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Rotaxanes of Cyclic Peptides

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Until recently the existence of mechanically interlocked peptide backbones in nature was far from clear-cut. However, in the past few years, first knots,[1] then catenanes[2] and, finally, rotaxanes[3] have been unambiguously characterized in naturally occurring peptides and proteins.[4] Although the implications of kinetically stable entanglements on peptide and protein structure, properties, function, and folding remain almost completely unknown, already studies on the few interlocked peptides available have shown them to possess a wealth of intriguing properties, including high resistance to peptidases,[5a] unique modes of antimicrobial and antiviral action,[5b−d] impressive membrane transport characteristics,[5e] and stability to thermal and chemical denaturing.[5f] However, few artificial or simpler analogues have been investigated to date since no methodology yet exists for synthetically interlocking peptide fragments.[6,7] En route to this goal, here we describe the preparation of rotaxanes from a class of well-known cyclic peptides and some nonpeptidic threads.

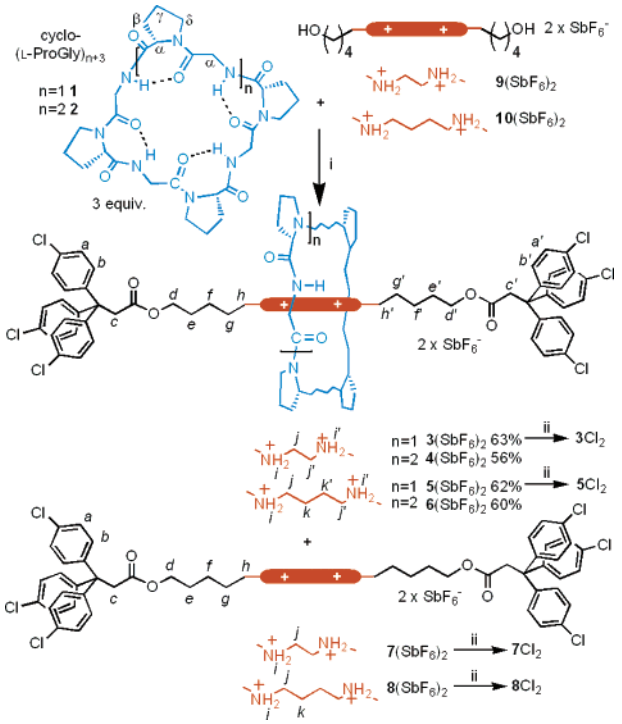
Efficient synthetic routes to catenanes and rotaxanes generally rely on strong-binding mutual recognition elements on each component to direct a threading or macrocyclization reaction.[8] The problem with applying such a strategy to peptides is that the amide groups of each free component will normally be largely self-satisfied in terms of hydrogen bonding through folding, and little driving force will exist for interlocking. Indeed, several attempts[3b,c] to synthesize microcin J25, a 21-residue oligopeptide with a rotaxane architecture, by conventional peptide synthesis strategies have failed. However, many natural and unnatural cyclic peptides are known to bind cations efficiently. Crown ether[9] and cucurbituril[10] organic cation binding provides some of the most effective rotaxane template syntheses known, and we wondered whether a similar interaction could also be used to promote rotaxane formation with peptidic macrocycles. As prototypical systems we investigated cyclic octa- (**1**) and deca (**2**)-peptides derived from the L-ProGly repeat unit.[11] High stability constants ($K_a \approx 10^3-10^5$ M^{-1}) have been reported for 1:1 metal and 1:1 and 1:2 protonated amino acid ester complexes of these cyclopeptides in acetonitrile.[11c] Accordingly, we constructed a series of cationic diol threads and examined their efficacy in rotaxane-forming "stoppering" reactions with a bulky acid chloride and macrocycles **1** and **2** (Scheme 1).[12]

