Supporting Information:

Self-Assembly of Fluorescent Inclusion Complexes in Competitive Media Including the Interior of Living Cells

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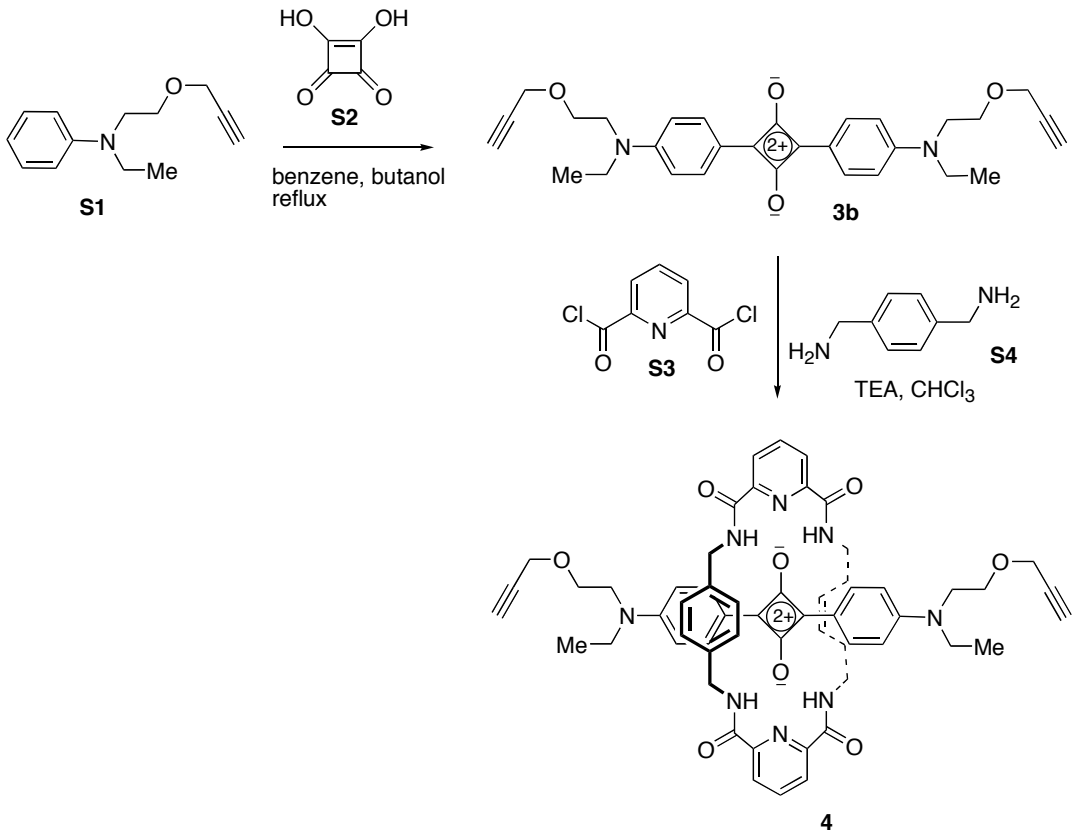
Contents

A. Synthesis



Scheme S1: Synthesis of squaraine **3b** and rotaxane **4**.

Procedure to synthesize aniline derivative S1

2-(*N*-Ethylanilino)ethanol (3.0 g, 0.018 mol) and propargyl chloride (4.92 g, 0.066 mol) were dissolved in benzene (100 mL). Tetrabutylammonium bisulfate (0.60 g, 1.77 mmol) was dissolved in 50% NaOH solution (60 mL) and the solutions were combined and the phase transfer reaction was gently stirred for 48 h at ambient temperature. The benzene layer was isolated, concentrated and the residue was taken up in chloroform (200 mL) and washed with water (2 x 100 mL), dried ($NaSO_4$) and concentrated to give a light yellow oil. Purification of the oil by column chromatography using silica gel (0-2% EtOAc/hexanes) gave the title compound **S1** as a light yellow oil (3.18 g, 86%); δ_H (500 MHz, $CDCl_3$): 7.21 (apparent t, J = 7.0 Hz, 2H), 6.70 (d, J = 8.0 Hz, 2H), 6.66 (t, J = 7.0 Hz, 1H), 4.17 (d, J = 2.0 Hz, 2H), 3.69 (t, J = 6.5 Hz,

2H), 3.53 (t, J = 6.5 Hz, 2H), 3.42 (q, J = 7.0 Hz, 2H), 2.43 (t, J = 2.0 Hz, 1H), 1.16 (t, J = 7.0 Hz, 3H); δ_C (125 MHz, $CDCl_3$): 147.5, 129.1, 115.7, 111.7, 79.5, 74.4, 67.5, 58.2, 49.7, 45.2, 12.0; *m/z* (FAB) 204 [$(M+H)^+$, 70%], 203 (79), 134 (100), 106 (62).

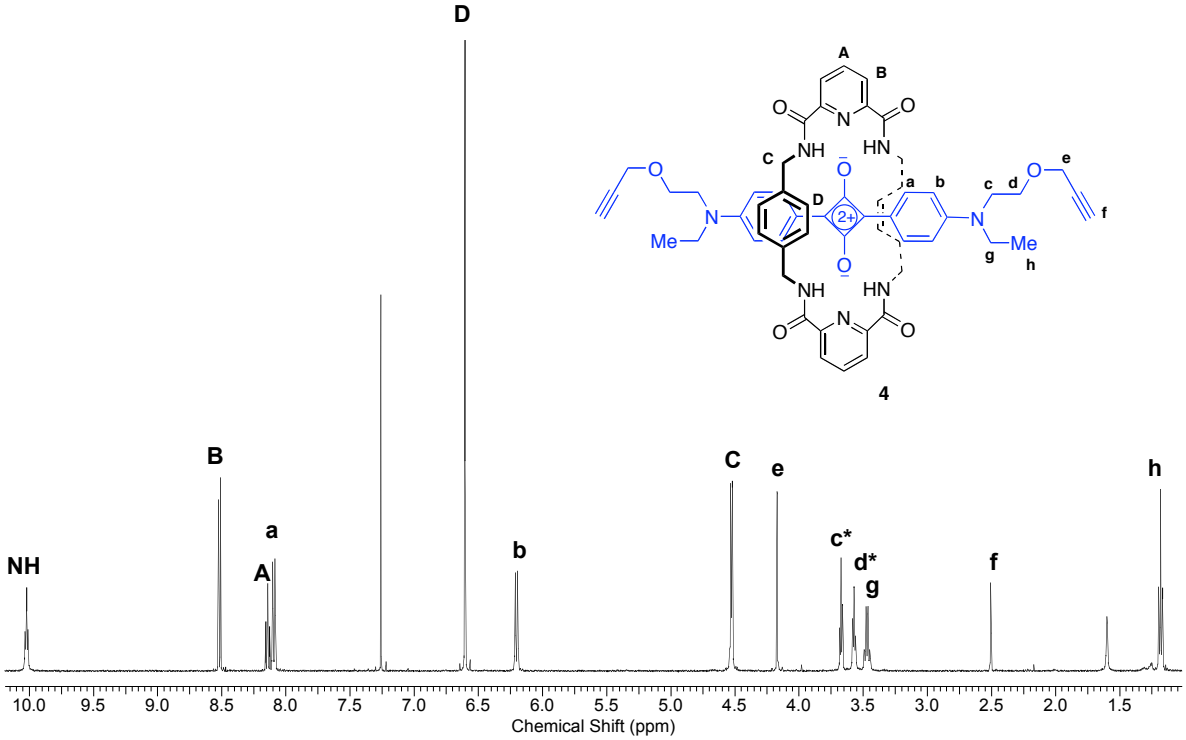
Procedure to synthesize squaraine dye 3b

Squaric acid **S2** (0.140 g, 1.23 mmol) was added to the aniline derivative **S1** (0.50 g, 2.46 mmol) dissolved in a mixture of benzene (15 mL) and *n*-butanol (8 mL). The reaction mixture was heated to reflux at 100° C for 20 h and any water formed during the reaction was trapped using a Dean and Stark apparatus. The resultant intense deep blue solution was concentrated and purification of the residue by column chromatography using silica gel (0-0.5% MeOH/$CHCl_3$) gave the squaraine dye **3b** as an intense green/blue solid that was dried *in vacuo* (0.262 g, 44%); m.p 149-150° C; λ_{max} (abs, $CHCl_3$) = 630 nm, ε = 447,000 $M^{-1}cm^{-1}$; δ_H (500 MHz, $CDCl_3$): 8.37 (d, J = 9.3 Hz, 4H), 6.77 (d, J = 9.3 Hz, 4H), 4.16 (d, J = 2.3 Hz, 4H), 3.75 (t, J = 5.5 Hz, 4H), 3.69 (t, J = 5.5 Hz, 4H), 3.59 (q, J = 7.0 Hz, 4H), 2.43 (t, J = 2.3 Hz, 2H), 1.25 (t, J = 7.0 Hz, 6H); δ_C (125 MHz, $CDCl_3$): 188.8, 183.3, 153.4, 133.3, 119.9, 112.3, 79.1, 75.0, 67.3, 58.6, 50.3, 46.4, 12.3; *m/z* (FAB) 485 [$(M+H)^+$, 12%], 484 (11); Found *m/z* (FAB) 485.2426. Calculated for $C_{30}H_{33}N_2O_4$ $(M+H)^+$ 485.2440.

