Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

Effect of stopper size on squaraine rotaxane stability

Na Fu [a]; Jeremiah J. Gassensmith [a]; Bradley D. Smith [a]
[a] Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, USA

Online Publication Date: 01 January 2009

To cite this Article Fu, Na, Gassensmith, Jeremiah J. and Smith, Bradley D.(2009)'Effect of stopper size on squaraine rotaxane stability',Supramolecular Chemistry,21:1,118 — 124
To link to this Article: DOI: 10.1080/10610270802468454
URL: http://dx.doi.org/10.1080/10610270802468454

Supramolecular Chemistry
Vol. 21, Nos. 1–2, January–March 2009, 118–124

Taylor & Francis
Taylor & Francis Group

Effect of stopper size on squaraine rotaxane stability

Na Fu, Jeremiah J. Gassensmith and Bradley D. Smith*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, USA

(*Received 16 July 2008; final version received 9 September 2008*)

A series of new squaraine rotaxanes have been synthesised with a tetralactam macrocycle and stopper groups of varying sizes and functionalities. In chloroform, the relative size of the stopper group appears to have little influence on the high mechanical stability of the rotaxane structure. There is no evidence of unthreading (sometimes referred to as deslipping), even in the presence of competing chloride salts or elevated temperatures. A difference in rotaxane stability emerges as the polarity of the organic solvent is increased. Squaraine rotaxanes with small stopper groups undergo unthreading in the polar aprotic solvent DMSO. However, a water-soluble tetracarboxylic acid derivative was found to be highly stable in aqueous solvents containing serum.

Keywords: fluorescence; rotaxane; synthesis; hydrogen bonding

Introduction

Near-infrared (NIR) organic fluorophores are increasingly used in modern bioimaging technologies, and are especially promising as probes for studies in living animals (*1–4*). This latter application requires the fluorescent dyes to possess certain characteristics. First, they must emit in the region 650–850 nm (approximately the NIR), where background autofluorescence from biomolecules and undesired absorption by tissue is reduced (*5–7*). Second, the dyes must be non-toxic and chemically inert in biological environments. Finally, the dyes must be amenable to conjugation with targeting ligands to produce imaging probes with high target selectivity (*8*, *9*).

The squaraines are a well-known class of NIR dyes with intense and narrow emission bands that are quite suitable for bioimaging. However, there are some technical drawbacks: they are susceptible to nucleophilic attack and, like many organic dyes, they undergo self-quenching upon aggregation (*10*, *11*). We have discovered that both problems are eliminated by encapsulating the dye inside a macrocycle to form a squaraine rotaxane (*12*). The macrocycle protects the C_4O_2 core of the squaraine thread from attack by nucleophiles and prevents interchromophore energy transfer upon aggregation. At the same time, the rotaxanes preserve the favourable photophysical properties of the precursor squaraines. The next step in our research is to determine how squaraine rotaxanes can be converted into high-performance fluorescent probes for bioimaging (*13*).

Our initial studies used rotaxanes such as **1a** (Figure 1) with large *N,N*-bisbenzyl stopper groups on the squaraine to ensure that unthreading (sometimes referred to as deslipping) did not occur (*12*). However, large hydrocarbon stopper groups are not desired for bioimaging probes, where the goal is to produce water-soluble compounds with low molecular weight. Additionally, we need to develop stopper groups that can be conjugated to targeting ligands using standard coupling reactions. Here, we report that squaraine rotaxanes **1b**–**1f** can be prepared with smaller and more flexible stopper groups. Spectroscopic studies in various solvents, in the presence of ions, and in biological solution, reveal exceptional mechanical stability even when using stopper groups that are relatively small compared to the macrocyclic cavity.

Result and discussion

Synthesis and structure

The precursor aniline derivatives, **2b**, **2d** and **2e**, were obtained by the straightforward synthetic methods shown in Scheme 1; whereas **2a** and **2c** are commercially available. In each case, the aniline compound was added to a benzene/butanol solution of squaric acid and heated under azeotropic distillation condition to afford squaraine dyes **3a**–**3e** in a yield of 35–45%. The dyes were converted into squaraine rotaxanes with yields around 20% by conducting Leigh-type clipping reactions (Scheme 2) (*14*). Rotaxane **1d** was hydrolysed by trifluoroacetic acid (TFA) to afford the water-soluble tetracarboxylic acid rotaxane **1f** (Scheme 3).

