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Rotaxanes and Biofunctionalized Pseudorotaxanes via Thiol-Maleimide Click Chemistry

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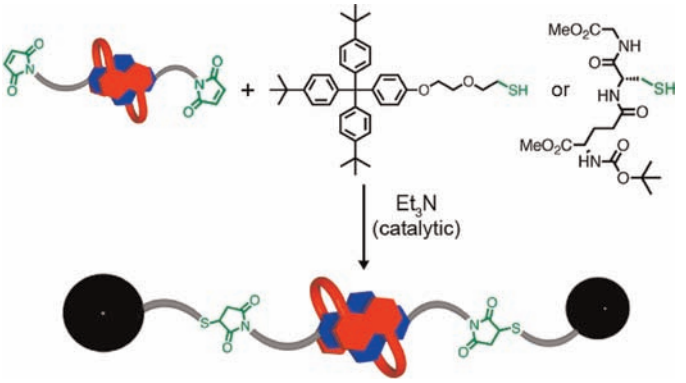
ABSTRACT



Base-catalyzed thiol-maleimide click chemistry has been applied to the synthesis of neutral donor−acceptor [2]rotaxanes in good yield. This method is extended further to the synthesis of a glutathione-functionalized [2]pseudorotaxane, a precursor to integrated conjugates of interlocked molecules with proteins and enzymes.

Molecules possessing mechanical bonds have attracted significant research interest for more than five decades.[1] Initial syntheses of mechanically interlocked catenanes and rotaxanes by Wasserman[2] and Harrison,[3] respectively, were achieved in very low yield (< 5%) owing to the statistical formation of interlocking species. Beginning in the 1980s, however, synthetic methods for the preparation of mechanically interlocked molecules have advanced apace, largely through the use of template-directed self-assembly protocols[4] used in conjunction with kinetic and thermodynamic covalent bond forming reactions[5] that "lock" individual components together. Increased synthetic efficiency has allowed increasingly complex mechanically interlocked structures to be synthesized, many of which have been designed to be stimuli-responsive[6] and form the basis of various molecular machines.[7] Toward the aim of increasing both synthetic ease and structural complexity,

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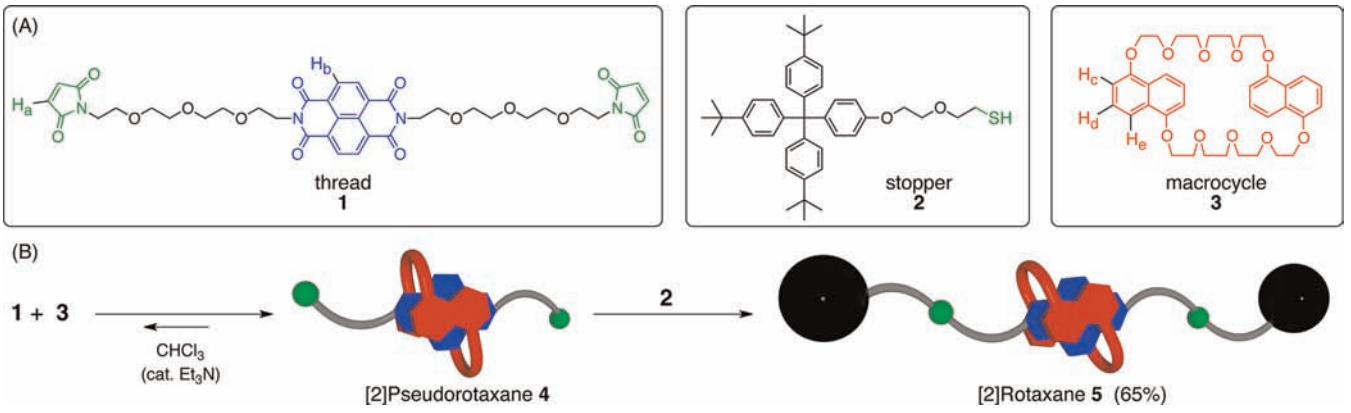
Scheme 1. Noncovalent Self-Assembly of [2]Pseudorotaxane **4** Followed by Thiol-Maleimide Click Chemistry Mediated Interlocking To Give [2]Rotaxane **5** in the Presence of Catalytic Et_3N



one of the most notable advances in the construction of mechanically interlocked molecules has been the introduction of highly efficient "click" reactions to their synthesis.[8] In 2006, the groups of Leigh,[9] Sauvage,[10] and Stoddart[11] each independently reported the efficient synthesis of interlocked rotaxanes utilizing copper(I) catalyzed alkyne–azide click (CuAAC) chemistry,[12] obtaining [2] and [3]rotaxanes in up to 94% isolated yield.[9]

The many attributes of click reactions, particularly the ability to tolerate low temperatures and high concentrations, make them particularly well suited to the threading followed by stoppering approach to mechanically interlocked molecules, as the thermodynamically controlled threading step is most favorable at low temperatures and high concentrations. While the vast majority of click chemical approaches to mechanically interlocked synthesis have used the CuAAC reaction, other click methods such as those using thiol-yne,[13] thiol-ene,[14] and nitrile-*N*-oxide[15] procedures have been successfully applied as well.

