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Synthesis and Photophysical Investigation of Squaraine Rotaxanes by "Clicked Capping"

Jeremiah J. Gassensmith, Lorna Barr, Jeffrey M. Baumes, Agelina Paek, Anh Nguyen, and Bradley D. Smith*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

smith.115@nd.edu

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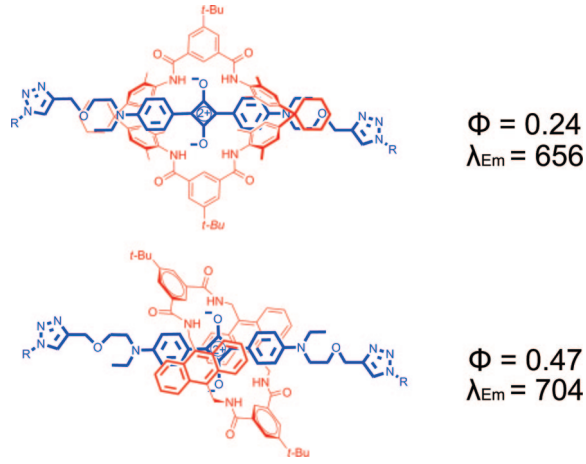
ABSTRACT



Pseudorotaxane complexes of squaraine dyes and tetralactam macrocycles are converted into permanently interlocked rotaxane structures using copper-catalyzed and copper-free cycloaddition reactions with bulky stopper groups. The photophysical properties of the encapsulated squaraine depend on the structure of the macrocycle. In one case, squaraine rotaxanes are produced in near-quantitative yields and with intense near-IR fluorescence. In another case, squaraine fluorescence is greatly diminished upon macrocyclic encapsulation but the signal can be restored by dye displacement with anions.

Many of the new and emerging biophotonic technologies require extremely bright and highly stable fluorescent dyes.[1] One way to improve dye performance is molecular encapsulation inside a host molecule or nanoparticle.[2] Our focus is on squaraine rotaxanes, which are produced by encapsulating highly fluorescent near-IR squaraine dyes inside surrounding macrocycles (Scheme 1).[3] This sterically protects the squaraine fluorophore such that squaraine rotaxanes

(1) (a) *Biophotonics: Optical Science and Engineering for the 21st Century*; Shen, X., Van Wijk, R., Eds.; Springer: New York, 2005. (b) *Optical Imaging Techniques in Cell Biology*; Cox, G. C., Ed.; CRC Press: Boca Raton, 2007. (c) Wolfbeis, O. S. *Fluorescence Methods and Applications: Spectroscopy, Imaging, and Probes*; Wiley-Blackwell: New York, 2008.

(2) (a) Arunkumar, E.; Forbes, C. C.; Smith, B. D. *Eur. J. Org. Chem.* **2005**, 4051–4059. (b) Buston, J. E. H.; Young, J. R.; Anderson, H. L. *Chem. Commun.* **2000**, 905–906. (c) Park, J. S.; Wilson, J. N.; Hardcastle, K. I.; Bunz, U. H. F.; Srinivasarao, M. *J. Am. Chem. Soc.* **2006**, *128*, 7714–7715. (d) Mohanty, J.; Pal, H.; Ray, A. K.; Kumar, S.; Nau, W. M. *ChemPhysChem* **2007**, *8*, 54–56. (e) Comes, M.; Marcos, M. D.; Martínez-Máñez, R.; Millán, M. C.; Ros-Lis, J. V.; Sancenón, F.; Soto, J.; Villaescusa, L. A. *Chem. Eur. J.* **2006**, *12*, 2162–2170. (f) Constantin, T. P.; Silva, G. L.; Robertson, K. L.; Hamilton, T. P.; Fague, K.; Waggoner, A. S.; Armitage, B. A. *Org. Lett.* **2008**, *10*, 1561–1564.

(3) Arunkumar, E.; Forbes, C. C.; Noll, B. C.; Smith, B. D. *J. Am. Chem. Soc.* **2005**, *127*, 3288–3289.

