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The Thread & Cut Method: Syntheses of Molecular Knot Precursors

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Dedicated to Professor Steven C. Zimmerman on the occasion of his 50th birthday

Keywords: Chemical Topology / Chirality / Macrocycles / Molecular knots / Synthesis design

A novel approach to molecular knots is described. This method may allow access to smaller and more complex knots. Two knot precursors, **1a** and **1b**, are efficiently prepared in overall yields of 9.6 % and 8.7 %, respectively. The convergent six-step syntheses utilize Fréchet-type etherifications, alkyne/azide click cycloadditions, and bis-macrolactonizations.

Introduction

There have been many notable achievements in chemical topology,[1,2] including the syntheses of molecular trefoil[3] and composite knots,[4] Solomon[5] and Borromean links,[6] and a Möbius strip.[7] In the DNA realm,[8] more complex topologies have been achieved, including the figure eight[9] and endless knots.[10] Proteins also form knots,[11] but many of these are not closed loops, so topologically they are not true knots.[12]

In the field of small-molecule topology, many open problems remain. Principal among these are: 1) what is the smallest knot that can be prepared? and 2) can prime knots more complex than the trefoil be prepared? Addressing these questions may help the development of new nanotechnologies.

Regarding the smallest knot that can still physically form, Wasserman used models to predict that a polyethylene trefoil requires at least 50 backbone atoms.[1] More recent calculations, however, suggested that the stability boundary for this knot may be 43 atoms or fewer.[13] The smallest known knot is an 80-atom trefoil prepared by Sauvage.[14] In terms of complexity, if one discounts DNA knots, the only prime knots to be deliberately prepared in molecular form are the unknot and trefoil (Figure 1). Synthetic efforts and strategies toward the next two prime knots, the figure eight[16] and pentafoil,[17] have been published, but these targets remain elusive.

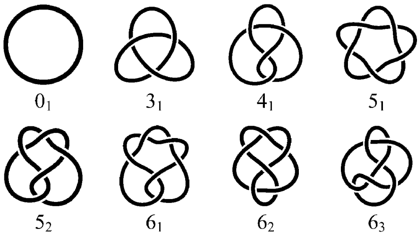

Figure 1. The first eight prime knots: unknot (0_1), trefoil (3_1), figure eight (4_1), pentafoil (5_1), three twist (5_2), and the six-crossing knots (6_1, 6_2, 6_3). The unknot and trefoil are the only non-DNA molecular examples deliberately synthesized. Billions of prime knots have been catalogued by mathematicians.[15]

Results and Discussion

Our thread & cut strategy[18] may allow smaller and more complex knots (i.e., the figure eight) to be prepared. The approach centers around knot precursor **1**, which is composed of two macrocycles and two tails (Figure 2). Key features of **1** include: 1) a fluorescent 2,5-dihydroxyterephthalic acid core[19] (blue ring) that exhibits an intense blue color under UV irradiation ($\lambda_{\max}$ = 334 nm); 2) a highly efficient and convergent synthetic route (vide infra) that employs etherifications (alkylations of green and blue rings) and an alkyne/azide click cycloaddition[20] to yield triazoles (red rings); 3) the ability to adjust the size of the knots produced simply by varying the length of linker 1, linker 2, or the tail; 4) robust functionality, except for purposely labile esters; and 5) the fact that only one bond is formed in the proposed knot-tying step, producing only isomeric products, not homologues as with other strategies.

The proposed knot-tying step is a ring-closing olefin metathesis[21] (RCM) of **1** under high dilution, during which threading of the tails through the macrocycles can occur.

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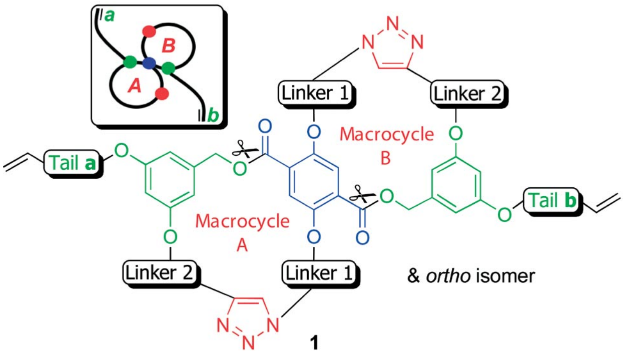


Figure 2. Chemical and schematic (inset) structures of the generalized form of molecular knot precursor **1**. The length of linkers and tails can be varied to place constraints on the RCM knot-tying step. The scissors indicate the ester bond to be hydrolyzed (cut) after the knot-tying step, see text and Table 1.