*Corresponding author. Email: smith.115@nd.edu

ISSN 1061-0278 print/ISSN 1029-0478 online

DOI: 10.1080/10610270802468454
http://www.informaworld.com

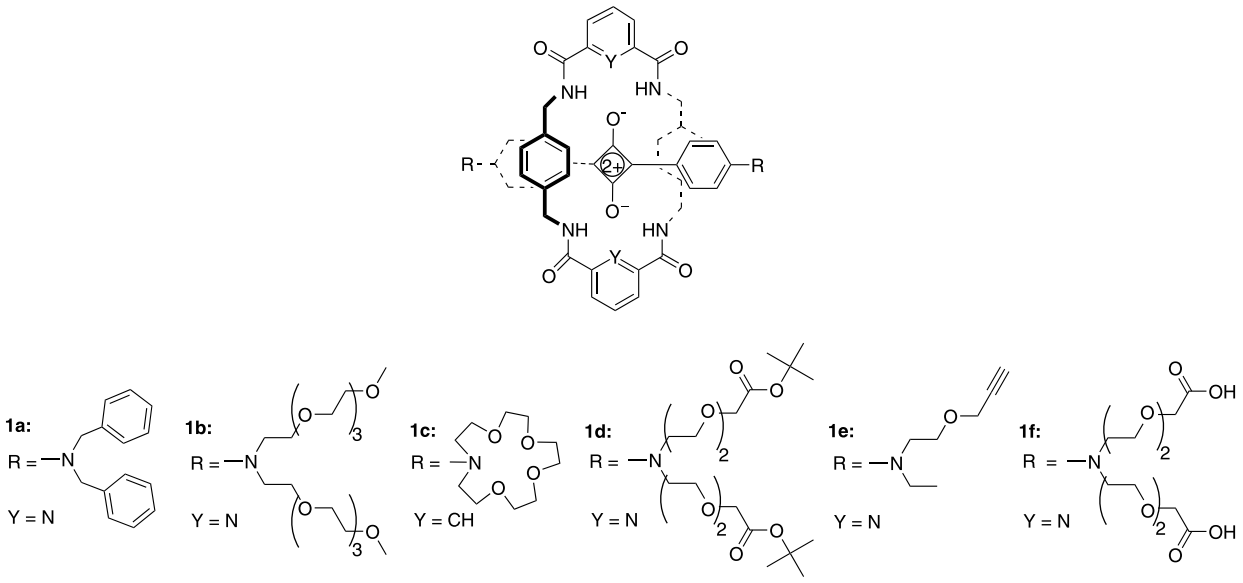
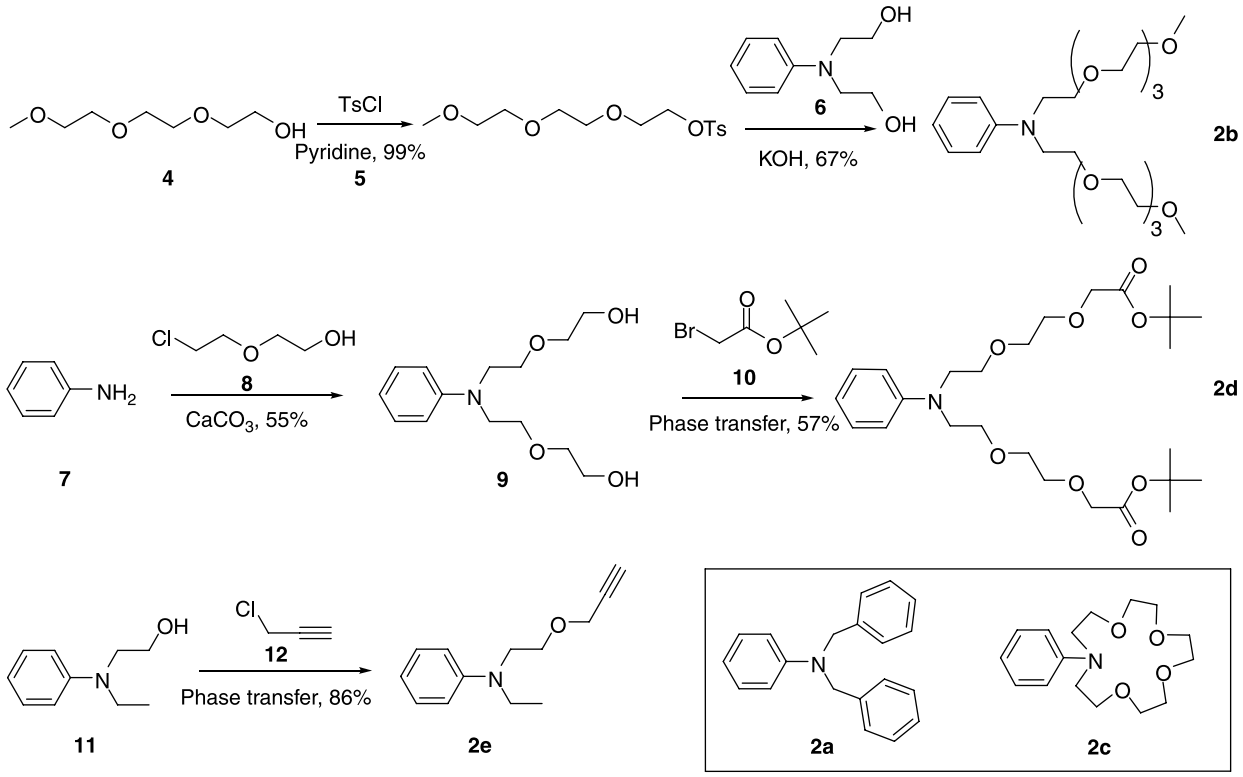
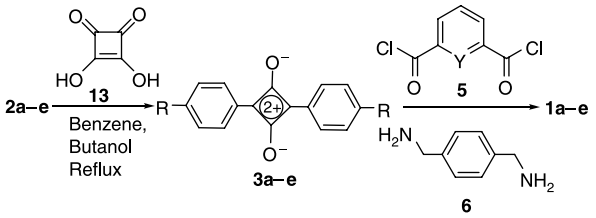


Figure 1. Squaraine rotaxanes.