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Scheme 1

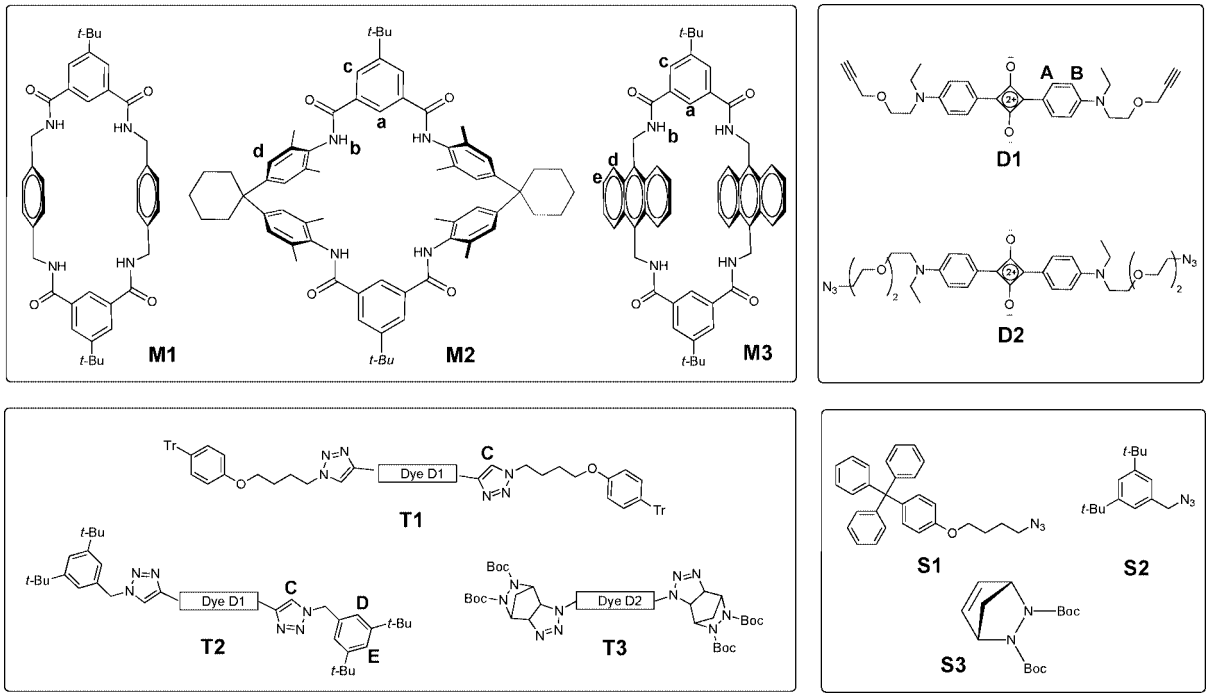
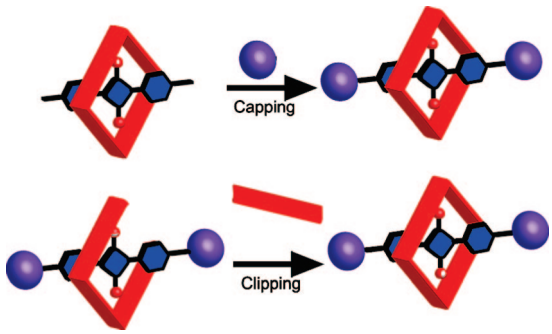


exhibit increased stability and decreased intermolecular quenching.[4] Furthermore, squaraine rotaxanes are well suited for structural conversion into molecular probes for bioimaging applications.[5] The first-generation squaraine rotaxanes were prepared in yields of 10−30% using a Leigh-type clipping method;[6] that is, a macrocyclization reaction that traps a squaraine template inside a tetralactam macrocycle. This synthetic strategy allows rapid production of prototype designs; however, the low yields limit the capacity to prepare more complex later generation structures. We have explored alternative methods of preparing squaraine rotaxanes, and we report here a series of related synthetic capping strategies.[7] In each case, a reversible pseudorotaxane complex is converted into a permanently interlocked rotaxane by covalent capping with bulky stopper groups (Scheme 2). In the best cases, squaraine rotaxanes are produced in >90% yield after column chromatography. We also find that different macrocycles alter squaraine photophysical properties in different ways; thus, the encapsulating macrocycle is a structural parameter that can be altered to control squaraine rotaxane function.

