Supporting Information for:

Rapid Macrocycle Threading by a Fluorescent Dye-Polymer Conjugate in Water with Nanomolar Affinity

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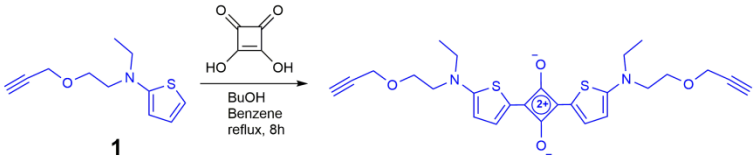
Contents

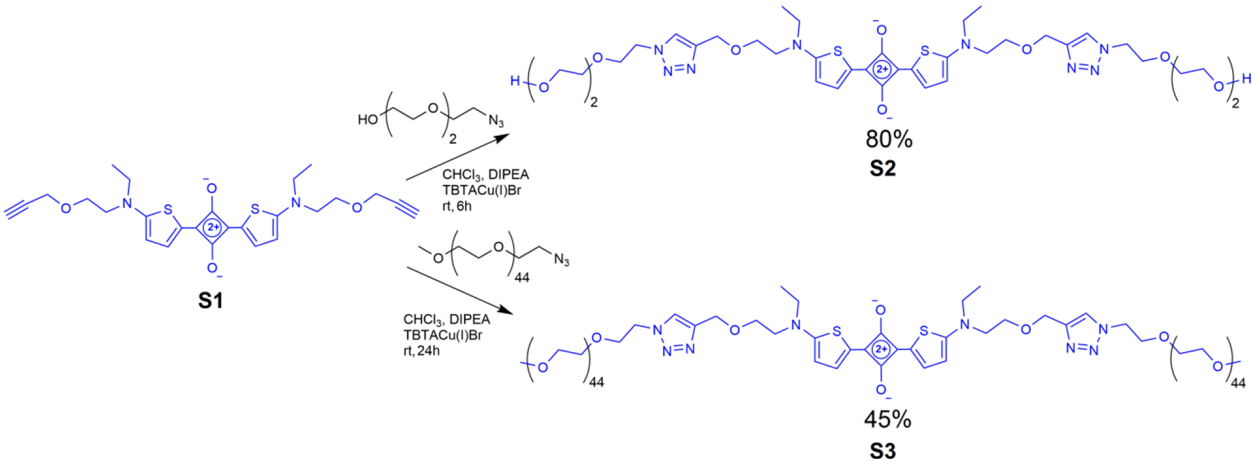
A. Synthesis and Characterization

Macrocycles **M1**[S1] and **M2**,[S2] alkyne-substituted aminothiophenes **1**[S3] and **2**,[S4] triethyleneglycol azide,[S5] and mPEG2000-azide[S5] were synthesized using previously reported methods.



1 77% **S1**

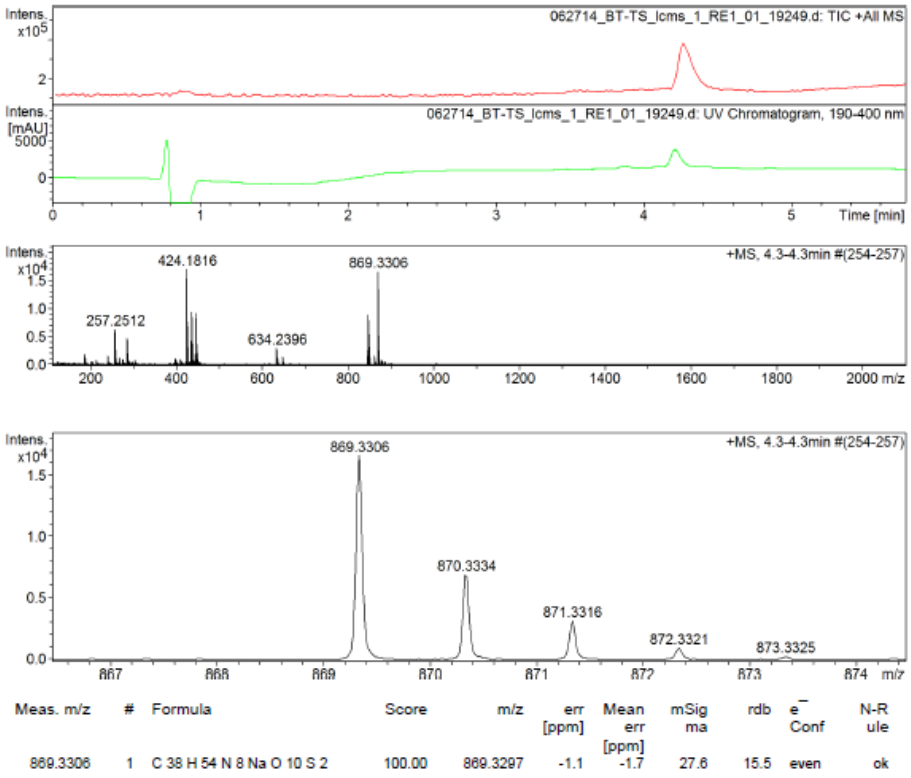
Compound **S1**[S6]: Aminothiophene alkyne **1** (401 mg, 1.9 mmol) and squaric acid (110 mg, 0.96 mmol) were dissolved in a mixture of anhydrous butanol (20 mL) and anhydrous benzene (60 mL). The reaction mixture was refluxed for 8 hours under argon, equipped with a Dean-Stark apparatus. The solvent was removed under reduced pressure and the residue was purified *via* column chromatography using 0-30% acetone in chloroform as eluent to yield **S1** (367 mg, 0.76 mmol, 77%) as a dark blue-green solid. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 7.97 (d, *J* = 4.6 Hz, 2H), 6.28 (d, *J* = 4.4 Hz, 2H), 4.18 (d, *J* = 2.8 Hz, 4H), 3.81 (t, *J* = 5.4 Hz, 4H), 3.69 (t, *J* = 5.1 Hz, 4H), 3.62 (q, *J* = 7.2 Hz, 4H), 2.45 (t, *J* = 2.4 Hz, 2H), 1.31 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C): δ 170.8, 169.5, 138.2, 115.4, 108.8, 79.0, 75.1, 66.9, 58.5, 53.4, 50.1, 12.0. HRMS (ESI-TOF): calculated for $C_{26}H_{28}N_2O_4S_2$ $[M+H]^+$ 496.1485, found 496.1462.



S1 80% **S2** 45% **S3**

Compound **S2**: Squaraine dye **S1** (10 mg, 0.02 mmol) and triethyleneglycol azide (9 mg, 0.05 mmol) were dissolved in chloroform (2 mL) and placed in a small vial. Copper catalyst (2.6 mg, 20 mol%), and *N*,*N*-diisopropylethylamine (10 µL, 0.08 mmol) were added to the vial and the reaction mixture was stirred at room temperature for 6 hours. The solvent was removed under reduced pressure and the residue was purified *via* column chromatography using 10% methanol in chloroform as eluent to yield pure **S2** (12 mg, 0.014 mmol, 80%), as a dark blue, sticky solid. ^{1}H NMR (500 MHz, Acetonitrile-d_3, 25°C): δ 7.80 (d, *J* = 4.7 Hz, 2H), 7.79 (s, 2H), 6.40 (d, *J* = 5.0 Hz, 2H), 4.58 (s, 4H), 4.47 (t, *J* = 5.1 Hz, 4H), 3.80 (t, *J* = 5.1 Hz, 4H), 3.77 (t, *J* = 5.3 Hz, 4H), 3.68 (t, *J* = 5.3 Hz, 4H), 3.47-3.59 (m, 16H), 3.41-3.44 (m, 4H), 2.93-3.01 (br s, 2H), 1.23 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR: Small quantity of material prevented ^{13}C analysis. HRMS (ESI-TOF):

calculated for $C_{38}H_{55}N_8O_{10}S_2$ $[M+H]^+$ 846.3399, found 846.3399. LC-MS (ESI-TOF): calculated for $C_{38}H_{54}N_8NaO_{10}S_2$ $[M+Na]^+$ 869.3297, found 846.3306.



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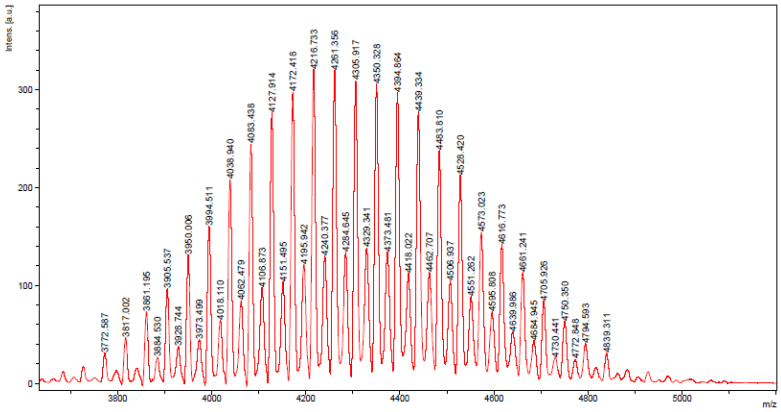
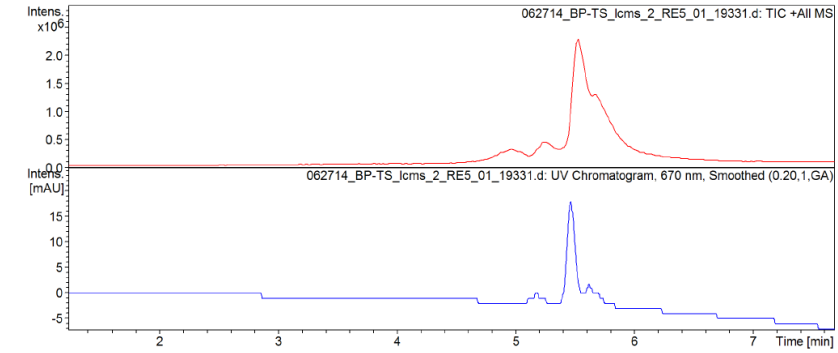
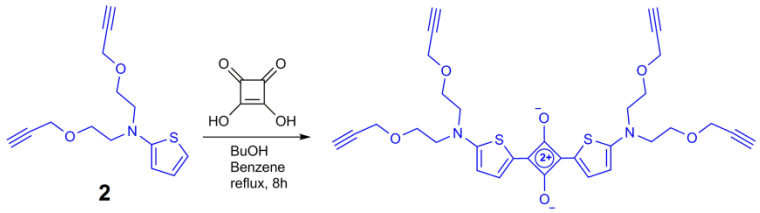
Compound **S3**: Squaraine dye **S1** (5 mg, 0.01 mmol) and mPEG2000-azide (50 mg, 0.03 mmol) were dissolved in chloroform (1.5 mL) and placed in a small vial. Copper catalyst (1.3 mg, 20mol%), and *N*,*N*-diisopropylethylamine (5 µL, 0.04 mmol) were added to the vial and the reaction mixture was stirred at room temperature for 24 hours. The solvent was removed under reduced pressure and the residue was purified *via* column chromatography using 10-20% methanol in chloroform as eluent to yield **S3** (20 mg, 4.4 µmol, 45%), as a dark blue solid. ^{1}H NMR (600 MHz, $CDCl_3$, 25°C): δ 7.94 (d, *J* = 4.7 Hz, 2H), 7.71 (s, 2H), 6.26 (d, *J* = 4.4 Hz, 2H), 4.64 (s, 4H), 4.54 (t, *J* = 5.0 Hz, 4H), 3.86 (t, *J* = 5.0 Hz, 4H), 3.45-3.85 (m, ~304H), 3.38 (s, 6H), 1.28 (t, *J* = 3.4 Hz, 6H). ^{13}C NMR: Small quantity of material prevented ^{13}C analysis. MS (MALDI-TOF): The compound is a distribution of molecular weights, due to polydispersion of the PEG lengths, centered around 4261.36.



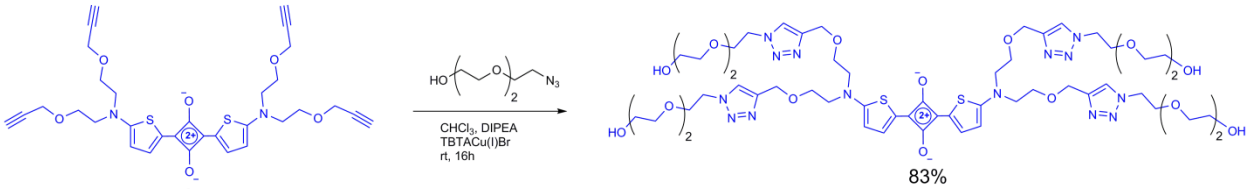
LC trace for **S3**:





2 → **3**: 73%

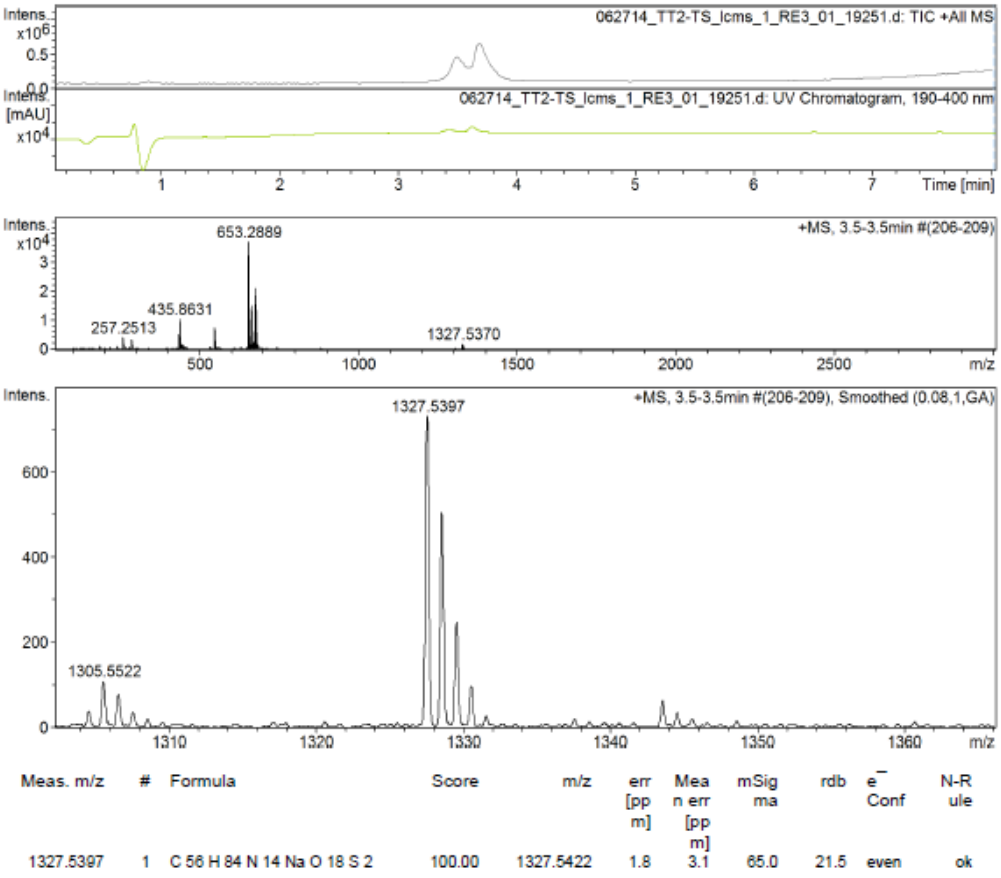
Compound **3**: Aminothiophene bisalkyne **2** (125 mg, 0.47 mmol) and squaric acid (28 mg, 0.24 mmol) were dissolved in a mixture of anhydrous butanol (10 mL) and anhydrous benzene (30 mL). The reaction mixture was refluxed for 8 hours under argon, equipped with a Dean-Stark apparatus. The solvent was removed under reduced pressure and the residue was purified *via* column chromatography using 0-30% acetone in chloroform as eluent to yield the product **2** (108 mg, 0.18 mmol, 73%) as a dark blue-green solid. ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ 7.95 (d, *J* = 4.6 Hz, 2H), 6.33 (d, *J* = 4.6 Hz, 2H), 4.15 (d, *J* = 1.8 Hz, 8H), 3.75-3.85 (m, 16H), 2.44 (t, *J* = 2.4 Hz, 4H). ^{13}C NMR (150 MHz, $CDCl_3$, 25°C): δ 171.4, 169.9, 138.4, 115.7, 109.3, 79.0, 75.1, 66.8, 58.5, 54.8. HRMS (ESI-TOF): calculated for $C_{32}H_{32}N_2O_6S_2$ $[M+H]^+$ 604.1696, found 604.1738.



3 → **S4**: 83%

Compound **S4**: Squaraine dye **3** (30 mg, 0.051 mmol) and triethyleneglycol azide (48 mg, 0.27 mmol) were dissolved in chloroform (5 mL) and placed in a small vial. Copper catalyst (6.4 mg, 20 mol%), and *N*,*N*-diisopropylethylamine (43 µL, 0.26 mmol) were added to the vial and the reaction mixture was stirred at room temperature for 16 hours. The solvent was removed under reduced pressure and the residue was purified *via* column chromatography using 90:10:0 $CHCl_3$:MeOH:NH_4OH to 80:19:1 $CHCl_3$:MeOH:NH_4OH as eluent to yield **S4** (53 mg, 0.041 mmol, 83%), as a dark blue, sticky solid. ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ 7.93 (d, *J* = 4.7 Hz, 2H), 7.77 (s, 4H), 6.30 (d, *J* = 5.0 Hz, 2H), 4.62 (s, 8H), 4.53 (t, *J* = 5.0 Hz, 8H), 3.85 (t, *J* =

5.1 Hz, 8H), 3.78 (t, *J* = 4.9 Hz, 8H), 3.70-3.76 (m, 16H), 3.58-3.63 (m, 16H), 3.56 (t, *J* = 4.7 Hz, 8H), 1.80-2.14 (br s, 4H). ^{13}C NMR: Small quantity of material prevented ^{13}C analysis. HRMS (ESI-TOF): calculated for $C_{56}H_{85}N_{14}O_{18}S_2$ $[M+H]^+$ 1305.5602, found 1305.5625. LC-MS (ESI-TOF): calculated for $C_{56}H_{84}N_{14}NaO_{18}S_2$ $[M+Na]^+$ 1327.5422, found 1327.5397, two observed peaks are *cis*/*trans* rotamers (identical mass).



Meas. m/z	#	Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
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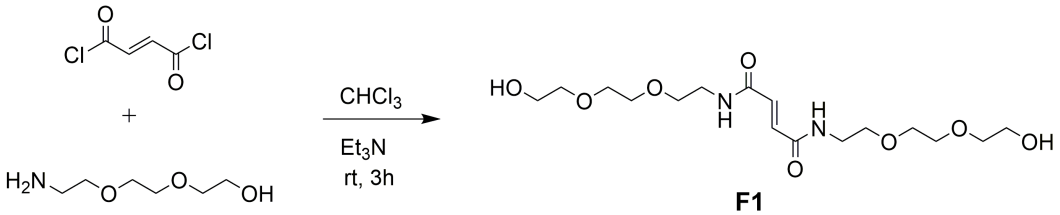
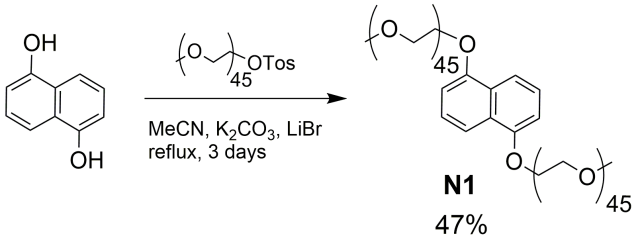


F1

39%

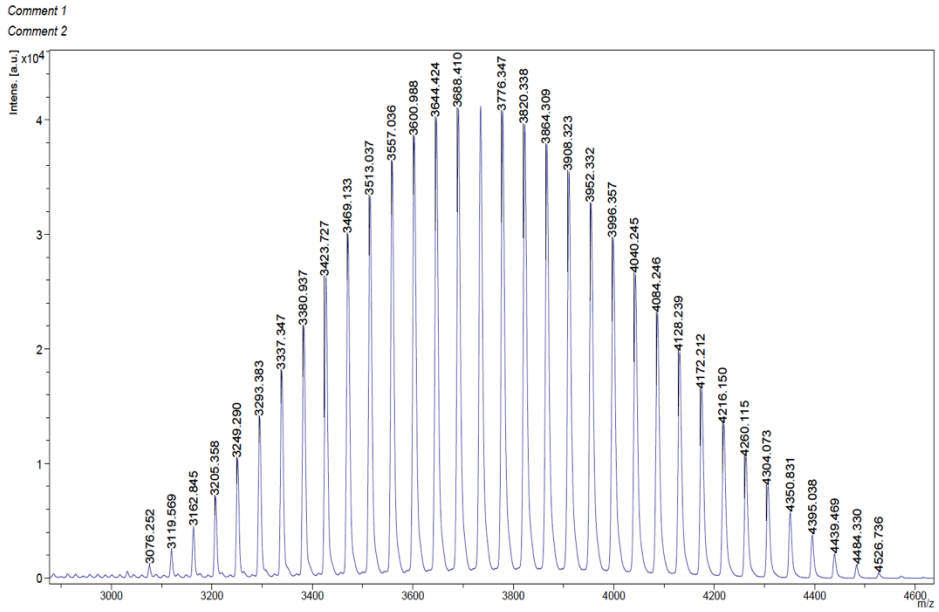
N^1,*N*^{4}-bis(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)fumaramide (**F1**): 2-(2-(2-aminoethoxy)ethoxy)-ethan-1-ol (0.67 g, 4.1 mmol) was dissolved in $CHCl_3$ (8 mL) under ice bath and fumaroyl dichloride (0.25 g, 1.63 mmol) dissolved in $CHCl_3$ (4 mL) was added over 0.5 hours. The resulting solution was titrated with Et_3N (1.1 mL) dissolved in $CHCl_3$ (3 mL) over 0.5 hour. After the titration, the reaction mixture was stirred at room temperature for another 2 hours. Solvent was removed under reduced pressure and the residue was purified *via* silica gel column chromatography by using 5% MeOH/$CHCl_3$ to elute the crude product as white solid. The crude product was further purified by recrystallization from hexane/$CHCl_3$ at -30 ˚C to obtain pure **F1** (463 mg, 39%). ^{1}H NMR (600 MHz, $CDCl_3$, 25°C): δ 7.09 (d, *J* = 9.3 Hz, 2H), 6.94–6.89 (m, 2H),

3.83–3.75 (m, 4H), 3.69–3.61 (m, 16H), 3.58 (t, *J* = 5.3 Hz, 2H), 3.55 (td, *J* = 5.4, 4.2 Hz, 4H). ^{13}C NMR (126 MHz, $CDCl_3$, 25°C): δ 165.2, 133.1, 72.8, 70.5, 70.5, 69.7, 61.7, 39.7. HRMS (ESI-TOF): calculated for $C_{16}H_{30}N_2NaO_8$ $[M+Na]^+$ 401.1894, found 401.1927.