Here we report the application of thiol-maleimide click chemistry[16] to the synthesis of mechanically interlocked rotaxanes and noncovalently associated pseudorotaxanes. The highly selective reactivity of thiols for maleimides has been utilized for many years in the context of bioconjugate chemistry.[17] In the presence of catalytic base the thiol-maleimide Michael addition product can be obtained rapidly (minutes) and often in quantitative yields. Indeed the extensive history of thiol-maleimide bioconjugate chemistry is what inspired us to explore the use of thiol-maleimide chemistry in the synthesis of interlocked molecules. Once suitable conditions are developed it is expected that stimuli-responsive mechanically interlocked molecules can be directly integrated with biological systems (e.g., proteins, enzymes) through thiol-maleimide click reactions involving maleimide-functionalized supramolecules and free thiol moieties of solvent assessible cysteine residues. Toward this aim we demonstrate the efficient synthesis and self-assembly of a glutathione-functionalized [2]pseudorotaxane. Such integration of artificial molecular machines with biological systems may open the door to external allosteric control of the functionality of biological molecules.

The design of maleimide-functionalized naphthalene diimide (NDI) guest **1** and thiol-functionalized sterically imposing "stopper" **2** is shown in Scheme 1. Noncovalent self-assembly of the electron poor naphthalene diimide with electron rich crown ethers such as DNP38C10 has been well studied,[18] particularly by Sanders[6f,19] and co-workers. The interaction between NDI derivatives and DNP38C10 ($K_a \approx 10^2\ M^{-1}$)[19h] is not typically as strong as the interactions between dibenzylammonium guests with

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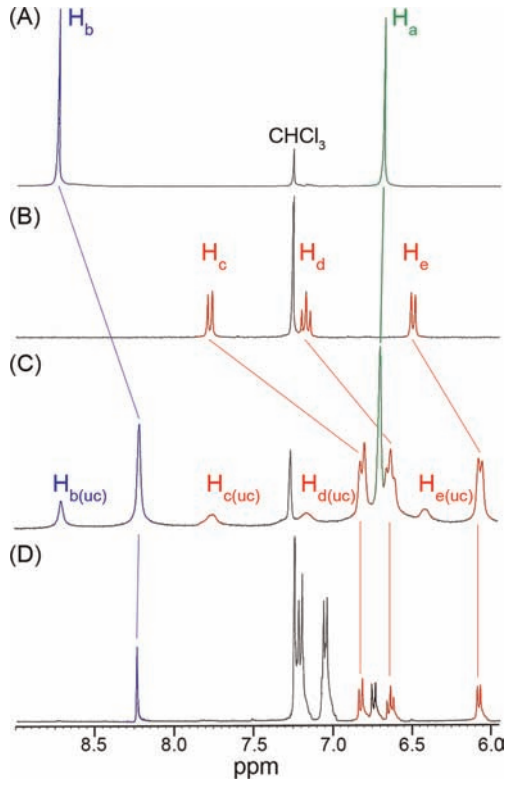

Figure 1. Partial ^{1}H NMR spectra (298 K unless otherwise noted) of thread **1** (A), macrocycle **3** (B), [2]pseudorotaxane **4** (C) (263 K), and [2]rotaxane **5** (D). Protons are labeled as in Scheme 1. Uncomplexed peaks are designated as "(uc)".

dibenzo-24-crown-8 hosts ($K_a = 10^2$–10^4 M^{-1})[20] or those between bipyridinium derivatives with DNP38C10 hosts ($K_a = 10^4$–10^5 M^{-1}).[21] NDI derivatives are, however, stable to base and therefore suitable for base catalyzed thiol-maleimide reactions whereas dialkylammonium and *N*-benzylbipyridinium guests are susceptible to deprotonation[6b] or nucleophilic attack,[22] respectively.