Capping strategies with phenylene-containing tetralactam macrocycles such as **M1** are not effective because of very poor macrocycle solubility.[8] To circumvent this problem, we investigated other macrocycles that have a similar isophthalamide tetralactam motif but exhibit more favorable solubility in organic solvents. We initially examined the well-known **M2**, which is soluble in methylene chloride and has been extensively studied as a macrocyclic component in various interlocked molecules.[9,10] NMR titration experiments showed that **M2** can encapsulate squaraine dye **D1** with an association constant

Scheme 2. Synthesis of Squaraine Rotaxanes via Capping (Top) and Clipping (Bottom)



(4) (a) Arunkumar, E.; Fu, N.; Smith, B. D. *Chem. Eur. J.* **2006**, *12*, 4684–4690. (b) Arunkumar, E.; Sudeep, P. K.; Kamat, K. V.; Noll, B.; Smith, B. D. *New J. Chem.* **2007**, *31*, 677–683.

(5) Johnson, J. R.; Fu, N.; Arunkumar, E.; Leevy, W. M.; Gammon, S. T.; Piwnica-Worms, D.; Smith, B. D. *Angew. Chem., Int. Ed.* **2007**, *46*, 5528–5531.

(6) (a) Leigh, D. A.; Murphy, A.; Smart, J. P.; Slawain, A. M. Z. *Angew. Chem., Int. Ed.* **1997**, *36*, 728. (b) Gatti, F. G.; Leigh, D. A.; Nepogodiev, S. A.; Slawin, A. M. Z.; Teat, S. J.; Wong, J. K. Y. *J. Am. Chem. Soc.* **2001**, *123*, 5983–5989. (c) Leigh, D. A.; Wong, J. K. Y.; Dehez, F.; Zerbetto, F. *Nature* **2003**, *424*, 174–179.

(7) (a) Klotz, E. J. F.; Claridge, T. D. W.; Anderson, H. L. *J. Am. Chem. Soc.* **2006**, *128*, 15374–15375. (b) Hübner, G. M.; Reuter, C.; Seel, C.; Vögtle, F. *Synthesis* **2000**, *1*, 103–108. (c) Dunnwald, T.; Jäger, R.; Vögtle, F. *Chem. Eur. J.* **1997**, *3*, 2043–2051.

(8) Inoue, Y.; Kanbara, T.; Yamamoto, T. *Tetrahedron Lett.* **2003**, *44*, 5167–5169.

(9) (a) Schalley, C. A.; Weilandt, T.; Bruggemann, J.; Vögtle, F. *Top. Curr. Chem.* **2004**, *248*, 141–200. (b) Affeld, A.; Hübner, G. M.; Seel, C.; Schalley, C. A. *Eur. J. Org. Chem.* **2001**, 2877–2890. (c) Fischer, C.; Nieger, M.; Mogck, O.; Böehmer, V.; Ungaro, R.; Vögtle, F. J. *Eur. J. Org. Chem.* **1998**, 155–161. (d) Handel, M.; Plevoets, M.; Gestermann, S.; Vögtle, F. J. *Angew. Chem., Int. Ed.* **1997**, *109*, 1248–1250.

(K_a) of 5 × 10^3 M^{-1} in methylene chloride at 25 °C.[11] We then covalently capped the protruding alkyne ends of the pseudorotaxane complex by conducting a copper mediated alkyne azide cycloaddition reaction.[12] After some experimentation, we found that mixing macrocycle **M2** with excess dye **D1** and stopper **S1** followed by treatment with $Cu(Ph_3P)_3Br$ catalyst generated the "clicked rotaxane" **M2⊃T1** with an isolated yield of 40%. The rotaxane structure was verified by standard spectroscopic methods including ^{1}H NMR (Figure 1). The downfield changes in