The rotaxane structures were characterised by standard spectrometric methods. The ^{1}H NMR spectra exhibited the expected changes in chemical shift due to anisotropic shielding by the interlocked rotaxane components. Another signature of rotaxane formation is a 10–20 nm red shift in absorption and emission maxima for the squaraine dye upon encapsulation inside the phenylene-containing tetralactam macrocycle. The bis(azacrown) squaraine rotaxane **1c** was obtained as single crystals that were suitable for analysis by X-ray diffraction. The molecule is located around a crystallographic inversion centre. As shown in Figure 2, the macrocycle



Scheme 1. Synthesis and structures of aniline precursors.

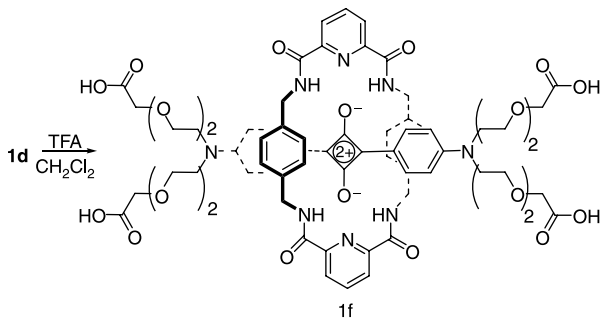


Scheme 2. Synthesis of squaraine rotaxanes.

adopts a *chair*-like conformation in the solid state. The four macrocyclic NH residues form bifurcated hydrogen bonds with the two squaraine oxygen atoms. The lengths and angles of the NH···O hydrogen bonds are 2.16(3) Å, 168.2(2)° and 2.22(3) Å, 174.7(3)°, respectively, for the centrosymmetric molecule. The parallel 1,4-xylylene units are stacked directly over both faces of the electron-deficient C_4O_2 core of the squaraine thread. The centroid-to-centroid distance between the two parallel phenyl rings in the macrocycle is 7.20 Å. A comparison with previously elucidated squaraine rotaxane structures such as **1a** (*12*) indicates that the more flexible crown ether stopper groups do not alter rotaxane co-conformation in the solid state.

Stability in a non-polar solvent

Since the encapsulating phenylene-containing macrocycle is highly insoluble when it is a free molecule (*15*), it seemed that a moderate amount of unthreading might manifest itself as an irreversible process that is driven by macrocycle precipitation. However, we do not see this phenomenon in chloroform solution. We find that rotaxanes **1a**–**1e** are quite soluble and completely stable with no spectroscopic evidence for unthreading. Even samples that were stored in $CDCl_3$ at 50°C for one week showed no changes in their ^{1}H NMR spectra. Furthermore, unthreading of **1c** could not be induced by the addition of chloride anions that potentially could form competing hydrogen bonds with the NH residues (*16*, *17*). The addition of excess lithium chloride in $CDCl_3$ or tetrabutylammonium chloride in CH_3CN did not induce any sign of unthreading as judged by ^{1}H NMR or absorption spectroscopy. We conclude that in a non-polar solvent, the surrounding macrocycle is held tightly to the squaraine



Scheme 3. Synthesis of squaraine rotaxane **1f**.

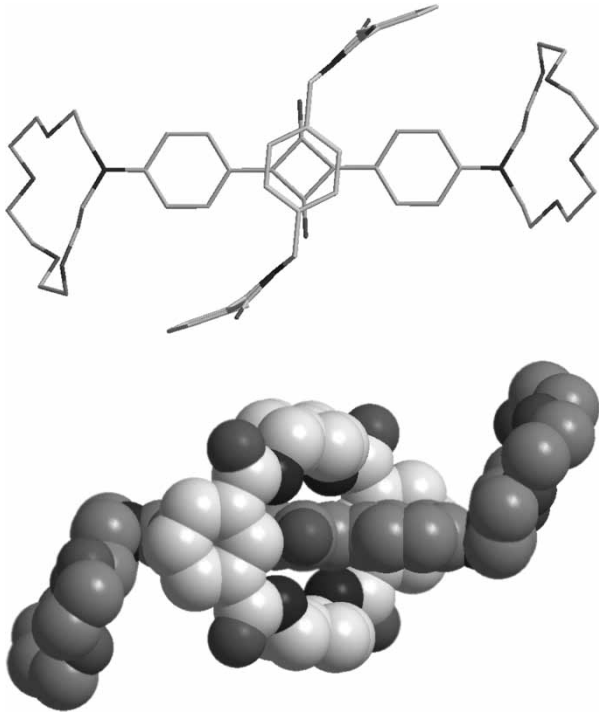


Figure 2. X-ray crystal structure of **1c** shown in the side view (top) and the top view (bottom).

thread by a combination of favourable aromatic stacking interactions between the electron-poor C_4O_2 core of the squaraine and the electron-rich 1,4-xylylene units in the macrocycle, and strong hydrogen bonds between the four macrocyclic NH residues and the two squaraine oxygen atoms.