Procedure to synthesize rotaxane 4 by "clipping" method

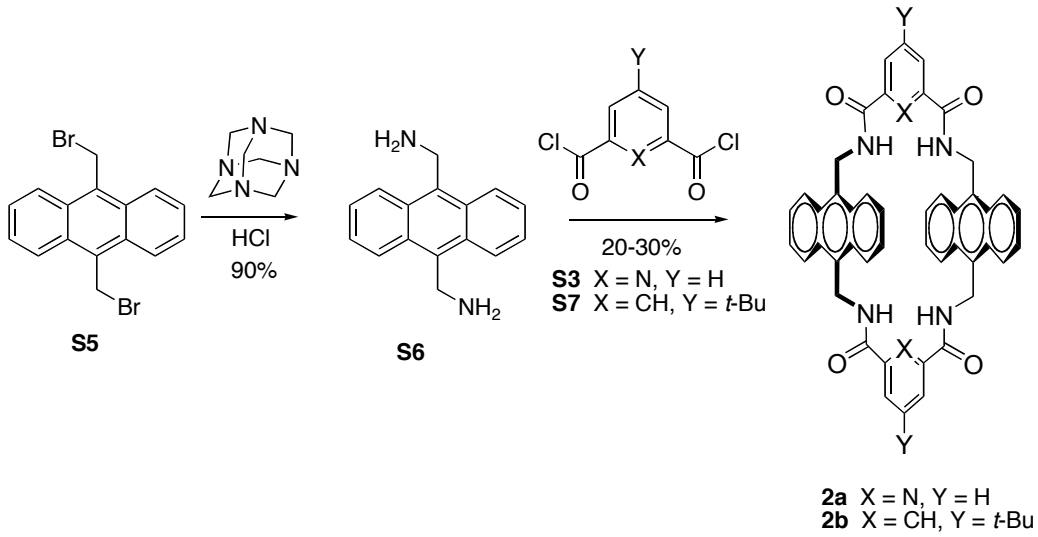
Solutions of *p*-xylylenediamine **S4** (0.297 g, 2.18 mmol) and 2,6-pyridinedicarbonyl dichloride **S3** (0.445 g, 2.18 mmol) in dry chloroform (5 mL) were simultaneously added over a period of 5 h to a solution of the squaraine **3b** (0.20 g, 0.413 mmol) and triethylamine (0.440 g, 4.36 mmol) in dry chloroform (20 mL) under a nitrogen atmosphere. The mixture was stirred for a further 20 h and then filtered through filter paper. The filter paper was washed with chloroform (100 mL) and the filtrate was concentrated. Purification of the crude material by column chromatography using silica gel (0-0.5% MeOH/$CHCl_3$) gave the rotaxane **4** as a dark blue solid (0.096 g, 22%); m.p 216-223° C (dec); λ_{max} (abs, $CHCl_3$) = 642 nm, ε = 501,000 $M^{-1}cm^{-1}$; δ_H (500 MHz, $CDCl_3$): 10.02 (t, J = 6.0 Hz, 4H), 8.51 (d, J = 8.0 Hz, 4H), 8.14 (t, J = 8.0 Hz, 2H), 8.08 (d, J = 9.0 Hz, 4H), 6.60 (s, 8H), 6.20 (d, J = 9.0 Hz, 4H), 4.52 (d, J = 6.0 Hz, 8H), 4.17 (d, J = 2.0 Hz, 4H), 3.67 (t, J = 6.0 Hz, 4H), 3.57 (t, J = 6.0 Hz, 4H), 3.46 (q, J = 7.0 Hz, 4H), 2.51 (t, J = 2.0 Hz, 2H), 1.18 (t, J = 7.0 Hz, 6H); δ_C (125 MHz, $CDCl_3$): 185.2, 184.5, 163.6, 153.5, 149.5, 138.7,

136.6, 133.6, 128.9, 125.2, 119.0, 111.6, 79.1, 75.2, 67.1, 58.6, 50.1, 46.3, 43.4, 12.2; *m/z* (FAB) 1019 [$(M+H)^+$, 12%], 613 (100). Found *m/z* (FAB) 1019.4443. Calculated for $C_{60}H_{59}N_8O_8$ $(M+H)^+$ 1019.4456.



* The assignment of protons **c** and **d** could be interchanged.

Figure S1: ^{1}H NMR spectrum of **4** in $CDCl_3$ (500 MHz).



Scheme S2: Synthesis of macrocycles **2a** and **2b**.

Procedure to synthesize 9,10-bis(aminomethyl)anthracene S6

9,10-Bis(bromomethyl)anthracene **S5**[§] (0.50 g, 1.37 mmol), and hexamethylenetetramine (0.43 g, 3.04 mmol) were mixed in chloroform (100 mL) and heated to reflux overnight. The yellow precipitate was removed by filtration and washed with chloroform. The solid was then added to an ethanolic HCl solution (80 mL ethanol and 10 mL conc. HCl) and vigorously refluxed for 48 hours. The flask was cooled to 0° C and the solid was removed by filtration, washed with cold ethanol and allowed to dry in the open air. The solid was dispersed into a 10% Na_2CO_3 solution (50 mL) and stirred. Chloroform (~ 20 mL) was added while stirring and the biphasic solution was separated and the aqueous layer extracted with chloroform (2 x 30 mL). The solution was dried ($MgSO_4$), concentrated and dried overnight under high vacuum to give the bisamine **S6** as a yellow solid (0.30 g, 93% yield); δ_H (300 MHz, $CDCl_3$): 4.85 (s, 4H), 7.57 (dd, J = 6.9 Hz, J = 3.2 Hz, 4H), 7.57 (dd, J = 6.9 Hz, J = 3.2 Hz, 4H); δ_C (75 MHz, $CDCl_3$): 38.4, 124.6, 125.8, 129.3.

[§]Aathimanikandan, S. V.; Sandanaraj, B. S.; Arges, C. G.; Bardeen, C.J.; Thayumanavan, S. *Org. Lett.* **2005**, *7*, 2809-2812.

Procedure to synthesize the macrocycle 2a

9,10-Bis(aminomethyl)anthracene **S6** (0.10 g, 0.42 mmol) was dissolved in a mixture of anhydrous chloroform (9.8 mL) and triethylamine (0.145 g, 1.44 mmol). Pyridine dicarbonyl dichloride **S3** (0.086 g, 0.42 mmol) was dissolved in anhydrous chloroform (10 mL). Both aliquots were added slowly over 10 hours by dual syringe pump with stirring into a solution of anhydrous dichloromethane (50 mL). The solvent was reduced and the residue was purified by column chromatography using silica gel (0-1% MeOH/$CHCl_3$) to afford the macrocycle **2a** as a pale yellow/green solid (0.038 g, 25%); δ_H (300 MHz, $CDCl_3$): 8.48 (d, J = 7.7 Hz, 4H), 8.26 (dd, J = 6.9 Hz, J = 3.2 Hz, 8H), 8.08 (t, J = 7.7 Hz, 2H), 7.56 (t, J = 5.5 Hz, 4H), 7.50 (dd, J = 3.2 Hz, J = 6.9 Hz, 8H), 5.59 (d, J = 5.5 Hz, 8H); δ_C (75 MHz, $CDCl_3$): 162.9, 148.7, 139.3, 130.5, 129.5, 126.9, 126.1, 124.4, 36.3; *m/z* (FAB) 735 [$(M+H)^+$, 99%]; Found *m/z* (FAB) 735.2706. Calculated for $C_{46}H_{34}N_6O_4$ $(M+H)^+$ 735.2720.