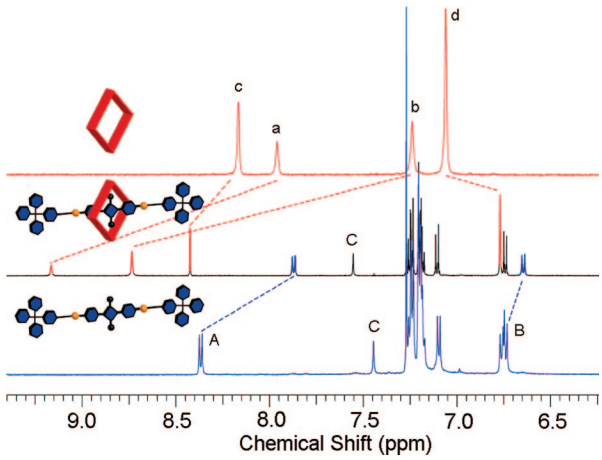


Figure 1. Partial ^{1}H NMR spectra of **M2** (top), **T1** (bottom), and the resulting clicked rotaxane **M2⊃T1** (middle).

chemical shift for the macrocycle amide and aryl protons are indicative of deshielding by the encapsulated squaraine thread **T1** and are consistent with previously observed changes in ^{1}H NMR spectra for squaraine rotaxanes.

The yield of squaraine rotaxane was sufficient to allow photophysical measurements. A direct comparison of squaraine thread **T1** and rotaxane **M2⊃T1** revealed a modest red shift in both absorption (+10 nm) and fluorescence emission (+7 nm) and approximately a 3-fold decrease in fluorescence quantum yield. It is also noteworthy that addition of two trizaole rings did not significantly alter the quantum yield in thread **T1** as compared to dye **D1** (Table 1). The macrocycle-induced quenching effect was verified by fluorescence titration experiments that added aliquots of **M2** to a solution of squaraine **D1** in methylene chloride. Addition

Table 1. Absorption, Emission, Extinction Coefficients, and Quantum Yields of Select Squaraine Derivatives in $CHCl_3$

compd	λ_{abs} (nm)	log ε	λem (nm)	Φ_f[a]
D1	631	5.65	651	0.68
T1	635	4.91	649	0.65
M2⊃[T1]	645	5.23	656	0.24
M3⊃[T2]	661	5.24	704	0.47

[a] Error ± 5%.

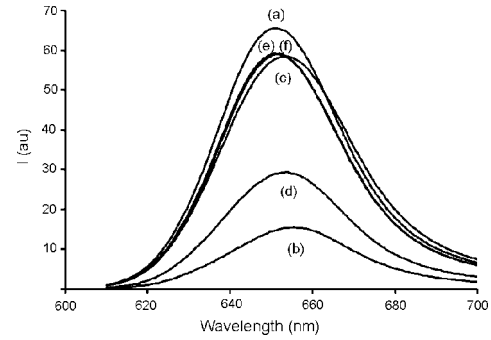


Figure 2. Fluorescence emissions of separate CH_2Cl_2 solutions of (a) **D1**, (b) **M2⊃D1**, and **M2⊃D1** in the presence of (c) TBA^+•Cl^-, (d) TBA^+•$H_2PO_4^-$, (e) TBA^+•CH_3COO^-, and (f) TBA^+•$C_6H_5COO^-$ at 5 mM for each anion. The concentrations of **D1** and **M2** were 1 μM and 3 mM, respectively.

of excess **M2** lowers the emission intensity of **D1** by about a factor of 4 (Figure 2 a vs b). Additional evidence that the quenching is due to inclusion of **D1** inside **M2** was gained by conducting anion displacement experiments.[13] As shown in Figure 2, treatment of the **M2⊃D1** psuedorotaxane system with the tetrabutylammonium salts of chloride, acetate, or benzoate leads to displacement of squaraine **D1** from the macrocyclic cavity and nearly complete restoration of its fluorescence intensity. These anions are known to bind strongly to the NH residues in **M2** and form hydrogen-bonded complexes (K_a > 10^5 M^{-1}).[14] Interestingly, tetrabutylammonium dihydrogenphosphate was less effective at squaraine displacement, suggesting that the **M2** cavity does not readily accommodate this larger anion. The fluorescence quenching induced by **M2** means that this system is unlikely to have utility as a bioimaging probe; however, the pseudorotaxane system **M2⊃D1** is an effective and selective anion sensor with near-IR fluorescence.[15]

