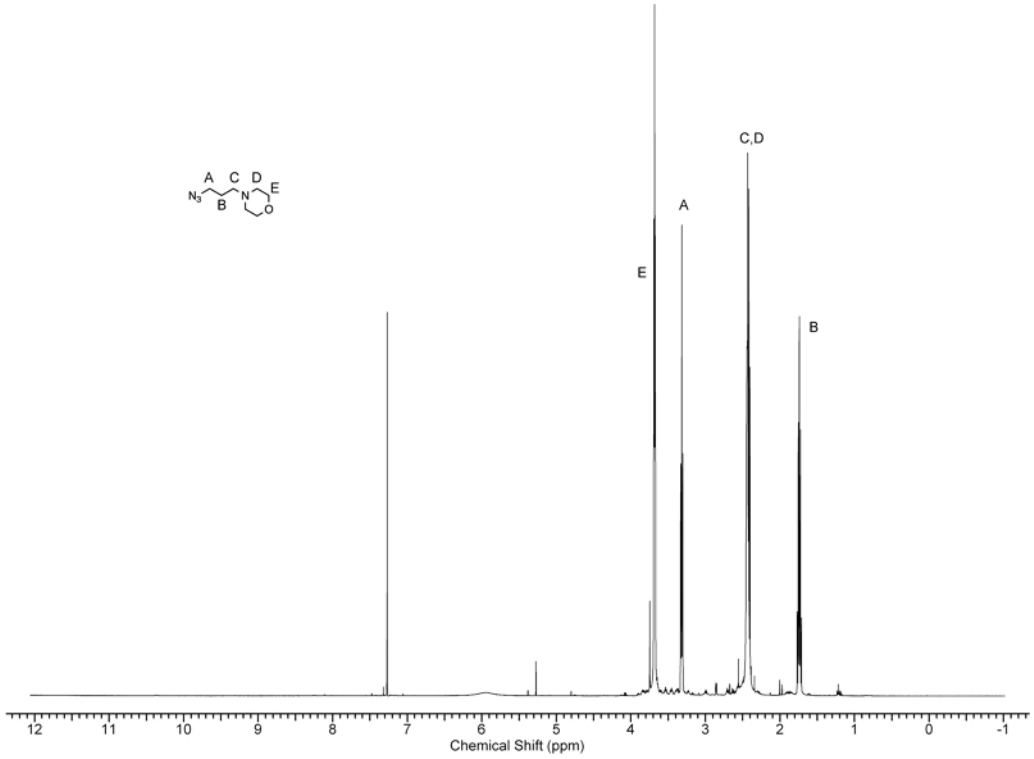
$^{1}$H NMR (500 MHz, $CD_3OD$) of Squaraine Rotaxane **4**



