Organic LETTERS

Letter

pubs.acs.org/OrgLett

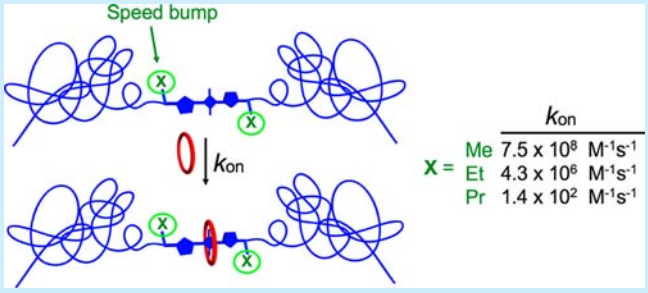
Sensitive Structural Control of Macrocycle Threading by a Fluorescent Squaraine Dye Flanked by Polymer Chains

Wenqi Liu, Evan M. Peck, Kevin D. Hendzel, and Bradley D. Smith*

Department of Chemistry and Biochemistry, 236 Nieuwland Science Hall, University of Notre Dame, Notre Dame, Indiana 46556, United States

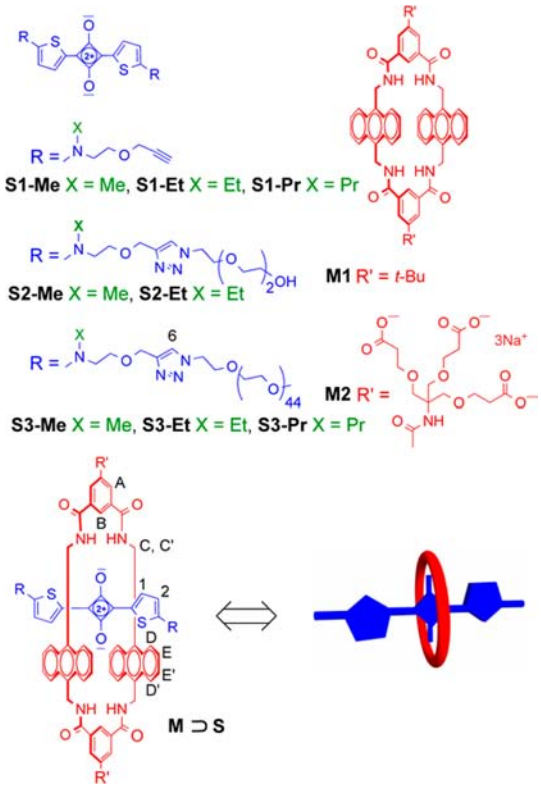
Supporting Information

ABSTRACT: A macrocyclic tetralactam is threaded by a complementary squaraine dye that is flanked by two polyethylene glycol chains to produce a pseudorotaxane complex with favorable near-infrared fluorescence properties. The association thermodynamics and kinetics were measured for a homologous series of squaraines with different *N*-alkyl substituents at both ends of the dye. The results show that subtle changes in substituent steric size have profound effects on threading kinetics without greatly altering the very high association constant.



Polymer appended rotaxanes or pseudorotaxanes are functionally attractive molecular architectures with unique structural and dynamic properties.[1] Successful rational design of these dynamic systems requires a precise understanding of the structural factors that control the underlying kinetics and thermodynamics.[2] We have previously reported that the macrocyclic tetralactam hosts in Scheme 1 can encapsulate highly fluorescent squaraine dyes to give structurally well-defined pseudorotaxane complexes that are well suited for fluorescence imaging of biological samples.[3] Squaraine binding studies in different solvents utilized the organic soluble macrocycle **M1** or the water-soluble version **M2**: the two structures vary only by the different appendages on the structural periphery. The macrocycles were threaded by the organic soluble bisalkyne squaraine **S1-Et**, or the water-soluble conjugates **S2-Et** and **S3-Et** with appended polyethylene glycol (PEG) chains, and very strong binding affinities were recorded due to the complementary fit of squaraine dye inside the macrocycle cavity.[3] The two oxygens on the encapsulated squaraine form hydrogens bonds to the four macrocycle NH residues, and there is coplanar stacking of the squaraine aromatic surfaces with the anthracene sidewalls of the macrocycle.[4] The macrocycle threading process produced a large ratiometric change in near-infrared optical properties, including turn-on fluorescence, and the pseudorotaxane complexes were stable enough to be visualized during live cell microscopy experiments. The rates of macrocycle threading were remarkably insensitive to the length of the PEG chains attached to the ends of the squaraine dye. For example, extending the length of the PEG chain from ∼7 atoms in **S2-Et** to ∼140 atoms in **S3-Et** only slowed the threading of macrocycle **M2** by a factor of 3 in water. These findings suggest that diffusion of the macrocycle along the PEG chain is not a rate limiting step in the threading process, and this has motivated us to further explore the sensitivity of the squaraine/macrocycle association system to steric effects. Here we report a comparative study of association thermodynamics and kinetics for homologous dye structures with slightly different *N*-alkyl substituents on the terminal nitrogen atoms at both ends