Compared to **M2**, the anthrylene macrocycle **M3** is a more promising building block for squaraine rotaxane fabrication and probe development. In a prior study, we showed that an admixture of **M3** and **D1** self-assembles quantitatively at millimolar concentration in chloroform solution (K_a of 1.8 × 10^5 M^{-1}) to produce an inclusion complex whose absorption

(10) (a) Hunter, C. A. *Chem. Commun.* **1991**, 749–751. (b) Hunter, C. A. *J. Am. Chem. Soc.* **1992**, *114*, 5303–5311.

(11) Macrocycle **M2** binds uncharged carbonyl-containing guests with association constants <10^3 M^{-1} in CH_2Cl_2. For further information, see: (a) Seel, C.; Parham, A. H.; Safarowsky, O.; Hübner, G. M.; Vögtle, F. *J. Org. Chem.* **1999**, *64*, 7236–7242.

(12) (a) Aucagne, V.; Hanni, K. D.; Leigh, D. A.; Lusby, P. J.; Walker, D. B. *J. Am. Chem. Soc.* **2006**, *128*, 2186–2187. (b) Dichtel, W. R.; Miljanić, O. S.; Spruell, J. M.; Heath, J. R.; Stoddart, J. F. *J. Am. Chem. Soc.* **2006**, *128*, 10388–10390. (c) Miljanić, O. S.; Dichtel, W. R.; Khan, S. I.; Mortezaei, S.; Heath, J. R.; Stoddart, J. F. *J. Am. Chem. Soc.* **2007**, *129*, 8236–8246.

(13) (a) Montalti, M.; Prodi, L. *Chem. Commun.* **1998**, 1461–1462. (b) Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963–972.

(14) Hübner, G. M.; Gläser, J.; Seel, C.; Vögtle, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 383–386.

(15) Preliminary anion titration experiments with the rotaxane **M2⊃T2** show that it acts as a fluorescent anion sensor. Full details will be reported when the studies are complete.

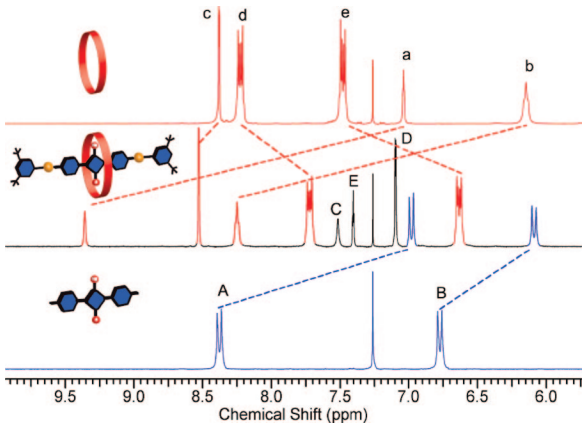


Figure 3. Partial ^{1}H NMR spectra of **M3** (top), **D1** (bottom), and the resulting clicked rotaxane **M3⊃T2** (middle).

and emission maxima are red-shifted by +40 nm.[16] We now report that "clicking" both ends of this pseudorotaxane with 2 molar equiv of stopper **S2** produces the squaraine rotaxane **M3⊃T2** in near-quantitative yield. The ^{1}H NMR spectra in Figure 3 exhibit the expected changes in chemical shift for a squaraine rotaxane, and the fluorescence spectra in Figure 4