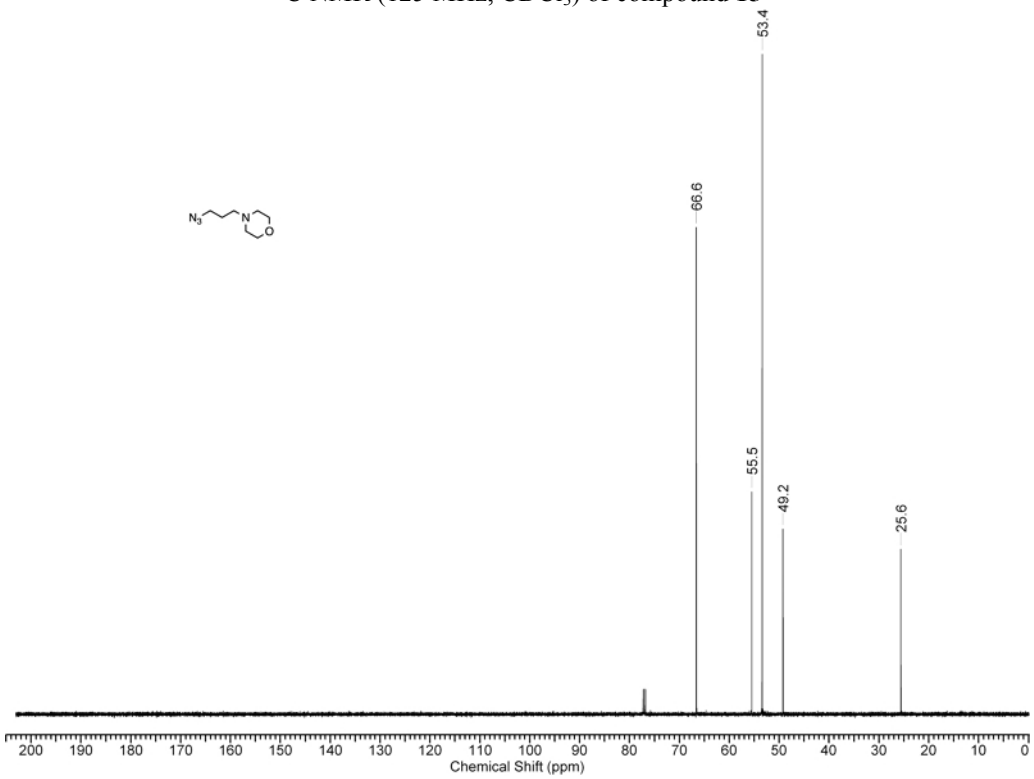

$^{13}$C NMR (500 MHz, $CD_3OD$) of Squaraine Rotaxane **4**



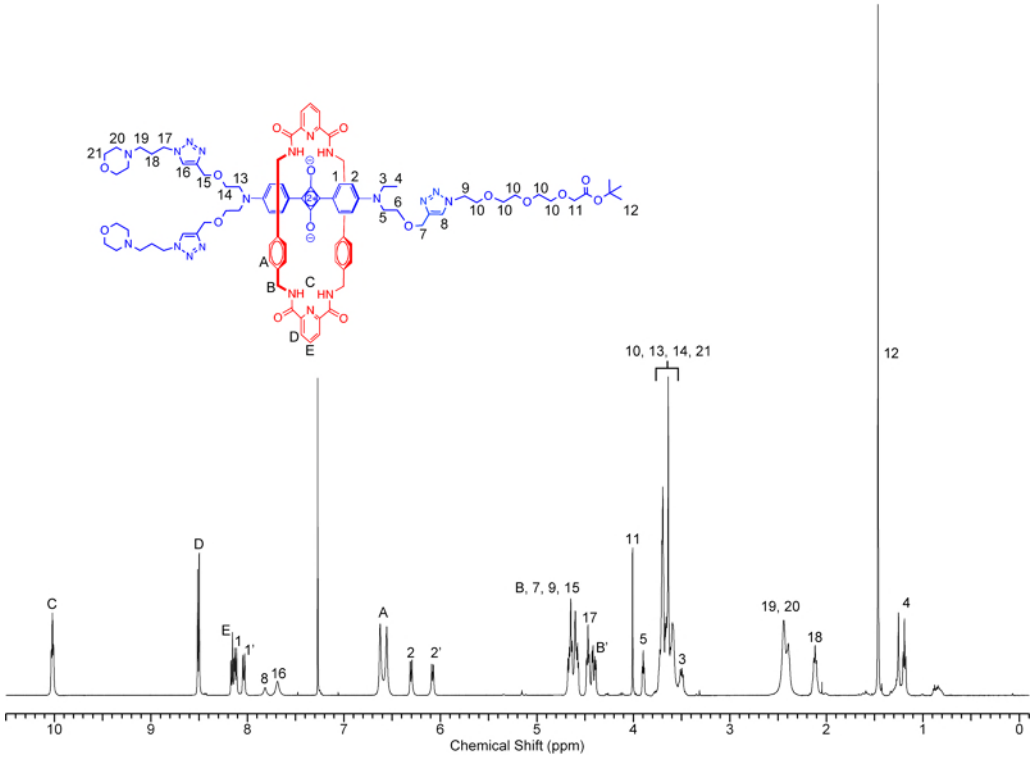
$^{1}$H NMR (500 MHz, $CDCl_3$) of compound **15**