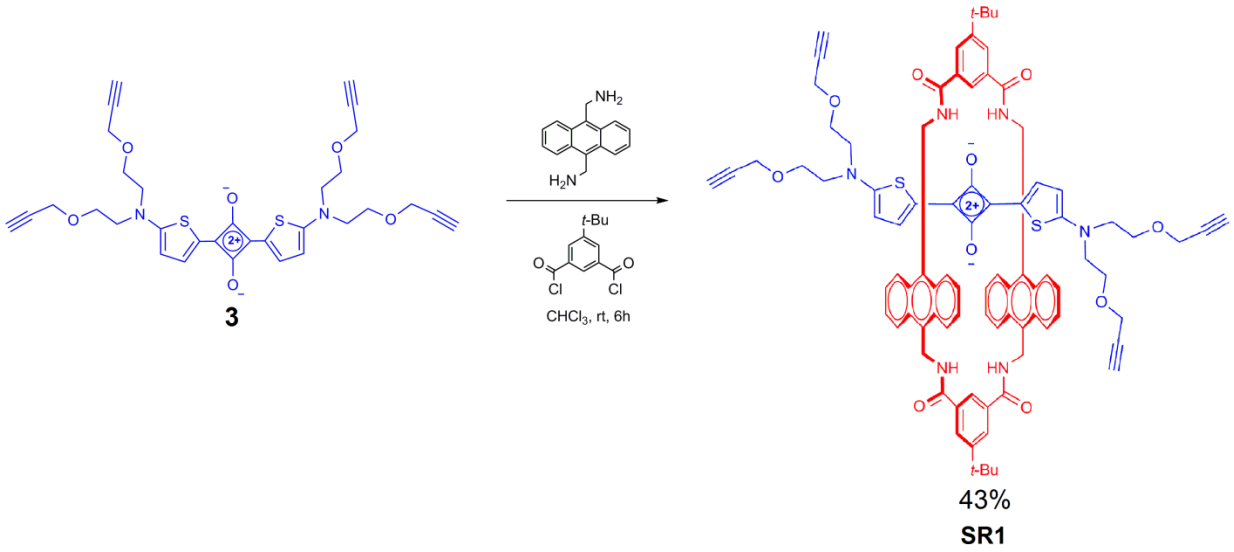
N1
47%

1,5-BisPEGylated Naphthalene (**N1**): 1,5-Bishydroxynaphthalene (36.8 mg, 0.23 mmol) was added to a degassed mixture of $mPEG_{2k}$-OTs (1.0 g, 0.46 mmol), K_2CO_3, (0.127 g, 129.2 mmol), and a catalytic amount of LiBr in dry MeCN (20 mL). The reaction mixture was heated under reflux for 3 days and filtered after cooling. The residue was dissolved in water (40 mL), and the resulting solution was extracted with CH_2Cl_2 (2 x 10 mL). The organic layers were combined and evaporated. The resulting brown solid was dissolved in CH_2Cl_2 (15 mL), and the solution was washed carefully with a mixture of brine and l0% aq. NaOH (3:1,3 x 20mL). The organic layer was dried over $MgSO_4$, and the solvent removed by rotary evaporation. The resulted solid was dissolved in H_2O and dialyzed (MWCO: 3500-5000) for 3 days. The water was removed by lyophilization to afford 0.44 g (47% yield) of **N1** as a brown powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 – 7.82 (m, 2H), 7.34 (dd, J = 8.5, 7.6 Hz, 2H), 6.84 (dd, J = 7.7, 0.9 Hz, 2H), 4.32 – 4.17 (m, 4H), 3.99 (dd, J = 5.5, 4.4 Hz, 4H), 3.82 – 3.46 (m, 458H), 3.38 (s, 6H). ^{13}C NMR: Small quantity of material prevented ^{13}C analysis. MS (MALDI-TOF): The compound is a distribution of molecular weights, due to polydispersion of the PEG lengths, centered around 3778.20 $[M+Na^+]$.



Isomerism of Encapsulated Squaraine Dye

To elucidate the source of the isomerism of the encapsulated squaraine dye (see section C below), the rotaxane structure **SR1** was prepared. The structure has a dye with symmetric disubstituted amino groups on the thiophene units. The NMR spectrum of **SR1** shows two sets of peaks for the encapsulated dye demonstrating that the isomerism is due to *cis* or *trans* orientation of the thiophene units and not due to hindered C-N bond rotation. It is worth noting that the *cis* or *trans* dye conformers are only observed as separate NMR signals when the dye is encapsulated by the macrocycle, presumably due to sterically restricted C-C bond rotation.



Compound **SR1**: Squaraine dye **3** (31 mg, 0.052 mmol) was dissolved in anhydrous $CHCl_3$ (100 mL). Solutions of 5-*tert*-butylisophthalic acid chloride (67 mg, 0.26 mmol) and a mixture of 9,10-anthracenemethylenediamine (59 mg, 0.25 mmol) and *N*,*N*-diisopropylethylamine (70 µL, 0.40 mmol) dissolved in anhydrous $CHCl_3$ (40 mL for each solution) were drawn into separate syringes. The components were added dropwise over a period of 6 hours by syringe pump to the solution containing squaraine dye **3**. At this time, the solvent was removed under reduced pressure and the residue was purified *via* column chromatography using 2-5% acetone in chloroform as eluent to yield **SR1** (32 mg, 0.022 mmol, 43%) as a dark green solid. ^{1}H NMR (600 MHz, $CDCl_3$, 25°C): δ 9.12-9.38 (m, 2H), 8.50-8.55 (m, 4H), 7.97-8.13 (m, 4H), 7.66-7.90 (m, 8H), 6.79-7.00 (m, 8H), 6.37-6.59 (m, 2H), 5.68-5.75 (m, 2H), 5.07-5.36 (m, 8H), 4.20-4.30 (m, 8H), 3.45-3.70 (m, 16H), 2.55-2.60 (m, 4H), 1.48-1.59 (m, 18H). ^{13}C NMR (150 MHz, $CDCl_3$, 25°C): δ 179.8, 178.8, 178.2, 169.8, 169.7, 166.8, 166.3, 166.0, 163.6, 163.5, 152.9, 152.5, 152.4, 139.3, 138.1, 133.0, 132.7, 132.6, 130.5, 130.4, 130.3, 130.2, 129.2, 129.1, 129.0, 128.7, 126.6, 126.1, 125.7, 125.4, 124.9, 124.4, 124.4, 124.0, 123.8, 123.2, 123.2, 122.0, 113.3, 112.5, 109.2, 109.1, 79.0, 79.0, 75.5, 75.5, 69.5, 66.8, 66.7, 58.7, 58.7, 54.6, 53.7, 37.7, 37.3, 35.3, 35.3, 31.5, 31.4, 29.3 [60 out of a theoretical 176 signals (two rotamers)]. HRMS (ESI-TOF): calculated for $C_{88}H_{85}N_6O_{10}S_2$ $[M+H]^+$ 1449.5763, found 1449.5766.

B. Photophysical Properties

All measurements were made at 25 °C, unless otherwise noted. Quantum yield measurements were performed in triplicate. An absorbance of 0.10 (3.0 μM of dye) and bis[4-(dimethylamino)phenyl]squaraine (Φ_f = 0.70 in chloroform) and methylene blue (Φ_f = 0.02 in H_2O) were used as standards for quantum yield measurements.[S7]

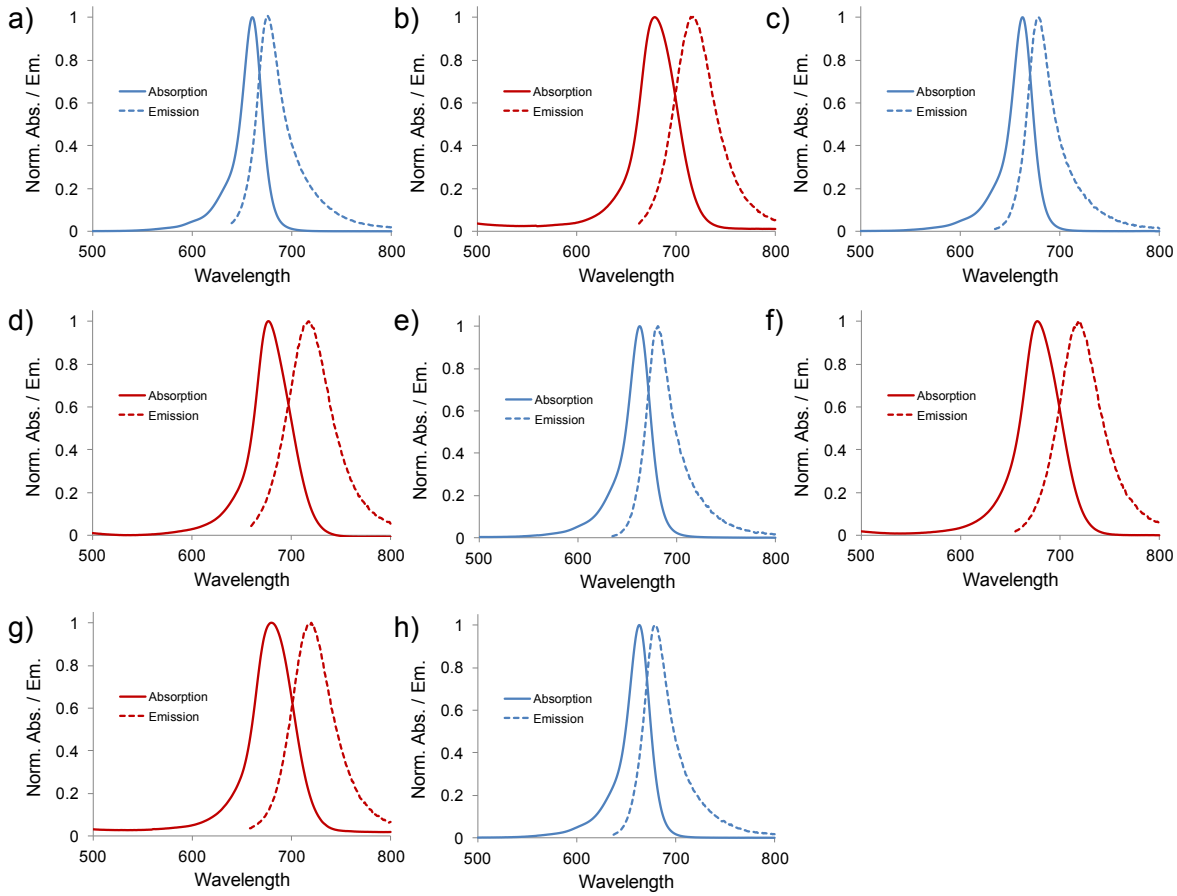


Figure S1: Absorption and emission maxima of a) squaraine dye **S1**; b) complex **M1⊃S1**; c) squaraine dye **S2**; d) complex **M1⊃S2**; e) squaraine dye **S3**; f) complex **M1⊃S3**; g) squaraine rotaxane **SR1**; and h) squaraine dye **S4** in $CHCl_3$ (3.0 μM).

Table S1: Photophysical data for squaraine dyes in $CHCl_3$ (3.0 μM).

Dye	λ_{abs} (nm)	log ε	λ_{em} (nm)	Φ_f
S1	660	5.51	678	0.15
M1⊃S1	678	5.14	715	0.34
S2	662	5.42	678	0.11
M1⊃S2	678	5.15	719	0.34
S3	662	5.36	679	0.09
M1⊃S3	677	5.13	718	0.26
S4	659	5.46	714	0.12
SR1	680	5.11	720	0.29

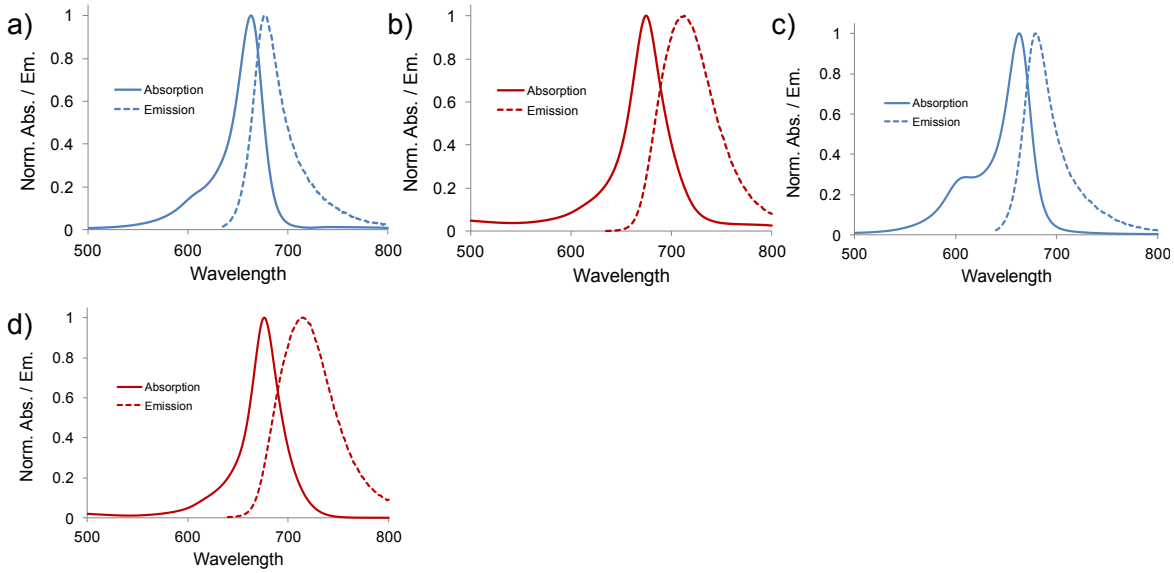


Figure S2: Absorption and emission maxima of a) squaraine dye **S2**; b) complex **M2⊃S2**; c) squaraine dye **S3**; and d) complex **M2⊃S3** in H_2O (3.0 μM).

Table S2: Photophysical data for squaraine dyes in H_2O (3.0 μM).

Compound	λ_{abs} (nm)	λ_{em} (nm)	Φ_f
S2	660	678	0.04
M2⊃S2	676	715	0.26
S3	663	679	0.03
M2⊃S3	678	714	0.27

C. Dye Isomerism and Computational Structure of Threaded Complex

The squaraine dyes exist as conformational isomers, based on the relative orientation of the thiophene units (*cis* or *trans*). When free in solution, the squaraine isomerism occurs rapidly on the NMR timescale at room temperature. Upon complexation within the macrocycle, the barrier to C-C bond rotation that exchanges the squaraine conformations is slowed and both conformations are observed in the ^{1}H NMR spectrum, with the *trans* isomer preferred.

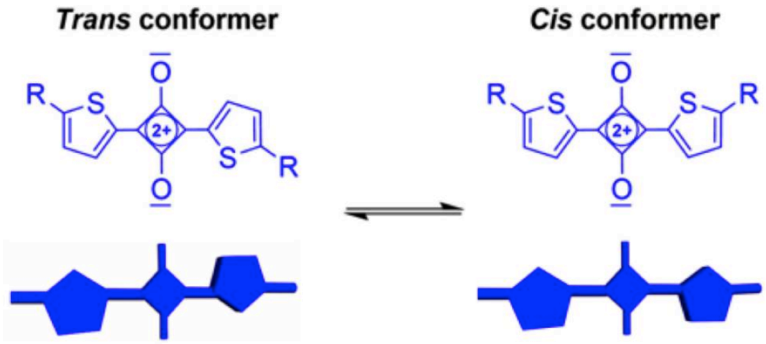


Figure S3: Cis/trans conformational isomers for thiophene-containing squaraine dyes.

A computational structure of the **M1⊃S1** analogue complex (the macrocycle lacks *tert*-Butyl groups) was optimized using the BLYP-gCP-D3 method in combination with def2-TZVP basis set and def2-TZVP/J auxiliary basis sets.[S12] The calculation was executed using the ORCA3.0.3 program. The structure is very similar to analogous aniline-containing squaraine rotaxane crystal structures, and shows bifurcated hydrogen bonds between the macrocycle amide NH residues and the squaraine oxygens. Furthermore, the macrocycle adopts a macrocyclic chair conformation with the two anthracene units stacked against the encapsulated squaraine dye.

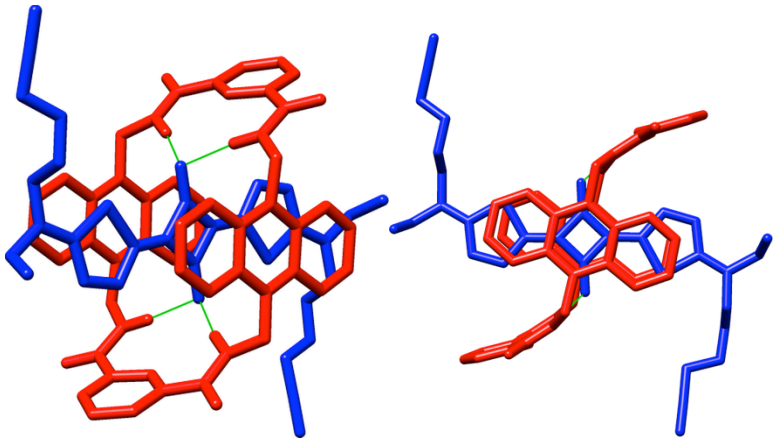


Figure S4: Computational structure (BLYP-gCP-D3/def2-TZVP) of **M1⊃S1** analogue (most hydrogens are hidden) highlighting the hydrogen bonding (*left*) and aromatic stacking (*right*).

D. Evidence for Host-Guest Complexation

^{1}H NMR Studies of Host-Guest Complexation

For complex formation studies, vials containing guest and macrocycle were dissolved in the solvent indicated. The samples were allowed to equilibrate for 10 minutes at room temperature, then ^{1}H NMR spectra of the complexes were acquired.