Cyclo(L-ProGly)$_4$ (**1**) and cyclo(L-ProGly)$_5$ (**2**) were prepared via a solid-phase backbone amide-linking approach (see Supporting Information (SI)). As they are water soluble, a simple aqueous extraction removes either macrocycle from a rotaxane-forming reaction mixture, and they can easily be recycled. In contrast, the chromatographic separation of the [2]rotaxanes from the corresponding threads is rather difficult, and it therefore proved convenient to use an excess of the cyclic peptide to maximize the ratio of rotaxane to thread. Although only traces of interlocked products were formed with monocationic ammonium or pyridinium threads, we were delighted to find that [2]rotaxanes **3**−**6**(SbF$_6$)$_2$ were formed in 56−63% yield from ethane-1,2-diammonium and butane-1,4-diammonium templates.[12]

The ^{1}H NMR spectra (Figure 1) of the cyclo(L-ProGly)$_4$-based [2]rotaxanes **3**Cl$_2$ and **5**Cl$_2$ and their free components (threads **7**Cl$_2$ and **8**Cl$_2$ and macrocycle **1**) provide some insights into the structure of the rotaxanes and the nature of the template interaction. Free cyclo(L-ProGly)$_4$ exists as a mixture of rotamers in acetonitrile,[11c] ~30% in the all-trans conformer form (shown in light blue in Figure 1c) in which four γ-turn Gly-to-Gly H-bonds give rise to a relatively planar structure with the glycine carbonyls pointing toward the center and the proline carbonyls directed away. However, a single set of signals is observed for each constitutionally distinct proton of the cyclic peptide in both **3**Cl$_2$ (Figure 1b) and **5**Cl$_2$ (Figure 1d). Cyclo(L-ProGly)$_4$ is known[11c] to adopt a geometry in 1:2 host−guest ammonium cation complexes which is also an all-trans rotamer, but is cylindrical rather than flat, with the glycine carbonyls rotated toward one face and the proline carbonyls to the other, destroying the internal H-bond network. The ^{1}H and ^{13}C (see ref 11c) NMR are consistent with a similar structure existing in rotaxanes **3**Cl$_2$ and **5**Cl$_2$. Rapid spinning of the thread in the cavity (the macrocycle resonances remain symmetrical even at 240 K in CD$_3$CN) enables each of the peptide carbonyls to interact with the two ammonium groups through an alternating network of hydrogen bonds and electrostatic C═O$^{\delta-}$···N$^+$ interactions.

Confirmation of the change in conformation of cyclo(L-ProGly)$_4$ upon rotaxane formation is seen in the changes in the resonances of the glycine methylene groups. The geminal GlyH$_\alpha$ protons in

Scheme 1. Synthesis of Cyclic Peptide [2]Rotaxanes **3**-**6**(SbF$_6$)$_2$[a]



[a] Reagents and conditions: i. (4-ClC$_6$H$_4$)$_3$CCH$_2$COCl, CHCl$_3$:CH$_3$CN 3:7, RT, 7 days. ii. sat. NaHCO$_3$, then 1 M HCl. For clarity, atom labels are only shown for one repeat unit of the cyclopeptide in [2]rotaxanes **3**−**6**(SbF$_6$)$_2$

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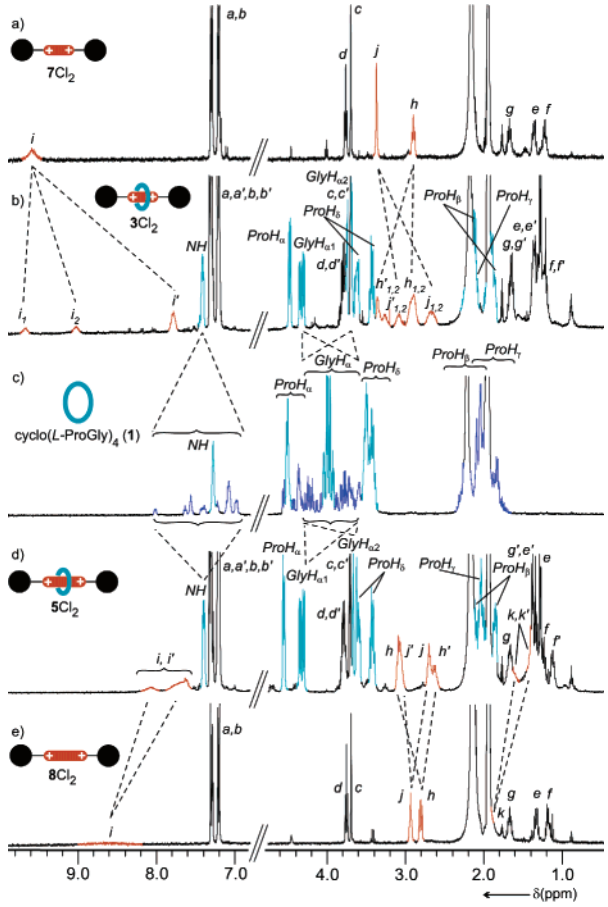