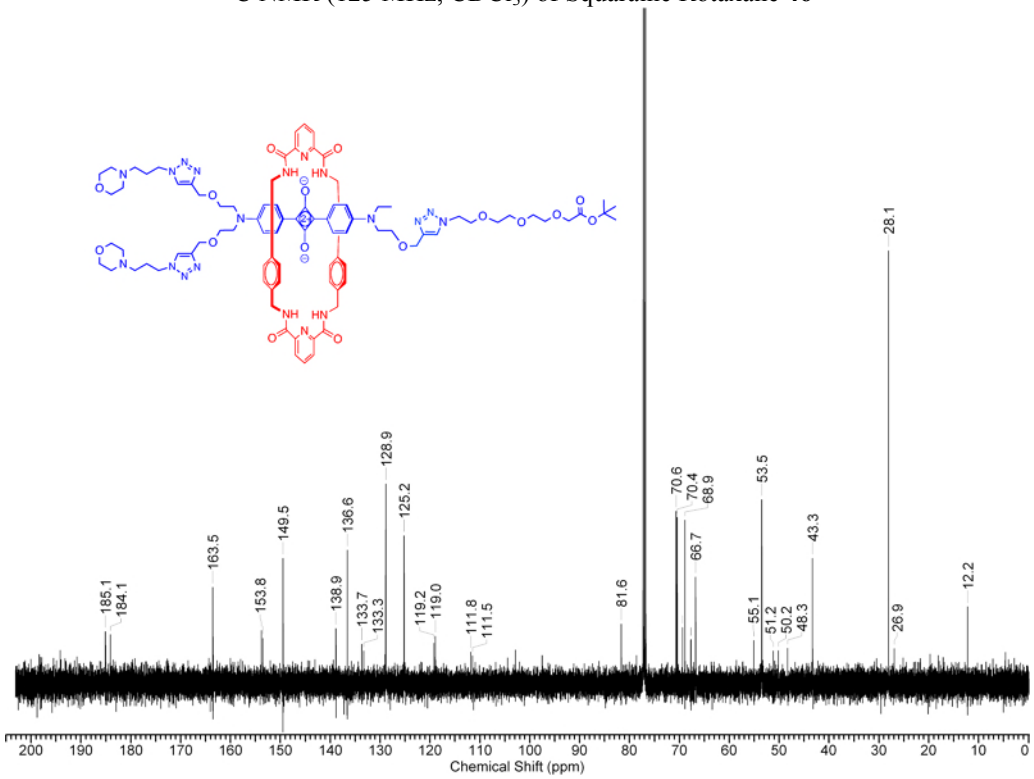

$^{13}$C NMR (125 MHz, $CDCl_3$) of compound **15**



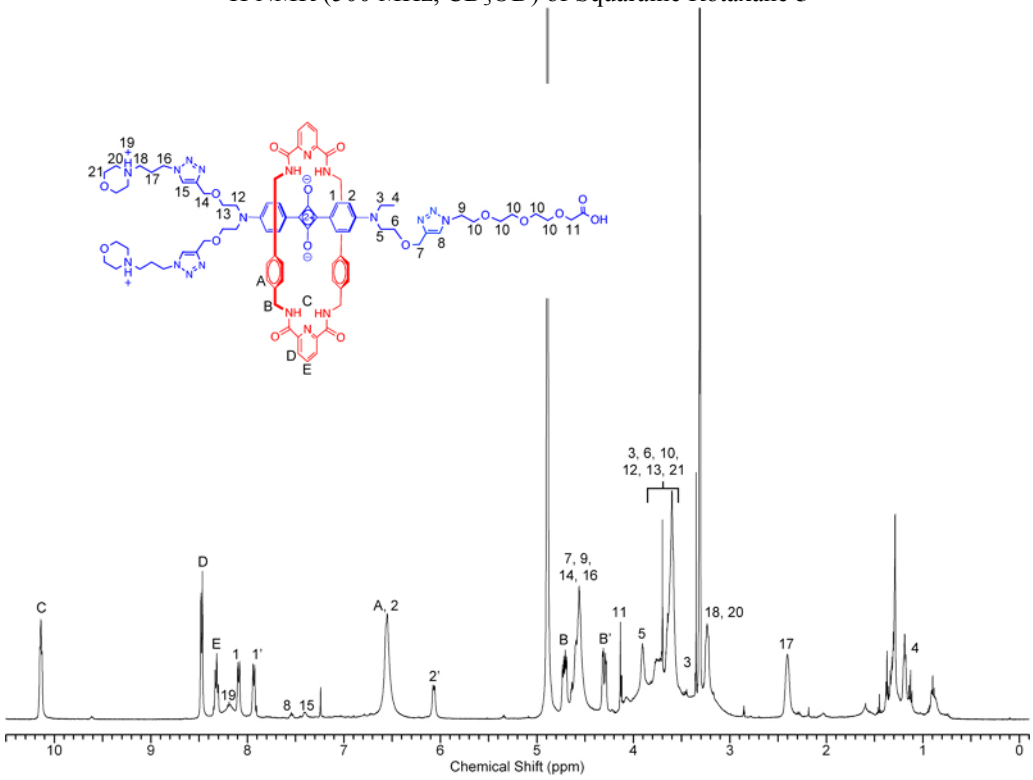
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine Rotaxane **46**