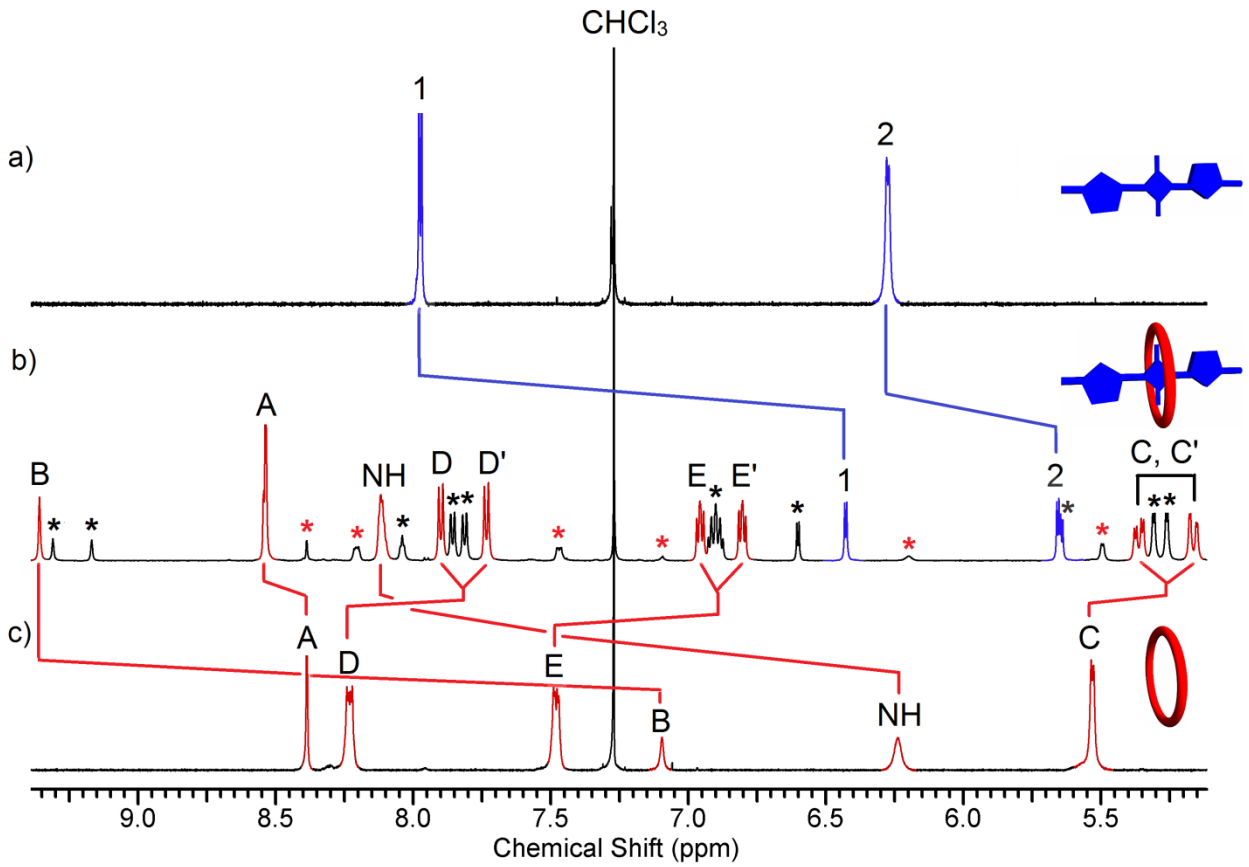


Figure *S5*: Partial ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) of a) **S1** (2.0 mM); b) **M1** (2.5 mM); and c) **M1⊃S1** (2.0 mM). Red and blue lines indicate major changes in chemical shift upon complexation. *indicates signals corresponding to the minor **M1⊃S1** complex with **S1** encapsulated as *cis* conformer (*trans*:*cis* ratio for encapsulated **S1** ~1.5:1). *indicates signals corresponding to a very slight excess of **M1**.

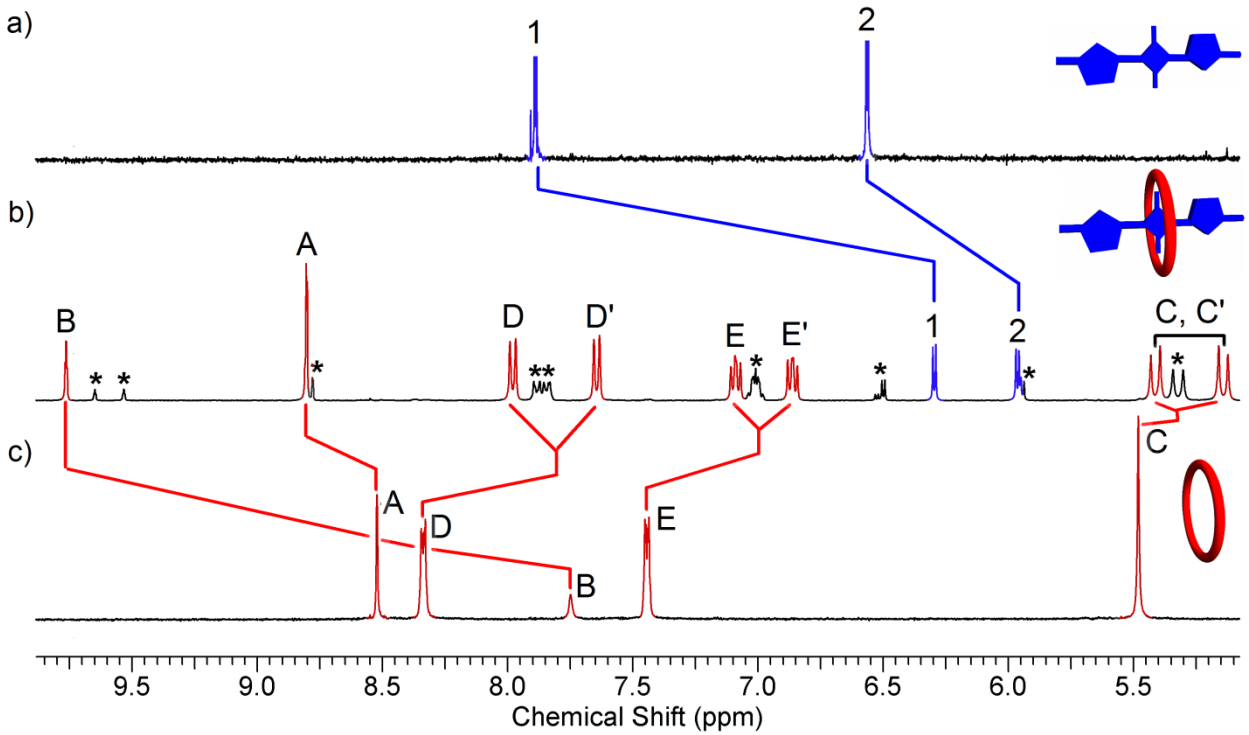


Figure *S6*: Partial ^{1}H-NMR (600 MHz, CD_3OD, 25 °C) of a) **S1** (2.0 mM); b) **M2** (2.0 mM); and c) **M2⊃S1** (2.0 mM). Red and blue lines indicate major changes in chemical shift upon complexation. *indicates signals corresponding to the minor **M2⊃S1** complex with **S1** encapsulated as *cis* conformer (*trans*:*cis* ratio for encapsulated **S1** ~3:1).

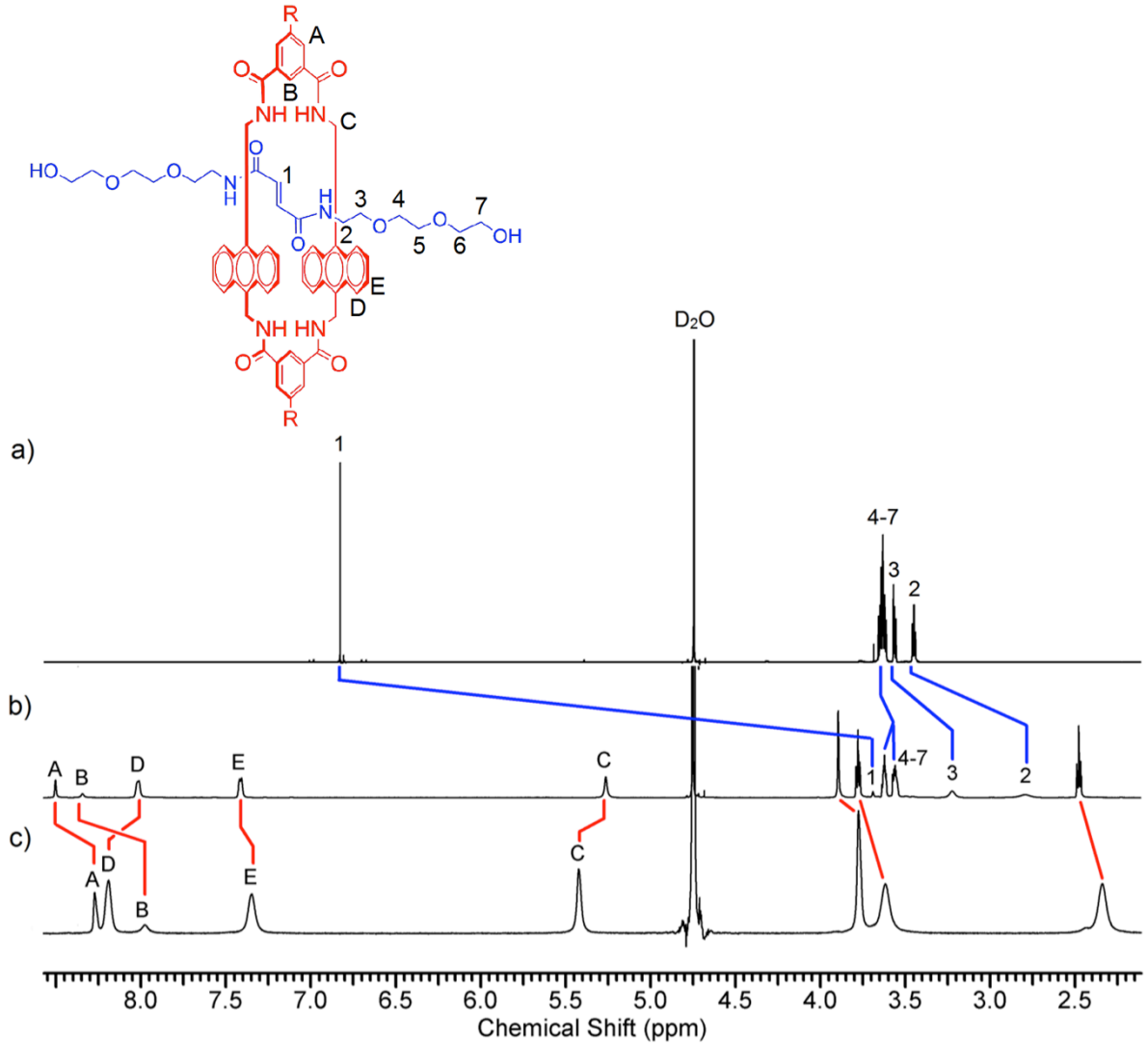


Figure *S7*: Partial ^{1}H NMR (500 MHz, D_2O, 25°C) of a) **F1** (2.0 mM); b) **M2⊃F1** (2.0 mM); and c) **M2** (2.0 mM). Red and blue lines indicate major changes in chemical shifts upon complexation.

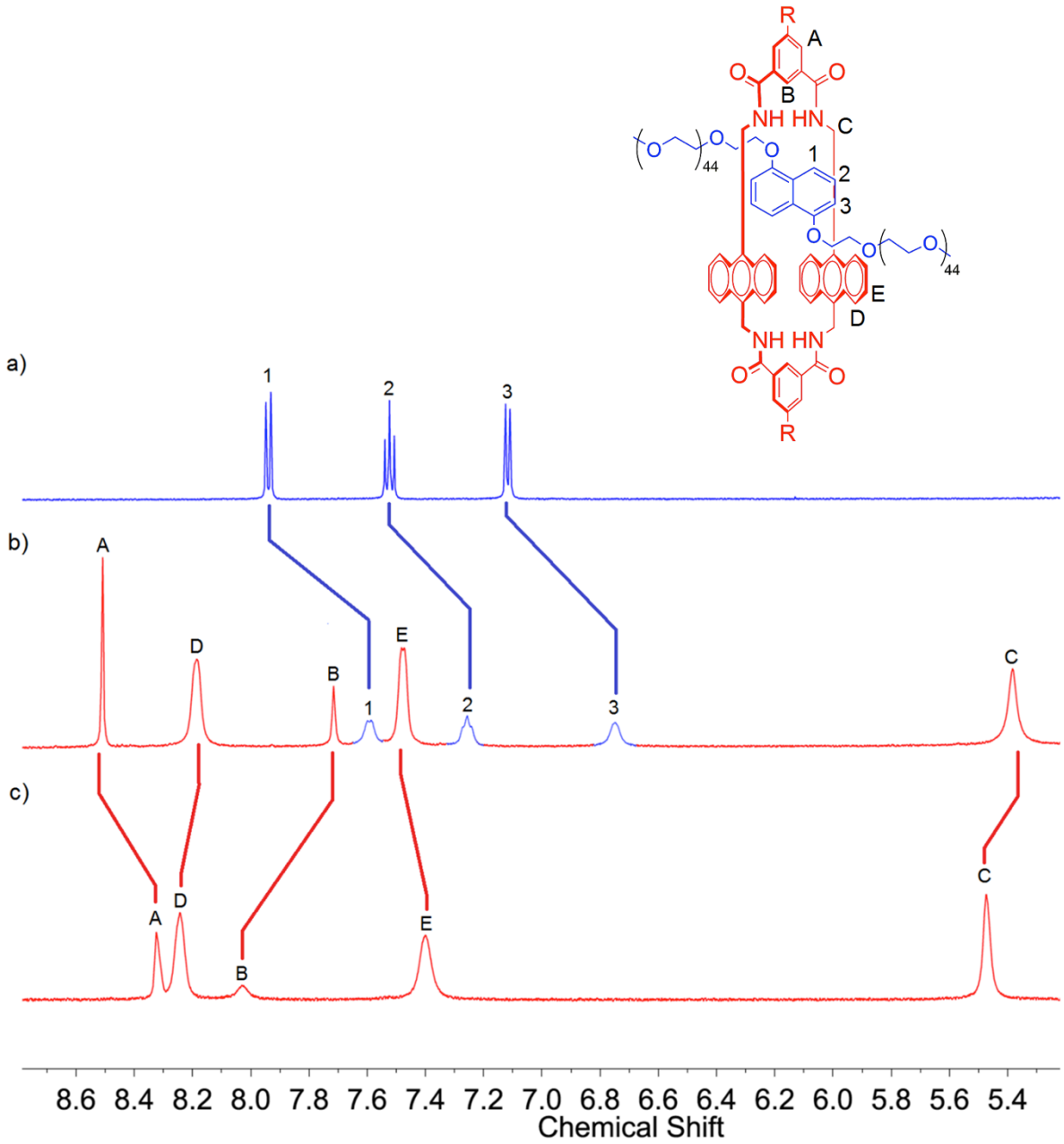


Figure *S8*: Partial ^{1}H NMR (500 MHz, D_2O, 25°C) of a) **N1** (2.0 mM); b) **M2⊃N1** (2.0 mM); and c) **M2** (2.0 mM). Red and blue lines indicate major changes in chemical shifts upon complexation.

Fluorescence Studies of Host-Guest Complexation and Job Plot

A solution of squaraine dye **S3** (3.0 μM) in water was placed in a cuvette and allowed to equilibrate for 20 minutes. A 1.0 mM stock solution of **M2** was prepared and aliquots of this stock solution were titrated into the cuvette containing **S3**; the sample was excited at 690 nm and the emission spectra was collected after each addition.

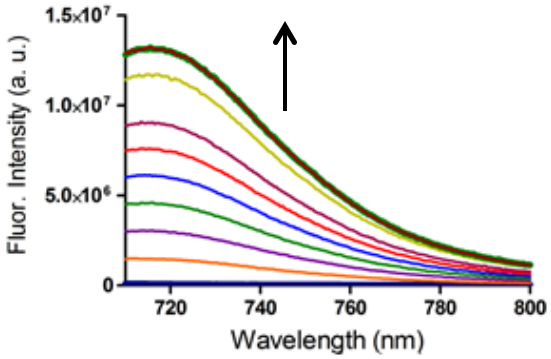


Figure *S9*: Fluorescence emission of **S3** (3.0 μM) upon addition of **M2** (up to 2 mol. equiv.) showing dramatic turn-on effect (25 °C, H_2O, Excitation = 690 nm).

Solutions containing **S3** + **M2** in H_2O at 25 °C were prepared from 250 μM stock solutions and placed in a 3.0 mL cuvette. In all cases, the total concentration [**S3**+**M2**] was kept constant at 2.0 μM, while the mole fraction of dye was increased in increments of 0.1 from 0.0 to 1.0. The solutions were excited at 710 nm and the emission at 725 nm was recorded. A plot of the fluorescence intensity at 725 nm versus the mole fraction yielded the Job Plot, confirming 1:1 binding between **S3** and **M2**.

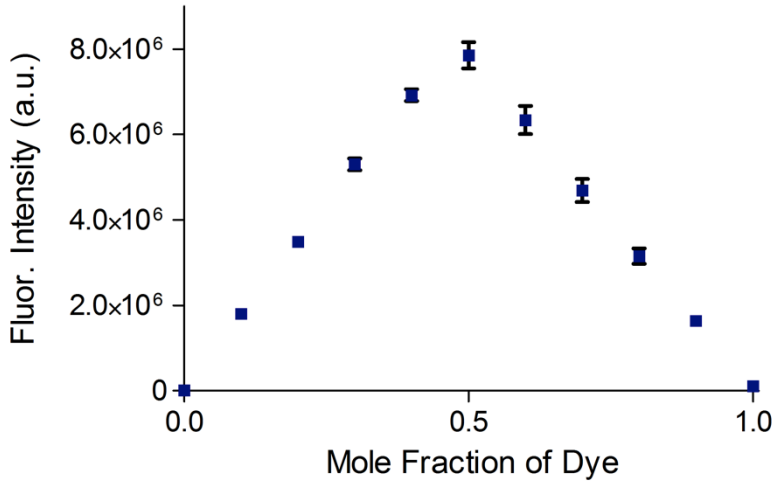


Figure *S10*: Job Plot indicating 1:1 binding between **S3** and **M2** ([**S3**+**M2**] = 2.0 μM, H_2O, 25 °C, Excitation = 710 nm, Emission = 725 nm).

Squaraine Stability Studies

Vials containing **S3** (5.0 μM) in 100% H_2O showed <5.0% decrease in absorption of maxima band after 24 hours. But absorption for a solution of **S3** (5.0 μM) in the presence of strong nucleophile Na_2S (10 mM) decreased by 55% after 20 minutes (curve B). In contrast, there was essentially no change in absorbance when Na_2S was added to a sample of **S3** that had been premixed with **M2** (forming **M2**⊃**S3**) (curve A).

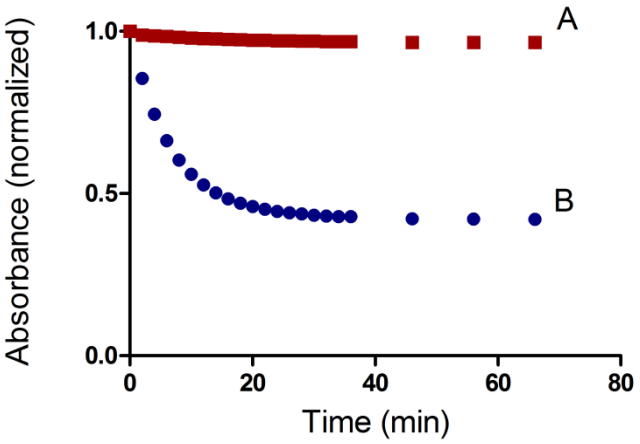


Figure *S11*: Change in absorbance of maxima band for solutions of A) complex **M2⊃S3** (5.0 μM **S3**, 7.5 μM **M2**) or B) squaraine dye **S3**, in the presence of external nucleophile Na_2S (10 mM) in water at 25°C.

TLC Studies of Squaraine/Macrocycle Complexation

Separate solutions of **S3**, **S3**+**M2**, **S4**, and **S4**+**M2** (100 μM each) in water were spotted on a silica gel plate and allowed to dry. The plate was eluted with methanol:chloroform (1:9) as the mobile phase. The plate was removed, allowed to dry, and observed under laboratory light and also under UV light. The presence of **M2** completely prevented elution of **S3** up the plate, indicating formation of a very polar **M2⊃S3** complex. In contrast, the presence of **M2** had no effect on the retention factor for control dye **S4**, indicating that a threaded complex was not formed.

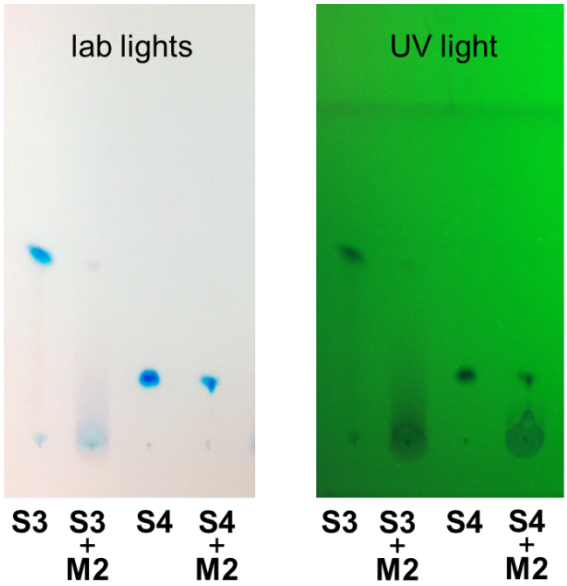


Figure *S12*: TLC plates after elution with methanol:chloroform (1:9) as the mobile phase.