Figure 1. ^{1}H NMR spectra (400 MHz, CD_3CN, 298 K) of (a) ethane-1,2-diammonium thread **7**Cl_2; (b) [2]rotaxane **3**Cl_2; (c) cyclo(L-ProGly)$_4$ (**1**); (d) [2]rotaxane **5**Cl_2; (e) butane-1,2-diammonium thread **8**Cl_2. The labeling corresponds to that shown in Scheme 1. The resonances of the all-trans rotamer of cyclo(L-ProGly)$_4$ are shown in light blue, and those from minor rotamers, only present in part (c), in dark blue.

the H-bonded all-trans rotamer of free **1** are in similar proximities to the shielding region of the adjacent proline carbonyl groups and appear at similar chemical shifts (Figure 1c). However, in both **3**Cl_2 and **5**Cl_2 H-bonding of the glycine carbonyls to the ammonium groups rotates the NHCO groups, causing the positions of the geminal methylene protons relative to the adjacent proline carbonyls to differ such that the four Gly$H_{\alpha 1}$ proton resonances are shifted *downfield* by 0.35 ppm, whereas the four Gly$H_{\alpha 2}$ proton resonances are shifted *upfield* by 0.30 ppm. Despite the loss of the internal H-bonding network, the amide resonances still experience a slight net upfield shift in the rotaxanes as a result of the inductive effect from the strong interaction of glycine carbonyls with the ammonium groups.

In the ethane-1,2-diammonium rotaxane, **3**Cl_2, it is clear that one ammonium group ($H_{i'}$, Figure 1b) H-bonds principally to the glycine carbonyls and one (H_{i1} and H_{i2}, Figure 1b) to the proline carbonyls. The greater shielding of the $H_{i'}$ protons (indicative of stronger H-bonding) is, again, consistent with the previous studies[12c] on cyclo(L-ProGly)$_4$−ammonium ion host−guest complexes. The fact that the $-CH_2N^+-$ protons internal to the template (H_j) are shielded in the rotaxane compared to the thread, while those external to the template (H_h) are deshielded, indicates that each ammonium group is largely H-bonded from just one direction, with the macrocycle located over the central ethane group.

While the shifts in the $-H_2N^+-$ signals in rotaxane **5**Cl_2 are less informative, the internal and external $-CH_2N^+-$ groups are both split into shielded and deshielded resonances, indicating that H-bonding with the longer template occurs to each ammonium group from both directions (obviously not simultaneously). In other words, the cyclopeptide is loosely held in **5**Cl_2 and able to access the full length of the thread.

The ability of cation−amide interactions to disrupt internal amide−amide H-bonding networks augurs well for their use to overcome peptide folding and provide a thermodynamic driving force for the formation of kinetically stable peptide and protein entanglements.

Supporting Information Available: Experimental details for the synthesis of the macrocycles, rotaxanes, threads, and precursors.

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(12) To maintain the ammonium template the "stoppering" reaction must be carried out in the absence of base. HCl is generated during the reaction, autocatalyzing ester formation. Since there is the potential for a mixture of counterions in the initially formed rotaxane salts, anion exchange was performed to ensure uniformity.

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