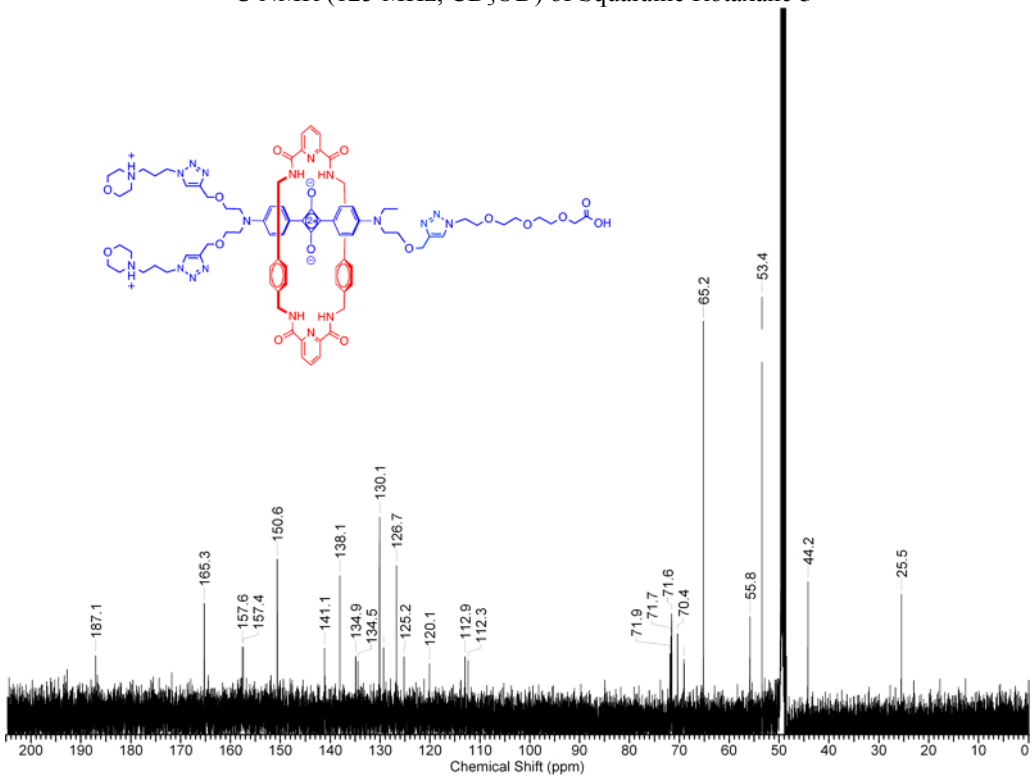

$^{13}$C NMR (125 MHz, $CDCl_3$) of Squaraine Rotaxane **46**



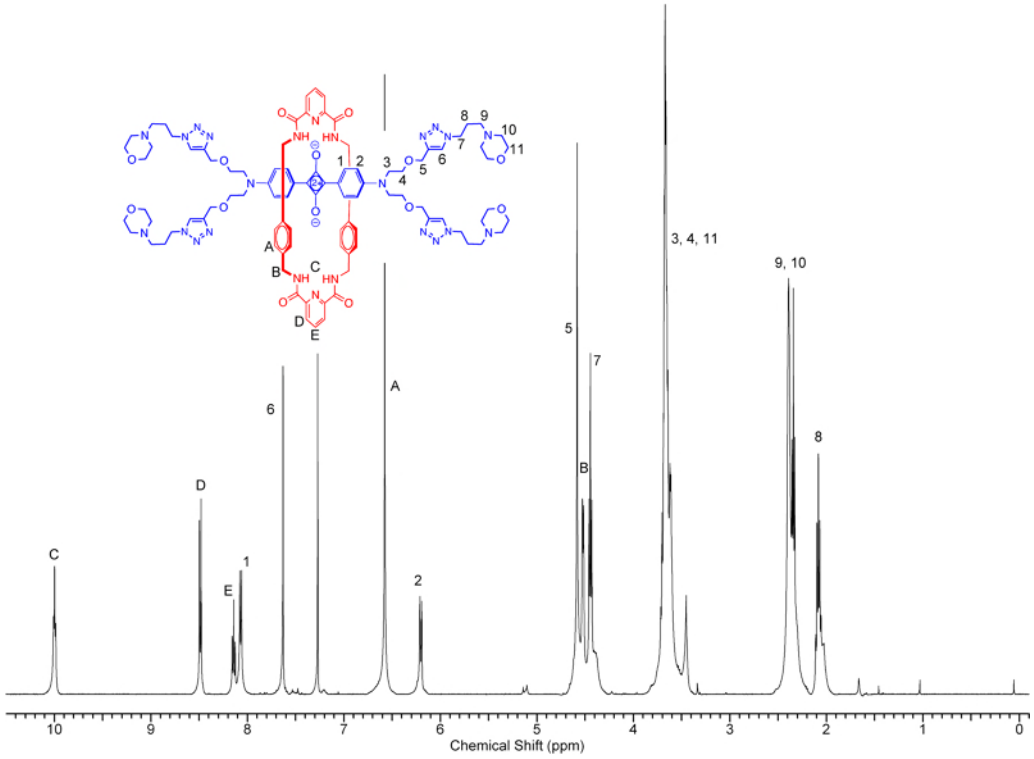
$^{1}$H NMR (500 MHz, $CD_3OD$) of Squaraine Rotaxane **5**



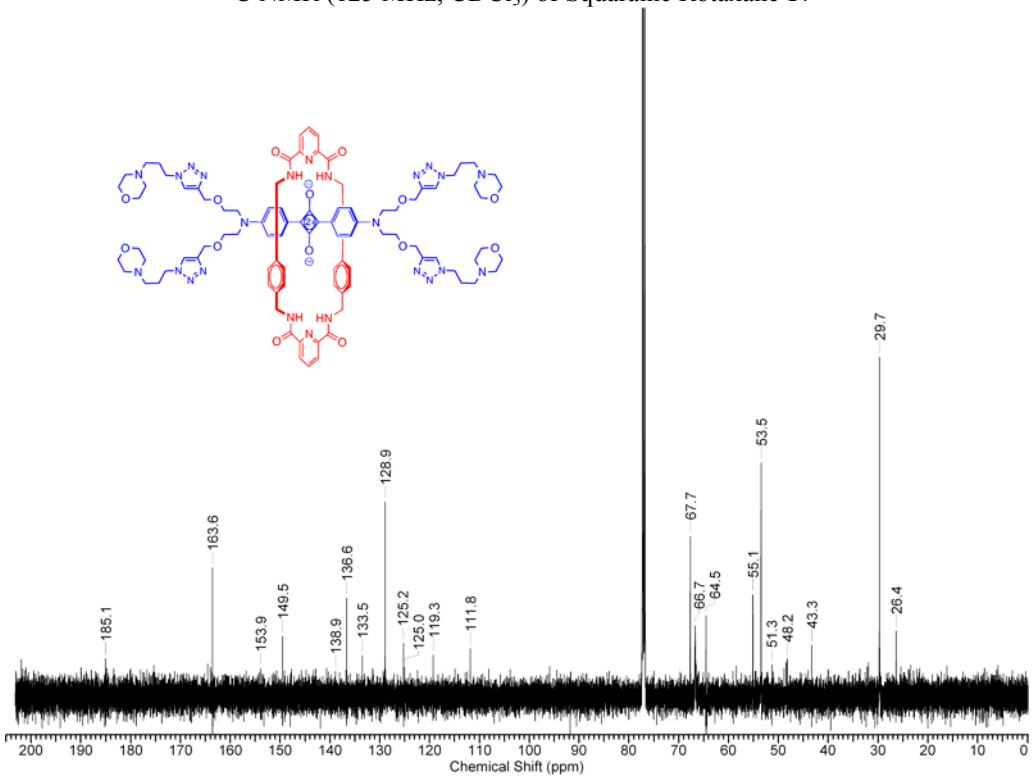

$^{13}$C NMR (125 MHz, $CD_3OD$) of Squaraine Rotaxane **5**



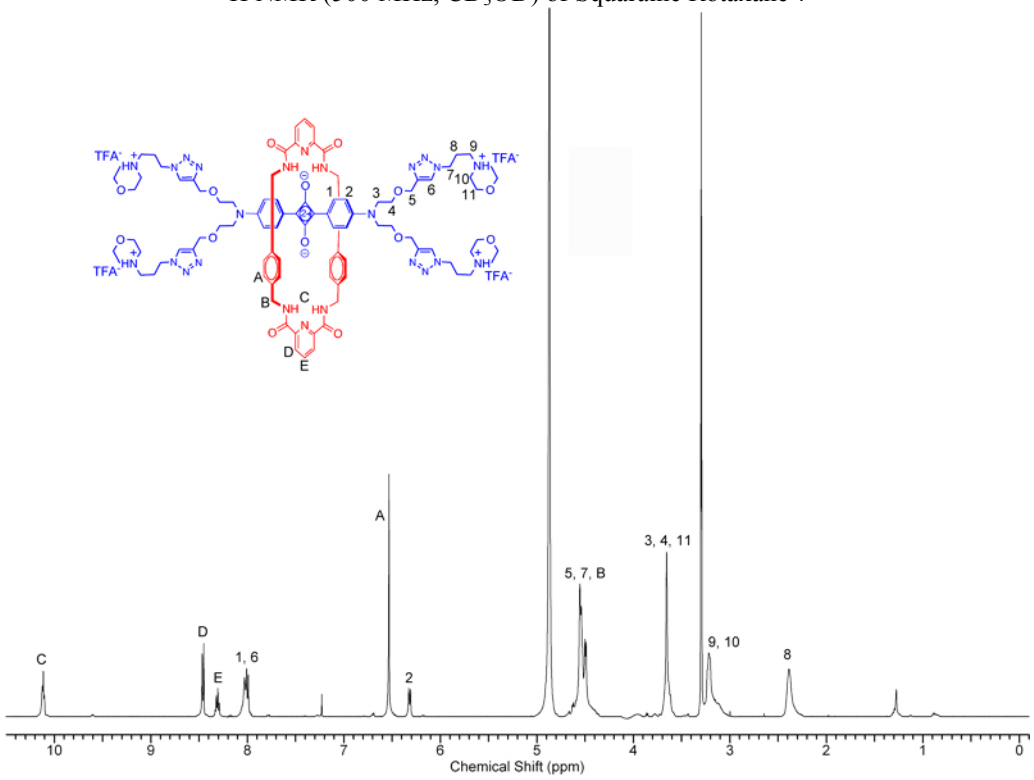
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine Rotaxane **17**