E. Determination of Association Constants (K_a)

Algorithms for determining association constants[S8- S11]

For 1:1 Host-Guest System:

$$H + G \underset{}{\overset{K_a}{\rightleftharpoons}} HG$$

The desired association constant is expressed in equation (1.1)

$$K_a = \frac{[HG]}{[H][G]} \quad (1.1)$$

Assigning the total concentration of host and guest as $[H]_0$ and $[G]_0$, respectively, gives mass balance equations (1.2) and (1.3)

$$[H]_0 = [H] + [HG] \quad (1.2)$$

$$[G]_0 = [G] + [HG] \quad (1.3)$$

Equation (1.2) is rearranged to define [H] and equation (1.3) is rearranged to define [G]. The newly defined [H] and [G] are used to replace [H] and [G] in equation (1.1) and the resulting equation is rearranged to yield equation (1.4)

$$[HG]^2 - ([H]_0 + [G]_0 + 1/K_a)[HG] + [H]_0[G]_0 = 0 \quad (1.4)$$

The real root of equation (1.4) is expressed in equation (1.5), which defines [HG] based on K_a and experimentally determined values ($[H]_0$ and $[G]_0$)

$$[HG] = 0.5\left\{\left([H]_0 + [G]_0 + 1/K_a\right) - \sqrt{\left([H]_0 + [G]_0 + 1/K_a\right)^2 - 4[H]_0[G]_0}\right\} \quad (1.5)$$

UV/Vis titration method

All absorbance measurements were made in triplicate on a Perkin-Elmer Lambda 25 spectrometer.

.According to Beer's law:

$$A = \varepsilon_H b[H] + \varepsilon_G b[G] + \varepsilon_{HG} b[HG] \quad (2.1)$$

If at the selected wavelength, ε_H=0, then equation (2.1) can be simplified as equation (2.2)

$$A = \varepsilon_G b[G] + \varepsilon_{HG} b[HG] \quad (2.2)$$

According to equation (1.3), [G] can be defined by $[G]_0$ and [HG], thus (2.2) gives equation (2.3)

$$A = \varepsilon_G b[G]_0 + (\varepsilon_{HG} - \varepsilon_G) b[HG] \quad (2.3)$$

At the beginning of the titration,

$$A_0 = \varepsilon_G b[G]_0 \quad (2.4)$$

Then

$$\Delta A = A - A_0 = (\varepsilon_{HG} - \varepsilon_G) b[HG] = \varepsilon b[HG] \quad (2.5)$$

Here $\varepsilon = \varepsilon_{HG} - \varepsilon_G$

From Equation (1.5) and (2.5), we get

$$\Delta A = f([H]_0, [G]_0, \varepsilon, K_a) \quad (2.6)$$

Here we established the relationship between absorption data ΔA and the concentration $[H]_0$, $[G]_0$, the absorption coefficients ε_{HG} and ε_G, and the association constant K_a. In a typical titration experiment, $[G]_0$ is kept constant and $[H]_0$ incrementally increased. OriginLab™ (version 8.6) software was used for nonlinear fitting. In our analysis, we treated either parameter G_0 or H_0 as slightly adjustable to reach the best fitting, since real molar concentration is inherently dependent on a variety of experimental conditions (polydispersity of polymer modified dye, balance error, presence of water as impurity both in our macrocycle and TEG or PEG modified molecule, photobleaching of squaraine dye). Varying this parameter, and related values in later analyses, greatly improves the accuracy of the fitting result. We define host guest ratio:

$$\frac{[H]_0}{[G]_0} = r \quad (2.7)$$

We further introduce concentration correction coefficient m into equation (2.7)

$$\frac{[H]_0}{m[G]_0} = r \quad (2.8)$$

From equation (2.6) and (2.8) we get

$$\Delta A = f([G]_0, r, m, \varepsilon, K_a) \quad (2.9)$$

Equation (2.9) was used for the nonlinear fitting. To better initialize the parameters, we bounded the fitted parameter within reasonable scope ($0.5<m<1.5$, $0<K_a<1000/[G]_0$). The resulting m value was used to correct the real concentration ratio.

Fluorescence titration method

All fluorescence measurements were made in triplicate on a Horiba-Yovan Fluoromax 4 spectrometer with a slit width of 2.0 nm.

$[G]_t$ was kept constant and $[H]_t$ incrementally increased. The fluorescence signal was related to the concentration of [G] and [HG], assuming that the host has negligible fluorescence at the wavelength studied. The fluorescence intensity can be described by equation (3.1)

$$F = k_1[G] + k_2[HG] \quad (3.1)$$

Combining equations (1.3) and (3.1) gives equation (3.2)

$$F = (k_2 - k_1)[HG] + k_1[G]_0 \quad (3.2)$$

When $[H]_t=0$,

$$F_0 = k_1[G]_0 \quad (3.3)$$

$$F / F_0 = 1 + \frac{(k_2 - k_1)[HG]}{k_1[G]_0} = 1 + \frac{k[HG]}{[G]_0} \quad (3.4)$$

Here $k=(k_2-k_1)/k_1$

Combining equations (3.4), (2.8) (1.5), we relate the fluorescence signal to concentration by

$$F / F_0 = f([G]_0, r, m, k, K_a) \quad (3.5)$$

Equation (3.5) is used in OriginLab™ (version 8.6) software for nonlinear fitting to obtain the association constant K_a.

Determination of Squaraine Association Constants in Chloroform

Solutions of dye (3.0 μM) with varying equivalents of macrocycle were equilibrated in chloroform in the dark for 24 hours at 20 °C, at which time the samples were placed in cuvettes and the absorbance at 660 nm was recorded. A plot of the change in absorbance versus the concentration of macrocycle was fitted to the 1:1 binding model expressed above:

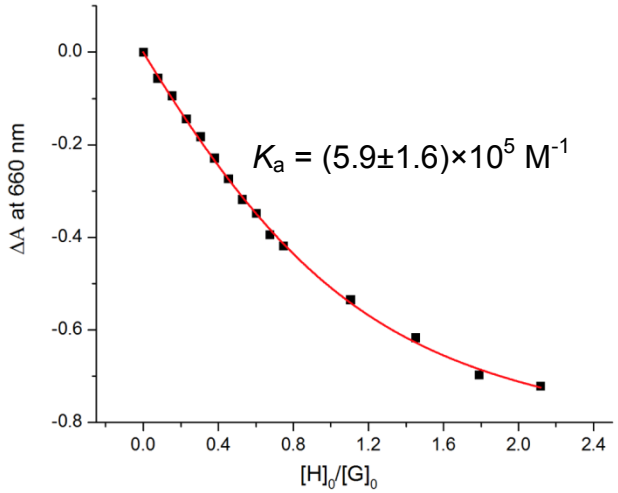


Figure *S13*: Determination of K_a for the association of squaraine **S1** with macrocycle **M1** in $CHCl_3$.

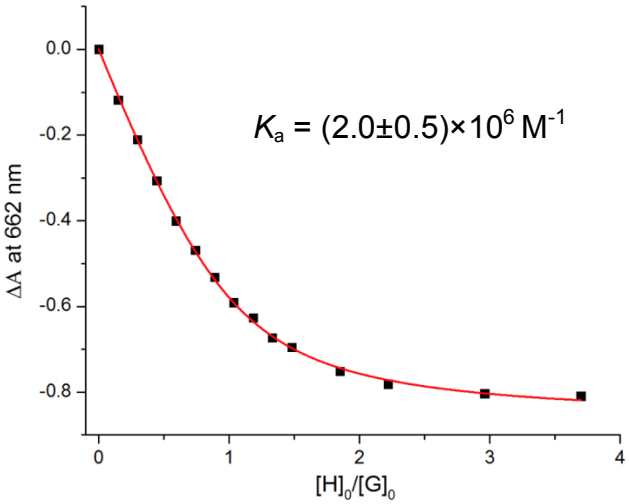


Figure *S14*: Determination of K_a for the association of squaraine **S3** with macrocycle **M1** in $CHCl_3$.

Determination of Squaraine Association Constants in Methanol

Solutions of dye (3.0 μM) with varying equivalents of macrocycle were equilibrated in methanol in the dark for 2 hours at 20 °C, at which time the samples were placed in cuvettes and the absorbance at 660 nm was recorded. A plot of the change in absorbance versus the H/G ratio was fitted to the 1:1 binding model expressed above:

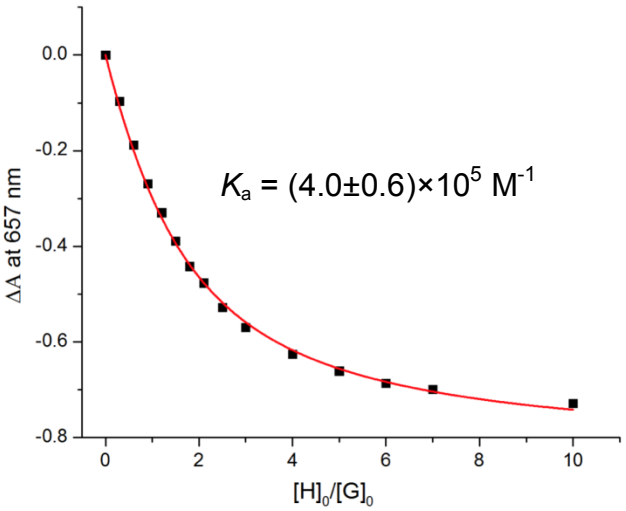


Figure *S15*: Determination of K_a for the association of squaraine **S1** with macrocycle **M2** in MeOH.

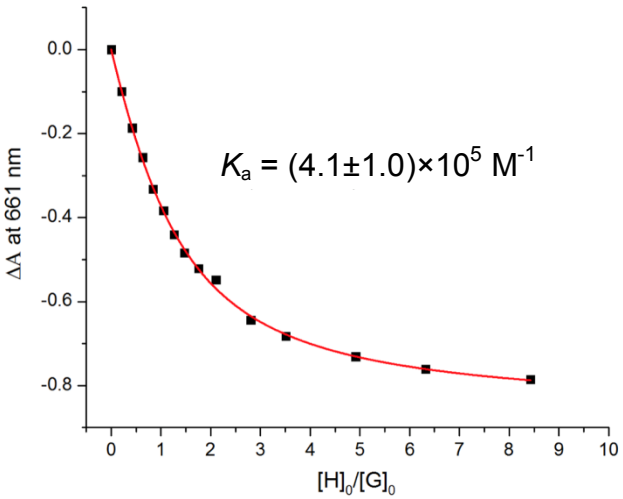


Figure *S16*: Determination of K_a for the association of squaraine **S3** with macrocycle **M2** in MeOH.

Determination of Squaraine Association Constants in Water

Solutions of dye (30 nM) with varying equivalents of macrocycle were equilibrated in H_2O for 5 minutes at 20 °C, at which time the samples were placed in cuvettes and the dye fluorescence intensity at 720 nm was recorded (Excitation = 710 nm, slit width 2 nm). A plot of the fluorescence (F/F_o) versus the H/G ratio was fitted to the 1:1 binding model expressed above:

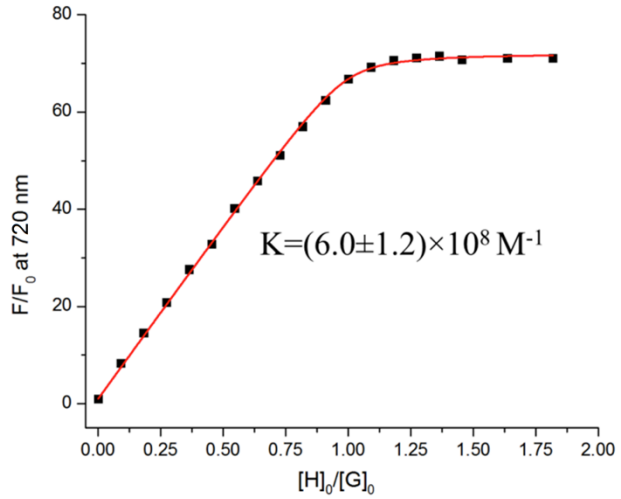


Figure *S17*: Determination of K_a for the association of squaraine **S2** with macrocycle **M2** in H_2O.

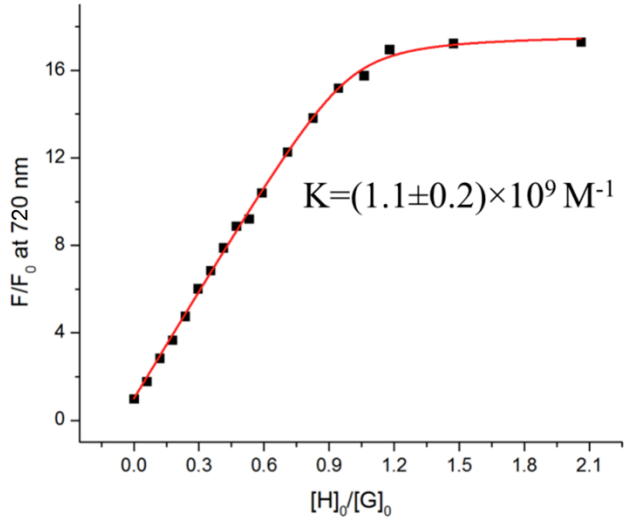


Figure *S18*: Determination of K_a for the association of squaraine **S3** with macrocycle **M2** in H_2O.

Fluorescence Studies of Fumaride (F1) Complexation

Macrocycle fluorescence is quenched upon association with **F1**.

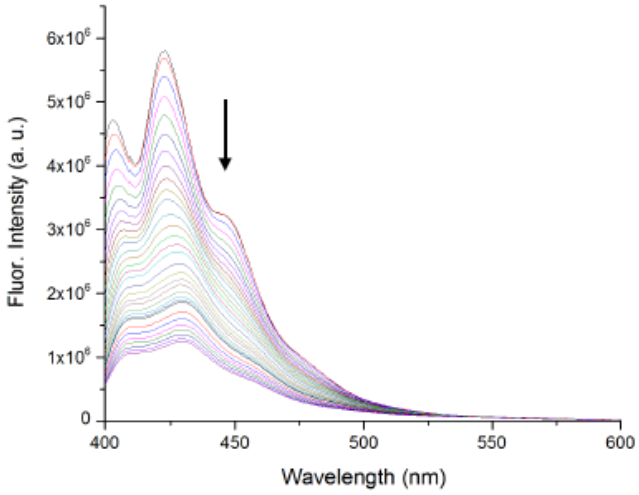


Figure *S19*: Quenching of anthracene band of **M2** (100 μM) upon complexation with **F1** (H_2O, 20 °C)

Determination of fumaride association constant by fluorescence titration.
In the fluorescence quenching titration experiment, $[H]_0$ was kept constant and $[G]_0$ incrementally increased. The macrocycle and complex were treated as fluorescent while the fumaride guest was assumed to be transparent at the selected excitation wavelength. The following equation was used for the nonlinear fitting.

$$[HG]=0.5\left\{\left([H]_0+[G]_0+1/K_a\right)-\sqrt{\left([H]_0+[G]_0+1/K_a\right)^2-4[H]_0[G]_0}\right\}$$

$$F/F_0=1-k\times[HG]/[H]_0$$

In the above equation, $[H]_0$ is the total concentration of macrocycle used for the titration, $[G]_0$ is the total concentration of fumaride guest used for the titration, [HG] is the concentration of complex in the titration, K_a is the association constant and k is the emission coefficient. The OriginLab™ 8.6 software was used for the nonlinear fitting.

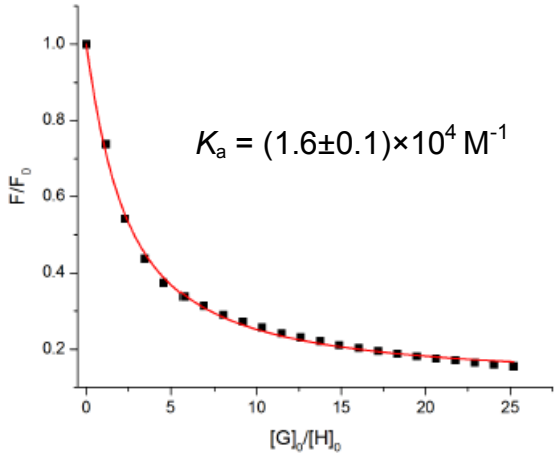


Figure *S20*: Determination of K_a for the association of fumaride **F1** with macrocycle **M2** in H_2O at 20 °C.

NMR Evidence for Displacement of Fumaride (F1) by Squaraine S2

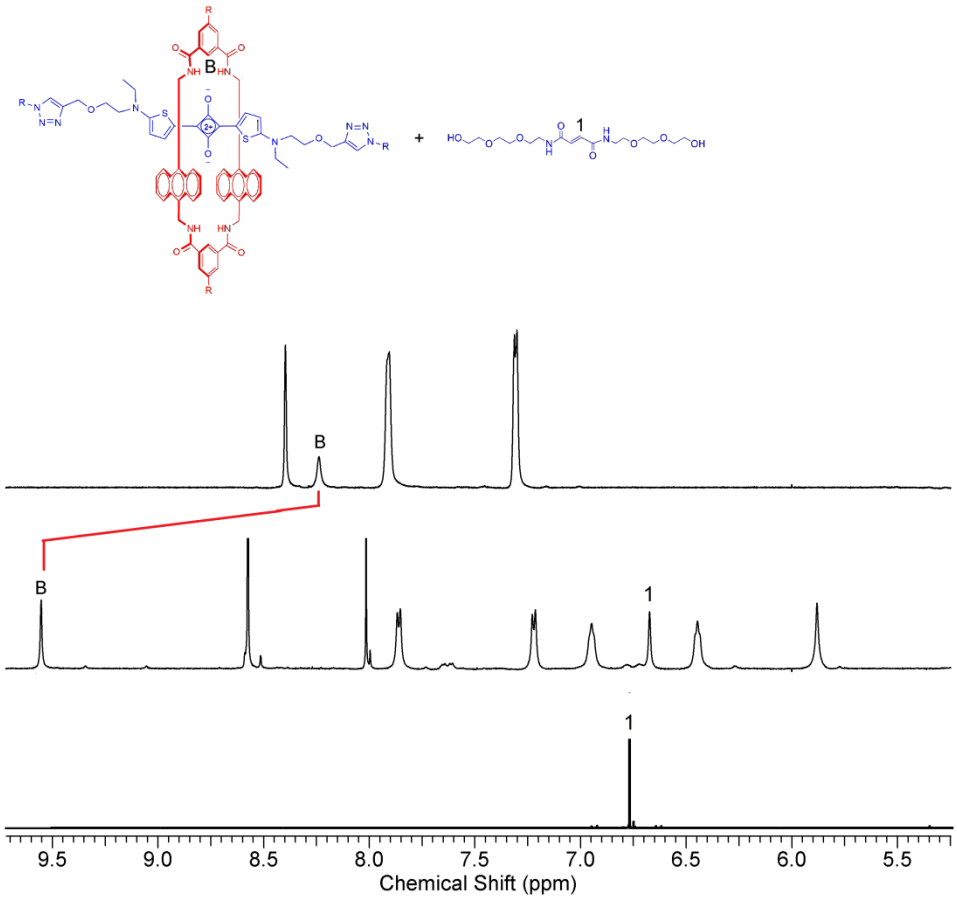


Figure *S21*: Partial ^{1}H NMR (600 MHz, D_2O, 25°C) of (*top*) **M2⊃F1** (2.0 mM); (*middle*) **M2⊃F1** (2.0 mM) treated with **S3** (2.0 mM) which displaced **F1**; (*bottom*) fumaride **F1** (2.0 mM). Red line indicates change in chemical shift for **M2** peak B upon displacement of **F1** by **S3**.

Determination of Squaraine Association Constant by Competitive Titration method

In the competitive titration experiment, **S2** or **S3** was titrated into a mixture of **F1** + **M2** (**F1**:**M2** molar ratio was 100:1). The squaraine/macrocycle complex was treated as fluorescent and the macrocycle and squaraine dye as transparent at the selected excitation wavelength. The association constant for **M2⊃F1** was predetermined by the fluorescence quenching titration above to be 1.6×10^4 M^{-1}. Newton's method was used to iterate the concentration of macrocycle ([H]) during the displacement titration.[S9]

The following equation was used for the nonlinear fitting:

$$F = k \times K_a \times [H] \times [G]_0 / (1 + K_a * [H])$$

In the above equation, $[G]_0$ is the total concentration of **S2** or **S3** used for the titration, [H] is the concentration of macrocycle in the titration, K_a is the association constant between **S2** or **S3** and **M2** and k is the emission coefficient. The nonlinear fitting was conducted using OriginLab™ (version 8.6) software.