Mixing a 1:1 molar ratio of **1** and DNP38C10 macrocycle **3** in $CHCl_3$ at ambient temperature results in the instantaneous formation of a deep red solution. ^{1}H NMR spectroscopy of this solution (Figure 1C) indicated the formation of thermodynamically stable [2]pseudorotaxane **4** through changes in the chemical shifts of diagnostic proton signals H_b-H_e. At 300 MHz and 298 K dethreading of macrocycle **3** onto and off of thread **1** is comparable to the ^{1}H NMR time scale, leading to significant broadening of resonances for both compounds. A better resolved spectrum of [2]pseudorotaxane **4** is obtained at 263 K (Figure 1C) where the dethreading rate is decreased. Further evidence of a self-assembling host–guest complex is obtained from UV/vis spectroscopy (see Supporting Information). A prominent charge-transfer (CT) band is observed at 487 nm for a 0.001 M $CHCl_3$ solution of **1** and **3** ($\varepsilon = 143$ M^{-1} cm^{-1}).

Adding 2.2 equiv of thiol-functionalized stopper **2** to a 0.1 M $CDCl_3$ solution of [2]pseudorotaxane **4** at 273 K in the presence of 0.03 equiv of Et_3N results in the formation of mechanically interlocked [2]rotaxane **5** (Scheme 1B) in 65% isolated yield. The formation of mechanically interlocked [2]rotaxane **5** was confirmed (see Supporting Information) by accurate mass APCI mass spectrometric analysis: $m/z = 2653.28$ $[M + Na]^+$ and 1338.13 $[M + 2Na]^{2+}$ compared with calculated values of 2653.28 and 1338.14, respectively. It is interesting to note that the relative intensity of the $[M + 2Na]^{2+}$ peak of [2]rotaxane **5** is twice that of the $[M + Na]^+$ peak. Super- and supramolecular assemblies of NDI derivatives with DNP38C10 are known[6f,19h] to interact with alkali metals in solution. Mass spectrometric analysis of [2]rotaxane **5** suggests these interactions persist in the gas phase as well. Mass spectra of the macrocycle and thread components in isolation show weak relative intensities of $[M + 2Na]^{2+}$ peaks (see Supporting Information).

The ^{1}H NMR spectrum of **5** (Figure 1D) displays well-resolved, sharp peaks communsurate with a kinetically stable, interlocked species. Characteristic upfield shifts of protons H_c, H_d, and H_e (0.92, 0.52, and 0.42 ppm, respectively) are observed and are indicative[18b,d,19g] of $[\pi\cdots\pi]$ stacking interactions. An upfield shift of 0.52 ppm is also observed for the aromatic proton H_b of the NDI guest. The disappearance of maleimide proton H_a and the formation of new signals in the 3.2–2.5 ppm region indicate the formation of a thiol-maleimide Michael adduct. Investigation of [2]rotaxane **5** by UV/vis spectroscopy reveals a charge-transfer band at 497 nm with a molar extinction coefficient of $\varepsilon = 714$ M^{-1} cm^{-1}, which is consistent[18c,19e] with other interlocked crown ether–naphthalene diimide systems.

One of the primary motivations of this work is the development of efficient methods for integrating stimuli-resonsive interlocked molecules with biological systems.

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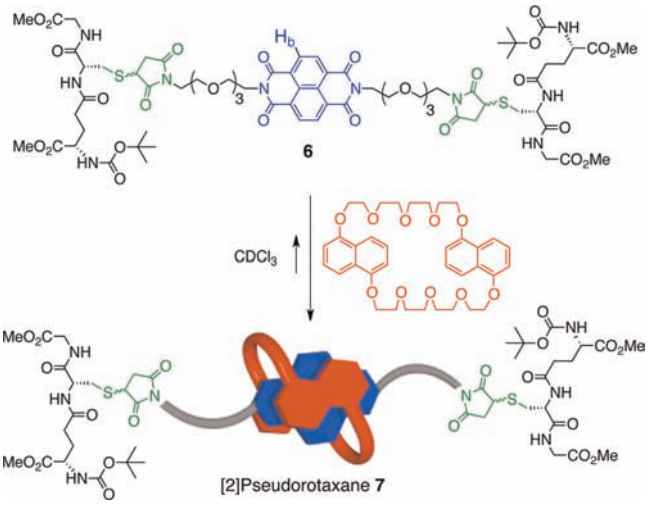
Scheme 2. Self-Assembly of *N*′-Boc-L-glutathione Dimethyl Ester-Functionalized [2]Pseudorotaxane **7**