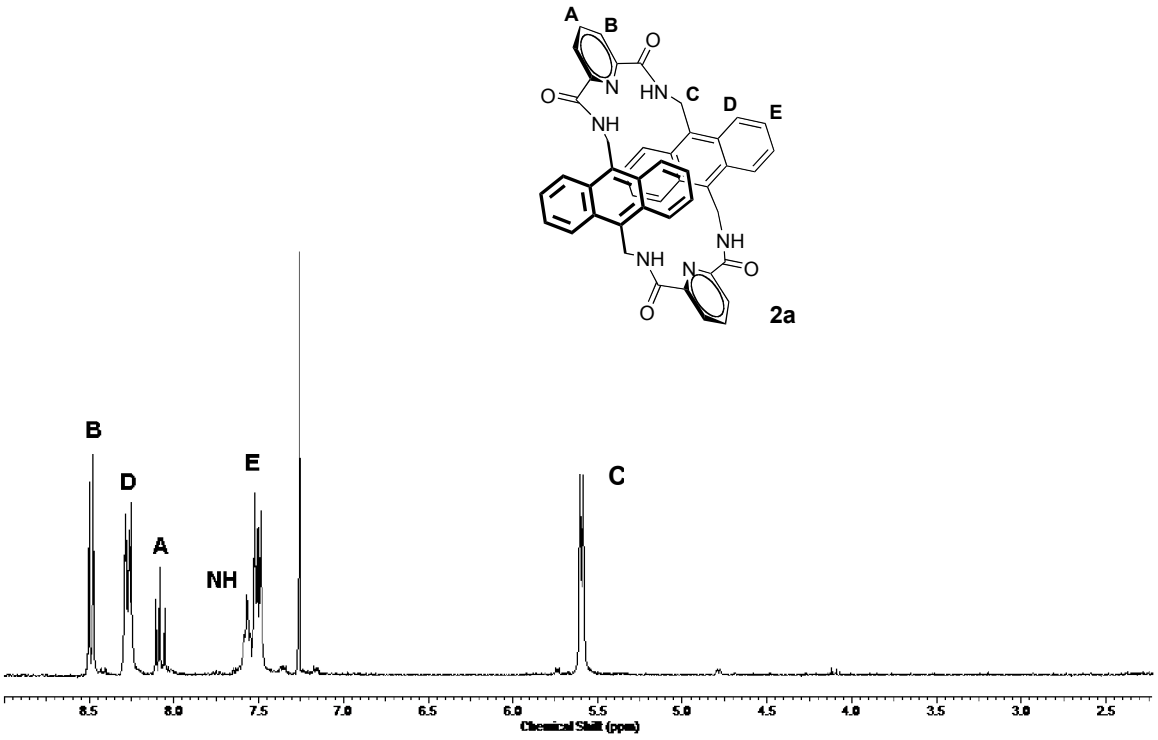


Figure S2: H^1 NMR spectrum of macrocycle **2a** in $CDCl_3$ (300 MHz).

Procedure to synthesize macrocycle 2b

9,10-Bis(aminomethyl)anthracene **S6** (0.30 g, 1.27 mmol) was dissolved in a solution containing anhydrous chloroform (60 mL) and triethylamine (0.51 g, 5.05 mmol). 5-*tert*-Butylisophthaloyl dichloride **S7** (0.329 g, 1.27 mmol) was dissolved in a solution of anhydrous chloroform (60 mL). Both aliquots were added over 10 hours by dual syringe pump with stirring into a solution of anhydrous chloroform (60 mL). The solvent was evaporated and the residue was purified by column chromatography using silica gel (0-1% MeOH/$CHCl_3$) to afford the macrocycle **2b** as a yellow solid (0.136 g, 25%); δ_H (300 MHz, $CDCl_3$): 8.38 (s, 4H), 8.22 (dd, J = 7.1 Hz, J = 3.2 Hz, 8H), 7.48 (dd, J = 7.1 Hz, J = 3.2 Hz, 8H), 7.04 (s, 2H), 6.15 (bs, 4H), 5.52 (d, J = 4.7 Hz, 8H), 1.45 (s, 18H); δ_C (75 MHz, $CDCl_3$): 166.6, 153.7, 133.4, 130.2, 129.9, 129.3, 126.8, 126.1, 124.4, 37.3, 31.2, 29.7; *m/z* (FAB) 845 [$(M)^+$, 21%]; Found *m/z* (FAB) 845.4091. Calculated for $C_{56}H_{52}N_4O_4$ $(M)^+$ 845.4060.

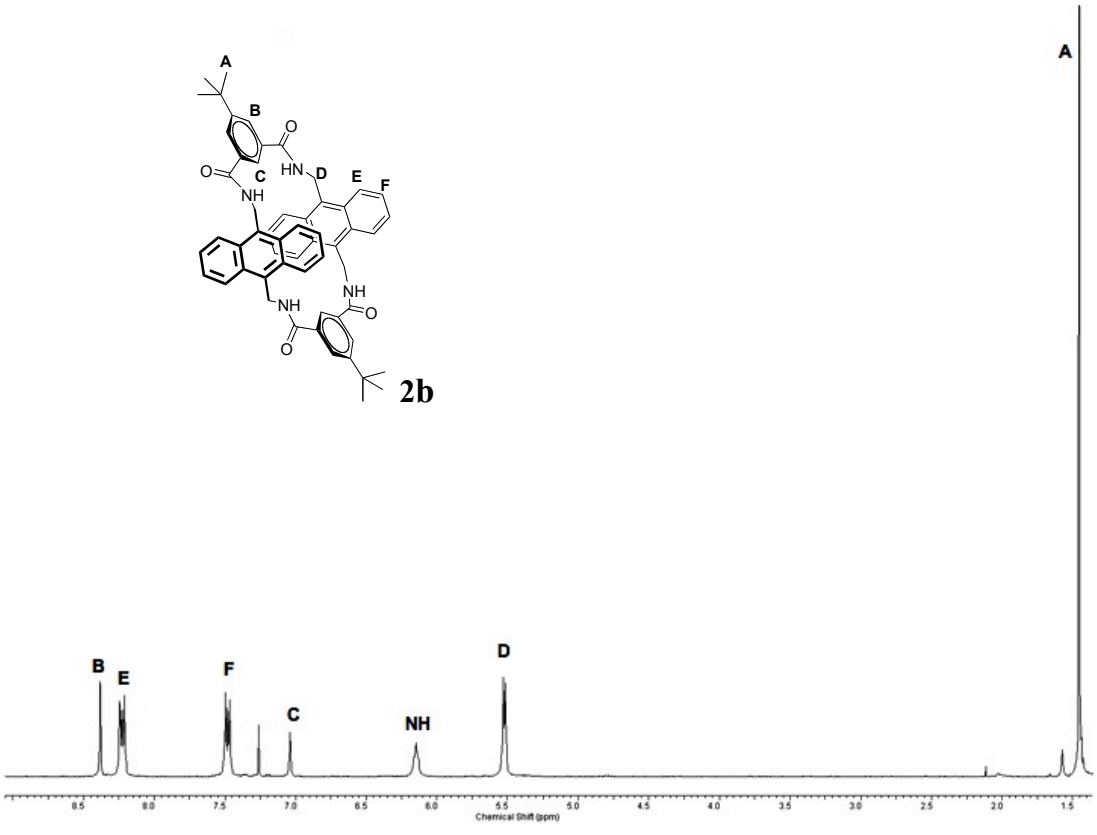


Figure S3: H^1 NMR spectrum of macrocycle **2b** in $CDCl_3$ (300 MHz).

Procedure to synthesize pseudo-rotaxane 2a⊃3a by "mixing" method

$CDCl_3$ (1.0 mL) was added to a mixture of *bis(N,N,*-dimethylanilino)squaraine dye **3a** (6.8 mg, 0.021 mmol) and macrocycle **2a** (6.5 mg, 0.009 mmol) and the suspension was stirred for 10 minutes, during which time the color darkened considerably. The mixture was filtered through a 0.45 µm syringe filter to rid the insoluble free dye and produce pure inclusion complex **2a⊃3a** in quantitative yield; δ_H (500 MHz, $CDCl_3$): 9.90 (t, J = 4.2 Hz, 4H), 8.66 (d, J = 8.0 Hz, 4H), 8.23 (t, J = 8.0 Hz, 2H), 7.72 (dd, J = 6.9 Hz, J = 3.2 Hz, 8H), 7.30 (d, J = 9.2 Hz, 4H), 6.64 (dd, J = 6.9, Hz, J = 3.2 Hz, 8H), 5.96 (d, J = 9.2 Hz, 4H), 5.32 (d, J = 4.2 Hz, 8H), 3.31 (s, 12H); δ_C (75 MHz, $CDCl_3$): 183.1, 182.3, 164.7, 154.5, 149.9, 136.6, 134.0, 130.5, 128.1, 125.9, 125.5, 123.9, 117.5, 110.9, 40.4, 37.6.

