Chemical Topology

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Active-Metal Template Synthesis of a Molecular Trefoil Knot**

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Although many different approaches to catenanes and rotaxanes have been introduced,[1] few strategies have been successfully developed for the synthesis of molecular knots.[2] Trefoil knots, the simplest prime knot other than the topologically trivial unknot (i.e., any ring or simple macrocycle),[3] have been found in DNA,[4] proteins,[5] and in synthetic polymers.[6] Sauvage and co-workers prepared the first synthetic molecular knot by using the preorganization of two ligand strands around two tetrahedral Cu^I centers as the key template interaction to generate the three crossing points required for a trefoil knot.[7] Subsequently, donor–acceptor interactions,[8] Watson–Crick base pairing,[9] amide hydrogen bonding,[10] and ligand folding around an octahedral metal ion[11] have all been used to template the formation of molecular trefoil knots.[12]

A few years ago a strategy for the synthesis of rotaxanes and catenanes was introduced in which metal ions play a dual role, acting as a template to entwine or thread the building blocks while also actively catalyzing the bond-forming reaction that covalently traps the interlocked structure.[13] This "active-template" approach has proven to be an effective route to various types of mechanically interlocked molecules and can be applied by using an increasing number of different transition-metal-catalyzed reactions.[13j] Herein we report an active-template reaction that occurs through a loop generated through classical "passive-template" coordination to synthesize the smallest trefoil knot reported to date. The trefoil knot was characterized by ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, and by drift tube ion mobility mass spectrometry (DT IM-MS) experiments that show that the molecular knot has a significantly smaller cross-sectional area (with a narrower distribution) than the corresponding open-chain and unknot-macrocycle isomers.

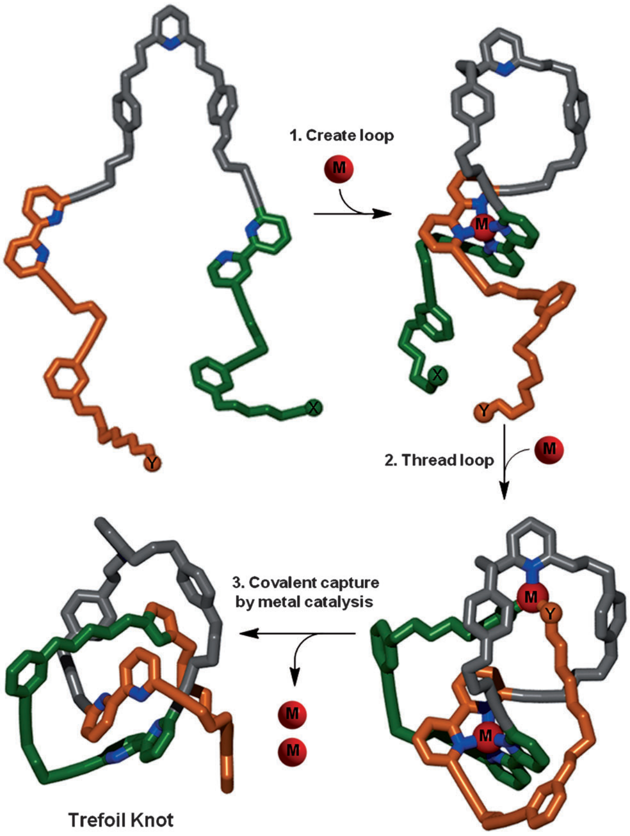

Figure 1. Schematic representation of the active-template synthesis of a molecular trefoil knot. A single-strand ligand with one monodentate and two bidentate binding sites (blue) and two functional end groups (X and Y) is knotted by the action of metal ions (red, M). Step 1: One metal ion creates a loop by coordination to the bidentate binding sites. Step 2: The other metal ion binds to the functional end groups and, through its preferred coordination geometry, threads the loop. Step 3: The knotted architecture is captured by metal-catalyzed covalent bond formation between X and Y.

To apply active-template synthesis to a trefoil knot architecture, we envisaged a system (Figure 1) in which a single molecular strand with reactive functional groups at each terminus (X and Y) could be geometrically manipulated and knotted through multiple interactions with metal ions (M). First, a loop in the strand would be formed by coordination of two bidentate binding sites in the strand to a tetrahedral metal ion (Figure 1, step 1). A second metal ion, bound endotopically within the loop by a monodentate ligating site, would then perform the twofold tasks of 1) gathering both functional end groups in a specific orientation that is dictated by the metal's preferred coordination geometry and places them on opposite sides of the loop (Figure 1, step 2), and 2) catalyzing a covalent-bond-forming reaction between the end groups to generate the molecular trefoil knot (Figure 1, step 3).