Stability in the polar aprotic DMSO

Polar aprotic organic solvents are known to greatly weaken the non-covalent association of host/guest complexes when the principal stabilising forces are hydrogen bonding (*18*, *19*), dispersion forces or aromatic stacking (*20*). Thus, it was not surprising to find that the highly polar DMSO promotes unthreading of squaraine rotaxanes. ^{1}H NMR spectroscopy was used to monitor the structural integrity of **1a** and **1e** in three solvent systems: pure $CDCl_3$; DMSO-d_6/$CDCl_3$ (1:9); and pure DMSO-d_6. For rotaxanes **1e**, with its slender alkyne stopper groups, there was no ^{1}H NMR evidence after 50 days at 23°C for unthreading in $CDCl_3$; however, unthreading was observed when the solvent was DMSO-d_6/$CDCl_3$ (1:9) and pure DMSO-d_6 with half-lives of 50 days and 12 h, respectively (Table 1). The same NMR experiments with squaraine rotaxane **1a** revealed the anticipated enhancement of mechanical stability afforded by the larger *N*,*N*-bisbenzyl stopper groups (*21*). In the highly disruptive DMSO-d_6, the half-life for squaraine rotaxane **1a** is approximately 40 days, which is 80 times longer than the half-life for squaraine rotaxane **1e**.

Table 1. Half-lives for rotaxane unthreading at 23°C.

Compound (5 mM)	Solvent	$t_{1/2}$
1a	$CDCl_3$	>50 days
	DMSO-d_6/$CDCl_3$ (1:9)	>50 days
	DMSO-d_6	40 days
1e	$CDCl_3$	>50 days
	DMSO-d_6/$CDCl_3$ (1:9)	50 days
	DMSO-d_6	12 h

Stability in water

The tetracarboxylic acid **1f** was synthesised and tested as a highly water-soluble squaraine rotaxane. In 10% serum solution, **1f** absorbs strongly at 651 nm ($\log \varepsilon \sim 5.4$) and emits at 679 nm with a quantum yield of 0.17 (Table 2). Not surprisingly, the quantum yield increases when THF is included as an organic co-solvent because squaraines are quenched by protic solvents (*19*). Previously, we have shown that biological nucleophiles can attack the electrophilic C_4O_2 core in squaraine dyes and bleach the dye's colour in a few minutes, but squaraine rotaxanes are highly resistant to this chemical attack (*12*). Thus, the loss of squaraine colour in biological solution is a convenient indicator of rotaxane unthreading. Figure 3 shows a comparison of the colour stability of water-soluble rotaxane **1f**, and the corresponding precursor squaraine dye **3d** in the presence of serum. As expected, the bleaching half-life for dye **3d** is only 1 min. Remarkably, the colour for rotaxane **1f** in 10% serum is unchanged after standing for many hours. Furthermore, there is no colour change when **1f** is in the more non-polar solvent mixture of 10% serum/THF (1:1). It appears that the mechanical stability of squaraine rotaxane **1f**, whose stopper groups each contain two ethyleneoxy chains, is extremely high in aqueous biological solution. This is a pleasing result for us because good water solubility and high stability are essential features for high-performance fluorescent bioimaging probes.

Table 2. Absorption and emission properties in different solvent mixtures.

Solvent	Compound	λ_{abs} (nm)	λ_{em} (nm)	Φ_f
H_2O	**1f**	651	679	0.15
10% serum	**1f**	651	679	0.17
10% serum/THF (1:1)	**1f**	646	673	0.34
THF	**1d**	640	665	0.54
	3d	633	652	0.74
10% serum/THF (1:1)	**1d**	648	674	0.33
	3d	645	673	–

Samples were excited at 580 nm and emission monitored in the region 600–850 nm. All quantum yields are relative to a standard solution of bis[4-(*N*,*N*-dimethylamino)phenyl]squaraine in $CHCl_3$ ($\Phi_f = 0.70$) and have an error of $\Phi_f \pm 5\%$.

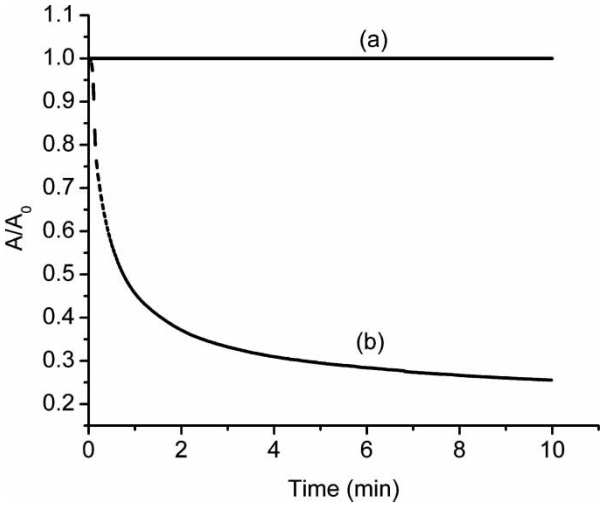


Figure 3. Change in absorption maxima for: (a) **1f** in 10% serum solution and (b) **3d** in 10% serum/THF (1:1).