After the RCM, the labile ester bonds are hydrolyzed (cut) to give the knot products. In this system, *productive* threading occurs when a tail threads through the opposing macrocycle;[22] e.g., tail **a** threads through macrocycle **B** (Figure 2, Table 1). The *meta* isomers[19] of knot precursors **1a** and **1b** (Scheme 2) each contain two 27-atom macrocycles. Compound **1a** has shorter tails (13 atoms) and CPK models suggest that steric constraints limit intramolecular products to the unknot and trefoil (Table 1). The trefoil would result if tail **a** threads top-down through macrocycle **B** and tail **b** threads top-down through macrocycle **A** followed by RCM and ester cleavage. The enantiomeric trefoil results if both threading events occur in a bottom-up manner. In **1a** the tails are too short to reach each other if they thread from opposite sides. CPK models suggest that the longer 22-atom tails of **1b** *do* permit RCM after threading from opposite sides of the macrocycle. This latter mode of threading would result in the figure eight knot after RCM and ester cleavage (Table 1). This knot is a particularly attractive target because of its unusual stereochemistry – it is a topological rubber glove.[23,24] This term, first introduced by Walba,[23] denotes a compound that contains no rigidly achiral representation but has a chiral enantiomerization pathway making it topologically achiral.[25,26]

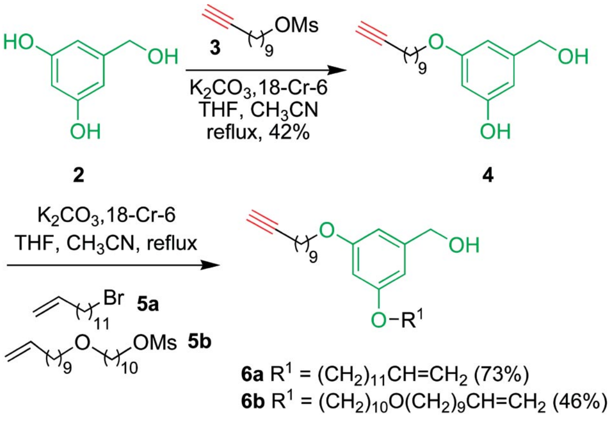
Table 1. Representative thread & cut outcomes for proposed RCM-ester hydroylsis reactions of knot precursors **1**.

Threading type[a,b]		Resulting knot after cutting
Tail **a**	Tail **b**	
none	none	unknot (0_1)
top-down **B**	none	unknot (0_1)
top-down **B**	top-down **A**	trefoil (3_1)
top-down **B**	bottom-up **A**	figure eight (4_1)

[a] Mode of tail threading through macrocycles **A** and **B**, see Figure 2.
[b] Degenerate and enantiomeric threading are not listed.

The preorganization of **1** should aid knot formation; e.g., RCM of **1a** only requires formation of a 42-atom macrocycle from which, after cutting, a 72-atom trefoil results. Furthermore, even though knot formation in polymers[27] and ropes[28] is spontaneous, conducting the RCM in a solvent in which **1** has low solubility, should give rise to solvophobic interactions which may in turn promote threading and increase the probability of knotting.

The synthetic routes commence with Fréchet-type etherifications. Alkylation of 3,5-dihydroxybenzyl alcohol (**2**) with one equivalent of mesylate **3** provided the alkyne **4** in good yield (42%), considering the symmetry of **2** is broken (Scheme 1). A second etherification with the bromide **5a** or the mesylate **5b** provided the eneynes **6** in modest yields.