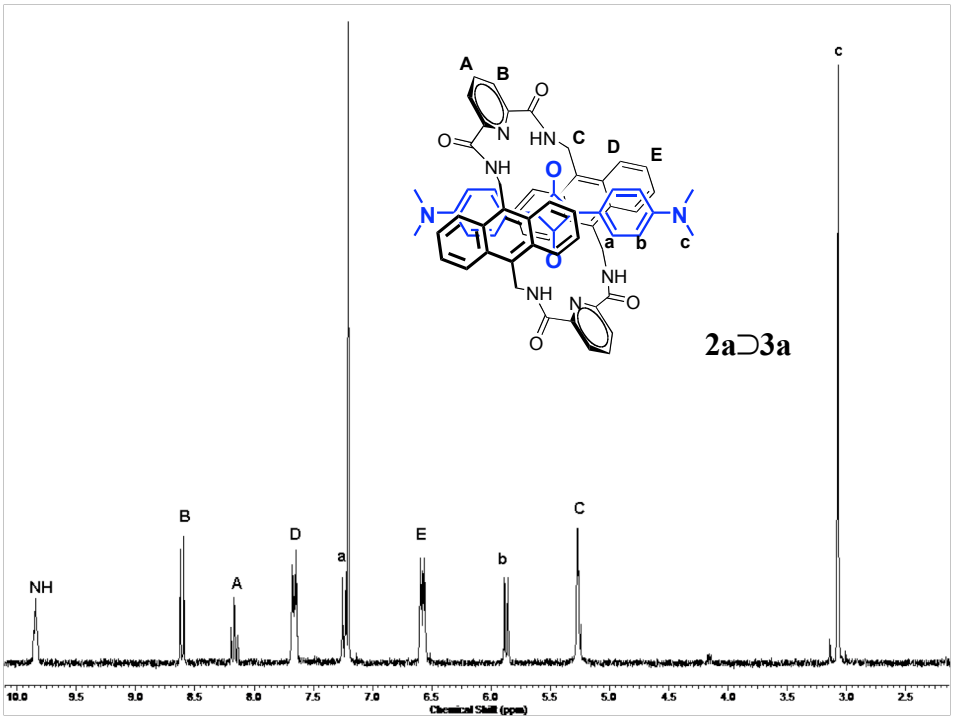


Figure S4: H^1 NMR spectrum of pseudo-rotaxane **2a⊃3a** (5 mM) in $CDCl_3$ (500 MHz).

Procedures to synthesize rotaxane 2a⊃3b

Clipping method: Separate solutions of 9,10-bis(aminomethyl)anthracene **S6** (0.097, 0.41 mmol), and 2,6-pyridinedicarbonyl dichloride **S3** (0.084 g, 0.41 mmol) in dry chloroform (5 mL) were simultaneously added by dual syringe pump over a period of 10 h to a solution of the squaraine **3b** (25 mg, 0.052 mmol) and triethylamine (0.080 g, 0.79 mmol) in dry chloroform (20 mL) under a nitrogen atmosphere. The solvent was evaporated and the residue was purified by column chromatography using silica gel (0-10% acetone/methylene chloride), to afford pure rotaxane **2a⊃3b** (0.015 g, 24%); δ_H (300 MHz, $CDCl_3$): 9.96 (t, J = 4.2 Hz, 4H), 8.66 (d, J = 8.0 Hz, 4H), 8.23 (t, J = 8.0 Hz, 2H), 7.71 (dd, J = 6.9 Hz, J = 3.2 Hz, 8H), 7.24 (d, J = 9.2 Hz, 4H), 6.66 (dd, J = 6.9 Hz, J = 3.2 Hz, 8H), 5.96 (d, J = 9.2 Hz, 4H), 5.31 (d, J = 4.2 Hz, 8H), 4.25 (d, J = 2.4 Hz, 4H), 3.75 (t, J = 5.7 Hz, 4H), 3.63 (t, J = 5.7 Hz, 4H), 3.53 (q, J =7.2 Hz, 4H), 2.56 (s, 2H), 1.28 (t, J = 7.2 Hz, 6H); δ_C (75 MHz, $CDCl_3$): 183.8, 182.0, 164.8, 153.0, 149.9, 138.6, 134.3, 130.5, 128.1, 125.9, 125.7, 123.9, 117.3, 110.9, 75.5, 67.4, 58.6, 50.1, 46.2, 37.8, 29.7, 12.6; *m/z* (FAB) 1219 [$(M-H)^+$, 87%].

Slippage method: Squaraine dye **3b** (10 mg, 0.0206 mmol) and macrocycle **2a** (14 mg, 0.0190 mmol) were placed in a thick-walled reaction vessel with anhydrous chloroform (10 mL). The solution was purged with argon, sealed tightly, and heated at 120° C for 5 days. NMR analysis of the crude sample revealed that it was 90% rotaxane **2a⊃3b**, the remainder being degraded material. The solvent was evaporated and the residue purified by column chromatography using silica gel (0-10% acetone/methylene chloride), to afford **2a⊃3b** (0.010 g, 43%).

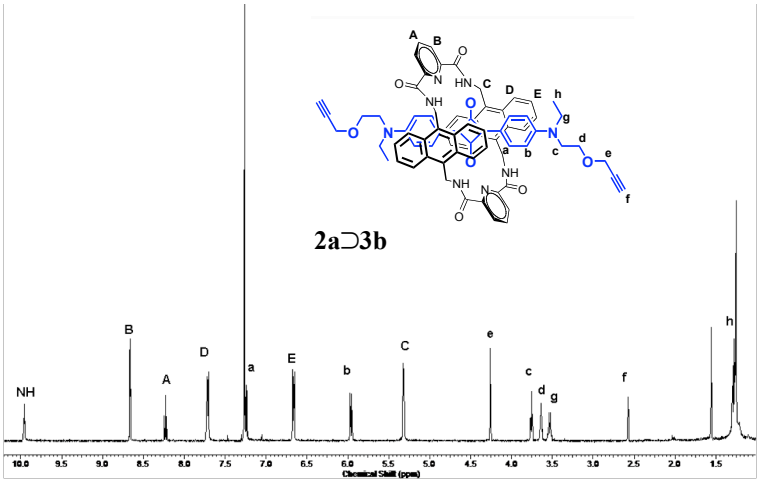


Figure S5: H^1 NMR spectrum of rotaxane **2a⊃3b** in $CDCl_3$ (500 MHz).

Procedure to synthesize pseudo-rotaxane 2b⊃3b by "mixing" method

A solution of squaraine dye **3b** (5.8 mg, 0.012 mmol) in chloroform (1 mL) was added to a solution of macrocycle **2b** (10.1 mg, 0.012 mmol) in chloroform (1 mL). An immediate analysis by NMR showed that pseudo-rotaxane formation was complete; however, small residual signals were observed due to imperfect stoichiometry. The sample was purified by column chromatography using silica gel (0.5% MeOH/$CHCl_3$) to give pseudo-rotaxane **2b⊃3b** (0.015 g, 94%); δ_H(300MHz, $CDCl_3$): 9.40 (s, 2H), 8.54 (s, 4H), 8.27 (bs, NH), 7.76 (dd, J = 6.8 Hz, J = 3.3 Hz, 8H), 7.02 (d, J = 9.4 Hz, 4H), 6.71 (dd, J = 6.8 Hz, J = 3.1 Hz, 8H), 6.15 (d, J = 9.4 Hz, 4H), 5.25 (d, J = 3.3 Hz, 8H), 4.28 (s, 4H), 3.81 (t, J = 5.3 Hz, 4H), 3.69 (t, J = 5.0 Hz, 4H), 3.59 (q, J = 7.1 Hz, 4H), 2.55 (t, J = 2.4 Hz, 2H), 1.56 (s, 18H), 1.33 (t, J = 7.1 Hz, 6H).

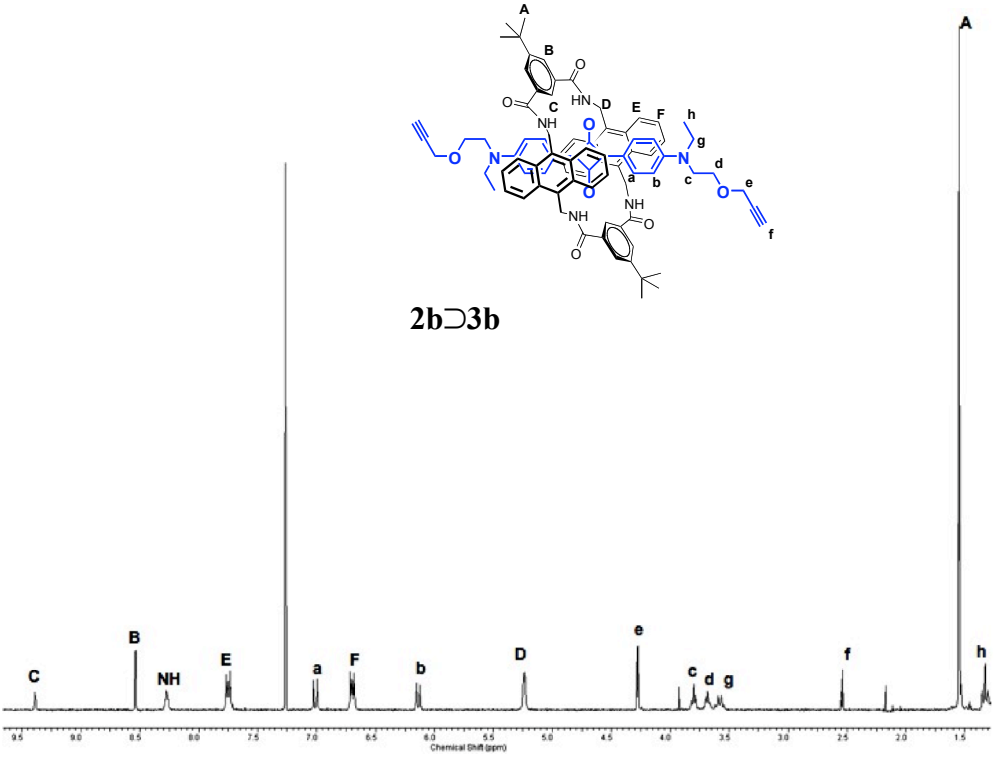
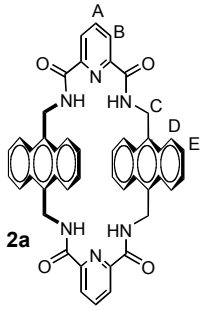
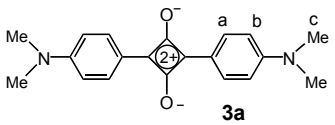


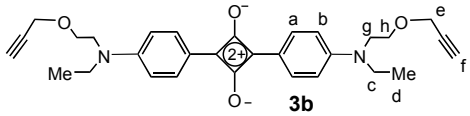
Figure S6: H^1 NMR spectrum of pseudo-rotaxane **2b⊃3b** (5 mM) in $CDCl_3$ (500 MHz).