Scheme 1. Chemical Structures and Atom Labels

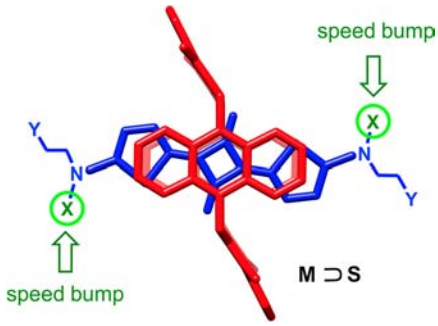


Received: September 11, 2015
Published: October 9, 2015

DOI: 10.1021/acs.orglett.5b02633
Org. Lett. 2015, 17, 5268−5271

of the squaraine dye (Scheme 2). We find that the substituent's steric size has almost no effect on the pseudorotaxane association

Scheme 2. Molecular Model of Macrocycle/Squaraine Pseudorotaxane Complex (M ⊃ S) Showing How the Speed Bump Substituents (X) on the Squaraine Are Located Outside the Macrocycle Cavity[a]



[a]For clarity the peripheral appendages on the surrounding macrocycle and the PEG chains (Y) that flank the squaraine are not shown.

constant, but there is a large difference in the assembly kinetics. Thus, the *N*-alkyl substituents act as highly effective "speed bumps" to control the rates of macrocycle threading and dethreading.

First, we compared the kinetics and thermodynamics for macrocycle threading by the original *N*-ethyl series of squaraine dyes (i.e., **S1-Et**, **S2-Et**, and **S3-Et**), with a new homologous *N*-methyl series (**S1-Me**, **S2-Me**, and **S3-Me**). The pseudorotaxane complexes were prepared by mixing separate solutions of **M1** or **M2** with the different dyes in chloroform, methanol, or water, and the self-assembled pseudorotaxane complexes were characterized using the same NMR, fluorescence, and UV/vis absorption methods that were employed previously.[3a] As expected, macrocycle threading was indicated by several diagnostic and independent spectral features. For example, in Figure 1 are the changes in ^{1}H NMR chemical shifts that

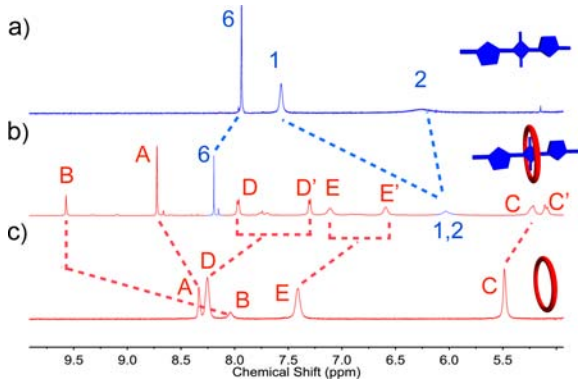


Figure 1. Partial ^{1}H NMR spectra (600 MHz, 25 °C, D_2O): (a) **S2-Me**, (b) 1:1 mixture of **M2** and **S2-Me** to form **M2** ⊃ **S2-Me**, (c) **M2**. Atom labels are provided in Scheme 1.

occurred when **S2-Me** was mixed with **M2** in D_2O to form **M2** ⊃ **S2-Me** (analogous ^{1}H NMR spectra supporting the formation of **M1** ⊃ **S1-Me** in $CDCl_3$ and **M2** ⊃ **S2-Me** in CD_3OD are shown in Figures S10 and S11). In agreement with the anisotropic chemical shielding induced by the pseudorotaxane structure, the thiophene proton 1 moved strongly upfield upon complexation, whereas the macrocycle proton B moved strongly downfield. Another common spectral change upon complexation is the 20–30 nm red shift in the squaraine absorption/emission maxima (Table S1). Additional compelling evidence for dye encapsulation by the macrocycle was the observation of efficient energy transfer from an excited anthracene sidewall in the macrocycle (ex: 385 nm) to the encapsulated squaraine dye (em: 704 nm) (Figure S20c).