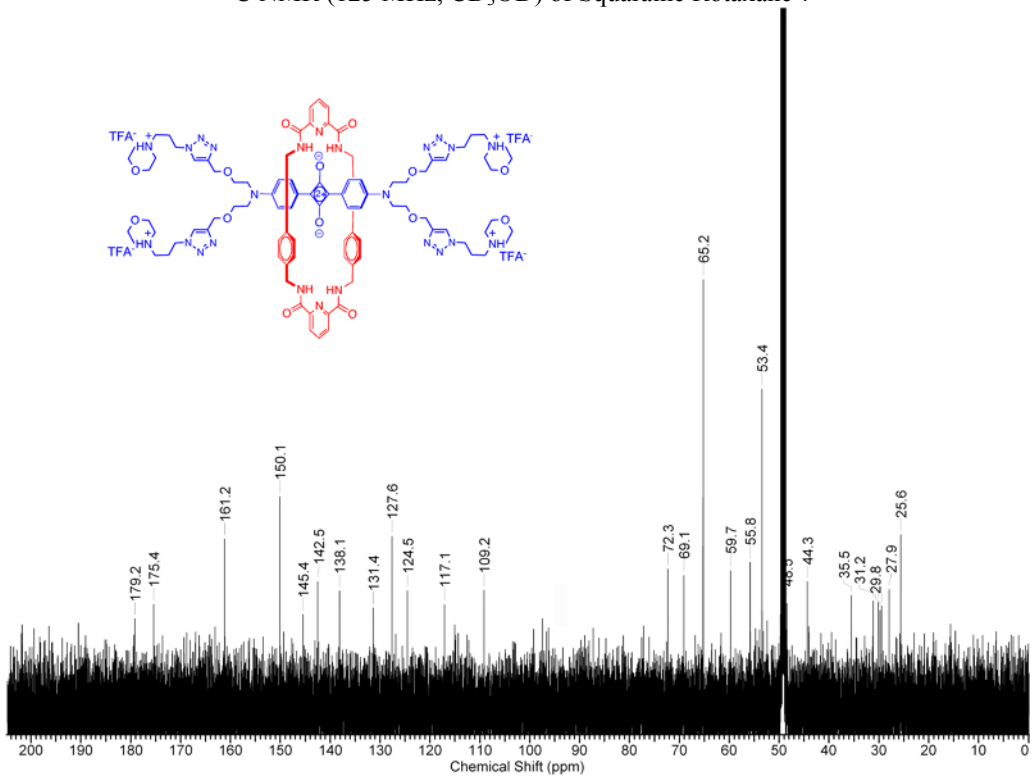

$^{13}$C NMR (125 MHz, $CDCl_3$) of Squaraine Rotaxane **17**



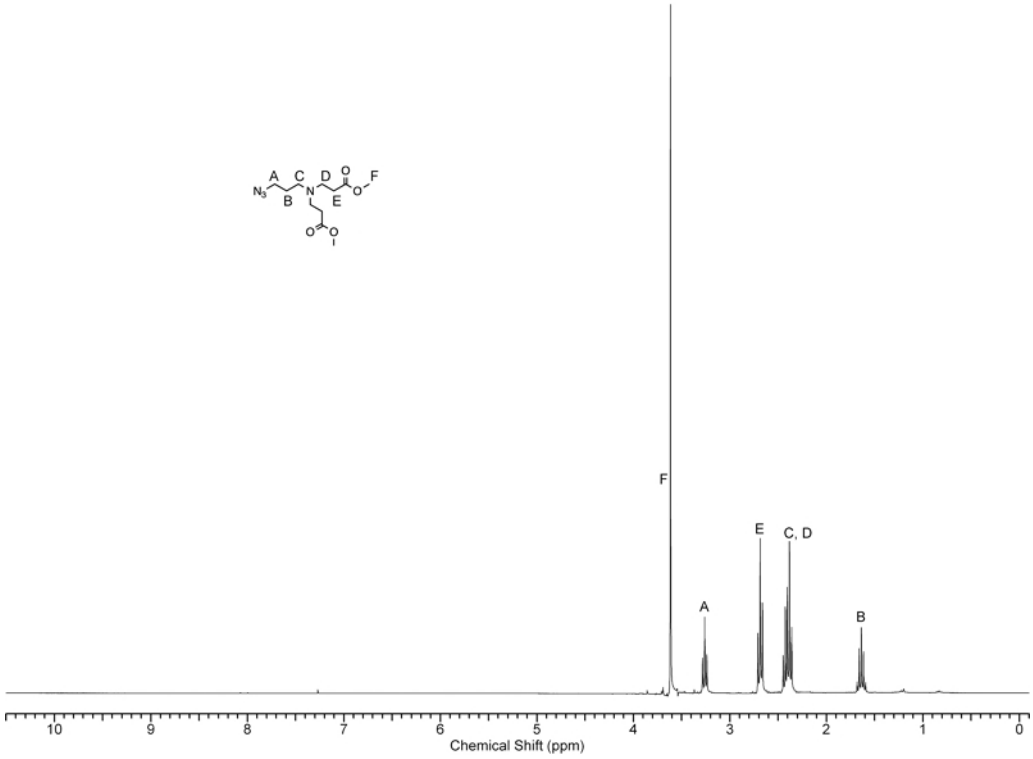
$^{1}$H NMR (500 MHz, $CD_3OD$) of Squaraine Rotaxane **7**