Ligand **1** (Scheme 1) was synthesized in nine steps from commercially available starting materials (for experimental

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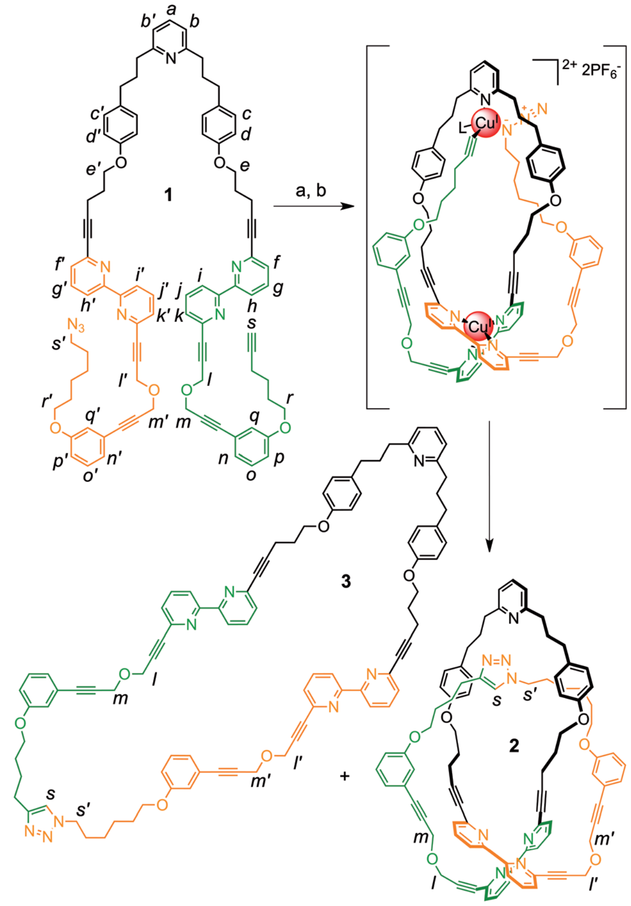

Scheme 1. Active-metal-template synthesis of trefoil knot **2**: a) $CHCl_3$/CH_3NO_2 (4:1), $[(CH_3CN)_4Cu]PF_6$ (1.5 mol per mol of **1**), 60 °C, 96 h; b) Na_2EDTA, NH_3. **2** 24 %, **3** 10 %. Trefoil knots are topologically chiral;[2,3] only one enantiomer of **2** is shown.

details see the Supporting Information). The single-strand molecule has three potential metal binding sites: two bipyridyl groups to create the loop (and one of the three required crossing points) by chelation to a tetrahedral Cu^I ion, and a 2,6-pyridine unit to bind the catalytically active metal center. The Cu^I-catalyzed azide–alkyne cycloaddition[14] (CuAAC) "click" reaction was chosen for the covalent-capture reaction that forms the remaining two crossing points, as it utilizes Cu^I ions (thus avoiding the complication of having different types of metal ions in the reaction) and because previous studies have shown this reaction to be highly effective in rotaxane- and catenane-forming active-template reactions.[13a,d,i,k,n,15] Molecular modeling[16] was used to estimate an appropriate length for the alkyl chain spacers between the functional end groups and the Cu^I binding sites.

We initially investigated reaction conditions for the active-metal-template knotting reaction of ligand **1** (Scheme 1) using dichloromethane, chloroform, and 1,2-dichloroethane, as these solvents had been employed in previously reported CuAAC active-template reactions.[13a,d,i,k,n] However, upon addition of $[Cu(CH_3CN)_4]PF_6$ to a dilute solution of **1** in any of these halogenated solvents, a precipitate formed immediately.[17] After screening a number of solvent mixtures, 4:1 chloroform-nitromethane was found to maintain the reactants and products in solution during the course of the reaction. An optimized concentration (1.5 mM) of **1**, with 1.5 molar equivalents[18] of $[Cu(CH_3CN)_4]PF_6$ at 60 °C, led to complete consumption of **1** after 4 days, as evidenced by the ^1H NMR spectrum of the crude reaction mixture.