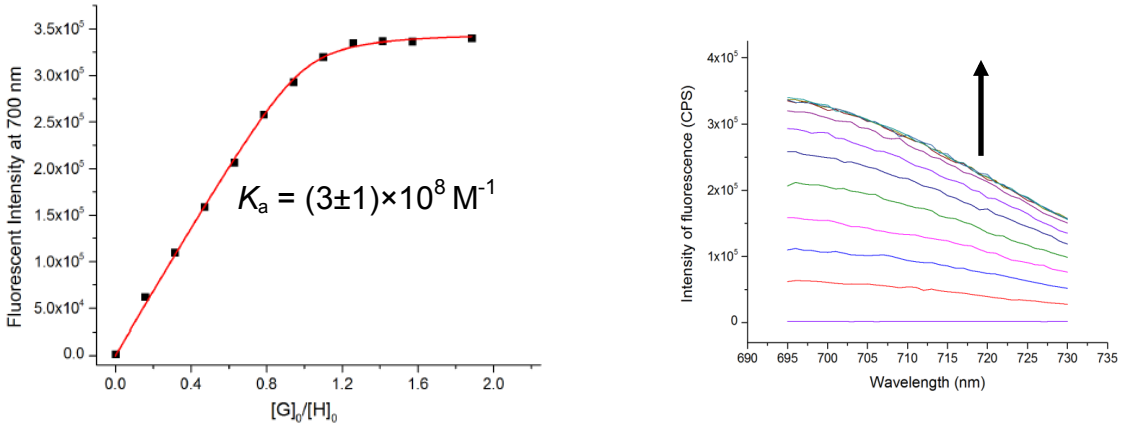


Figure *S22*: Determination of K_a by the competitive displacement of **F1** from **M2⊃F1** by addition of squaraine **S2** to a mixture of **M2**+ **F1** (**F1**:**M2** molar ratio was 100:1) in H_2O at 20 °C.

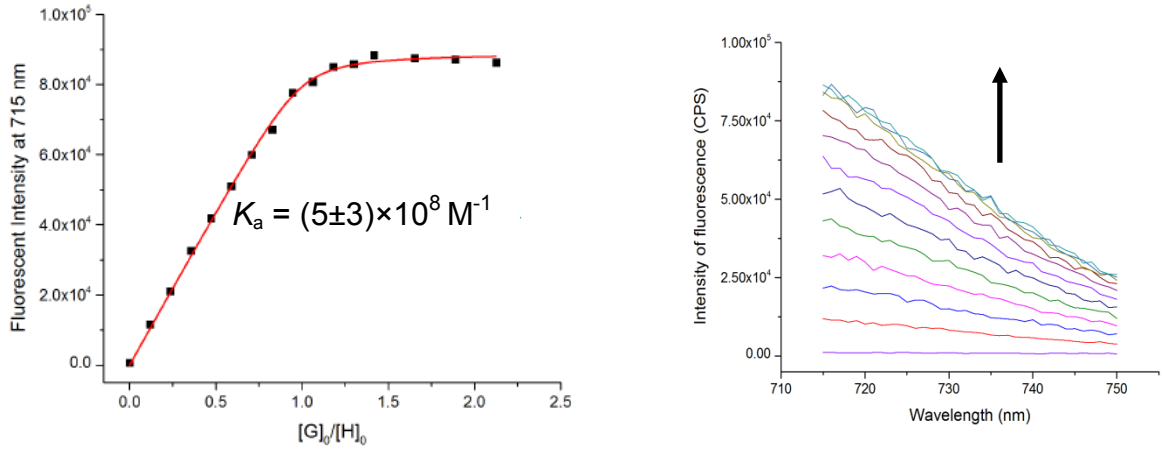


Figure *S23*: Determination of K_a by the competitive displacement of **F1** from **M2⊃F1** by addition of squaraine **S3** to a mixture of **M2**+ **F1** (**F1**:**M2** molar ratio was 100:1) in H_2O at 20 °C.

F. Thermodynamic Measurements

ITC Titration of M2 and F1

An aqueous solution of **M2** (300 µL, 0.1-0.5 mM) was injected into the sample cell of a Malvern Microcal iTC200 Microcalorimeter. An aqueous solution of **F1** (30-35 µL, 2-10 mM) was loaded into the titration syringe and 20-25 injections (1 µL each) were added stepwise into the sample cell at 27 ˚C. The heat generated was monitored over time. To adjust for the dilution effect, the **F1** solution was titrated into the pure H_2O and heat generated by this process was also monitored over time. The net heat was obtained by subtracting this dilution value from the heat of complexation. The resulting data was analyzed using the Origin™ program to fit a 1:1 binding model and values for K_a, ΔH, and ΔS were determined directly.

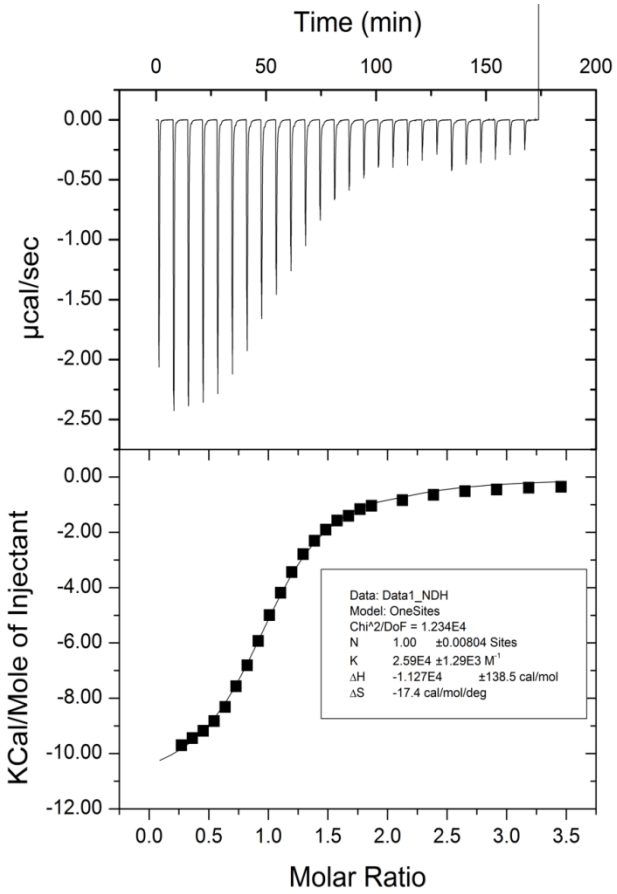


Figure *S24*: ITC titration of **F1** into an aqueous solution containing **M2** at 27 °C.

ITC Titration of M2 and S3

The association constant for **M2** and **S3** was too high for accurate measurement using our microcalorimeter (C>1000) but the step-like isotherm did confirm that N=1. A single injection method was used to measure the enthalpy of complexation. A single aliquot of **S3** (5 µL of 2.0 mM stock solution) was injected into the cell containing **M2** (50 µM, 200 µL) and the generated heat was monitored over time. To adjust for the dilution effect, an identical aliquot of **S3** solution was added to pure H_2O and heat generated was monitored over time. The net heat effect was obtained by appropriate subtraction.

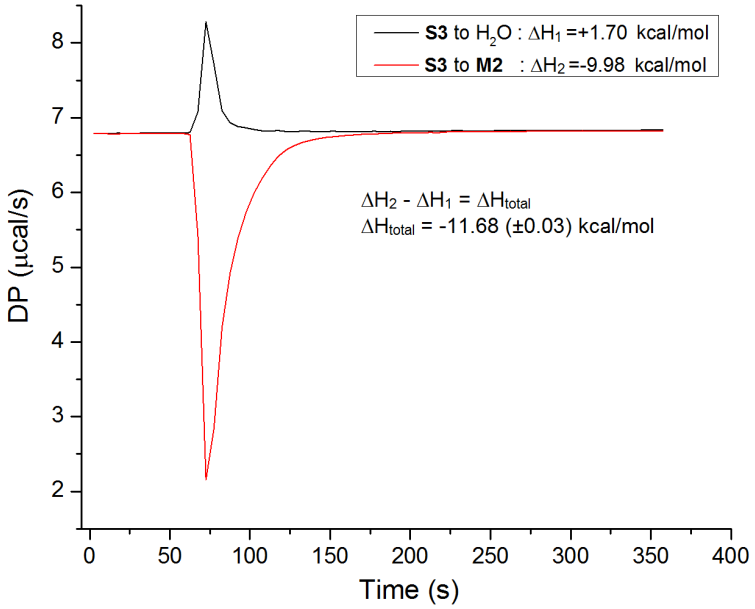


Figure *S25*: ITC single injection of **S3** into H_2O or an aqueous solution containing **M2** (50 µM, 27 °C) enabled determination of the enthalpy of complexation.

The association constant at 27 °C was determined by fluorescence titration. Separate aliquots of **S3** (300 nM) and varying molar equivalents of **M2** were equilibrated in H_2O for 5 minutes and the fluorescence intensity at 720 nm was recorded (ex: 710 nm) in temperature controlled cuvettes. The isotherm was fitted to the 1:1 binding model expressed above:

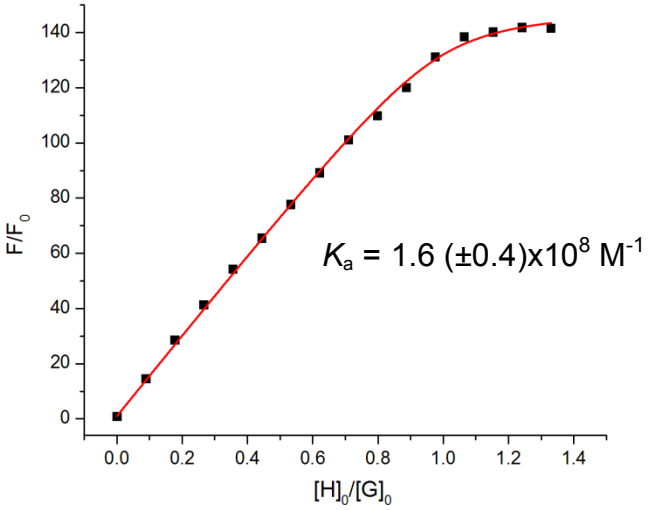


Figure *S26*: Determination of K_a for the association of **S3** with **M2** in H_2O at 27 °C.

A competitive ITC titration experiment that added **S3** to a solution of **M2⊃F1** produced very little heat and the titration isotherm could not be fitted reliably to a binding model. The small change in heat for this displacement equilibrium is consistent with the similar values of ΔH for each guest (**S3** or **F1**) binding to **M2**.

ITC Titration of M2 and N1

An aqueous solution of **M2** (300 µL, 0.5 mM) was injected into the sample cell of a Malvern Microcal iTC200 Microcalorimeter. An aqueous solution of **N1** (30-35 µL, 2-10 mM) was loaded into the titration syringe and 20-25 injections (1 µL each) were added stepwise into the sample cell at 27 ˚C. The heat generated was monitored over time. To adjust for the dilution effect, the **N1** solution was titrated into the pure H_2O and heat generated by this process was also monitored over time. The net heat was obtained by subtracting this dilution value from the heat of complexation. The resulting data was analyzed using the Origin™ program to fit a 1:1 binding model and values for K_a, ΔH, and ΔS were determined directly.

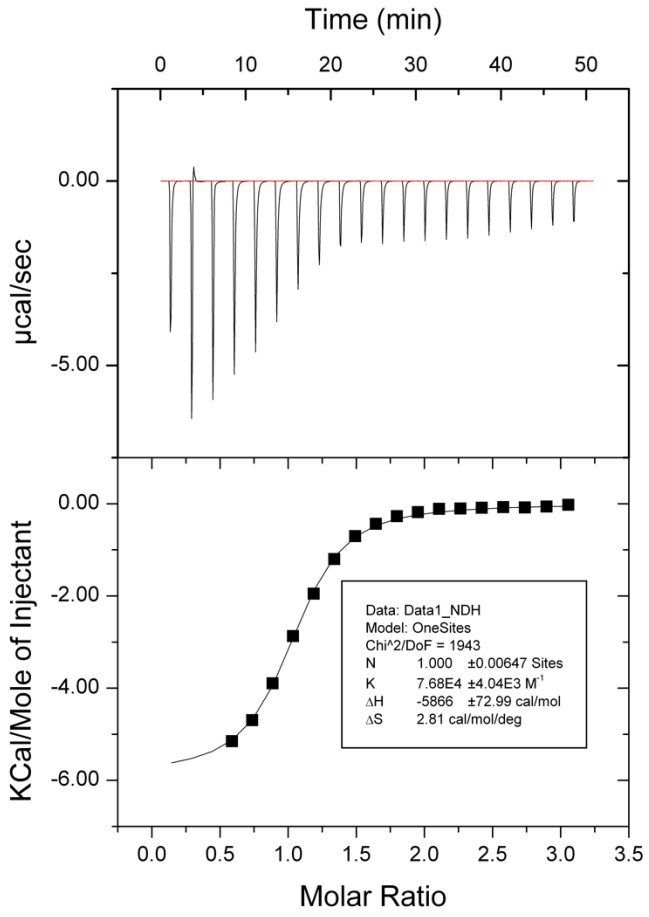


Figure *S27*: ITC titration of **N1** into an aqueous solution containing **M2** in H_2O at 27 °C.

G. Kinetic Studies

Determination of Association Rate Constant (k_{on}) by Absorption Method

Molar absorptivities at 690 nm were determined from absorbance of the dye before association ($\varepsilon_{\lambda Dye}$) and after association with macrocycle ($\varepsilon_{\lambda Complex}$).

$\varepsilon_{\lambda S1}$ (690 nm, $CHCl_3$): 6.02×10^4
$\varepsilon_{\lambda M1 \supset S1}$ (690 nm,$CHCl_3$): 1.41×10^5
$\varepsilon_{\lambda S3}$ (690 nm, $CHCl_3$): 1.66×10^4
$\varepsilon_{\lambda M2 \supset S3}$ (690 nm,$CHCl_3$): 1.17×10^5

When [D] = 5.0 μM; [M] = 15.0 μM ($CHCl_3$ study)

Because the equilibrium constant is high ($K_a > 10^5$ M^{-1}) the formation process is treated as an irreversible second-order reaction that obeys the following relationship:

$$\ln\left(\frac{[\mathrm{M}] \cdot [\mathrm{D}]_0}{[\mathrm{D}] \cdot [\mathrm{M}]_0}\right) = ([\mathrm{M}]_o - [\mathrm{D}]_0) \cdot k\mathrm{t}$$

Rearrangement leads to:

$$\frac{\ln\left(\frac{[\mathrm{M}] \cdot [\mathrm{D}]_0}{[\mathrm{D}] \cdot [\mathrm{M}]_0}\right)}{([\mathrm{M}]_o - [\mathrm{D}]_0)} = k\mathrm{t}$$

The [M] is given by:

$$[\mathrm{M}] = 3[\mathrm{D}]_0 - [\mathrm{R}]$$

Since b = 1 cm, the [R] at a specific wavelength **λ** is given by the modified Beer's Law:

$$[\mathrm{R}] = \frac{\mathrm{A}_\lambda - \varepsilon_{\lambda \mathrm{D}}[\mathrm{D}]_0}{\varepsilon_{\lambda \mathrm{R}} - \varepsilon_{\lambda \mathrm{D}}}$$

Substitution into the equation gives:

$$\frac{\ln\left(\frac{(3[\mathrm{D}]_0 - [\mathrm{R}]) \cdot [\mathrm{D}]_0}{([\mathrm{D}]_0 - [\mathrm{R}]) \cdot [\mathrm{M}]_0}\right)}{([\mathrm{M}]_o - [\mathrm{D}]_0)} = k\mathrm{t}$$

The plot of the ratio versus time yields a linear relationship whose slope = k_{on}.

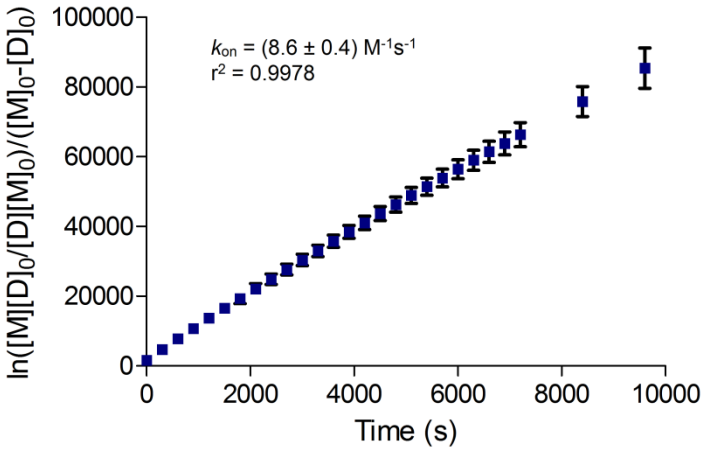


Figure *S28*: Second-order kinetic plot for the rate of association between **S1** and **M1** in $CHCl_3$ at 20 °C. N = 3.

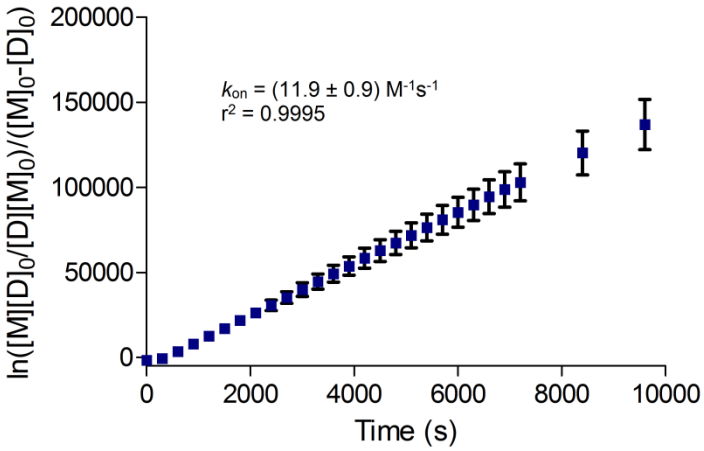


Figure *S29*: Second-order kinetic plot for the rate of association between **S3** and **M1** in $CHCl_3$ at 20 °C. N = 3.

Determination of Association Rate Constant (k_{on}) by Stopped-flow Method

All stopped-flow experiments were run in triplicate at 20 °C and averaged to obtain kinetic curves which were fitted according to the following method:

Algorithms for the determination of k_{on}

In a second order reaction, we have

$$H + G \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} HG$$

The reaction rate can be written as

$$Rate = -\frac{d[G]}{[G]} = k_{on}[H][G] - k_{off}[HG] = k_{on}[H][G] - \frac{k_{on}}{K_a}[HG] \quad (4.1)$$

For strong association system where K_a >100 M^{-1}, the contribution of reverse reaction on reaction rate is negligible. So equation (4.1) can be simplified as (4.2)

$$Rate = -\frac{d[G]}{[G]} = k_{on}[H][G] \quad (4.2)$$

In the case $[H]_0=[G]_0$, since H and G react with a 1:1 stoichiometry, $[H]= [H]_0-x$ and $[G]=[G]_0-x$. At any time t, [H]=[G] and equation (4.2) can be further simplified as (4.3)

$$Rate = -\frac{d[G]}{[G]} = k_{on}[G]^2 \quad (4.3)$$

Solving the differential rate equation (4.3) gives the integrated rate equation (4.4)

$$\frac{1}{[G]_t} - \frac{1}{[G]_0} = k_{on}t \quad (4.4)$$

Measuring k_{on} by fluorescence

Equimolar solutions of the host and guest were mixed, and the fluorescence signal was related to the concentration of [G] and [HG], assuming that the host has negligible fluorescence at the wavelength studied. The fluorescence intensity can be described by equation (5.1)

$$F = k_1[G] + k_2[HG] \quad (5.1)$$

When t=0,

$$F_0 = k_1[G]_0 \quad (5.2)$$

Combining equations (1.3), (5.1) and (5.2) gives equation (5.3)

$$F / F_0 = 1 + \frac{(k_2 - k_1)[HG]}{k_1[G]_0} \quad (5.3)$$

Combining equation (1.3) and equation (4.4) gives equation (5.4)

$$\frac{1}{[G]_0 - [HG]} - \frac{1}{[G]_0} = k_{on}t \quad (5.4)$$

Replacing [HG] in equation (5.4) by equation (5.3) gives the equation (5.5), which describes the relationship between fluorescence and time

$$F / F_0 = 1 + \frac{k_2 - k_1}{k_1}\left(1 - \frac{1}{k_{on}t[G]_0 + 1}\right) \quad (5.5)$$

Equation (5.5) was used in OriginLab™ 8.6 version for the nonlinear fitting. The association rate k_{on} can be determined by using the stopped-flow data and nonlinear regression analysis.

In the case where the guest molecule is also transparent, especially at nanomolar concentration, the fluorescence intensity can be described by equation (5.6)

$$F = k_2[HG] \quad (5.6)$$

Replacing [HG] in equation (5.4) by equation (5.6) gives the equation (5.7)

$$F = k_2 G_0\left(1 - \frac{1}{k_{on}t[G]_0 + 1}\right) \quad (5.7)$$

Equation (5.7) is used in OriginLab™ (version 8.6) for nonlinear fitting to determine the association rate k_{on}.

Determination of k_{on} in Methanol

In methanol, $[D]_o = [M]_o = 0.5$ μM, and the fluorescence response at 720 nm (Excitation = 710 nm) was monitored as a function of time.