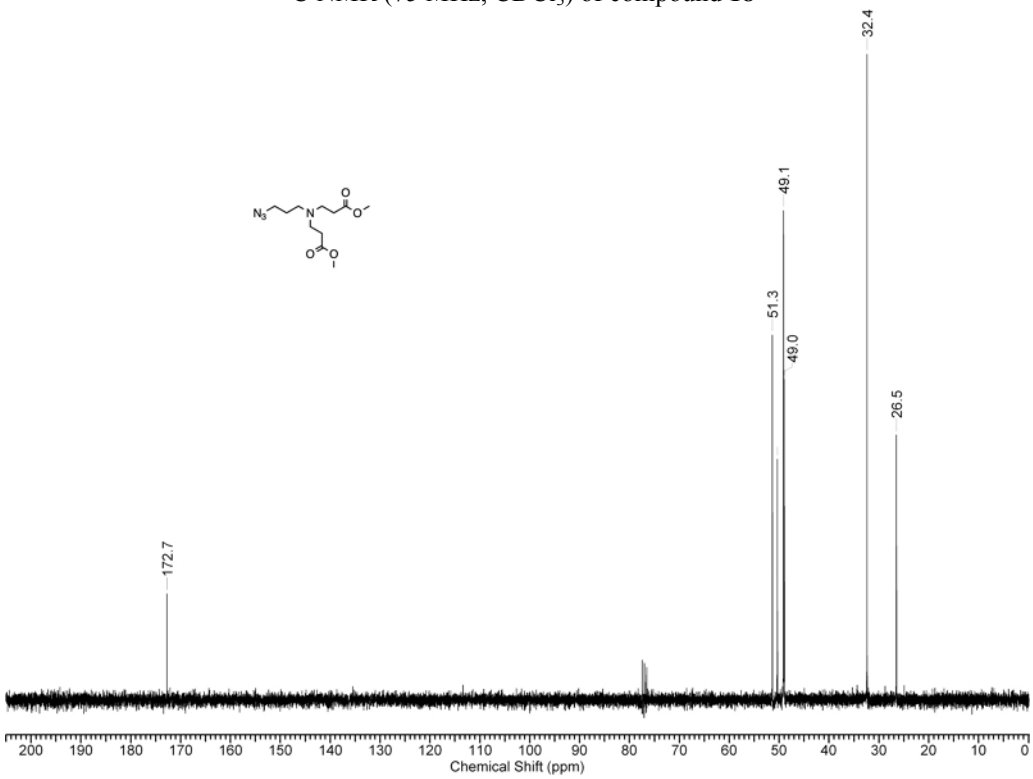

$^{13}$C NMR (125 MHz, $CD_3OD$) of Squaraine Rotaxane **7**



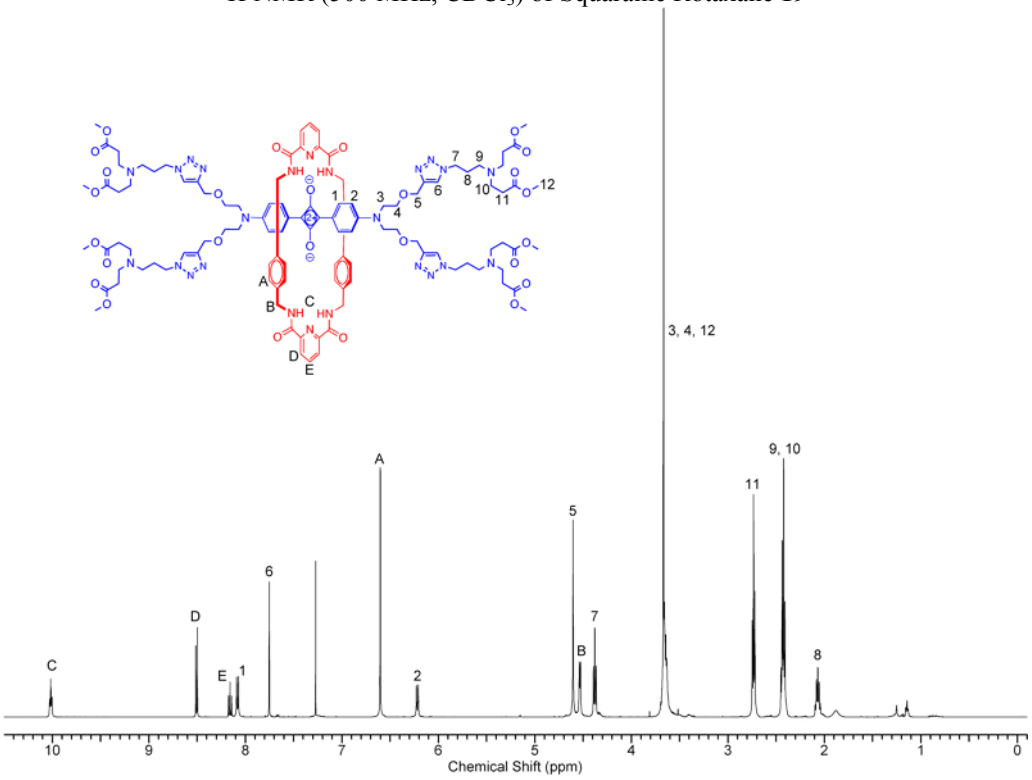
$^{1}$H NMR (300 MHz, $CDCl_3$) of compound **18**