Complexation Induced Changes in Chemical Shift for Squaraine/Macrocycle Inclusion

Table S1. Change in chemical shift (Δδ, ppm) upon formation of **2a⊃3a** and **2a⊃3b** in $CDCl_3$.



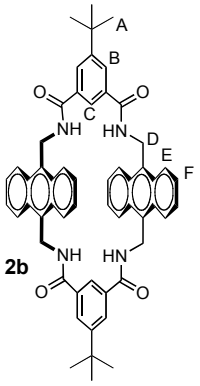


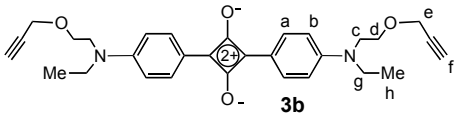


	A	B	C	D	E	NH	a	b	c	d	e	f	g	h
2a⊃3a Δδ, ppm	+0.15	+0.18	-0.27	-0.55	-0.86	+2.34	*	*	*					
2a⊃3b Δδ, ppm	+0.15	+0.18	-0.28	-0.55	-1.42	+1.70	-1.13	-0.81	-0.06	+0.03	+0.09	+0.13	0.00	-0.06

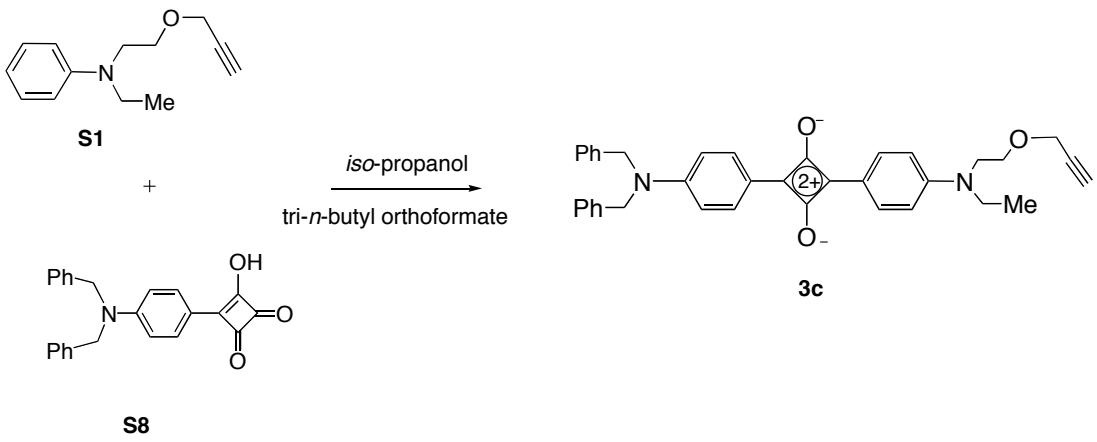
*Poor solubility prohibits acquisition of the spectroscopic data for free squaraine **3a**.

Table S2. Change in chemical shift (Δδ, ppm) upon formation of **2b⊃3b** in $CDCl_3$.





	A	B	C	D	E	F	NH	a	b	c	d	e	f	g	h
2b⊃3b Δδ, ppm	+0.11	+0.16	+2.36	-0.27	-0.46	-0.77	+2.12	-1.35	-0.62	+0.06	0.00	+0.12	+0.12	0.00	+0.08



Scheme S3: Synthesis of squaraine **3c**.

Procedure to synthesize squaraine dye 3c

The dehydrating agent tri(*n*-butyl)orthoformate (1 mL) was added to a solution of the semi-squaraine **S8**[¶] (0.526 g, 1.63 mmol) and the aniline derivative **S1** (0.30 g, 1.48 mmol) in dry *iso*-propanol (20 mL). The mixture was heated to reflux at 95° C for 6 h. The resulting intense deep blue solution was concentrated and purification of the residue using silica gel (0-0.5% $MeOH/CHCl_3$) gave the squaraine **3c** as a dark blue solid (0.375 g, 46%); m.p 169-170° C; λ_{max} (abs, $CHCl_3$) = 630 nm, ε = 358, 000 $M^{-1}cm^{-1}$; δ_H (500 MHz, $CDCl_3$): 8.38 (d, *J* = 9.0 Hz, 2H), 8.35 (d, *J* = 9.0 Hz, 2H), 7.35 (apparent t, *J* = 7.0 Hz, 4H), 7.28 (t, *J* = 7.0 Hz, 2H), 7.20 (d, *J* = 7.5 Hz, 4H), 6.88 (d, *J* = 9.0 Hz, 2H), 6.78 (d, *J* = 9.0 Hz, 2H), 4.78 (s, 4H), 4.15 (d, *J* = 2.5 Hz, 2H), 3.75 (t, *J* = 5.8 Hz, 2H), 3.69 (t, *J* = 5.8 Hz, 2H), 3.59 (q, *J* = 7.0 Hz, 2H), 2.43 (t, *J* = 2.5 Hz, 1H), 1.25 (t, *J* = 7.0 Hz, 3H); δ_C (125 MHz, $CDCl_3$): 190.5, 188.5, 183.1, 154.5, 153.9, 136.0, 133.7, 133.0, 129.0, 127.6, 126.4, 120.8, 119.8, 112.9, 112.5, 79.0, 75.0, 67.2, 58.5, 54.0, 50.3, 46.5, 12.3; *m/z* (FAB) 555 [$(M+H)^+$, 2%], 554 (2). Found *m/z* (FAB) 555.2673. Calculated for $C_{37}H_{35}N_2O_3$ $(M+H)^+$ 555.2648.

[¶]Compound **S8** was prepared by modifying the standard procedure described in, Keil, D.; Hartmann. H. *Dyes Pigm*. **2001**, *49*, 161.

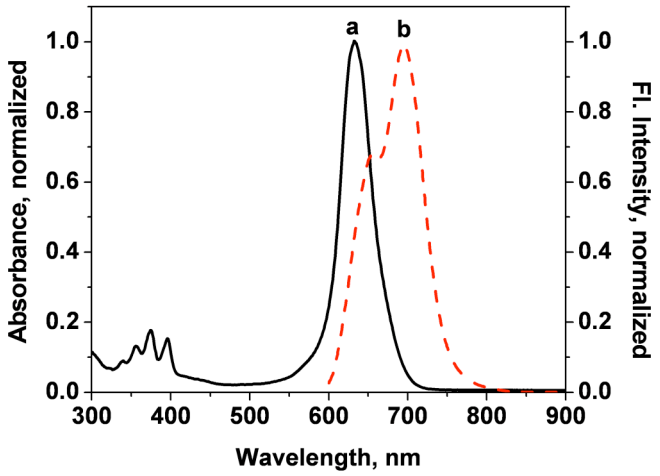


Figure S7: (**a**) Absorption spectrum of pseudo-rotaxane **2a⊃3a** in chloroform (6 μM), λ_{max} (abs) = 633 nm; (**b**) fluorescence emission spectrum of **2a⊃3a** in chloroform (6 μM), excited at 580 nm, λ_{max} (em) = 692 nm. The shoulder peak at 647 nm is due to the free squaraine dye. Spectrum **b** was unchanged after sitting for 1 h.