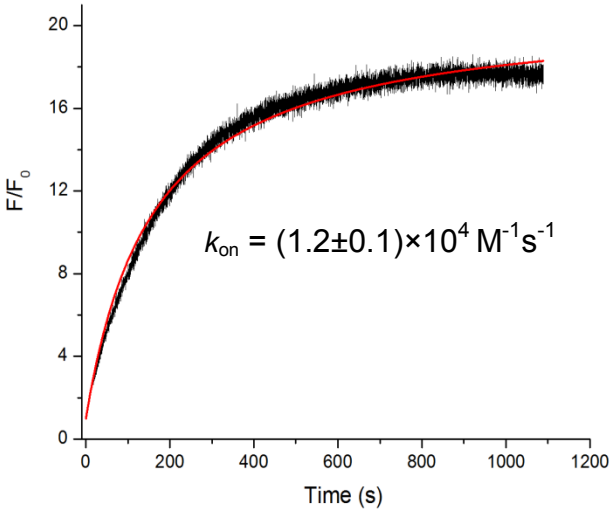


Figure *S30*: Determination of k_{on} for association of squaraine **S1** with macrocycle **M2** in MeOH at 20 °C.

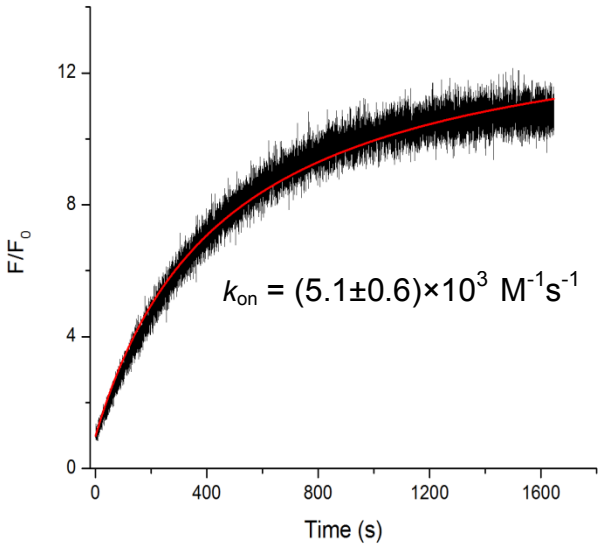


Figure *S31*: Determination of k_{on} for the association of squaraine **S3** with macrocycle **M2** in MeOH at 20 °C.

Determination of k_{on} in Water

In water, $[D]_o = [M]_o = 30$ nM and the fluorescence response at 720 nm (Excitation = 710 nm) was monitored as a function of time.

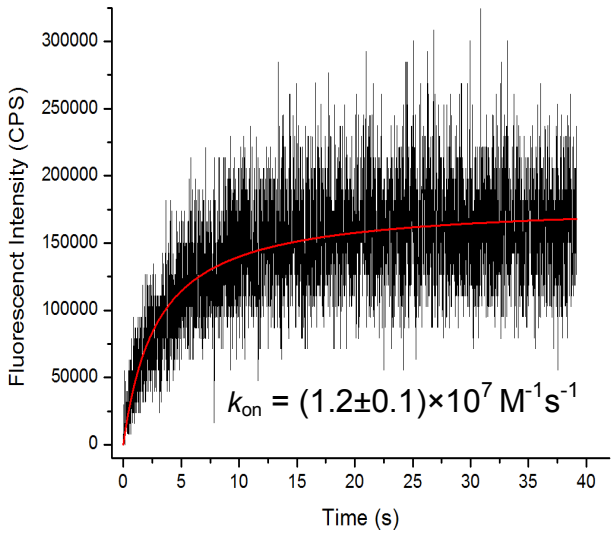


Figure *S32*: Determination of k_{on} for association of squaraine **S2** with macrocycle **M2** in H_2O at 20 °C.

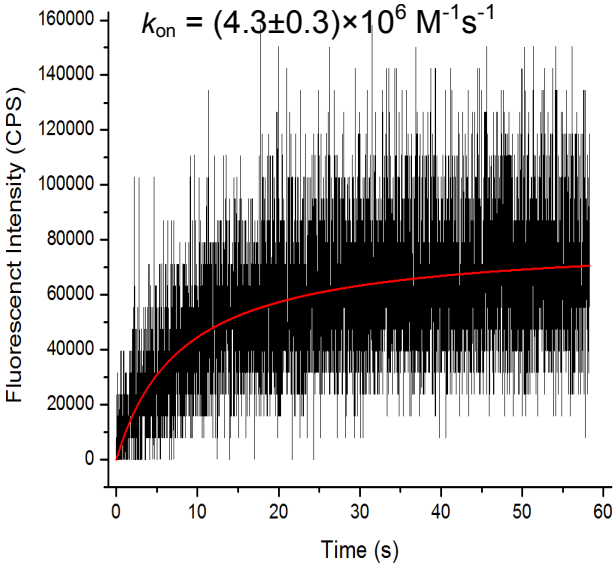
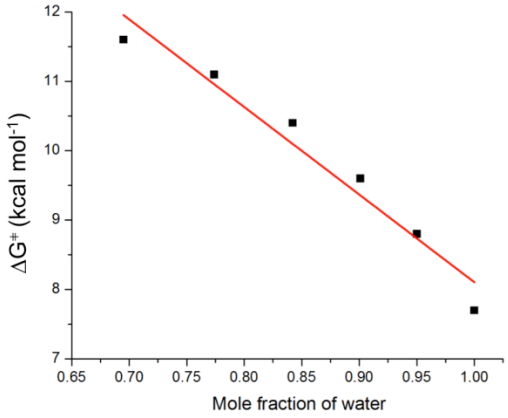


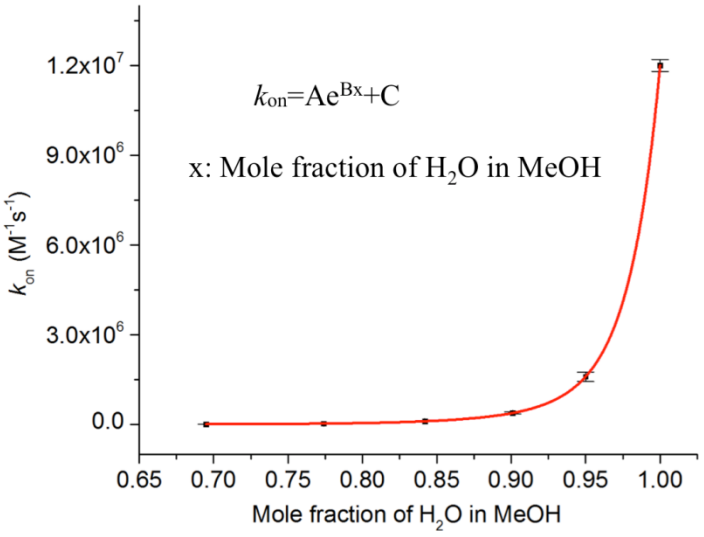
Figure *S33*: Determination of k_{on} for association of squaraine **S3** with macrocycle **M2** in H_2O at 20 °C.

Determination of k_{on} in H_2O/Methanol Mixtures

Table *S3*: Influence of solvent on k_{on} for **M2⊃S3** at 20 °C.

Mole Fraction H_2O in MeOH	$k_{on}(M^{-1}s^{-1})$	$\Delta G^{\ddagger}$ (kcal mol^{-1})
0.69	1.3×10^4	11.6
0.77	3.4×10^4	11.1
0.84	11×10^4	10.4
0.90	39×10^4	9.6
0.95	160×10^4	8.8
1.00	1200×10^4	7.7





$$k_{on}=Ae^{Bx}+C$$

Model	Winstein (User)		
Equation	y=A*exp(B*x)+C		
Reduced Chi-Sqr	5.84277E9		
Adj. R-Square	0.99974		
		Value	Standard Error
F	A	2.59791E-11	2.63893E-11
	B	40.66802	0.91503
	C	70955.36089	41272.50588

Figure *S34*: Influence of solvent on k_{on} for **M2⊃S3** at 20 °C. Also shown is a fit of the data to an exponential equation that reflects the dependence on mole fraction of water.

H. Cell Studies

Cell Toxicity

Cell toxicity was measured using the common MTT cell viability assay. CHO-K1 (Chinese hamster ovary) cells (ATCC CCL-61) were cultured following ATCC protocols and maintained at 37 °C, in a humidified incubator with 5% CO_2 atmosphere. The cells were seeded into 96-microwell plates, and grown to confluence using F-12K medium (supplemented with 10 % fetal bovine serum; 1 % penicillin streptomycin) in a humidified incubator (37 °C; 5 % CO_2). The Vybrant MTT cell proliferation assay kit (Invitrogen, Eugene, USA) was performed according to the manufacturer's protocol. The cells were incubated with different concentrations of each compound in F-12K media for 24 h at 37 °C, 5% CO_2. The medium was removed and replaced with 110 µL of F-12K medium containing [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT, 1.2 mM) and the sample incubated for 4 h at 37 °C and 5 % CO_2. An SDS−HCl detergent solution was added and the samples incubated for an additional 12 h. The absorbance of each well was measured at 570 nm (n = 4), and the readings were normalized relative to untreated cells.

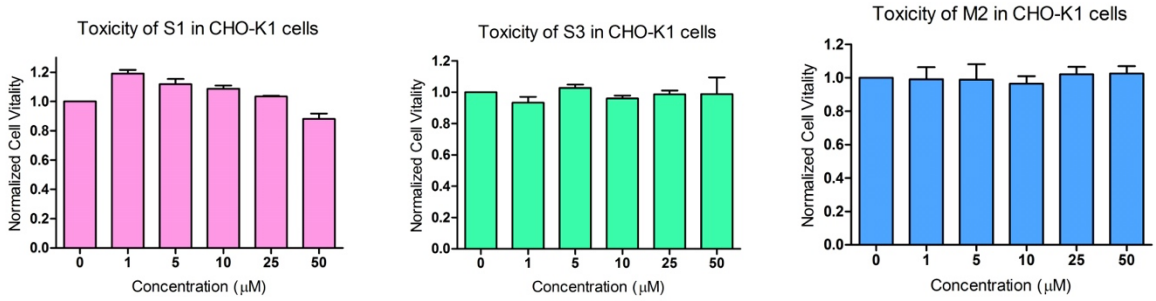


Figure *S35*: MTT assay of toxicity against CHO-K1 cells.

Fluorescence Microscopy

Cultured CHO-K1 cells were plated out on 8-well microscope chambered slides (Nunc) in F-12K medium and allowed to grow to approximately 70% confluence. The medium was replaced with solutions of **S3** (10 µM) or **S3** + **M2** (10 µM) in OptiMEM media (Life Technologies, catalogue number 11058-021) and the cells allowed to incubate for 8 hours at 37 º C. The cells were then washed twice with PBS and imaged under OptiMEM media. Fluorescence microscopy was conducted on a Nikon Eclipse TE-2000U epifluorescence microscope equipped with an X-cite 120 fluorescence illumination system and either: (a) red filter set (Nikon C53375, Exciter: HQ620/60X, Dichroic: 660LP, Emitter: HQ700/75m), (b) near-infrared filter set (Nikon C81153, Exciter: HQ710/75X, Dichroic: 750LP, Emitter: HQ810/90m), (c) blue excitation, red emission filter set (Exciter: D360/40x, Dichroic: 400DCLP, Emitter: 610LP). Images were collected in NIS-Elements using a Hamamatsu ORCA-Flash4.0 V2 Digital CMOS camera with 2x2 binning and 2 second integration times. Images were processed in ImageJ, and a 100 pnt rolling background was subtracted.

Shown in Figure S36 are representative micrographs of living CHO-K1 cells containing either **S3** or **M2⊃S3** complex and imaged using the blue excitation, red emission filter set (c) described above. The images show that the **S3** dye cannot be imaged using this filter set (although it can be visualized using using the red excitation/emission filter set (a) described above), whereas the cells containing **M2⊃S3** are easily observed. The Supporting Information contains a movie of live cells <ja5b03573_si_002.avi> imaged for 2 minutes at 100x speed and 90x magnification. The movie shows time resolved tracking of endosomes containing the **M2⊃S3** complex which is selectively visualized using the energy transfer imaging filter set.

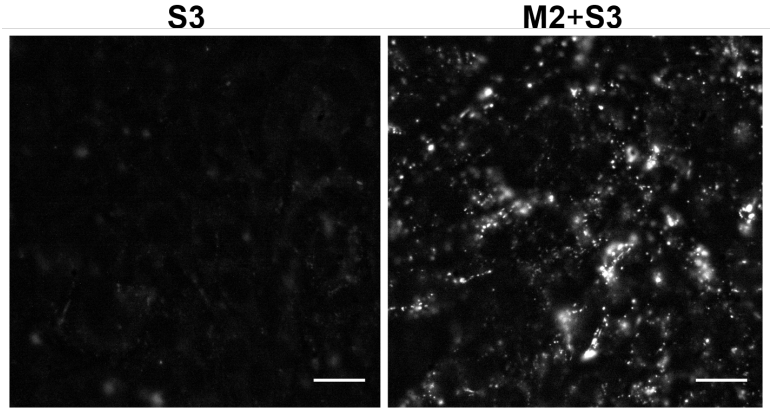


Figure *S36***.** Epifluorescence micrographs of live CHO cells after incubation for 8 hours with **S3** (10 μM) or **M2** + **S3** (10 μM each) in culture media. The micrographs were acquired using a blue excitation, red emission filter set (Exciter: D360/40x, Dichroic: 400DCLP, Emitter: 610LP) and the same acquisition settings; thus, the image intensities are directly comparable. The length bar is 10 μm.

The same type of energy transfer imaging (blue excitation, red emission) was achieved on the mesoscale using a Multispectral FX Pro *in vivo* imaging system equipped with a CCD camera (filter set: 410/10 excitation; 700/17.5 emission). Shown in Figure S37 is an image (30 second exposure) of a microwell plate with separate solutions (200 μL) of culture media containing; no additives; **M2** (10 μM); **S3** (10 μM); or a mixture of **M2**+**S3** (10 μM each). The fluorescence intensity of the last well was 20 times brighter than any other due to formation of the **M2⊃S3** complex which enables energy transfer from the surrounding macrocycle anthracene units to the encapsulated squaraine dye.

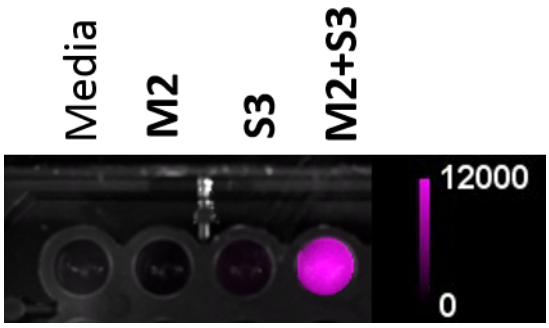


Figure *S37***.** Image of a microwell plate using a CCD camera and an illumination filter set that allowed blue excitation (410/10 nm) and captured red emission (700/17.5 nm). Each microwell (4 mm diameter) contained a solution of additive (10 μM) in culture media (200 μL), and the image is an overlay of a photograph and a fluorescence intensity pixel map. The fluorescence intensity bar is in arbitrary units.

I. ^{1}H and ^{13}C NMR Spectra

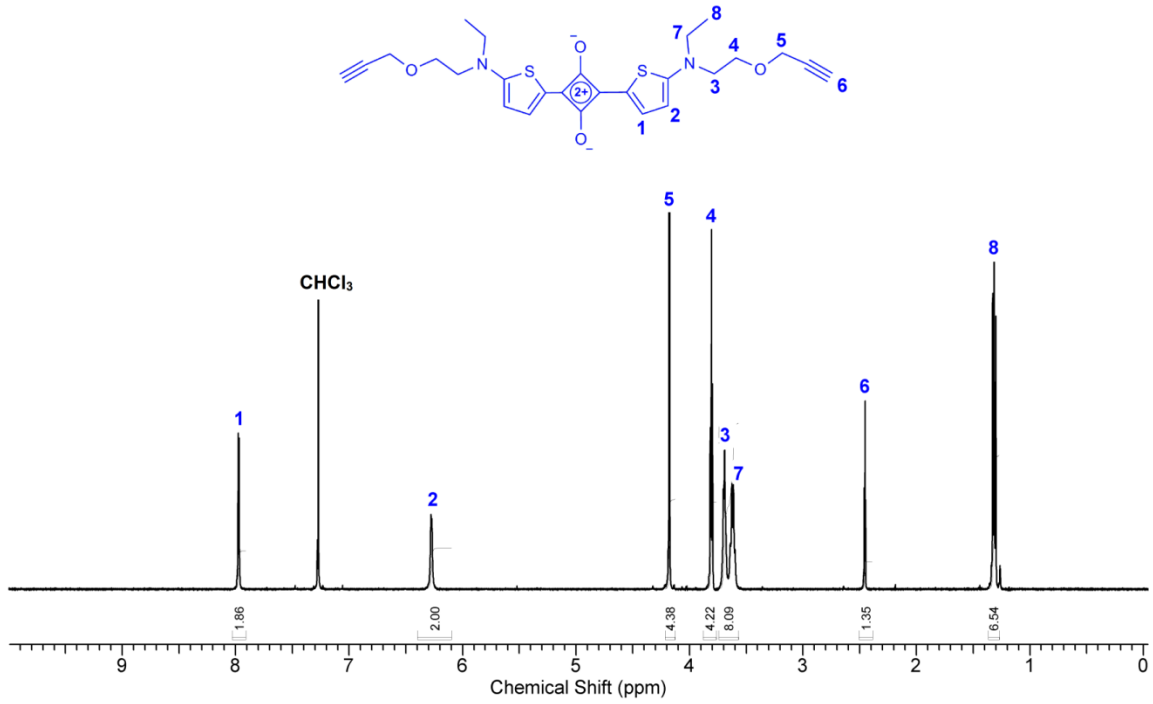


Figure *S38*: ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) spectrum of squaraine dye **S1**.

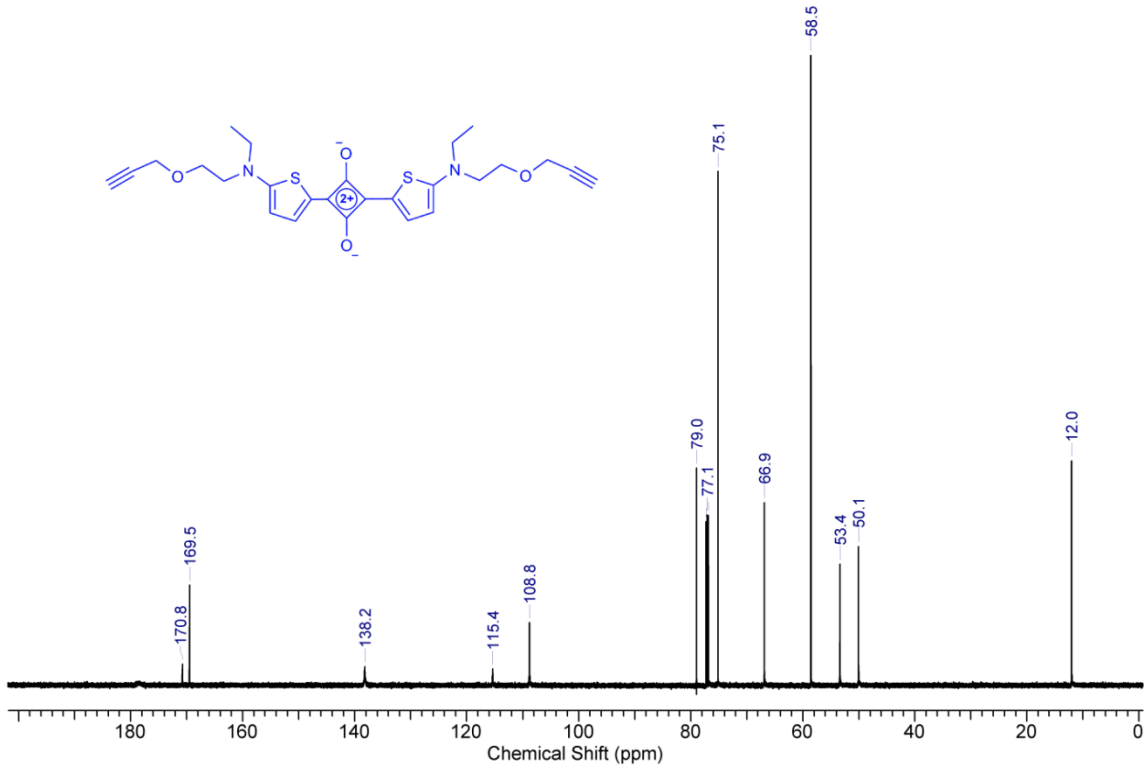


Figure *S39*: ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C) spectrum of squaraine dye **S1**.