The association constants listed in Table 1 were determined by conducting fluorescence titration experiments at 20 °C followed by nonlinear fitting of the data to a 1:1 stoichiometry model (the stoichiometry was confirmed by titration experiments using Job's method; see Figure S20b). By focusing on the most biomedically relevant results in water (entries 5 and 6), the very high association constants for threading of **M2** by dyes **S2-Me**, **S2-Et**, **S3-Me**, and **S3-Et** are all within a factor of 2.[5] The different *N*-alkyl substituents that flank the encapsulated squaraine dyes are located beyond the macrocycle cavity (Scheme 2) and appear to have very little effect on pseudorotaxane complex stability in water. The thermodynamic factors controlling the association of **M2** with **S2-Me** or **S2-Et** in water at 27 °C were determined by a combination of fluorescence titration and isothermal titration calorimetry. The values listed in Table S2 show that macrocycle threading to form a very stable pseudorotaxane complex in water is driven by a highly favorable enthalpic change.

Table 1 also lists the rate constants for macrocycle threading (k_{on}) which were measured by conducting stopped flow experiments and fitting the kinetic profile to a second order reaction model. Consistent with previous observations, the rate constants are highly solvent dependent and increase significantly in the order chloroform < methanol ≪ water.[3a] There is a remarkable dependence on the steric size of the *N*-alkyl substituents that flank the squaraine dye. As illustrated in Figure 2, threading of **M2** in water by squaraine **S2-Me** is almost diffusion controlled with $k_{on} = 1.1 \times 10^9$ M^{-1} s^{-1}. Extending the length of the *N*-alkyl-PEG chain (colored blue) in **S2-Me** by adding about 127 atoms to create **S3-Me** only slowed the threading of **M2** by a factor of 1.4 in water, in agreement with the previous trend seen in the *N*-ethyl series (**S2-Et** vs **S3-Et**). But adding one carbon to the *N*-alkyl substituent (colored green) to create **S2-Et** decreased k_{on} by a factor of 100.[6] This suggests that translocation of the macrocycle over the substituents that are attached to the sp^2 hybridized nitrogen atoms on the ends of the squaraine dye is a rate limiting step for the macrocycle threading process. This highly sensitive "speed bump" effect is reminiscent of similar observations by different groups studying steric effects within relatively short rotaxane shuttle or pseudorotaxane threading systems.[7] But it has not been reported for systems that thread a macrocycle onto a long polymer chain.[2]

The extremely rapid and high affinity threading of **M2** in water by squaraine dyes that are flanked by very long PEG chains is notable,[8] and the possibility of eventual biomedical applications prompted us to characterize the process in fetal bovine serum (FBS). Inspection of entries 7 and 8 in Table 1 shows that the macrocycle/squaraine association constant decreases by ~10^2–10^3 when the solvent is changed from pure water to FBS. Moreover, there is more than a 1000-fold decrease in k_{on}. Serum proteins are known to associate with molecules that have lipophilic surfaces, and presumably the serum proteins compete for the separated molecular partners which decreases k_{on} and K_a. The lower pseudorotaxane stability in serum leads to slow dissociation of a preassembled complex. For example, an aliquot of **M2** ⊃ **S3-Et** stock solution (preassembled by mixing the two

DOI: 10.1021/acs.orglett.5b02633
Org. Lett. 2015, 17, 5268–5271

Table 1. Association Constants (K_a) and Threading Rates (k_{on}) for Association of Squaraine and Macrocycle at 20 °C[a,b]