After demetalation by washing with a basic ethylenediaminetetraacetic acid disodium salt/ammonia (Na_2EDTA-NH_3) solution,[13g] the products were purified using a combination of size-exclusion (SEC) and high-performance liquid (HPLC) chromatographies. SEC enabled facile removal of the oligomeric by-products and the resulting mixture was separated into its individual components by reverse-phase preparative HPLC. Two products were isolated, in 24 % and 10 % yields, both of which were shown to be isomers of the acyclic starting material **1** by high-resolution electrospray ionization-mass spectrometry (HRESI-MS; see Figure 2 caption and the Supporting Information). The isomer formed in lower yield (10 %) was identified as the simple macrocycle **3** (i.e., a cyclic structure with unknot topology) by comparison of its ^1H NMR spectrum (Figure 2 b) with that of the starting

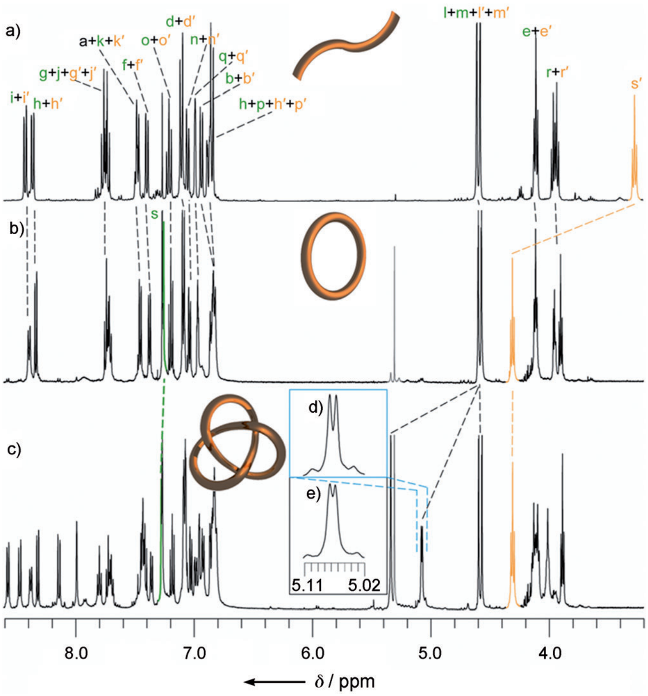

Figure 2. Partial ^1H NMR spectra (400 MHz, $CDCl_3$, 300 K) of three isomers (**1**, **2**, and **3**) of molecular formula $C_{88}H_{84}N_8O_6$: building block (**1**) and the two products (**2** and **3**) isolated from the reaction of **1** shown in Scheme 1. The signals of the $H_{s'}$ protons, associated with the -NCH_2- group (adjacent to the azide in **1** and the triazole ring in **2** and **3**), are shown in orange. The signals of the H_s protons, associated with the -CH- of the triazole ring in **2** and **3**, are shown in green. a) Open-chain building block **1**, HRESI-MS: m/z 1361.6613 $[M+H]^+$ (calcd for $C_{88}H_{85}N_8O_6$ 1361.6597). b) Unknot-macrocycle **3**, HRESI-MS: m/z 1361.6603 $[M+H]^+$. c) Trefoil knot **2**, HRESI-MS: m/z 1361.6601 $[M+H]^+$. Expansion of the region between $\delta = 5.11$ and 5.02 ppm of the d) 500 MHz and e) 400 MHz ^1H NMR spectra of **2** showing a propargylic methylene ($H_{l,l',m\,or\,m'}$) AB system ($J_{AB} = 13.2$ Hz). The protons are diastereotopic as a consequence of the chirality of the trefoil knot. The lettering corresponds to that shown in Scheme 1.

material **1** (Figure 2a). The spectrum of the flexible 76-membered-ring macrocycle (**3**) is very similar to that of the open-chain isomer (**1**), the only significant differences being the shift of the $H_{s'}$ (-NCH_2-) and H_s protons (the latter from $\delta = 2.0$ ppm in **1** to $\delta = 7.25$ ppm in **3**) following conversion of the azide and terminal alkyne to the triazole ring.