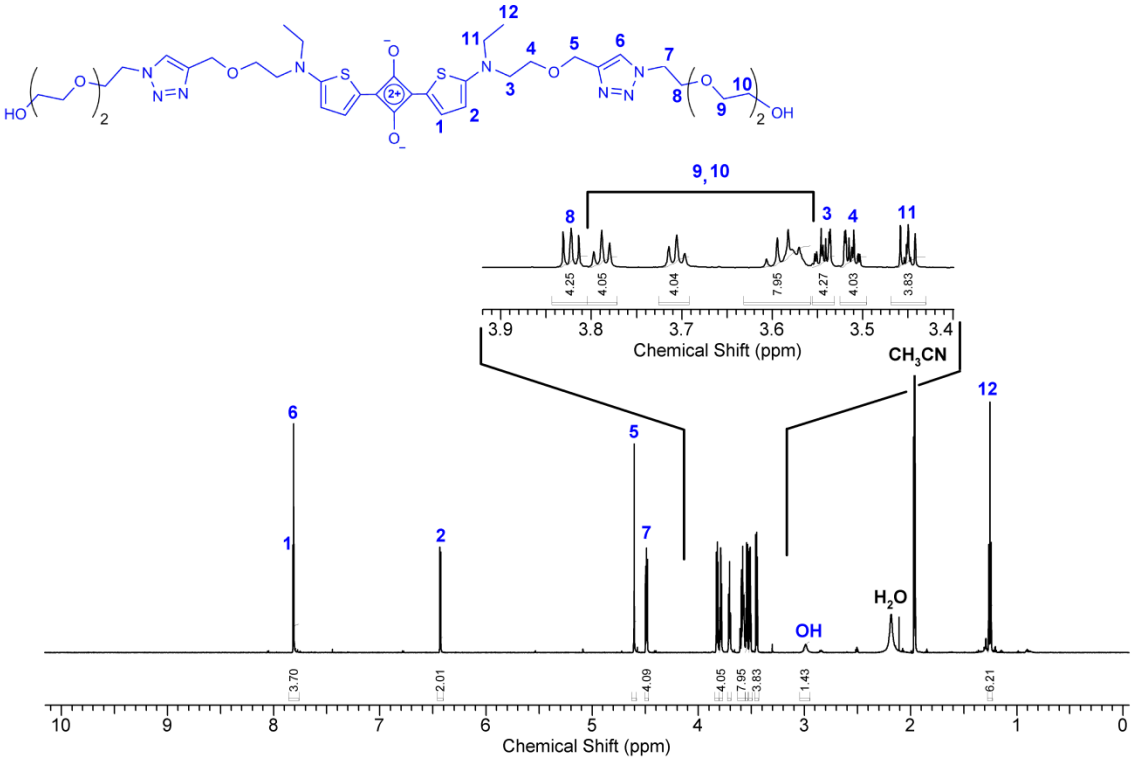


Figure *S40*: ^{1}H NMR (500 MHz, CD_3CN, 25 °C) spectrum of squaraine dye **S2**.

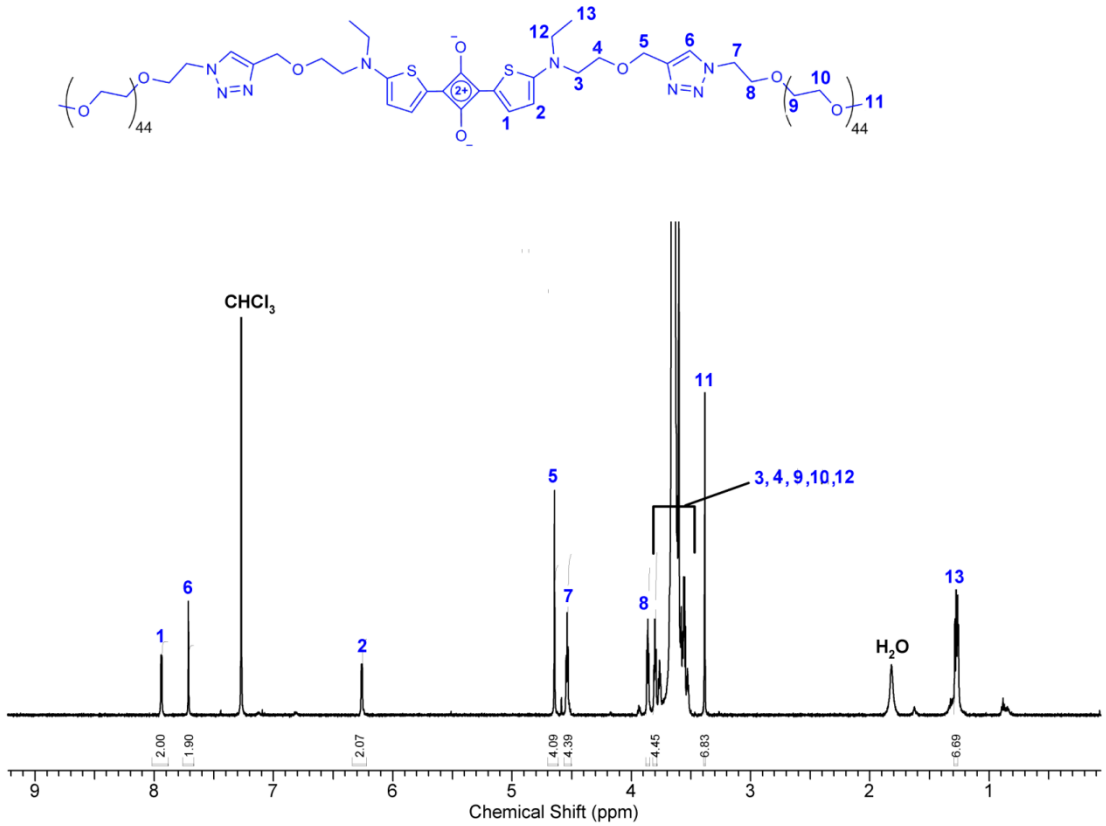


Figure *S41*: ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) spectrum of squaraine dye **S3**.

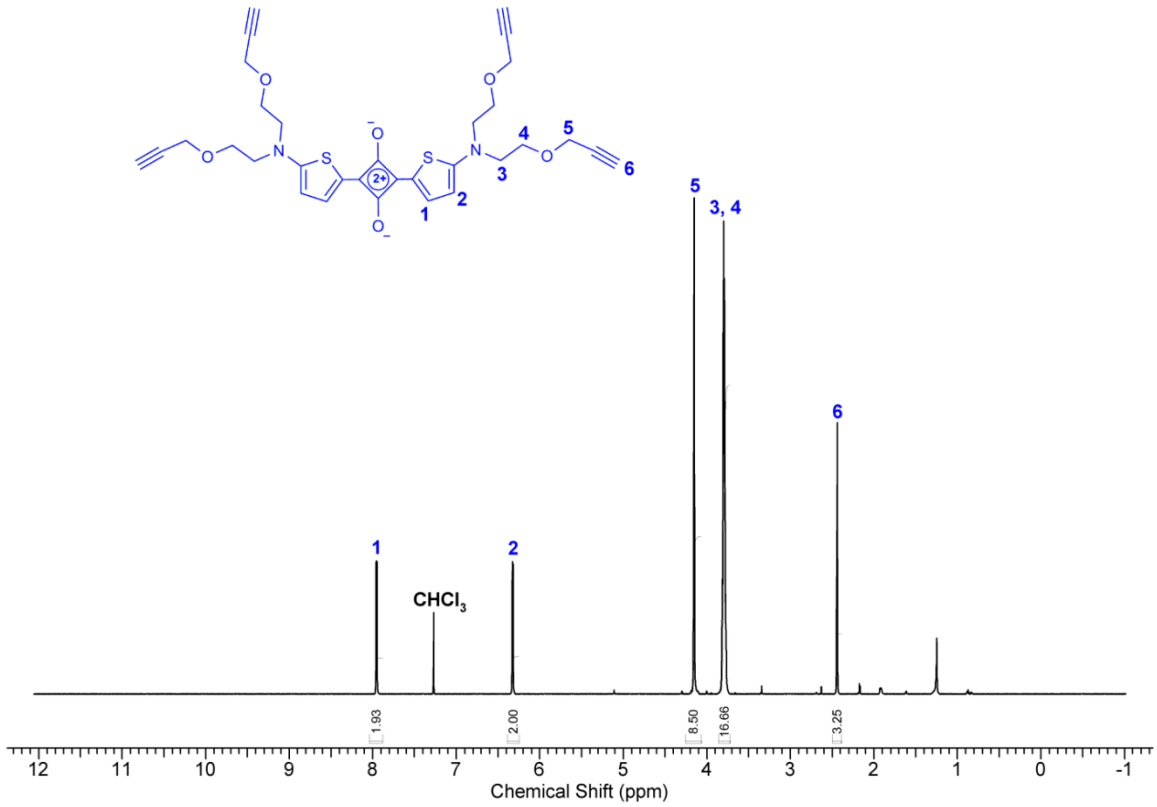


Figure *S42*: ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) spectrum of squaraine dye **3**.

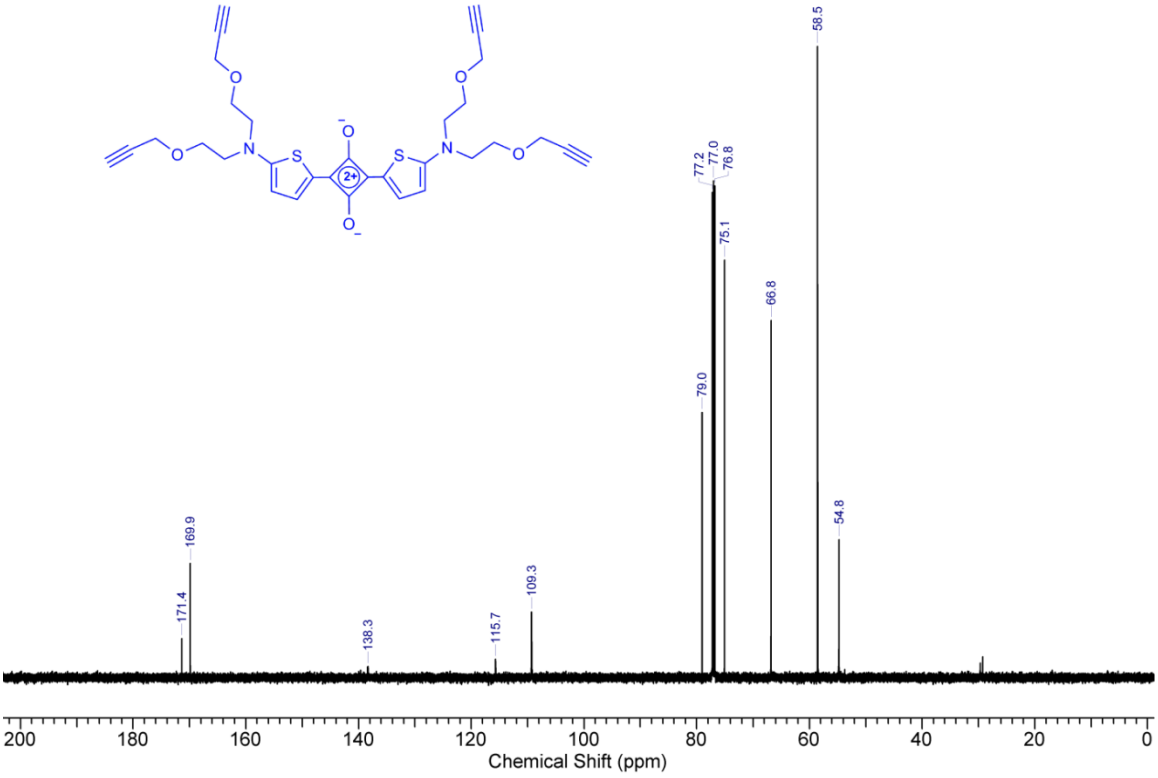


Figure *S43*: ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C) spectrum of squaraine dye **3**.

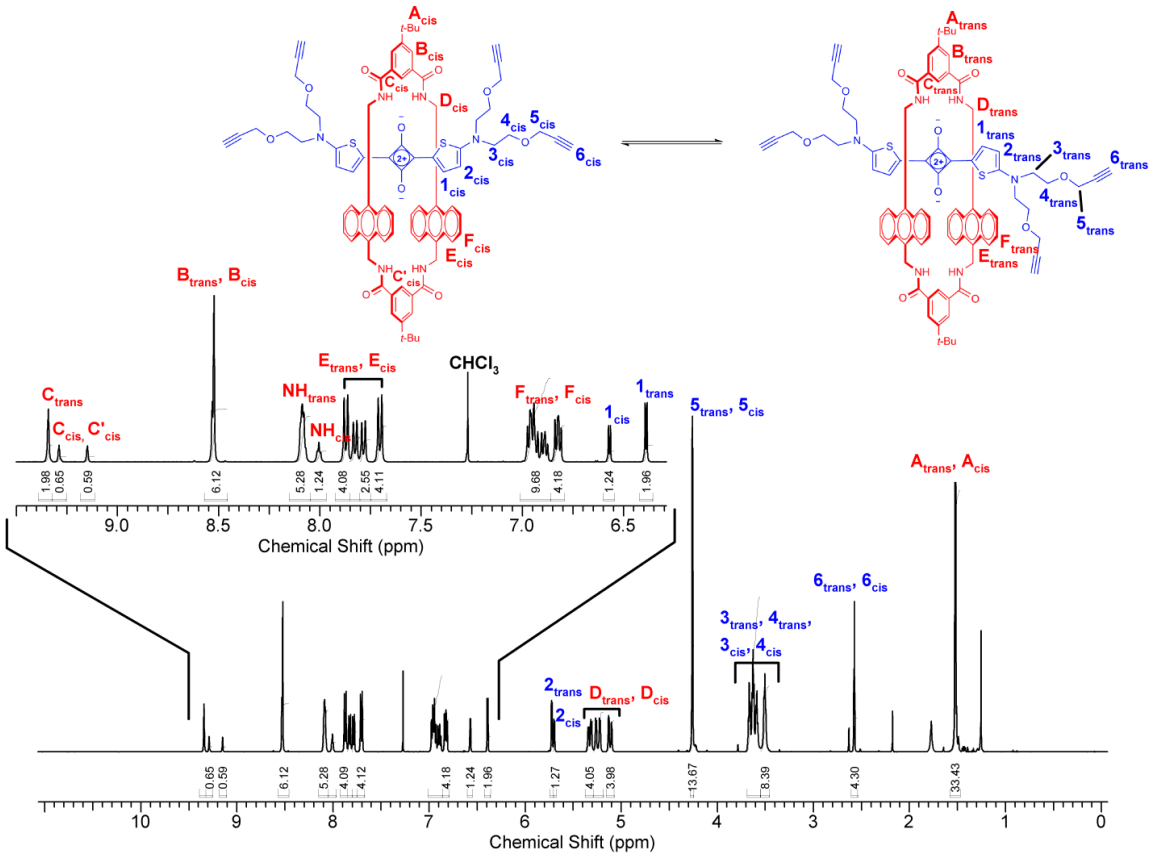


Figure *S44*: ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) spectrum of squaraine rotaxane **SR1**.

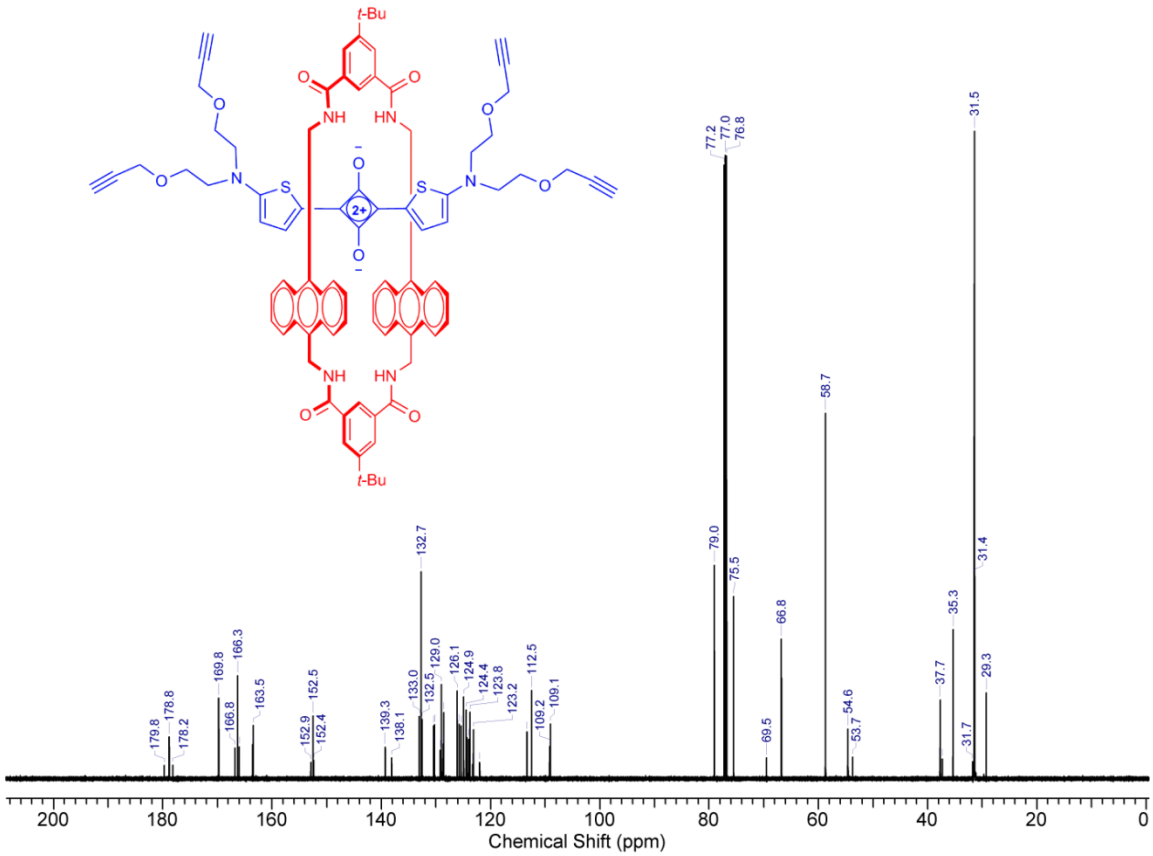


Figure *S45*: ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C) spectrum of squaraine dye **SR1**.

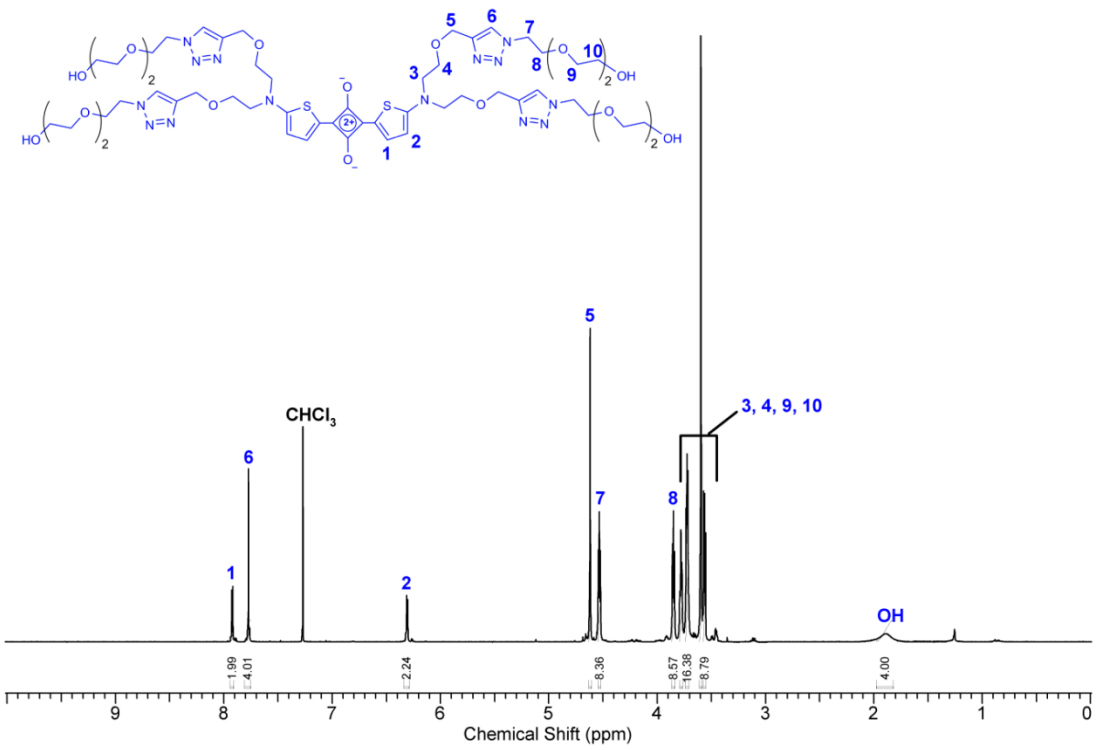


Figure *S46*: ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) spectrum of squaraine dye **S4**.