As an initial, though important, first step toward this goal, glutathione-functionalized thread **6** (Scheme 2) was synthesized and its self-assembly with DNP38C10 was investigated. A solvent mixture capable of solubilizing both L-glutathione and macrocycle **3** could not be found; therefore *N*′-Boc-L-glutathione dimethyl ester,[23] which is soluble in a range of organic solvents, was prepared. Reacting this soluble glutathione derivative with thread **1** in $CHCl_3$ and catalytic Et_3N gave glutathione-functionalized thread **6**. Addition of 1.0 equiv of **3** to thread **6** in $CDCl_3$ resulted in a red solution indicating the formation of [2]pseudorotaxane **7** (Scheme 2). [2]Pseudorotaxane **7** can also be obtained in one pot by adding catalytic Et_3N to a solution of 1:1 thread **1** and DNP34C10 macrocycle **3**. UV/vis analysis of **7** (see Supporting Information) reveals a CT band at 492 nm (ε = 137 M^{-1} cm^{-1}). Diagnostic protons of thread **7** and macrocycle **3** were shifted upfield relative to their free components as observed by ^{1}H NMR spectroscopy (Figure 2A). Shifts of 0.51, 0.96, 0.53, and 0.37 ppm were observed for protons H_b, H_c, H_d, and H_e, respectively. Notably, signals corresponding to both complexed and uncomplexed species are well resolved in the ^{1}H NMR spectrum obtained at 298 K and broadening is essentially nonexistent at 268 K (Figure 2A). This contrasts sharply with the significant line broadening observed for [2]pseudorotaxane **4**. It is hypothesized that the bulkier glutathione moieties of thread **7** impose a higher free energy barrier to threading/dethreading in [2]pseudorotaxane **7** than the maleimide moieties of thread **1** in the case of [2]pseudorotaxane **4**. Furthermore, the greater kinetic stability of [2]pseudorotaxane **7** enabled analysis by ESI/APCI mass spectrometry which indicated a peak of m/z = 2305.88 $[M + Na]^+$ compared to a calculated value of 2305.87 (Figure 2B). Peaks for both

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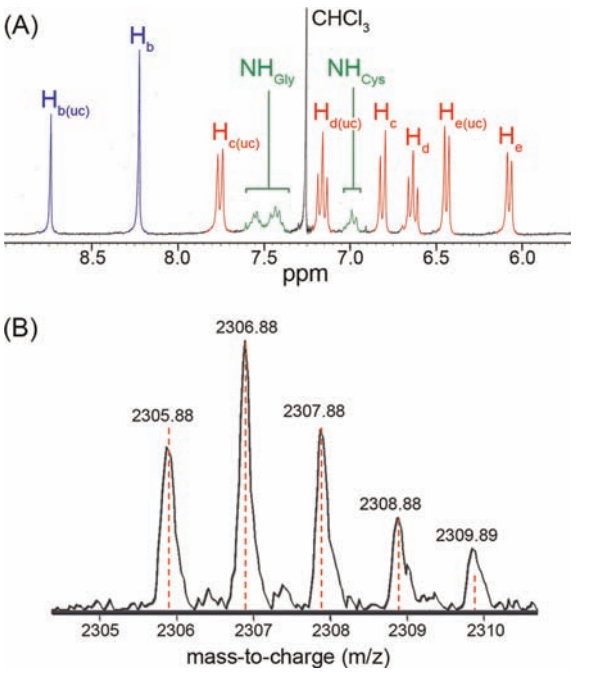

Figure 2. (A) Partial ^{1}H NMR spectrum ($CDCl_3$, 268 K) of glutathione-functionalized [2]pseudorotaxane **7**. Protons are labeled as in Scheme 1. Uncomplexed peaks are designated as "(uc)". (B) experimental (black solid line) and theoretical (dashed red lines) ESI/APCI-MS isotopic distribution of **7**.

macrocycle **3** and thread **6**, however, could also be observed in the same spectrum (see Supporting Information for the full ESI/APCI-MS).

The results reported here highlight the utility of thiol-maleimide click chemistry in the synthesis of mechanically interlocked molecules. Some of the greatest prospects for this chemistry stem from the potential to use thiol-maleimide click chemistry as a means to incorporate mechanically interlocked molecules into biological systems, opening the possibility of using stimuli-responsive interlocked molecules to impart allosteric control over the functions of proteins and enzymes. The demonstration of glutathione-functionalized [2]pseudorotaxane **7** represents a first step toward this goal. Another key step to extending the chemistry presented herein to the facile hybridization of interlocked molecules with biological systems will be the synthesis of aqueous-soluble derivatives of macrocycle **3**. We are currently working toward these goals in order to develop the chemistry of water-soluble protein−rotaxane conjugates.

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Supporting Information Available. Synthetic procedures, ^{1}H and ^{13}C NMR, mass spectroscopy, and UV/vis spectroscopy. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.