entry	solvent	squaraine	macrocycle	K_a (M^{-1})	k_{on} (M^{-1} s^{-1})	squaraine	macrocycle	K_a (M^{-1})	k_{on} (M^{-1} s^{-1})
1	$CHCl_3$	**S1-Me**	**M1**	$(7.7 \pm 0.8) \times 10^7$	$(8.0 \pm 0.3) \times 10^4$	**S1-Et**	**M1**	$(5.9 \pm 1.6) \times 10^5$	(8.6 ± 0.4)
2	$CHCl_3$	**S3-Me**	**M1**	$(2.5 \pm 0.7) \times 10^7$	$(3.3 \pm 0.7) \times 10^4$	**S3-Et**	**M1**	$(2.0 \pm 0.5) \times 10^6$	(11.9 ± 0.9)
3	MeOH	**S1-Me**	**M2**	$(2.9 \pm 0.1) \times 10^5$	$(2.0 \pm 0.5) \times 10^6$	**S1-Et**	**M2**	$(4.0 \pm 0.6) \times 10^5$	$(1.2 \pm 0.1) \times 10^4$
4	MeOH	**S3-Me**	**M2**	$(1.0 \pm 0.1) \times 10^5$	$(5.7 \pm 0.1) \times 10^5$	**S3-Et**	**M2**	$(4.1 \pm 0.1) \times 10^5$	$(5.1 \pm 0.6) \times 10^3$
5	H_2O	**S2-Me**	**M2**	$(1.2 \pm 0.5) \times 10^9$	$(1.1 \pm 0.1) \times 10^9$	**S2-Et**	**M2**	$(6.0 \pm 1.2) \times 10^8$	$(1.2 \pm 0.1) \times 10^7$
6	H_2O	**S3-Me**	**M2**	$(7.5 \pm 2.7) \times 10^8$	$(7.2 \pm 0.3) \times 10^8$	**S3-Et**	**M2**	$(1.1 \pm 0.4) \times 10^9$	$(4.3 \pm 0.3) \times 10^6$
7	FBS	**S2-Me**	**M2**	$(2.2 \pm 0.2) \times 10^6$	$(7.8 \pm 0.8) \times 10^5$	**S2-Et**	**M2**	$(1.2 \pm 0.3) \times 10^7$	$(1.7 \pm 0.1) \times 10^3$
8	FBS	**S3-Me**	**M2**	$(1.4 \pm 0.1) \times 10^6$	$(6.3 \pm 0.8) \times 10^5$	**S3-Et**	**M2**	$(2.2 \pm 0.4) \times 10^6$	$(2.1 \pm 0.2) \times 10^3$

[a]The data for **S1-Et**, **S2-Et**, and **S3-Et** in entries 1−6 taken from ref 3a. [b]The free energy of association and threading free energy of activation are listed in Table S3.

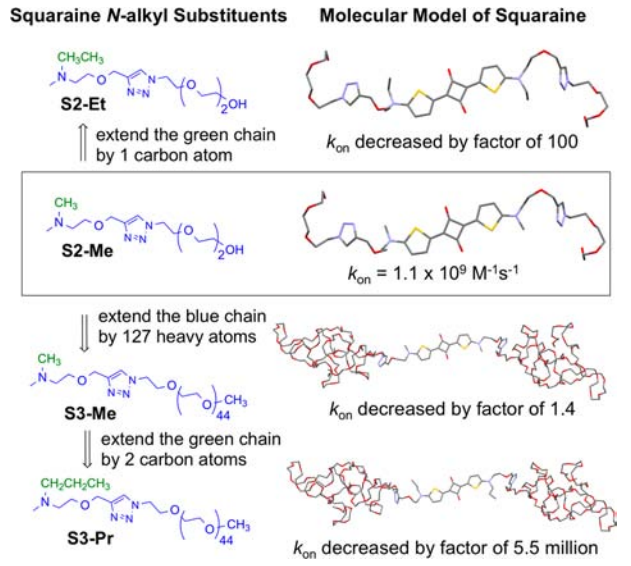


Figure 2. Different structural changes in the *N*-alkyl substituents that flank the squaraine have distinctive effects on the rate constant k_{on} for threading of **M2** in water at 20 °C. Squaraine molecular models were optimized using the semiempirical method at PM7 level.

partners at high micromolar concentration in water) was diluted to 500 nM in FBS and observed by fluorescence to partially dissociate over 1 h (Figure 3).[9] We reasoned that the problem of

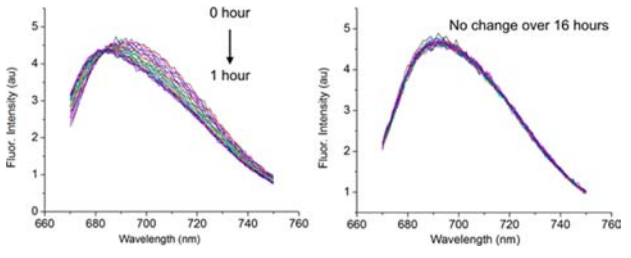


Figure 3. (Left) Fluorescence spectra (ex: 650 nm) indicating partial dissociation of **M2** ⊃ **S3-Et** (500 nM) in FBS over 1 h (spectra acquired every 3 min). (Right) Fluorescence spectra (ex: 650 nm) indicating no dissociation of **M2** ⊃ **S3−Pr** (500 nM) in FBS over 16 h (spectra acquired every 30 min).