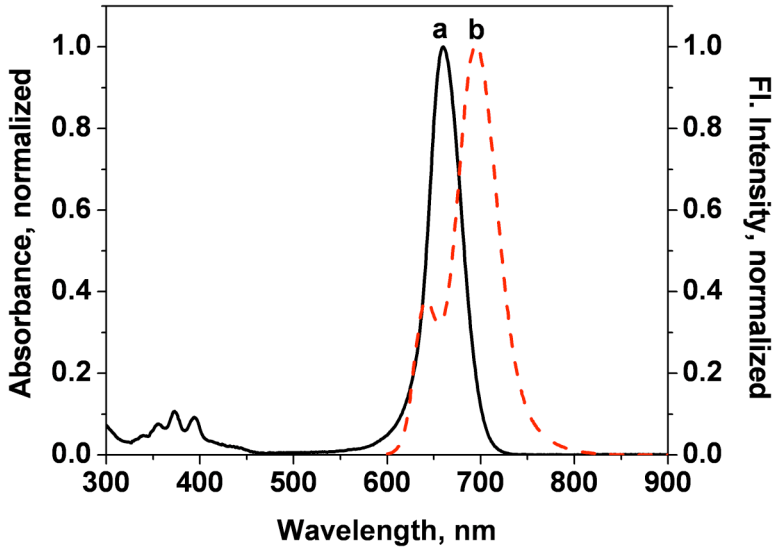


Figure S8: (**a**) Absorption spectrum of pseudo-rotaxane **2b⊃3b** in chloroform (6 μM), λ_{max} (abs) = 658 nm; (**b**) fluorescence emission spectrum of **2b⊃3b** in chloroform (6 μM), excited at 590 nm, λ_{max} (em) = 695 nm. The shoulder peak at 642 nm is due to free squaraine dye. Spectrum **b** was unchanged after sitting for 1 h.

Resistance to Quenching by Methanol

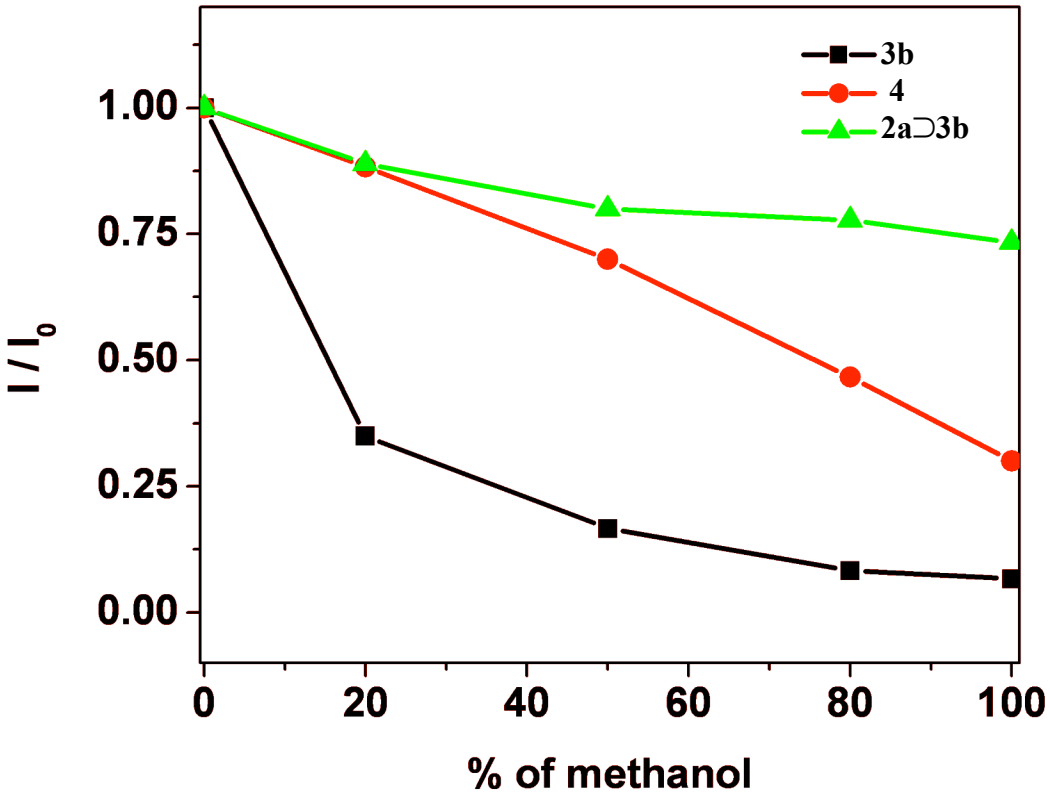


Figure S9: Fluorescence intensities for **3b**, anthrylene rotaxane **2a⊃3b** and phenylene rotaxane **4** (at respective emission maxima) in chloroform (each sample was 6 μM) with increasing methanol concentration.

B. X-ray Crystal Structure of 2a⊃3a

Single crystals were obtained as follows: a solution of **2a⊃3a** in chloroform was prepared in a 2 mL vial and placed inside a 20 mL vial filled with hexanes. The 20 mL vial was sealed to allow slow diffusion of hexanes into chloroform. Crystallographic summary: triclinic, $C_{72}H_{60}Cl_{18}N_8O_6$, FW = 1771.38, $P\bar{1}$, Z = 1 in a cell of dimensions a = 10.5116(3) Å, b = 13.4898(4) Å, c = 13.9436(4) Å, α = 104.169(2)°, ß = 90.708(2)°, γ = 105.057(2)°, V =1845.13(9) Å^3, D_{alc} = 1.164 Mg/m^3, F(000) = 902. The structure was refined on F^2 to $wR2$ = 0.1712, conventional $R1$ = 0.0560 [8864 reflections with $I > 2\sigma(I)$], and a goodness of fit = 1.113 for 402 refined parameters. Where:

1) $wR2 = \frac{\sqrt{\sum[w(F_o^2 - F_c^2)^2]}}{\sum[w(F_o^2)^2]}$

2) $R1 = \frac{\sum \left| |F_o| - |F_c| \right|}{\sum |F_o|}$

3) $w = [\sigma^2(F_o^2) + (aP)^2 + bP]$

4) $GooF = S = \sqrt{\frac{\sum\left[w(F_o^2 - F_c^2)^2\right]}{(n-p)}}$ where n is the number of reflections and p is the number of parameters refined.

Five chemical moieties are found within the asymmetric unit, one half of a tetralactam macrocycle, one half of a squaraine dye thread and three molecules of chloroform. The complete structure is generated by inversion. The crystal readily solved in the $P\bar{1}$ space group using direct methods. After convergence of macrocycle, thread, and solvent molecule *C1s*, considerable residual density was still present in the difference Fourier map. Attempts were made to model this as two molecules of chloroform but this did not allow for adequate refinement, thus PLATON/SQUEEZE was used to correct the data and calculate a solvent accessible volume of 432.8 Å^3 containing 190 electrons, which corresponds to approximately two molecules of chloroform. All non-hydrogen atoms were refined with anisotropic parameters but H atoms were placed in idealized positions.

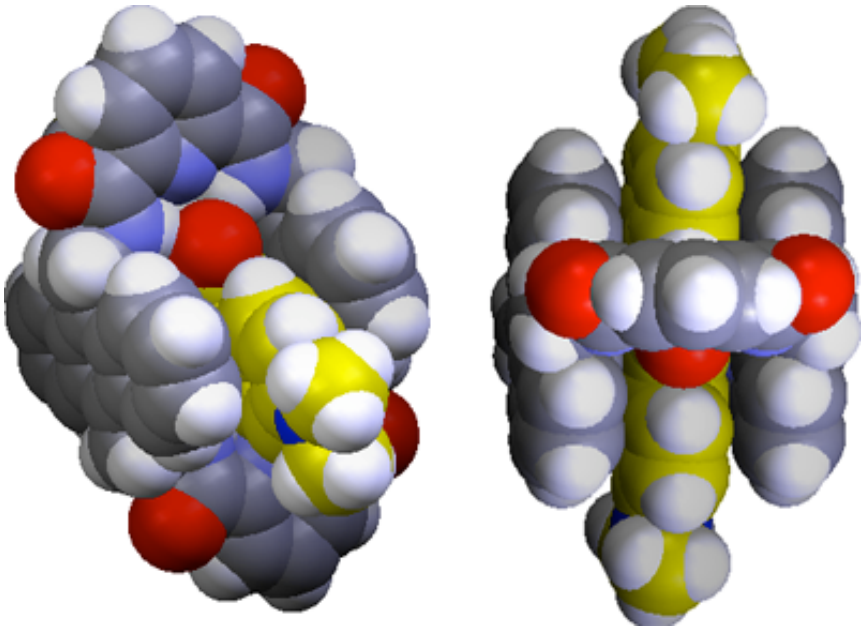


Figure S10: Spacefilling models of **2a⊃3a**. The yellow thread structure is the dye.