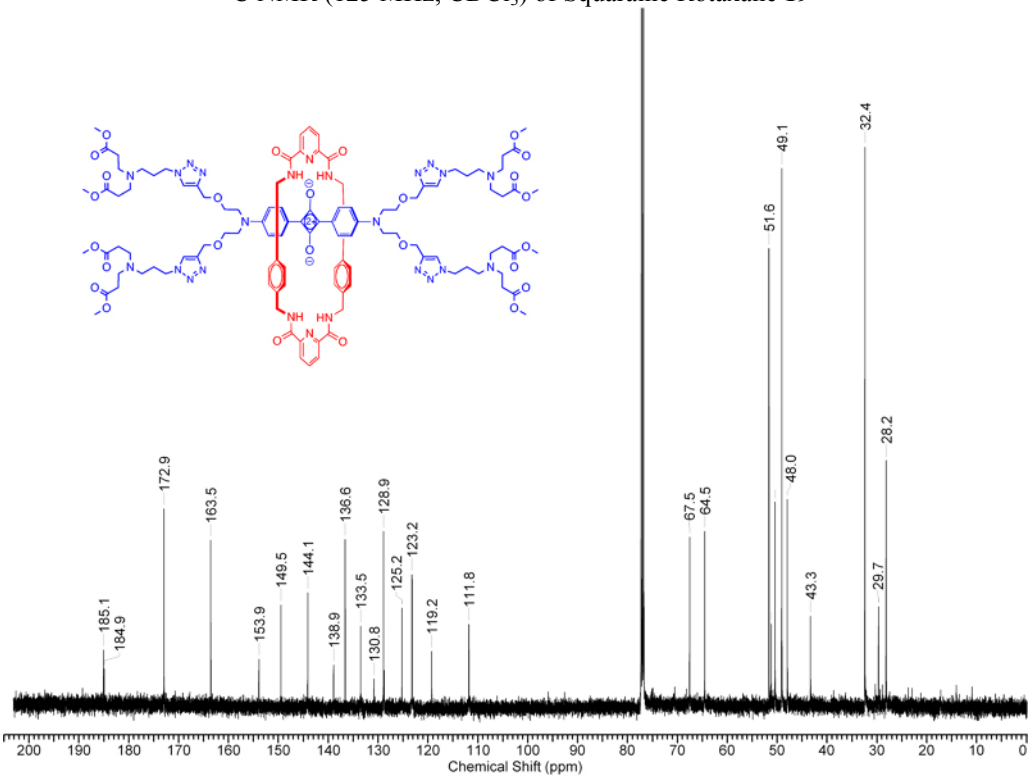

$^{13}$C NMR (75 MHz, $CDCl_3$) of compound **18**



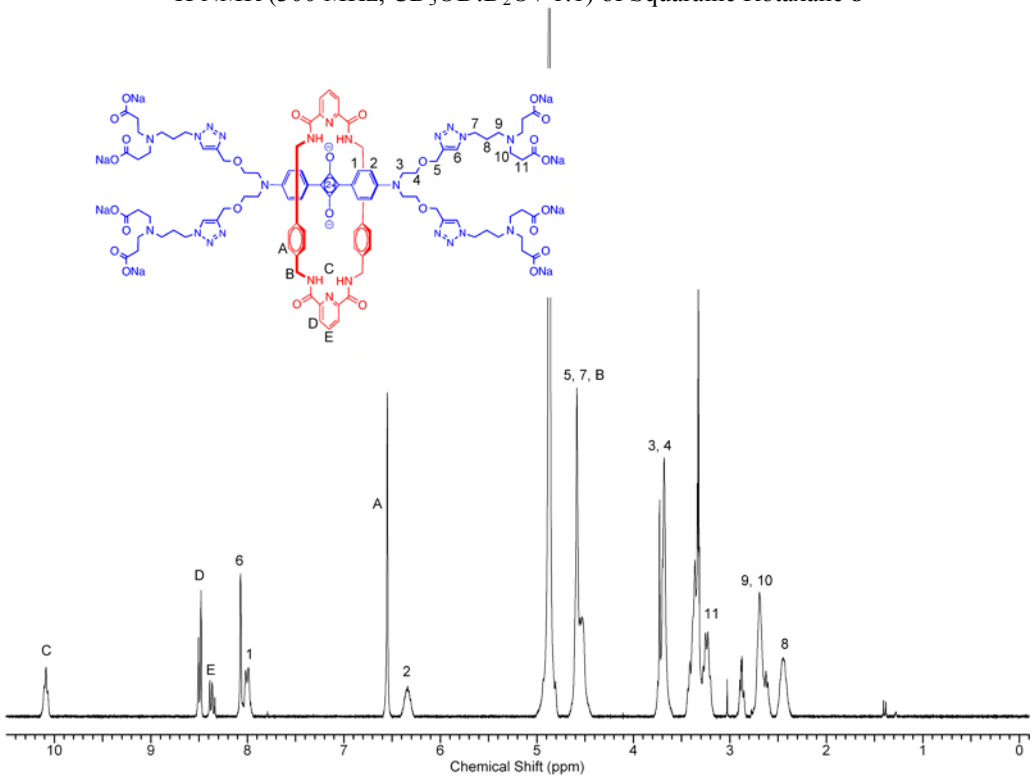
$^{1}$H NMR (500 MHz, $CDCl_3$) of Squaraine Rotaxane **19**