Conclusion

The very high host/guest complementarity between squaraine dyes and the encapsulating tetralactam macrocycle provides excellent mechanical stability and little propensity for unthreading. In a non-polar organic solvent, the rotaxanes are stabilised by strong hydrogen bonds and in water by hydrophobic aromatic stacking interactions. Only in high polar aprotic solvents such as DMSO is there evidence for rotaxane unthreading, which can be countered by employing sterically large stopper groups. The high rotaxane stability in aqueous solution means that the size of the stopper group is not a major design constraint, and it should be possible to attach a wide range of targeting groups to these dyes and produce a diverse portfolio of bioimaging agents.

Experimental section

Unless otherwise stated, all starting materials and reagents were purchased from commercial suppliers and used without further purification. ^1H and ^{13}C NMR spectra were recorded by using Varian Unity Plus spectrometers. Fast-atom-bombardment (FAB) mass spectra (MS) were recorded on a double sector JEOL JMS-AX 505 HA instrument. Electron spray ionisation (ESI)-MS were recorded on a Micromass Quattro LC triple quadrupole mass spectrometer (Waters).

Synthesis of tosylate ***5***. Tri(ethylene glycol) monomethyl ether (16.4 g, 0.1 mol) and pyridine (15 ml) were added to a round bottom flask, which was cooled in an ice bath. *p*-Toluenesulphonyl chloride (22.8 g, 0.12 mol) was slowly added into the flask. The solution was allowed to warm to room temperature, and stirred for 8 h. The solution was neutralised with 1 M hydrochloric acid to pH 7.

The solution was extracted with dichloromethane (3 × 100 ml); the combined organic layers were washed with water (3 × 100 ml), dried with anhydrous Na_2SO_4 and concentrated *in vacuo* to afford the product quantitatively as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.72 (d, 2H, $J = 9.3$), 7.27 (d, 2H, $J = 9.3$), 4.14 (t, 2H, $J = 4.8$), 3.45–3.64 (m, 10H), 3.30 (s, 3H), 2.45 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.0, 133.1, 130.0, 128.1, 72.0, 70.8, 70.7, 69.5, 68.8, 59.1, 21.8; FAB-MS, calculated for $C_{14}H_{23}O_6S^+$ $(M + H)^+$319, found 319.

Aniline ***2b***. *N*-Phenyldiethanolamine (2 g, 0.011 mol), excess KOH (8 g, 0.14 mol) and compound **5** (8.1 g, 0.025 mol) in THF (100 ml) were refluxed for 24 h. Excess solvent was removed and the residue obtained was dissolved in water and extracted with dichloromethane (3 × 100 ml). The organic layer was separated and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography using a silica column that was eluted with a mixture of ethyl acetate/hexane (1:19) to afford a yellow oil (3.5 g, 67% yield). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.19 (m, 2H), 6.71 (m, 3H), 3.65 (m, 32H), 3.38 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 148.0, 129.5, 116.2, 111.9, 72.1, 70.9, 70.8, 70.7, 70.6, 68.7, 59.2, 51.1; FAB-MS, calculated for $C_{24}H_{44}NO_8^+$ $(M + H)^+$474, found 474.

Aniline ***9***. Aniline (10 g, 0.1 mol), 2-(2-chloroethoxy) ethanol (37.2 g, 0.3 mol) and excess $CaCO_3$ (15 g, 0.15 mol) were heated in water under reflux with stirring until the mixture turned transparent after about 4 days. The liquid was cooled and extracted with ether (3 × 100 ml). The ether solution was collected and the solvent was removed *in vacuo* to give the crude product. The crude product was purified by column chromatography using a silica column that was eluted with a mixture of methanol/chloroform (1:19) to afford the pure product as a yellow oil (14.8 g, 55% yield). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.20 (m, 2H), 6.73 (m, 3H), 3.53–3.68 (m, 16H), 3.23(bs, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 148.3, 129.6, 117.1, 112.9, 72.8, 69.1, 61.8, 51.7; FAB-MS, calculated for $C_{14}H_{24}NO_4^+$ $(M + H)^+$270, found 270.

Aniline ***2d***. Compound **9** (1 g, 3.7 mmol) and *tert*-butyl bromoacetate (4.3 g, 20 mmol) were dissolved in benzene (50 ml). Tetrabutylammonium bisulphate (0.37 mmol) was dissolved in 50% NaOH solution (50 ml) and the solutions were combined and stirred for 24 h at room temperature. The organic layer was isolated and washed with saturated NaCl solution (100 ml) and dried over anhydrous Na_2SO_4 and concentrated to give a yellow oil. The crude product was purified by column chromatography using a silica column that was eluted with a mixture of methanol/chloroform (1:49) to afford a yellow oil as the product (1.1 g, 57% yield). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.17 (m, 2H), 6.68 (m, 3H), 4.00 (s, 4H), 3.55–3.70 (m, 16H), 1.47 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.8, 147.9, 129.5, 116.2, 111.9, 81.8, 70.9, 70.8, 69.3, 68.7, 51.1, 28.4; FAB-MS, calculated for $C_{26}H_{44}NO_8^+$ $(M + H)^+$498, found 498.