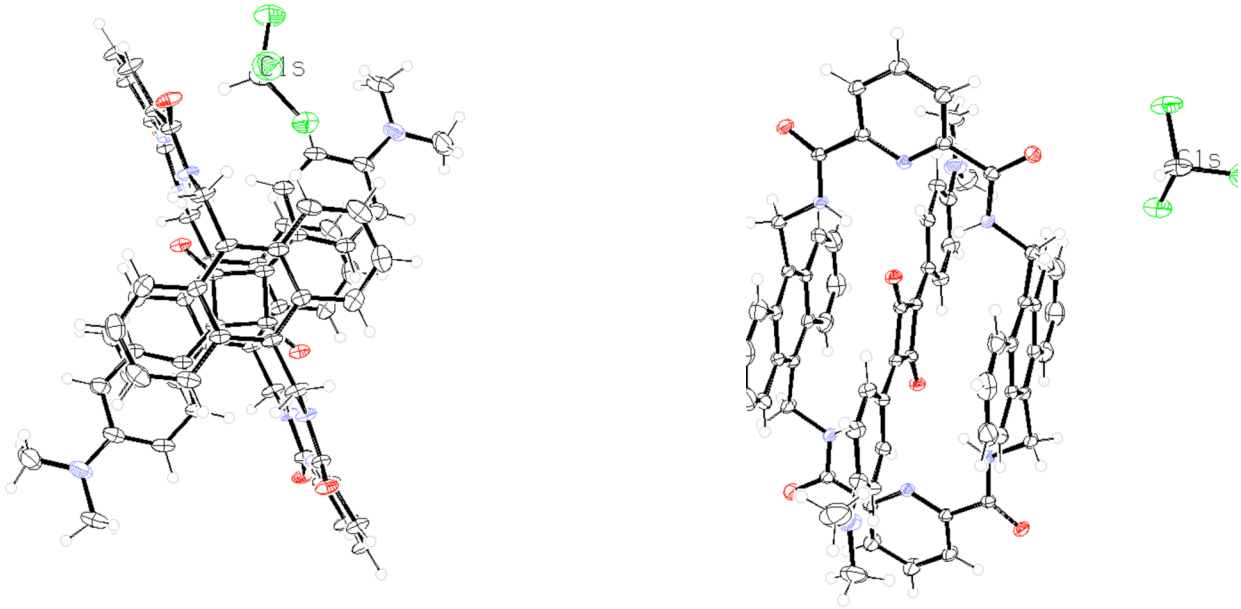


Figure S11: Inclusion complex **2a⊃3a** as ORTEPs with ellipsoids at 50% probability.

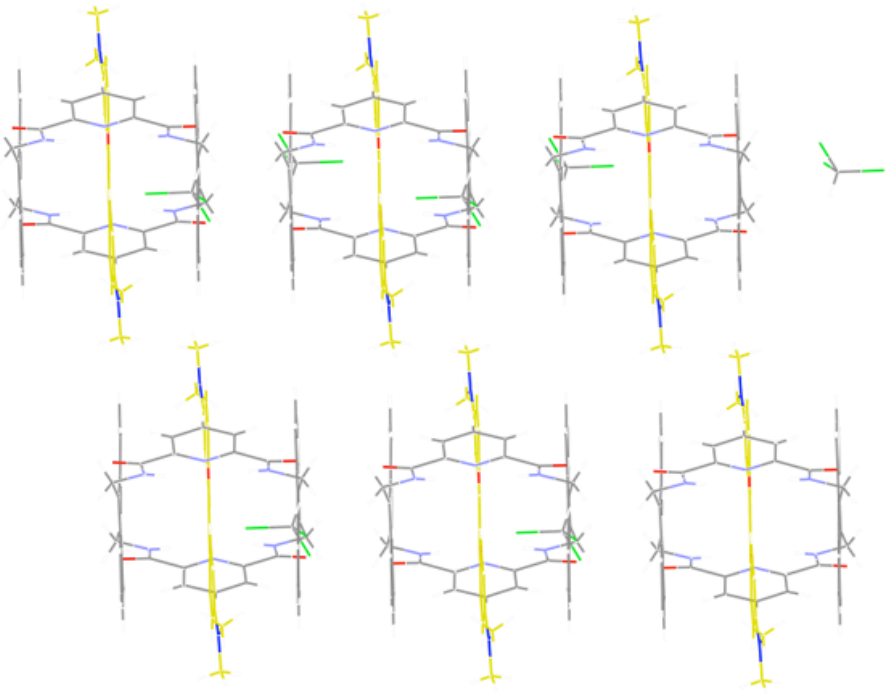


Figure S12: Lattice packing of **2a⊃3a** viewed down reciprocal cell axis b* (*i.e.*, perpendicular to ac plane).

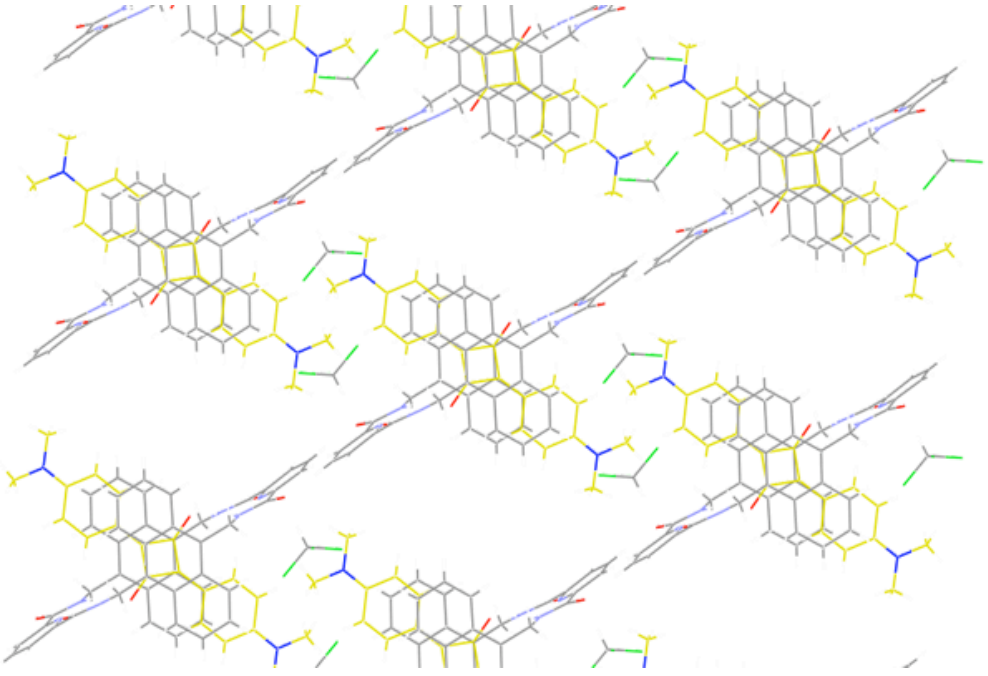


Figure S13: Lattice packing of **2a⊃3a** viewed down the crystallographic a axis.

C. Analytical Measurements

Association constant (K_{eq}) for pseudo-rotaxane 2b⊃3b

The association equilibrium is:

$$[\text{Dye}] + [\text{Macrocycle}] \overset{K_{eq}}{\rightleftharpoons} [\text{Rotaxane}]$$

The absorbance maxima for free Dye (630 nm) and Rotaxane (658 nm) overlap. Thus, the absorbance at each wavelength is due to contributions from both chromophores, according to Beer's law.

$$A_\lambda = (\varepsilon_{\lambda 1} \cdot b \cdot c_1) + (\varepsilon_{\lambda 2} \cdot b \cdot c_2)$$

$$A_\lambda = \varepsilon_{\lambda R}\, b\, c_R + \varepsilon_{\lambda D}\, b\, c_D$$

Where the cell length (b) is 1 cm, and:

$$\varepsilon_{D630} = 4.56\text{x}10^5\ \text{M}^{-1}\text{cm}^{-1} \qquad \varepsilon_{D658} = 2.80\text{x}10^4\ \text{M}^{-1}\text{cm}^{-1}$$

$$\varepsilon_{R630} = 6.67\text{x}10^4\ \text{M}^{-1}\text{cm}^{-1} \qquad \varepsilon_{R658} = 2.76\text{x}10^5\ \text{M}^{-1}\text{cm}^{-1}$$

The initial concentrations of Dye and Macrocycle were:

$$C_{Do} = [D]_o = 4.2\mu\text{M}$$

$$C_{Mo} = [M]_o = 2 \cdot [D]_o = 8.4\mu\text{M}$$

At equilibrium:

$$[R] = [D]_o - [D]$$

$$[R] = [M]_o - [M] = 2[D]_o - [M]$$

Since b = 1 cm, the absorption at a specific wavelength λ is:

$$A_\lambda = \varepsilon_{\lambda R} \cdot [R] + \varepsilon_{\lambda D} \cdot ([D]_o - [R])$$

which rearranges to give:

$$[R] = \frac{A_\lambda - \varepsilon_{\lambda D}[D]_o}{\varepsilon_{\lambda R} - \varepsilon_{\lambda D}}$$

since:

$$K_{eq} = \frac{[R]}{[D] \cdot [M]}$$

The above is simplified to the following:

$$K_{eq} = \frac{[R]}{([D]_o - [R]) \cdot (2[D]_o - [R])} = \frac{[R]}{2 \cdot [D]_o^2 - 3 \cdot [R] \cdot [D]_o + [R]^2}$$

The absorption measured at 630 nm provides the association constant to form **2a**⊃**3b** at 298 K in chloroform as K_{eq} = 1.81x10^5 M^{-1}

Determination of the second-order rate constants (*k*) for formation of 2a⊃3b and 2b⊃3b

Since the association constants are very large (log K > 5), the formation processes is treated as an irreversible second-order reactions that obey the following relationship:

$$\ln\left(\frac{[M] \cdot [D]_o}{[M]_o \cdot [D]}\right) = ([M]_o - [D]_o) \cdot kt$$

Rearrangement leads to:

$$\frac{\ln\left(\frac{[M] \cdot [D]_o}{[M]_o \cdot [D]}\right)}{([M]_o - [D]_o)} = kt$$

A plot of the ratio versus time yields a straight line through zero whose slope is the second-order rate constant, *k*. The rate constant for association of **2b** and **3b** (k = 80 M^{-1}s^{-1}) was measured by absorption spectroscopy in chloroform at 298 K and the rate constant for association of **2a** and **3b** (k = 2 x 10^4 M^{-1}s^{-1}) was measured by ^{1}H NMR in $CDCl_3$ at 298 K.