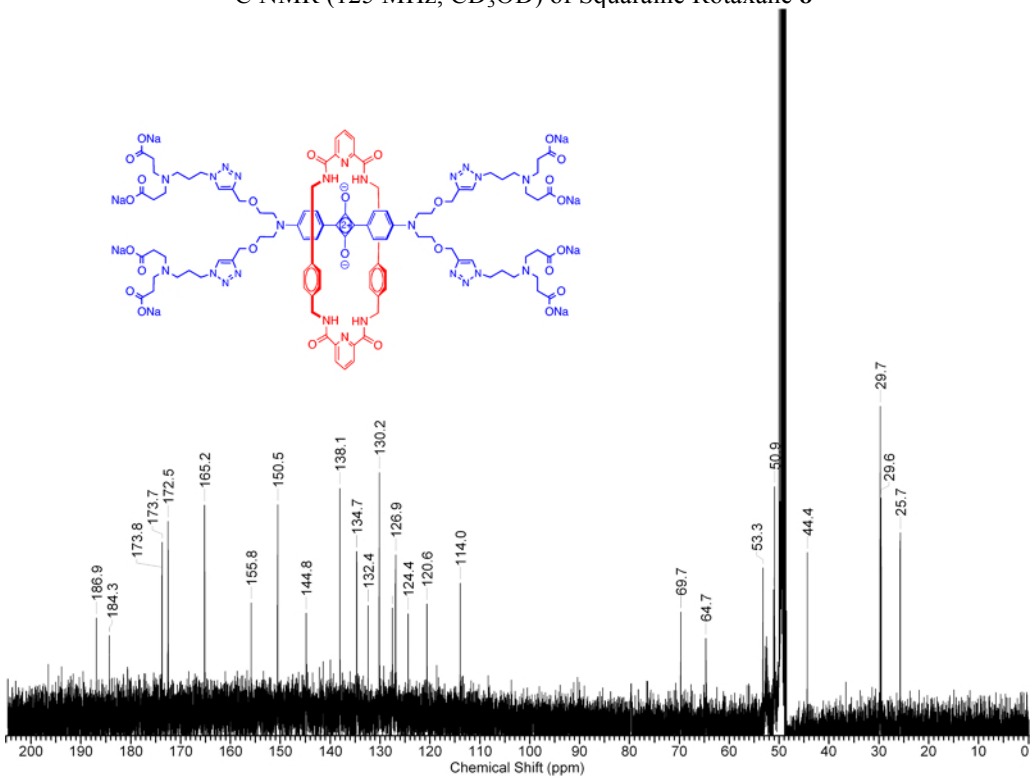

$^{13}$C NMR (125 MHz, $CDCl_3$) of Squaraine Rotaxane **19**



$^{1}$H NMR (300 MHz, $CD_3OD$:$D_2O$ / 1:1) of Squaraine Rotaxane **8**




$^{13}$C NMR (125 MHz, $CD_3OD$) of Squaraine Rotaxane **8**



**6. Animal Imaging**

All animal procedures were approved by the University of Notre Dame Institutional Animal Care and Use Committee. Three cohorts of 6-week old female hairless mice (strain: SKH-1, 25 g, n = 3-4) were injected intravenously with 40 pmol/g of either **7**, **8** or **ICG** (Cardiogreen, Sigma-Aldrich, St. Louis, MO) (100 μL in $H_2O$). Two hours post-probe injection, the mice were anesthetized by isoflurane inhalation (1.5 %), sacrificed, and subjected to ex vivo imaging on an optical imaging station. The skin surrounding the abdominopelvic area was removed thus exposing the internal organs. Fluorescence images for mice treated with **7** and **8** were acquired using the same acquisition parameters including 630 nm ±10 nm excitation filter and 700 nm ± 20 nm emission filter. Fluorescence images for mice treated with **ICG** were acquired using a 750 ± 10 nm excitation filter and an 830 nm ± 20 nm emission filter. With each digital image, a region of interest (ROI) was drawn around the bladder, intestines, liver, and exposed skin and the mean pixel intensities (MPI) were recorded. Selected tissues were excised from each animal and placed on a transparent imaging tray for ex vivo biodistribution imaging using the same filter sets. ROI analysis was performed on the digital images by manually drawing a shape around each excised tissue and calculating the MPI. The resulting ROI values were plotted in Fig. S2 using Graphpad Prism 4.

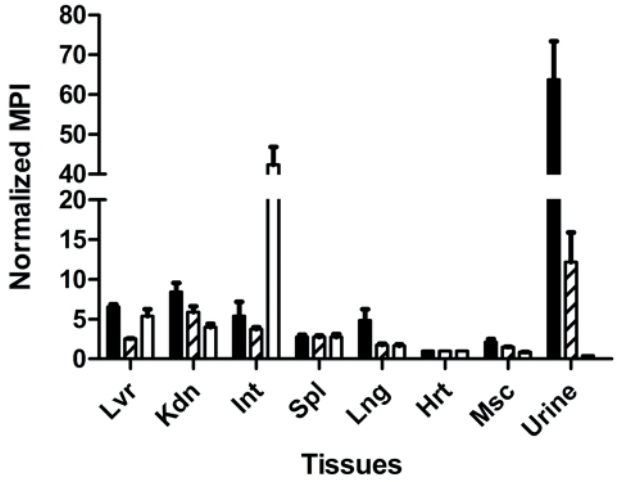



**Fig. S2.** Bar graph showing ex vivo tissue distribution of **7** (black bars), **8** (dashed bars), and **ICG** (white bars). For each digital image, a region of interest was drawn around each excised tissue and the mean pixel intensity (MPI) was plotted as a normalized value relative to the MPI for heart tissue. MPI ± SEM. n = 3-4

**7. References**

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