Aniline ***2e***. 2-(*N*-Ethylanilino)ethanol (3 g, 0.018 mol) and propargyl chloride (4.9 g, 0.066 mol) were dissolved in benzene (60 ml). Tetrabutylammonium bisulphate (0.6 g, 1.77 mmol) was dissolved in 50% NaOH solution (60 ml) and the solutions were combined and stirred for 48 h at room temperature. The organic layer was isolated and washed with saturated NaCl solution (100 ml) and dried over anhydrous Na_2SO_4 and concentrated to give a light yellow oil. The crude product was purified by column chromatography using a silica column that was eluted with a mixture of ethyl acetate/hexane (1:49) to afford a light yellow oil (3.18 g, 86% yield); ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ 7.21 (m, 2H), 6.70 (m, 3H), 4.17 (d, 2H, $J = 2.0$), 3.69 (t, 2H, $J = 6.5$), 3.53 (t, 2H, $J = 6.5$), 3.42 (q, 2H, $J = 7.0$), 2.43 (t, 1H, $J = 2.0$), 1.16 (t, 3H, $J = 7.0$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 147.5, 129.1, 115.7, 111.7, 79.5, 74.4, 67.5, 58.2, 49.7, 45.2, 12.0; FAB-MS, calculated for $C_{13}H_{18}NO^+$ $(M + H)^+$204, found 204.

General procedure to synthesise squaraine dye

Aniline derivatives **2a**–**2e** (1.83 mmol) were added to a solution of squaric acid **13** (0.92 mmol) in a mixture of *n*-butanol (15 ml) and benzene (30 ml) in a 100 ml round bottom flask equipped with a Dean–Stark apparatus. After refluxing for 12 h at 95°C, the solvent was removed *in vacuo* and the crude product was obtained. The crude product was purified by column chromatography using a silica column that was eluted with a mixture of methanol/chloroform (1:49).

Squaraine dye ***3b***. (0.33 g, 35% yield): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 8.35 (d, 4H, $J = 9.0$), 6.80 (d, 4H, $J = 9.0$), 3.54–3.76 (m, 64H), 3.37 (s, 12H); ^{13}C (75 MHz, $CDCl_3$): δ 188.9, 183.4, 154.2, 133.5, 120.3, 112.9, 72.2, 71.1, 70.9, 70.8, 70.7, 68.7, 59.2, 51.6, 29.9; ESI-MS, calculated for $C_{52}H_{85}N_2O_{18}^+$ $(M + H)^+$1025, found 1025.

Squaraine dye ***3c***. (0.25 g, 40% yield): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 8.38 (d, 4H, $J = 9.0$), 6.77 (d, 4H, $J = 9.0$), 3.63–3.82 (m, 40H); ^{13}C (75 MHz, $CDCl_3$): δ 188.9, 183.5, 153.9, 133.5, 120.4, 112.9, 71.5, 70.7, 70.3, 68.5, 53.6; FAB-MS, calculated for $C_{36}H_{49}N_2O_{10}^+$ $(M + H)^+$669, found 669.

Squaraine dye ***3d***. (0.45 g, 45% yield): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 8.36 (d, 4H, $J = 9.0$), 6.81 (d, 4H, $J = 9.0$), 3.99(s, 8H), 3.66–3.77 (m, 32H), 1.47 (s, 36H); ^{13}C (75 MHz, $CDCl_3$): δ 188.8, 183.4, 169.7, 154.2, 133.4, 120.4, 112.9, 81.9, 71.1, 71.0, 69.2, 68.7, 51.8, 28.4; ESI-MS, calculated for $C_{56}H_{85}N_2O_{18}^+$ $(M + H)^+$1074, found 1074.

Squaraine dye ***3e***. (0.2 g, 44% yield): ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ 8.37 (d, 4H, $J = 9.3$),

6.77 (d, 4H, $J = 9.3$), 4.16 (d, 4H, $J = 2.3$), 3.75 (t, 4H, $J = 5.5$), 3.69 (t, 4H, $J = 5.5$), 3.59 (q, 4H, $J = 7.0$), 2.43 (t, 2H, $J = 2.3$), 1.25 (t, 6H, $J = 7.0$); ^{13}C (125 MHz, $CDCl_3$): δ 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; FAB-MS, calculated for $C_{30}H_{33}N_2O_4^+$ $(M + H)^+$485, found 485.

General procedure to synthesise squaraine rotaxanes

Clear solutions of the corresponding 2,6-pyridinedicarbonyl dichloride/isophthaloyl dichloride (2.56 mmol) and *p*-xylylenediamine (2.56 mmol) in anhydrous chloroform (20 ml) were simultaneously added dropwise using a mechanical syringe pump apparatus over 5 h to a stirred solution of squaraine dyes **3a**–**3e** (0.32 mmol) and triethylamine (6.4 mmol) in anhydrous $CHCl_3$ (40 ml). After stirring overnight, the reaction mixture was filtered through a pad of celite to remove any precipitation. The crude product was purified by column chromatography using a silica column that was eluted with a mixture of methanol/chloroform (1:49).