The isomer isolated in greater yield (24 %) was confirmed as the trefoil knot **2** through a series of NMR, mass spectrometry, and DT IM-MS experiments. The ^{1}H NMR spectrum of **2** (Figure 2c) is very different to those of its open-chain **1** and unknot-macrocycle **3** isomers (Figure 2a and b). Many of the resonances in the aromatic region of **2** are separated into two sets of inequivalent signals. The increase in the overall number of resonances observed is a result of the loss of the pseudosymmetry of **1** (and **3**) upon formation of the conformationally contorted trefoil knot structure—the 76-atom entwined loop is the smallest knot reported to date[11c]—and molecular modeling[16] and DT IM-MS results (see below) indicate it to be tightly wound. Two of the propargylic methylene resonances (two of $H_{l/l'/m/m'}$) are shifted significantly downfield in **2** compared to the unknot-macrocycle **3** (from $\delta = 4.59$ ppm to $\delta = 5.33$ and 5.07 ppm). It appears that these protons spend a significant amount of time edge-on to an aromatic ring in low-energy conformations of the knot, and are deshielded through ring current effects. Notably, the propargylic methylene resonance at $\delta = 5.07$ ppm appears as an AB system (Figure 3d,e), thus indicating that the protons are diastereotopic.[19] This behavior is a consequence of the inherent chirality of a trefoil knot.[11d,20]

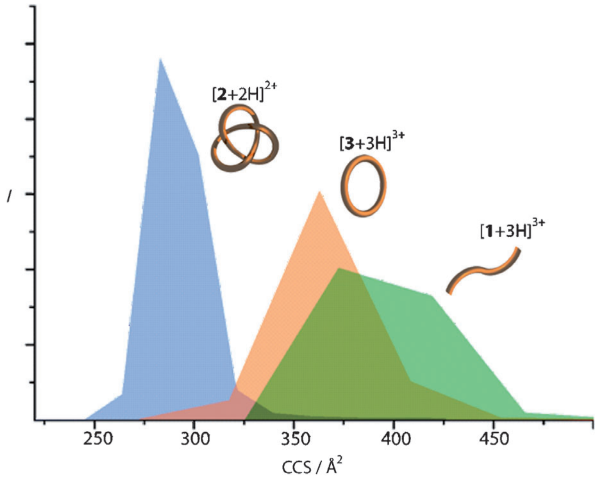

Figure 3. DT IM-MS spectra of building block **1** (green, $[M+3H]^{3+}$), and products **2** (blue, $[M+2H]^{2+}$) and **3** (orange, $[M+3H]^{3+}$) isolated from the reaction of **1** shown in Scheme 1. The CCS area distributions were calculated from the measured arrival times for the highest observed charge state of each isomer. Data shows the arrival times at a drift voltage of 50 V. Intensities (*I*) are normalized to the peak areas. Ligand strand **1**, which is expected to have a large degree of flexibility, exhibits the broadest distribution and largest CCS. Unknot-macrocycle **3** has a smaller CCS and narrower distribution and trefoil knot **2** displays the smallest CCS and the narrowest distribution, reflecting its compact structure and persistent size and shape.

Further insight into the structure of trefoil knot **2** was provided by drift tube ion mobility mass spectrometry (DT IM-MS). In these experiments, the velocity with which an ion travels through a cell containing a buffer gas (commonly helium), under the influence of a weak electric field, depends on the collision cross section (CCS) of the ion with the buffer gas (averaged over all possible orientations of the ion).[21,22] A larger ion with few conformational restrictions takes longer to traverse the drift cell, undergoing more collisions with the buffer gas, than a smaller, more compact, structure. This behavior has previously been demonstrated for naturally occurring antimicrobial peptides[21] and for synthetic cyclic and linear peptides.[22] With low charge numbers, flexible molecules may wrap tightly around the charged regions in order to solvate them with heteroatoms and aromatic rings. However, as the number of charges on a molecular ion increases, the size of the adopted conformations increases as electrostatic repulsions try to force the largest distance between the charges that the molecule will allow. In general, the observed CCS increases with the amount of charge that a flexible structure carries. In addition to the magnitude of the CCS, which gives information about the size of the molecular ion, the broadness of the distribution indicates the flexibility (the number of differently sized and shaped conformations adopted) of the molecular structure.