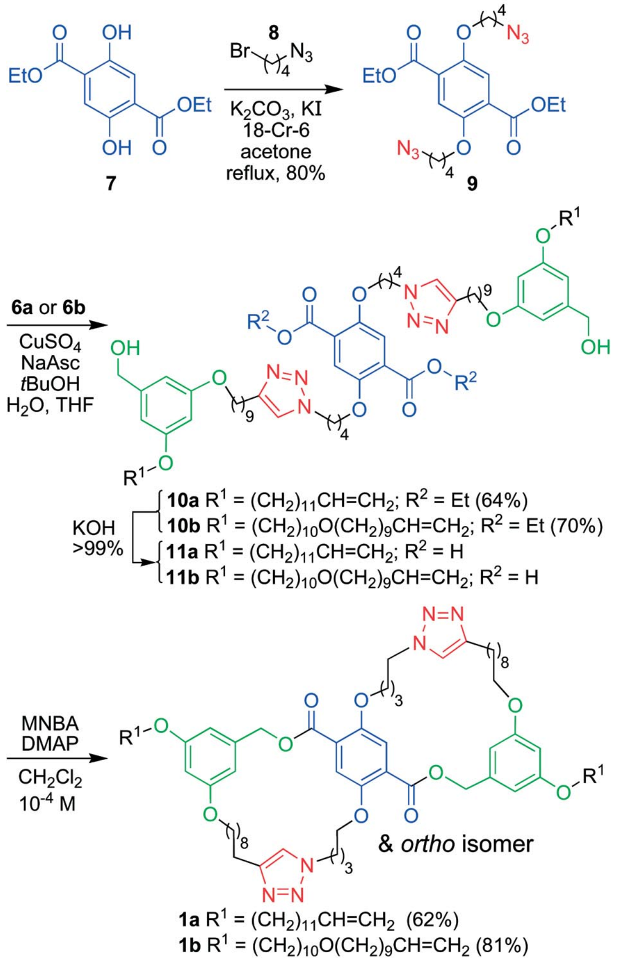


6a $R^1 = (CH_2)_{11}CH{=}CH_2$ (73%)
6b $R^1 = (CH_2)_{10}O(CH_2)_9CH{=}CH_2$ (46%)

Scheme 1. Syntheses of the eneynes **6**.

Preparation of the diazide **9** proceeded smoothly upon etherification of diethyl 2,5-dihydroxyterephthalate (**7**) with the bromide **8** (Scheme 2). A copper-catalyzed alkyne/azide click cycloaddition of **6** and **9** provided the bis-triazoles **10** in moderate yields. The IR spectra of **10** lacked the alkyne (2118 cm^{-1}) and azide (2093 cm^{-1}) absorptions of **6** and **9**, respectively, and the ^1H NMR spectra showed a new aromatic resonance (δ = 7.33 ppm) for the triazole rings. Saponification of **10** provided the diacids **11** in quantitative yields. Finally, the knot precursors **1** were furnished in moderate to good yields via bis-macrolactonization of **11** using Shiina's mixed-anhydride method under high dilution.[29] Evidence for the structure of **1** includes the downfield shift of the benzylic protons in the ^1H NMR spectra by ca. 0.66 ppm, in agreement with ester formation. The electrospray mass spectra also support the structures with molecular ions at 1297.9 and 1555.2 Da for **1a** and **1b**, respectively. Furthermore, for the first time in the syntheses, the NMR spectra of the purified products indicated a mixture of two isomers, as expected. Integration of the benzylic proton peaks indicated a 64:36 isomer ratio for **1a** and a nearly identical ratio for **1b**. The isomers proved to be inseparable and it is unknown whether the *ortho* or *meta* isomer is the major product.[19]

Preliminary experiments on the thread & cut sequence have produced noteworthy results. For example, RCM of **1b** with Grubb's first generation catalyst (10^{-4} M in acetone) followed by ester hydrolysis produced two products. Preparatory TLC purification gave 3.8 mg (2.5%, two steps) of the less polar product and 6.3 mg (4.5%, two steps) of the more polar product. Negative ion electrospray mass spec-



Scheme 2. Syntheses of the knot precursors **1**.

trometry indicated that the products were isomeric, with each giving rise to a molecular ion (M – 1) peak at 1560.9 Da. The molecular ion isotope pattern excluded the possibility of a doubly charged dimer. IR and ^{1}H NMR spectroscopic data of the products were quite similar to each other but some distinct differences were noted. Peaks for *E* and *Z* internal alkenes were observed in both isomers. One possible interpretation of these data is that one of the products is the unknot (macrocycle) and the other is either the trefoil or figure eight knot.[30] When **1a** was subjected to a similar RCM/ester hydrolysis sequence, five products were isolated from a preparatory TLC plate. Electrospray mass spectrometry showed that the products were isomeric (M + 1 peak at 1306 Da) and the IR and ^{1}H NMR spectroscopic data were quite similar to each other with all showing peaks for both *E* and *Z* internal alkenes. Further structure elucidation experiments on the thread & cut products from precursors **1a** and **1b** are in progress.

Conclusions

A novel approach to molecular knots is described. The bis-macrocyclic knot precursors **1a** and **1b** are efficiently prepared via convergent six-step syntheses with overall yields of 9.6% and 8.7%, respectively. Preliminary results on the RCM/ester hydrolysis sequence suggest that this strategy may afford products with non-trivial topologies. The method may allow access to smaller knots; e.g., precursor **1a** would produce a 72-atom trefoil, 10% smaller than the current record.[14] The knot precursor **1b** may allow access to a figure eight knot. A non-DNA version of this knot would be the first example of a chemically achiral molecular knot that is also a topological rubber glove.[23,24] Further characterization of the products from the thread & cut experiments are ongoing and will be reported in due course. Meanwhile, second-generation iterations of the method are also being pursued. These systems encourage tail threading by employing more rigid macrocycles, which should maintain an open conformation, and incorporation of molecular recognition elements between the tail and the macrocycle.