*Squaraine rotaxane **1b***. (99 mg, 20% yield): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 10.03 (t, 4H, $J = 6.0$), 8.50 (d, 4H, $J = 7.5$), 8.16 (t, 2H, $J = 8.5$), 8.06 (d, 4H, $J = 9.0$), 6.60 (s, 8H), 6.20 (d, 4H, $J = 9.0$), 4.51 (d, 8H, $J = 6.0$), 3.53–3.66 (m, 64H), 3.37 (s, 12H); ^{13}C (75 MHz, $CDCl_3$): δ 188.4, 184.9, 163.4, 153.9, 149.6, 138.8, 136.4, 133.6, 129.0, 125.0, 119.5, 111.5, 71.9, 70.8, 70.7, 70.6, 70.5, 68.3, 59.0, 51.3, 43.5; ESI-MS, calculated for $C_{82}H_{111}N_8O_{22}^+$ $(M + H)^+$1559, found 1559.

*Squaraine rotaxane **1c***. (111 mg, 29% yield): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 9.39 (s, 2H), 8.31 (d, 4H, $J = 9.0$), 8.23 (t, 4H, $J = 6.0$), 7.65 (m, 6H), 6.70 (s, 8H), 6.40 (d, 4H, $J = 9.0$), 4.43 (d, 8H, $J = 9.0$), 3.63–3.79 (m, 40H); ^{13}C (75 MHz, $CDCl_3$): δ 185.5, 181.8, 166.4, 154.4, 136.6, 134.3, 133.1, 131.9, 129.5, 125.1, 118.4, 116.5, 113.0, 71.4, 70.7, 69.9, 68.3, 53.9, 50.4, 44.6; ESI-MS, calculated for $C_{68}H_{77}N_6O_{14}^+$ $(M + H)^+$1201, found 1201.

*Squaraine rotaxane **1d***. (77 mg, 15% yield): ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 10.03 (t, 4H, $J = 5.6$), 8.52 (d, 4H, $J = 8.5$), 8.17 (t, 2H, $J = 8.5$), 8.09 (d, 4H, $J = 8.5$), 6.62 (s, 8H), 6.25(d, 4H, $J = 8.5$), 4.53(d, 8H, $J = 8.5$), 4.00 (s, 8H), 3.69 (m, 32H), 1.48 (s, 36H); ^{13}C (75 MHz, $CDCl_3$): δ 186.3, 185.1, 169.5, 163.6, 153.9, 149.6, 138.9, 136.7, 133.3, 128.7, 125.3, 119.5, 111.8, 81.7, 70.8, 69.0, 68.4, 51.3, 43.4, 28.1; ESI-MS, calculated for $C_{86}H_{111}N_8O_{22}^+$ $(M + H)^+$1607, found 1607.

*Squaraine rotaxane **1e***. (71 mg, 22% yield): ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ 10.02 (t, 4H, $J = 6.0$), 8.51 (d, 4H, $J = 8.0$), 8.14 (t, 2H, $J = 8.0$), 8.08 (d, 4H, $J = 9.0$), 6.60 (s, 8H), 6.20 (d, 4H, $J = 9.0$), 4.52 (d, 8H, $J = 6.0$), 4.17 (d, 4H, $J = 2.0$), 3.67 (t, 4H, $J = 6.0$), 3.57 (t, 4H, $J = 6.0$), 3.46 (q, 4H, $J = 7.0$), 2.51 (t, 2H, $J = 2.0$), 1.18 (t, 6H, $J = 7.0$); ^{13}C (125 MHz, $CDCl_3$): δ 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; ESI-MS, calculated for $C_{60}H_{59}N_8O_8^+$ $(M + H)^+$1019, found 1019.

*Squaraine rotaxane **1f***. The precursor **1d** (20 mg) was dissolved in dichloromethane (3 ml) and TFA (1 ml) was added. The solution was stirred at room temperature for 6 h, and then the solvent was removed. The residue was taken up in chloroform (100 ml) and washed with water (2 × 50 ml), dried with anhydrous Na_2SO_4 and concentrated to give **1f** quantitatively as a blue solid that was dried *in vacuo*. ^{1}H NMR (300 MHz, DMSO): δ 9.89 (t, 4H, $J = 6.0$), 8.30–8.42 (m, 5H), 7.89 (d, 4H, $J = 9.0$), 6.46 (s, 8H), 6.26 (d, 4H, $J = 9.0$), 4.38 (d, 8H, $J = 6.0$), 4.02 (s, 8H), 3.39–3.62 (m, 32H); ^{13}C (75 MHz, DMSO): δ 184.6, 182.7, 171.7, 162.5, 153.9, 148.9, 139.9, 136.4, 132.6, 128.2, 124.9, 118.3, 111.8, 69.9, 67.6, 67.5, 50.4, 42.3, 40.4; ESI-MS, calculated for $C_{70}H_{79}N_8O_{22}^+$ $(M + H)^+$1383, found 1383.