pseudorotaxane dissociation in FBS could be circumvented by adjusting the steric barrier controlling the assembly kinetics.[7] We decided to slightly increase the size of the speed bump substituent at each end of the squaraine dye so that it was still small enough to allow efficient macrocycle threading at relatively high concentrations in water, but large enough to slow pseudorotaxane dissociation in FBS. The obvious first choice was an *N*-propyl substituent, and thus we prepared the squaraine dye **S3-Pr** (Scheme 1) and characterized its association with macrocycle **M2**. We were pleased to find that **S3-Pr** and **M2** formed a highly stable pseudorotaxane complex in water with $K_a = (0.9 \pm 0.3) \times 10^8$ M^{-1}. The measured value of $k_{on} = (1.3 \pm 0.2) \times 10^2$ M^{-1} s^{-1} in water at 20 °C was a remarkable 5.5 million times slower than the threading of **M2** by **S3-Me** (Figure 2). Although this is a substantial decrease in the rate of self-assembly, the **M2** ⊃ **S 3-Pr** complex still formed quantitatively within a few minutes when both components were mixed at 500 μM each in water at 20 °C. This aqueous stock solution of the **M2** ⊃ **S3−Pr** complex was stable when stored at 4 °C. There was also no measurable dissociation or chemical decomposition when an aliquot of the stock solution was diluted to 500 nM in FBS, as judged by repeated fluorescence scans over 16 h (Figure 3). Confirmation that the interlocked structure of **M2** ⊃ **S3−Pr** was retained in FBS was gained by irradiating the sample with 385 nm light and observing efficient energy transfer from the anthracene sidewalls of the surrounding macrocycle to the encapsulated squaraine dye which produced strong emission at 695 nm (Figure S30).[10] This finding of high chemical and mechanical stability in FBS is very encouraging and suggests that pseudorotaxane complexes with *N*-propyl "speed bumps" can be rapidly preassembled in quantitative yield in water, then diluted into complex biological media, and used as stable, high performance, near-infrared fluorescent molecular probes.

In summary, the results of these studies show that subtle changes in steric size of the *N*-alkyl substituents at either end of the squaraine structure can produce profound changes in the kinetics of macrocycle threading without greatly altering the mechanical stability of the associated pseudorotaxane complex. This new insight should allow development of macrocycle threading systems with kinetic features that enable specific applications. For example, polymer self-assembly or surface adhesion experiments that require extremely rapid and high-affinity association in water should use PEG conjugated squaraines with *N*-methyl substituents (such as **S3-Me**), whereas molecular imaging applications that require a long-lived preassembled fluorescent probe in biological media should employ PEG conjugated squaraines with *N*-propyl substituents (such as **S3-Pr**) and appropriately modified versions of **M2** with appended biomarker targeting ligands. Studies that employ these supramolecular systems for new and interesting applications will be reported in due course.

DOI: 10.1021/acs.orglett.5b02633
Org. Lett. 2015, 17, 5268−5271

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02633.

Synthesis and characterization, thermodynamic and kinetics data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: smith.115@nd.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support for this work was provided by the NSF (CHE1401783).

■ REFERENCES

(1) (a) Van Dongen, S. F. M.; Cantekin, S.; Elemans, J. A. A. W.; Rowan, A. E.; Nolte, R. J. M. *Chem. Soc. Rev.* **2014**, *43*, 99. (b) Aoki, D.; Uchida, S.; Takata, T. *Polym. J.* **2014**, *46*, 546. (c) Aoki, D.; Uchida, S.; Takata, T. *Angew. Chem., Int. Ed.* **2015**, *54*, 6770. (d) De Bo, G.; De Winter, J.; Gerbaux, P.; Fustin, C. A. *Angew. Chem., Int. Ed.* **2011**, *50*, 9093. (e) van Dongen, S. F. M.; Elemans, J. A. A. W.; Rowan, A. E.; Nolte, R. J. M. *Angew. Chem., Int. Ed.* **2014**, *53*, 11420.