Following nanoelectrospray ionization, DT IM-MS showed significant differences in the rotationally averaged CCS areas of ions of the three isomers **1**–**3** (Figure 3).[23] The largest average CCS areas of the open-chain isomer **1** ($[\mathbf{1}+3H]^{3+}$ ion) and unknot-macrocycle **3** ($[\mathbf{3}+3H]^{3+}$ ion) were (395 ± 3.5) Å^2 and (368 ± 5.3) Å^2, respectively. The largest average CCS area observed for the trefoil knot **2** ($[\mathbf{2}+2H]^{2+}$ ion) was (292 ± 1) Å^2. Therefore for the highest charge state observed for each species, open-chain isomer **1** has a larger molecular cross-section than unknot-macrocycle **3** which, in turn, has a much larger cross-sectional area than trefoil knot **2**. Furthermore, the open-chain isomer **1** has the broadest CCS distribution, followed by the unknot-macrocycle **3**, with the trefoil knot **2** having the narrowest range. These results indicate that the trefoil knot has a much more compact and inflexible structure than the unknot-macrocycle, which is more compact and less flexible than the acyclic strand. Calculations of the expected CCS values from the Spartan-minimized[16] structures of the most extended form of each molecular species support the observed experimental trend (see the Supporting Information).

In conclusion, we have demonstrated an active-template approach to the synthesis of molecular knots based upon the cooperative manipulation of a ligand with reactive end groups by two metal ions. One of the metal centers creates a loop in the ligand whilst the other catalyzes a covalent-bond-forming reaction that links the end groups through the cavity. The resulting trefoil knot and its unknot and acyclic isomers were characterized by NMR spectroscopy, mass spectrometry, and DT IM-MS experiments. The latter technique is able to discriminate between the isomers through both the size and relative flexibility of their multiply charged molecular ions. Active-template strategies for entangling molecular chains, and novel methods for the structural characterization of the resulting products,[24] may prove useful for the synthesis of other complex molecular structures.

Experimental Section

Active-template trefoil knot synthesis: In a typical procedure, a solution of $[(CH_3CN)_4Cu]PF_6$ (14.7 mg, 39.4 μmol) in CH_3NO_2 (2.5 mL) was added to a solution of **1** (35.8 mg, 26.3 μmol) in $CHCl_3$ (14 mL) and CH_3NO_2 (1.0 mL), and the reaction mixture was heated at 60°C for 96 h. The solution was allowed to cool to RT and diluted with CH_2Cl_2 (30 mL). A 17.5% aqueous solution of NH_3 saturated with Na_2EDTA (30 mL) was added and the mixture stirred vigorously for 30 min. The phases were separated and the organic phase was further extracted with a 17.5% aqueous solution of NH_3 saturated with Na_2EDTA (30 mL), H_2O (30 mL), and brine (30 mL), then dried ($MgSO_4$) and concentrated under reduced pressure. The resulting residue was purified by size-exclusion chromatography (CH_2Cl_2 mobile phase) followed by preparative HPLC (reverse-phase column, gradient elution: 1) MeOH with 5→0% H_2O, 2) MeOH with 0→10% CH_2Cl_2 to give trefoil knot **2** as a colorless film (8.6 mg, 24%) and macrocycle **3** as a yellow film (3.5 mg, 10%). Full details of experimental procedures and compound characterization are given in the Supporting Information.

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[17] Upon adding $[(CH_3CN)_4Cu]PF_6$ to a solution of **1** in chloroform a red, gummy solid formed instantaneously. The solid was insoluble in all common laboratory solvents and was thus assumed to be a result of rapid polymerization.

[18] The use of less than a stoichiometric amount of copper is to try to minimize the amount of Cu^I ions not coordinated to **1**. “Free” Cu^I ions could catalyze the CuAAC reaction without the end groups passing through the ligand strand loop, thus generating unknot macrocycle **3**. Cu^I ions can turn over during active-template CuAAC reactions [Ref 13 a,d,i,l] and therefore stoichiometric amounts of the metal are unnecessary.

[19] The assignment is confirmed as an AB system by a coupling constant value ($J_{AB} = 13.2$ Hz) that is unchanged when measured at 500 MHz (Figure 2 d) and 400 MHz (Figure 2 e), and ^1H–^1H COSY experiments that show that the two protons coupled in the signal are not coupled to other protons in the molecule.

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[23] For all three isomers (**1**–**3**) the $[M+2H]^{2+}$ ion was the dominant charge state observed in the DT IM-MS experiments (see the Supporting Information). The absence of the $[M+3H]^{3+}$ species for the trefoil knot may be indicative of a compact conformation unable to support the electrostatic repulsions between three protons.

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