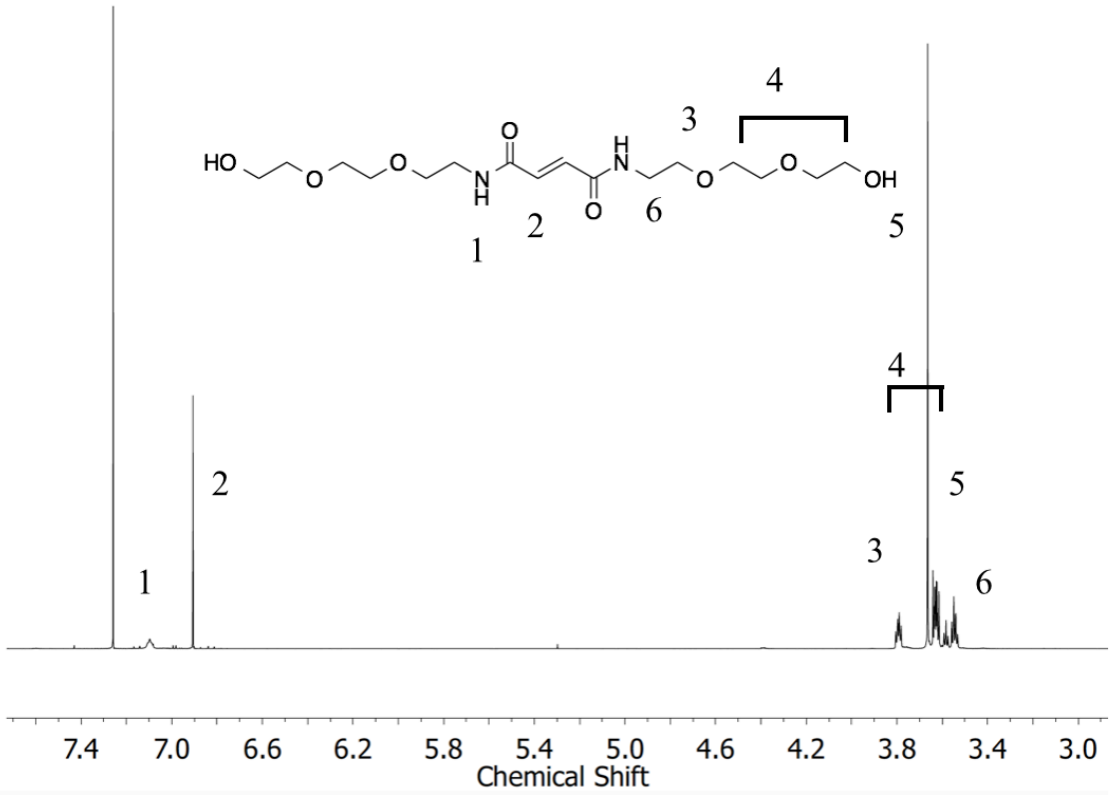


Figure *S47*: ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) spectrum of fumaride **F1**.

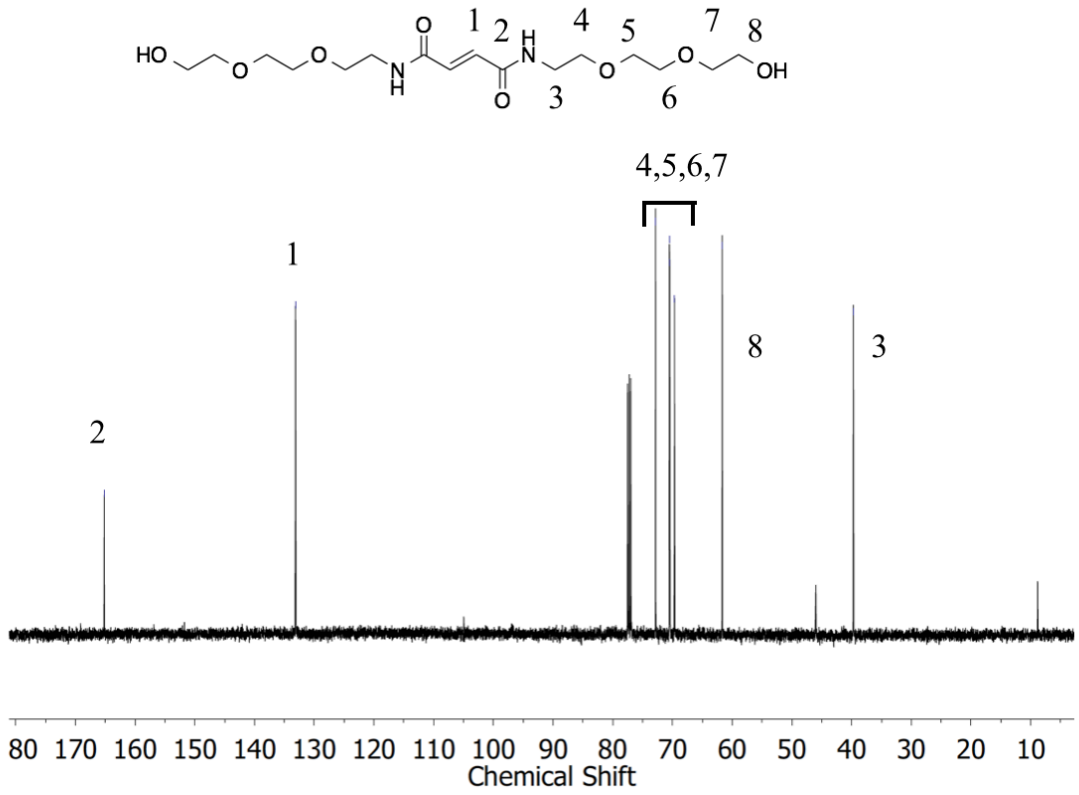


Figure *S48*: ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C) spectrum of fumaride **F1**.

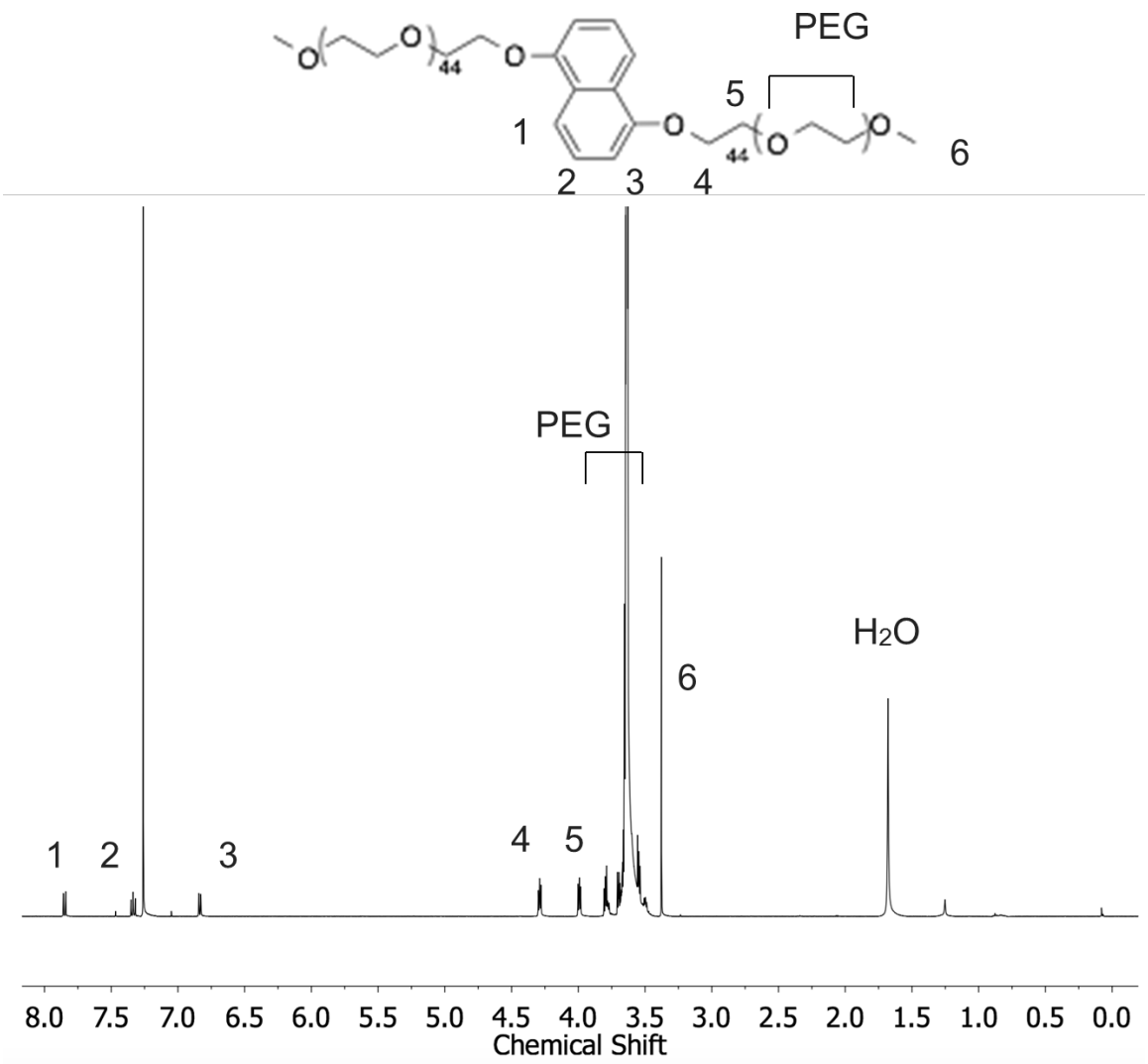


Figure *S49*: ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) spectrum of **N1**.

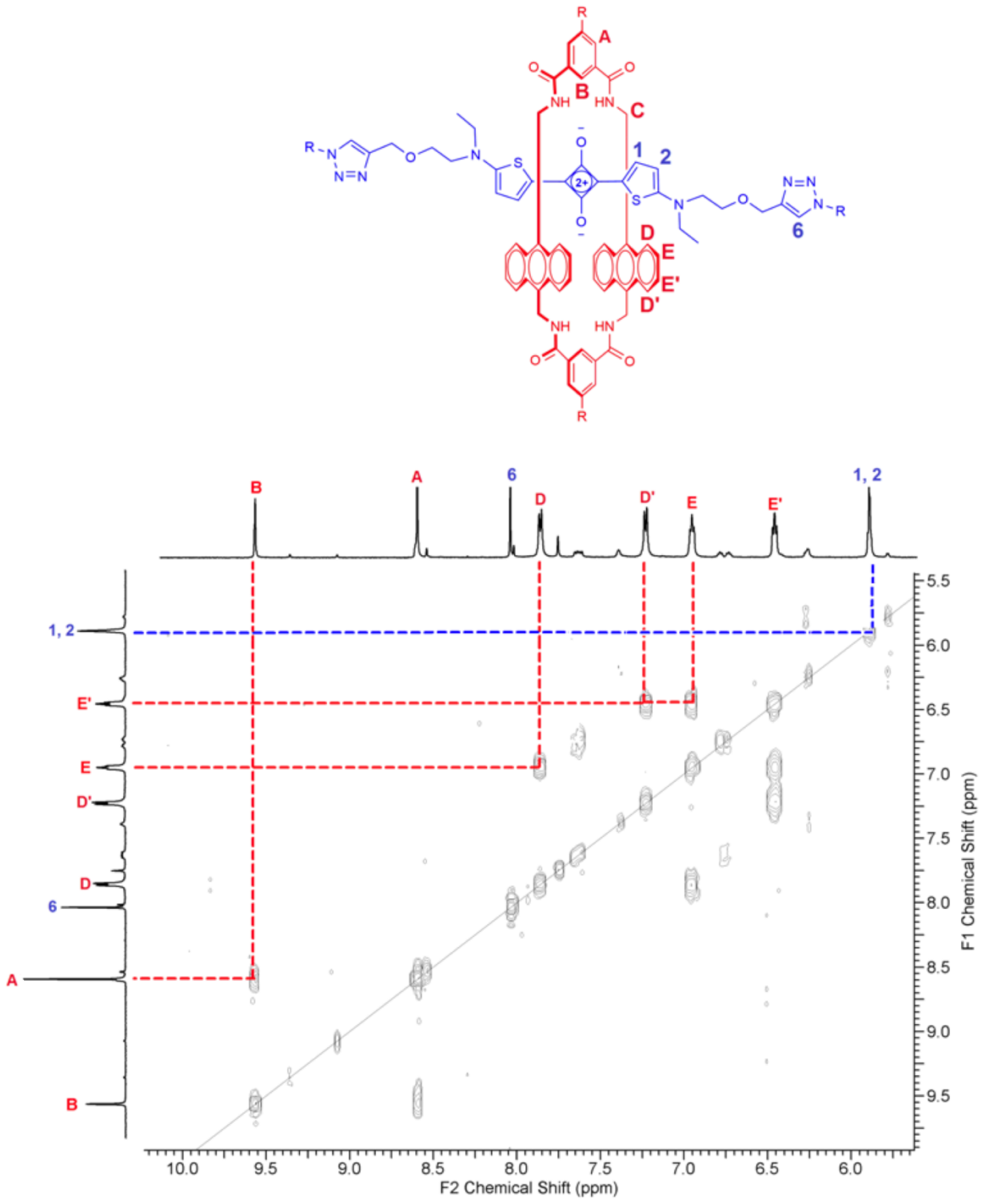


Figure *S50*: Partial COSY spectrum (600 MHz, D_2O, 25°C) of complex **M2⊃S3**.

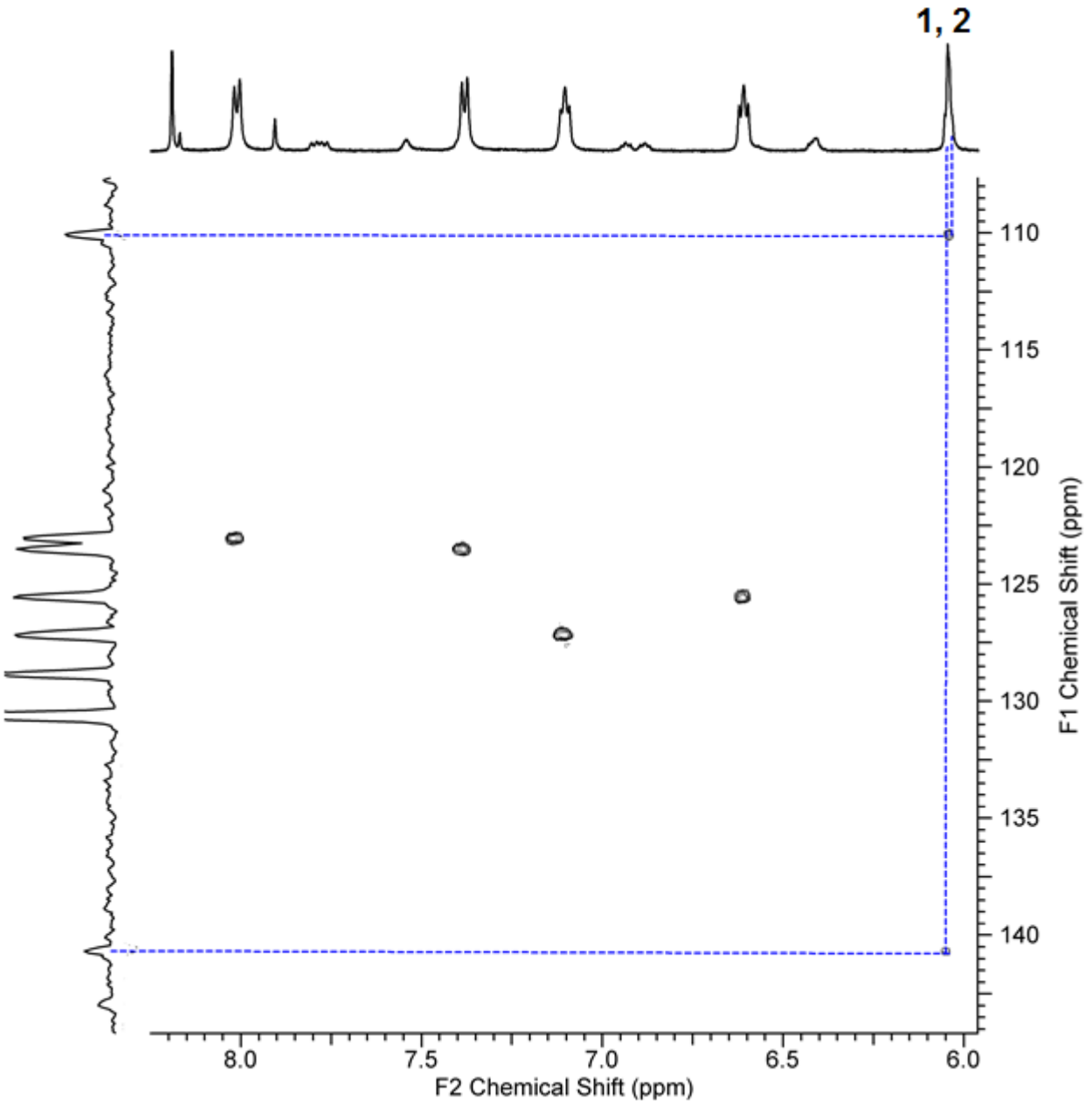


Figure *S51*: Partial HSQC spectrum (600 MHz, D_2O, 25 °C) of complex **M2⊃S3**.

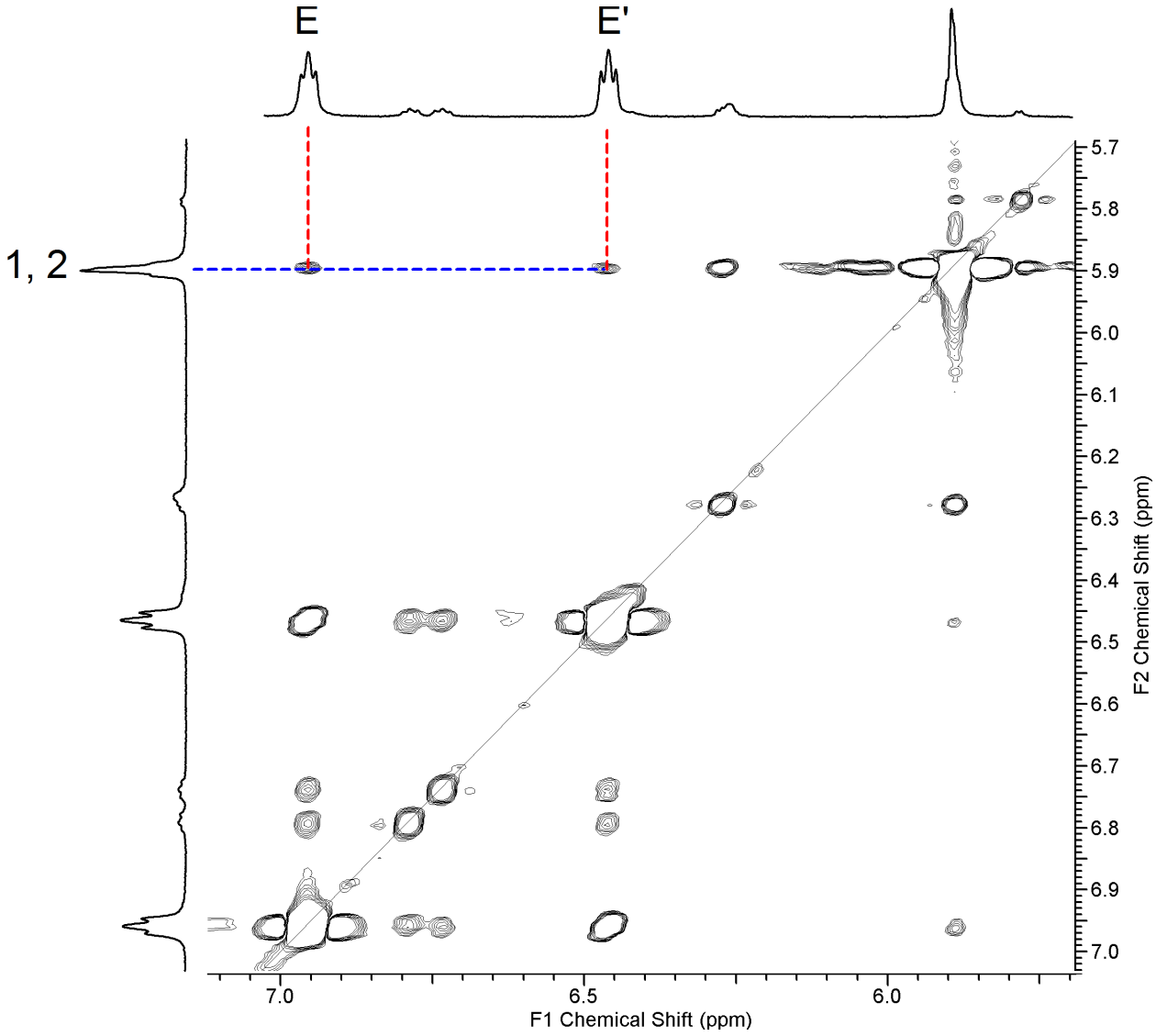


Figure *S52*: Partial ROESY spectrum (600 MHz, D_2O, 25 °C) of complex **M2⊃S3**.

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