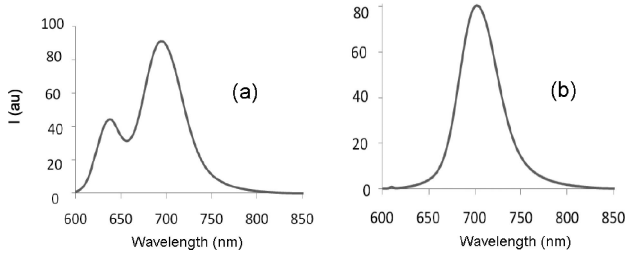
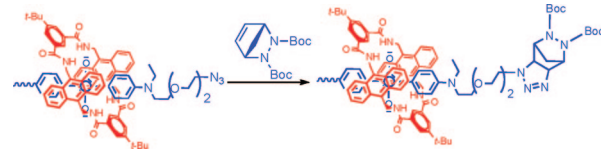


Figure 4. Fluorescence spectra in chloroform (ex: 590 nm); (a) pseudorotaxane **M3⊃D1** (6 μM) yields free **D1** (638 nm) and **M3⊃D1** (694 nm); (b) **M3⊃T2** (6 μM).

demonstrate that it is a permanently interlocked structure. Pseudorotaxane **M3⊃D1** partially dissociates at micromolar concentrations in chloroform and produces two emission peaks, one at 638 nm which corresponds to free squaraine **D1** and one at 694 nm, corresponding to pseudorotaxane. In contrast, the squaraine rotaxane **M3⊃T2** does not dissociate under these conditions or in more polar solvents such as pure methanol (see the Supporting Information).

One of our long-term research goals is to assemble highly stable squaraine rotaxane probes in complex biological environments such as the interior of cells. This requires the development of covalent capping methods that are biocompatible; that is, they do not use cytotoxic copper(I). One possible approach is to employ strain-promoted cycloadditions with cyclic alkynes that undergo uncatalyzed reactions at practically useful rates.[17] We were attracted to the related idea of a strained bicyclic alkene azide cycloaddition which produces five membered triazoline ring structures.[18] As a biocompatible method for capping pseudorotaxanes, the reaction is appealing because it is perfectly atom economical and does not require copper catalysis. As a proof of concept, we heated a mixture of bis-azide dye **D2**, macrocycle **M3**, and stopper **S3** (1:1:2 molar ratio) in chloroform for 3 days and produced the squaraine rotaxane **M3⊃T3** in near-quantitative yield (Scheme 3). The compound is stable

Scheme 3. Capping of Pseudorotaxane **M3⊃D2** To Produce **M3⊃T3**



enough for immediate characterization but slowly decomposes if left standing under laboratory lights.[19] Thus, the capping reaction is quite efficient but the product instability may possibly limit applications.

In summary, we report that squaraine rotaxanes with the anthrylene-containing macrocycle **M3** can be prepared in high yield by capping the alkyne groups that protrude from pseudorotaxane precursors using either copper catalyzed azide−alkyne cycloaddition or uncatalyzed azide−alkene cycloaddition reactions. The resulting squaraine rotaxanes exhibit intense near-IR absorption/emission maxima, and it should be possible to develop them into molecular probes for many types of photonic and bioimaging applications. In contrast, squaraine fluorescence intensity is greatly diminished when the dye is encapsulated by macrocycle **M2**. The fluorescence is restored when a suitable anionic guest is used to displace the squaraine dye from a pseudorotaxane complex; thus, the multicomponent system can be employed as a fluorescent anion sensor.

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Supporting Information Available: Experimental procedures and spectral data for all new products This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) 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.

(17) (a) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046–15047. (b) Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzit, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16793–16797. (c) Ning, X. H.; Guo, J.; Wolfert, M. A.; Boons, G. J. *Angew. Chem., Int. Ed.* **2008**, *47*, 2253–2255.

(18) (a) Bodnar, B. S.; Miller, M. J. *J. Org. Chem.* **2007**, *72*, 3929–3932. (b) Shea, K. J.; Kim, J. S. *J. Am. Chem. Soc.* **1992**, *114*, 4846–4855. (c) Stout, D. M.; Takaya, T.; Meyers, A. I. *J. Org. Chem.* **1974**, *40*, 563–569.

(19) Triazolines can liberate N_2 producing acid-sensitive aziridines. See ref 18c.