(2) (a) Deutman, A. B. C.; Varghese, S.; Moalin, M.; Elemans, J. A. A. W.; Rowan, A. E.; Nolte, R. J. M. *Chem. - Eur. J.* **2015**, *21*, 360. (b) Deutman, A. B. C.; Cantekin, S.; Elemans, J. A. A. W.; Rowan, A. E.; Nolte, R. J. M. *J. Am. Chem. Soc.* **2014**, *136*, 9165. (c) Biedermann, F.; Elmalem, E.; Ghosh, I.; Nau, W. M.; Scherman, O. A. *Angew. Chem.* **2012**, *124*, 7859. (d) Deutman, A. B. C.; Monnereau, C.; Elemans, J. A. A. W.; Ercolani, G.; Nolte, R. J. M.; Rowan, A. E. *Science* **2008**, *322*, 1668.

(3) (a) Peck, E. M.; Liu, W.; Spence, G. T.; Shaw, S. K.; Davis, A. P.; Destecroix, H.; Smith, B. D. *J. Am. Chem. Soc.* **2015**, *137*, 8668. (b) 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.

(4) (a) Arunkumar, E.; Forbes, C. C.; Noll, B. C.; Smith, B. D. *J. Am. Chem. Soc.* **2005**, *127*, 3288. (b) Gassensmith, J. J.; Baumes, J. M.; Smith, B. D. *Chem. Commun.* **2009**, 6329. (c) Peck, E. M.; Oliver, A. G.; Smith, B. D. *Aust. J. Chem.* **2015**, *68*, 1359.

(5) Association constants for pseudorotaxane formation in methanol (entries 3 and 4) are also all quite similar (within a factor of 4), whereas the systems in chloroform (entries 1 and 2) show a larger difference in association constants (variation of one hundred). The larger difference in chloroform is likely due to substituent-induced variations in macrocycle/squaraine hydrogen bonding which is a dominant interaction for assembly in the weakly polar organic solvent.

(6) The threading rate constants (k_{on}) in methanol (entries 3 and 4) also show a 100-fold decrease when the *N*-alkyl group is changed from *N*-methyl to *N*-ethyl, whereas the systems in chloroform (entries 1 and 2) show a much larger difference (decrease of almost 10 000). The larger decrease in chloroform is likely due to a higher activation barrier for passage of the macrocycle over the ends of the squaraine due to stronger hydrogen bonding interactions in the weakly polar organic solvent. There is no kinetic evidence for a major polymer chain entanglement effect in any of the solvents.

(7) (a) Senler, S.; Cheng, B.; Kaifer, A. E. *Org. Lett.* **2014**, *16*, 5834. (b) Dey, S. K.; Coskun, A.; Fahrenbach, A. C.; Barin, G.; Basuray, A. N.; Trabolsi, A.; Botros, Y. Y.; Stoddart, J. F. *Chem. Sci.* **2011**, *2*, 1046. (c) Carrasco-Ruiz, A.; Tiburcio, J. *Org. Lett.* **2015**, *17*, 1858. (d) Jeppesen, J. O.; Becher, J.; Stoddart, J. F. *Org. Lett.* **2002**, *4*, 557. (e) Young, P. G.; Hirose, K.; Tobe, Y. *J. Am. Chem. Soc.* **2014**, *136*, 7899.

(8) (a) Verdejo, B.; Gil-Ramírez, G.; Ballester, P. *J. Am. Chem. Soc.* **2009**, *131*, 3178. (b) Ferrand, Y.; Klein, E.; Barwell, N. P.; Crump, M. P.; Jiménez-Barbero, J.; Vicent, C.; Boons, G.-J.; Ingale, S.; Davis, A. P. *Angew. Chem., Int. Ed.* **2009**, *48*, 1775. (c) Ke, C.; Destecroix, H.; Crump, M. P.; Davis, A. P. *Nat. Chem.* **2012**, *4*, 718. (d) Hunter, C.; Thomas, J.; Bernad, P., Jr. *Chem. Commun.* **1998**, *22*, 2449.

(9) As a general trend, the rate of complex dissociation (k_{off}) in FBS was observed to be about four times slower than k_{off} in water (calculated from the K_a and k_{on} data in Table 1 and confirmed in some cases by independent measurements) presumably due to association of the complex with the serum proteins (Figure S27).

(10) In contrast, a control experiment that mixed equimolar amounts of the two partners (**M2** and **S3-Pr**) at 500 nM in FBS for 24 h showed no energy transfer from the anthracene sidewalls to the squaraine, indicating that macrocycle threading had not occurred (Figure S30).

DOI: 10.1021/acs.orglett.5b02633
Org. Lett. 2015, 17, 5268−5271