Crystallography

Crystal data for **1c**. $C_{32}H_{28}N_4O_4$, $C_{36}H_{48}N_2O_{10}$, 2.46 $CHCl_3$, 1.54 C_2H_3N, Mr = 1558.22, $T = 100$ K, monoclinic, $P2_1/c$, $a = 15.1808(3)$ Å, $b = 13.1032(3)$ Å, $c = 19.7252(4)$ Å, $\beta = 106.479(1)°$, $V = 3762.51(14)$ Å^3, $Z = 2$, $R1 = 0.0856$, $wR2 = 0.1819$. Crystals were grown by slow diffusion of hexane into acetonitrile/chloroform solution. The structure was solved with three unique elements in the asymmetric unit, two components as the wheel and thread of the rotaxane and four crystallisation solvents with partial occupancy between 2.46 molecules of chloroform and 1.54 molecules of acetonitrile. The X-ray data can be retrieved free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/conts/retrieving.html and quoting CCDC 700171.

Quantum yields

All quantum yields were determined using a previously described method with an optically matched standard solution of bis[4-(*N*,*N*-dimethylamino)phenyl]squaraine in $CHCl_3$ (*22*).

Acknowledgements

We are grateful for funding support from the NIH (USA) and the Elsa U. Pardee Foundation.

References

(1) Massoud, T.F.; Gambhir, S.S. *Genes Dev.* **2003**, *17*, 545–580.

(2) Becker, A.; Hessenius, C.; Licha, K.; Ebert, B.; Sukowski, U.; Semmler, W.; Wiedenmann, B.; Grotzinger, C. *Nat. Biotechnol.* **2001**, *19*, 327–331.
(3) Chen, X.Y.; Conti, P.S.; Moats, R.A. *Cancer Res.* **2004**, *64*, 8009–8014.
(4) Leevy, W.M.; Gammon, S.T.; Jiang, H.; Johnson, J.R.; Maxwell, D.J.; Jackson, E.N.; Marquez, M.; Piwnica-Worms, D.; Smith, B.D. *J. Am. Chem. Soc.* **2006**, *128*, 16476–16477.
(5) Frangioni, J.V. *Curr. Opin. Chem. Biol.* **2003**, *7*, 626–634.
(6) Mahmood, U.; Weissleder, R. *Mol. Cancer Ther.* **2003**, *2*, 489–496.
(7) Ntziachristos, V.; Ripoll, J.; Weissleder, R. *Opt. Lett.* **2002**, *27*, 1652–1652.
(8) Pham, W.; Choi, Y.D.; Weissleder, R.; Tung, C.H. *Bioconj. Chem.* **2004**, *15*, 1403–1407.
(9) Cheng, Z.; Levi, J.; Xiong, Z.M.; Gheysens, O.; Keren, S.; Chen, X.Y.; Gambhir, S.S. *Bioconj. Chem.* **2006**, *17*, 662–669.
(10) Chen, H.J.; Law, K.Y.; Whitten, D.G. *J. Phys. Chem.* **1996**, *100*, 5949–5955.
(11) Ros-Lis, J.V.; Garcia, B.; Jimenez, D.; Martinez-Manez, R.; Sancenon, F.; Soto, J.; Gonzalvo, F.; Valldecabres, M.C. *J. Am. Chem. Soc.* **2004**, *126*, 4064–4065.
(12) Arunkumar, E.; Forbes, C.C.; Noll, B.C.; Smith, B.D. *J. Am. Chem. Soc.* **2005**, *127*, 3288–3289.
(13) Johnson, J.R.; Fu, N.; Arunkumar, E.; Leevy, W.M.; Gammon, S.T.; Piwnica-Worms, D.; Smith, B.D. *Angew. Chem. Int. Ed. Engl.* **2007**, *46*, 5528–5531.
(14) Leigh, D.A.; Murphy, A.; Smart, J.P.; Slawin, A.M.Z. *Angew. Chem. Int. Ed.* **1997**, *36*, 728–732.
(15) Johnston, A.G.; Leigh, D.A.; Murphy, A.; Smart, J.P.; Deegan, M.D. *J. Am. Chem. Soc.* **1996**, *118*, 10662–10663.
(16) Lin, C.F.; Lai, C.C.; Liu, Y.H.; Peng, S.M.; Chiu, S.H. *Chem. Eur. J.* **2007**, *13*, 4350–4355.
(17) Sambrook, M.R.; Beer, P.D.; Wisner, J.A.; Paul, R.L.; Cowley, A.R.; Szemes, F.; Drew, M.G.B. *J. Am. Chem. Soc.* **2005**, *127*, 2292–2302.
(18) Lane, A.S.; Leigh, D.A.; Murphy, A. *J. Am. Chem. Soc.* **1997**, *119*, 11092–11093.
(19) Gassensmith, J.J.; Arunkumar, E.; Barr, L.; Baumes, J.M.; DiVittorio, K.M.; Johnson, J.R.; Noll, B.C.; Smith, B.D. *J. Am. Chem. Soc.* **2007**, *129*, 15054–15059.
(20) Wu, A.X.; Mukhopadhyay, P.; Chakraborty, A.; Fettinger, J.C.; Isaacs, L. *J. Am. Chem. Soc.* **2004**, *126*, 10035–10043.
(21) Affeld, A.; Hubner, G.M.; Seel, C.; Schalley, C.A. *Eur. J. Org. Chem.* **2001**, 2877–2890.
(22) Arun, K.T.; Ramaiah, D. *J. Phys. Chem. A* **2005**, *109*, 5571–5578.