Experimental Section

Diethyl 2,5-Bis(4-azidobutoxy)terephthalate (9): A mixture of diethyl 2,5-dihydroxyterephthalate (**7**) (1.00 g, 3.9 mmol), 1-azido-4-bromobutane[31] (**8**) (2.134 g, 12.0 mmol), potassium carbonate (3.34 g, 24.2 mmol), potassium iodide (49 mg, 0.30 mmol), 18-crown-6 (32 mg, 0.12 mmol) and dry acetone (30 mL) was heated at 70 °C for 27 h. The reaction mixture was cooled and concentrated under reduced pressure. The mixture was then partitioned between EtOAc and water. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine (1×), dried (Na_2SO_4), and concentrated under reduced pressure. The resulting brown oil was purified by flash chromatography (SiO_2, 10% EtOAc/PE) to afford 1.407 g (80%) of **9** as a pale yellow oil. The product was crystallized as colorless needles by cooling an ethereal solution that had been layered with petroleum ether (PE) to –30 °C in a freezer; m.p. 37–38 °C. $C_{20}H_{28}N_6O_6$ (448.48): calcd. C 53.56, H 6.29, N 18.74; found C 53.63, H 6.03, N 18.60.

Click Cycloaddition Product 10b:[20] Sodium ascorbate (77 mg, 0.39 mmol) was added to a mixture of **6b** (904 mg, 1.51 mmol), **9** (334 mg, 0.74 mmol), copper sulfate pentahydrate (19.6 mg, 0.078 mmol), THF (12 mL), *tert*-butyl alcohol (5 mL), and water (5 mL). The mixture was stirred vigorously at ambient temperature for 23 h, after which time additional copper sulfate pentahydrate (28 mg, 0.11 mmol) and sodium ascorbate (88 mg, 0.44 mmol) were added and the mixture was stirred for an additional 3 h. The reaction mixture was then diluted with water, Et_2O, and EtOAc. The organic layer was collected and the aqueous layer was extracted with Et_2O. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by flash chromatography (SiO_2, compound loaded as a CH_2Cl_2 solution: 30→100% EtOAc/PE) afforded 859 mg (70%) of **10b** as a waxy white solid; m.p. 48.5–50.5 °C. $C_{98}H_{160}N_6O_{14}$ (1646.37): calcd. C 71.49, H 9.80, N 5.10; found C 71.63, H 9.70, N 5.13.

Terephthalic Acid Derivative 11b: A solution of KOH (214 mg, 3.82 mmol) in water (2 mL) was added to a solution of **10b** (134 mg, 0.081 mmol) in THF (6 mL) and ethanol (1 mL). The resulting solution was heated at reflux for 2 h, cooled to ambient temperature, and diluted with saturated aqueous ammonium chloride, Et_2O, and EtOAc. The organic layer was collected, the aqueous layer was made acidic with 0.5 M HCl and then extracted with

CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to give 132.9 mg (quantitative) of **11b** as a white solid that was a single spot via TLC (10% MeOH/CH_2Cl_2); m.p. 66.5–68 °C.

Knot Precursor *o*-1b & *m*-1b: A solution of **11b** (109 mg, 0.069 mmol) in CH_2Cl_2 (150 mL) was added to a mixture of DMAP (58 mg, 0.47 mmol) and 2-methyl-6-nitrobenzoic anhydride[29] (71 mg, 0.21 mmol), 4-Å molecular sieves, and CH_2Cl_2 (150 mL) over a period of 100 min. The resulting mixture was stirred at ambient temperature for 45 h. The reaction mixture was then concentrated under reduced pressure and the product was purified by flash chromatography (SiO_2, 5% MeOH/CH_2Cl_2) to yield 86.3 mg (81%) of ***o*-1b & *m*-1b** as a white solid. On the basis of ^{1}H NMR integrations of the benzylic protons, the ratio of the two isomers is approximately 63:37. The isomers proved to be inseparable and it is unknown whether the *ortho* or *meta* is the major isomer; m.p. 126–127 °C.

Supporting Information (see also the footnote on the first page of this article): Experimental procedures and full characterization data for all new compounds.

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[19] The 2,5-dihydroxyterephthate core leads to *ortho* and *meta* isomers of **1**. However, both isomers are capable of undergoing threading. Furthermore, after ester hydrolysis, both the *ortho* and *meta* isomers of a particular topological isomer yield a single product.

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