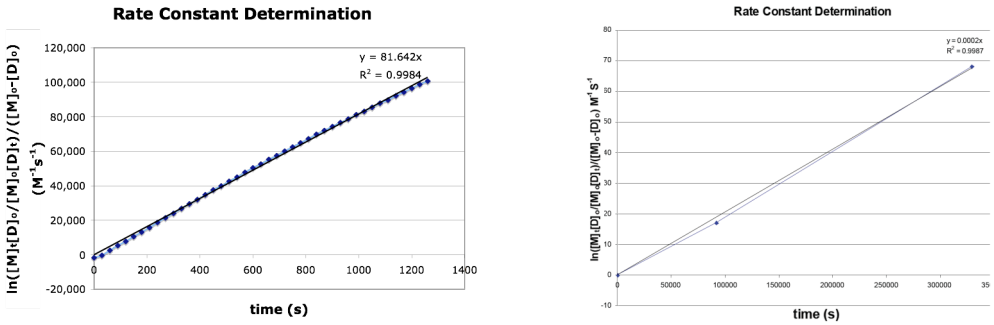


Figure S14: Second-order kinetic plots for rates of bimolecular association of: (left) **2b** with **3b**; (right) **2a** with **3b**.

D. Vesicle Studies

1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) in $CHCl_3$ was purchased from Avanti Polar Lipids and stored at -20° C. A chloroform solution of phospholipid with or without macrocycle (**2b**) was dried *in vacuo* for 1 h. A stock solution of 10 mM vesicles was made by rehydration at room temperature with TES buffer (5 mM TES, 100 mM NaCl, pH 7.4). Multilamellar vesicles were extruded to form unilamellar vesicles with a Basic LiposoFast device purchased from Avestin, Incorporated. The vesicles were extruded 29 times through a 19-mm polycarbonate Nucleopore filter with 200 nm diameter pores.

An aliquot of vesicle stock solution (110 μL) was added to a cuvette containing 2.90 mL of TES buffer. Initial fluorescence and absorbance scans were taken. To this cuvette was added **3b** (13.75 μL of a stock solution, 0.2 mg/mL in ethanol) and spectra were acquired at the indicated time points. The fluorescence spectra (excitation at 370 nm; slit width 5 nm) employed a Horiba Jobin Yvon Fluoromax-3 and the absorbance scans were taken on a Perkin Elmer UV/Vis Lambda 2 spectrometer, with the temperature set at 25° C. The vesicle experiments were repeated and the data shown is from one trial.

E. Cell Studies

Eukaryotic cell culture

A549, CHO, COS-7, HeLa, and BSC1 cell lines were cultured following ATCC protocols and maintained at 37º C, 5% CO_2 atmosphere in a humidified incubator. For microscopy, cells were plated out on 8-well microscope chambered slides (Nunc) and allowed to grow to approximately 60% confluence. Cells were prepared for flow cytometry by growing to 80% confluence in 6 well cell culture plates. Live cells were analyzed in phenol-free Dublecco's Minimum Essential Medium containing 2 mM L-glutamine, 4.5 g/L Glucose, and supplemented with 10% fetal bovine serum.

Fluorescence microscopy

For microscopy, cells were treated with an aliquot of squaraine dye (10 μM of **3b** or **3c**) and incubated for 1 hour in growth media. The cells were then rinsed with fresh growth media and treated with 10 μM of macrocycle **2b** for one hour, or otherwise given fresh growth media. At

the appropriate time after macrocycle addition, the growth media was removed, rinsed twice with fresh growth media and immediately imaged. Fluorescence microscopy was conducted on a Nikon Eclipse TE-2000 U epifluorescence microscope equipped with Blue (Exciter: D360/40x, Dichroic: 400DCLP, Emitter: 460/50m), Red (Exciter: HQ620/60X, Dichroic: 660LP, Emitter: HQ700/75m) and Far-Red (Exciter: HQ710/75X, Dichroic: 750LP, Emitter: HQ810/90m) filter sets. Excitation light was provided by an X-cite 120 fluorescence illumination system. 16-bit images were acquired using a Photometrics 512 B black and white digital camera using Metamorph Software V6.2. Images were acquired using a 100 ms integration time.

The supporting information contains two movies in .mpg format. The first movie (14 MB) is entitled <BSC1cells_2b_3c.mpg> and shows BSC1 cells (monkey kidney cells) that have been treated with separate aliquots of macrocycle **2b** and squaraine **3c**. The movie is twice real time and indicates trafficking of vesicle-like structures emitting far-red fluorescence due to **2b⊃3c** (Filter Set; Exciter: HQ710/75X, Dichroic: 750LP, Emitter: HQ810/90m). The second movie (3 MB) is entitled <Close_up.mpg> and shows a close-up view of one region of the above movie, with the structures exhibiting vesicle-like adhesion behavior.

Flow Cytometry

Flow cytometry analysis was conducted on a Beckman Coulter MPL 500 flow cytometer. Cell fluorescence was monitored in the Fl5 fluorescence channel (755 nm band pass emission, 710 nm dichroic long pass optical filters), excited by uniphase red Helium-Neon laser (633 nm, 20 mW output). Data was prepared using the instrument's MPL and CXP software packages. The cells were prepared by growing to 80% confluence in 6 well growth plates. The cells were treated with an aliquot of squaraine dye (10 μM of **3b** or **3c**) and incubated for 1 hour in growth media. The cells were then rinsed with fresh growth media, and treated with 10 μM of macrocycle **2b**, or otherwise given fresh growth media. After macrocycle addition, the growth media was removed. The cells were rinsed with PBS, trypsinized to detach them from the cell growth plates, and analyzed.

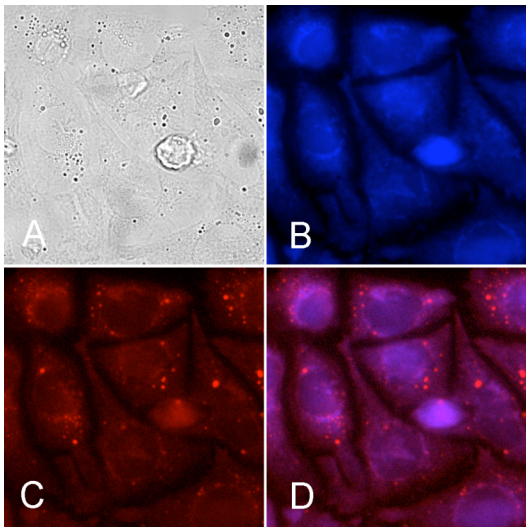


Figure S15: Fluorescence microscopy images of live Chinese hamster ovary (CHO) cells treated with separate aliquots of **3b** (10μM) and **2b** (10μM). Panel A: Phase contrast image of the treated CHO cells. Panel B: Blue emission of **2b**. Panel C: Red emission of pseudo-rotaxane **2b⊃3b** that was formed. Panel D: Overlay of panels B and D.

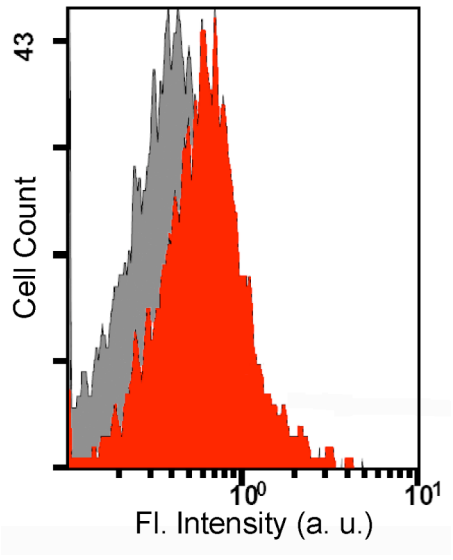


Figure S16: Flow cytometry of CHO cells at time zero (grey), and 24 h (red) after treatment with squaraine **3b** and macrocycle **2b**. The filter set only detects cells that emit far-red fluorescence due to time-dependent formation of **2b⊃3b**.