Thermoregulated Optical Properties of Peptidic Pseudorotaxanes**

Jean-Christophe Meillon, Normand Voyer,* Éric Biron, François Sanschagrin, and J. Fraser Stoddart

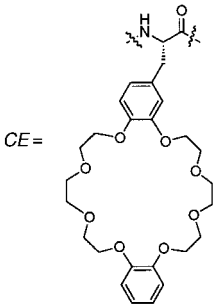
The importance of self-assembly in biological processes has led many chemists to design and build superstructures assembled through noncovalent interactions.[1] Thus, catenanes, rotaxanes, and pseudorotaxanes have been prepared using a variety of different approaches.[2] The main objective of this scientific effort is the development of nanoscale supramolecules with entirely new and specific properties. It is noteworthy, however, that polypeptides have not been used in such supramolecular devices, even though many researchers have reported the use of peptides in the preparation of receptors, enzyme mimics, artificial ion channels, and dendrimers.[3] Herein, we describe the preparation and the unusual optical properties of the first synthetic pseudorotaxanes involving a chiral peptidic framework.[4]

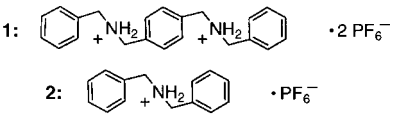
The bis-crown peptide **A**, incorporating two amino acids modified with a crown ether (dibenzo-24-crown-8), was designed and synthesized according to a procedure we have developed.[5] The heptapeptide features a terminal *tert*-butoxycarbonyl (Boc) protective group, a leucine unit, then the first crown ether substituted amino acid, the three amino acid groups (-X-Y-Z-), the second crown ether–amino acid, and ends with leucine *n*-propylamide. The lateral dibenzo-24-crown-8 has been shown to form "threaded" complexes with polyammonium ions, such as **1**, that have been fully characterized.[6]

A peptide–diammonium host–guest complex of 1:1 stoichiometry between **A** and **1** with PF_6^- counterions (**[A·1]**) has been successfully prepared in two different ways. In one approach, **A** is dissolved in CH_2Cl_2 and then **1** is added. After a brief sonication period, the suspension was stirred for 10

A, B: Boc-Leu-*CE*-X-Y-Z-*CE*-Leu-NH-*n*Pr

A: X,Y,Z = Leu
B: X = Ile, Y = Pro, Z = Ala





hours. In another approach, an aqueous solution of **1** was extracted by a solution of **A** in CH_2Cl_2. In both cases, the solvent was removed under vacuum and the resulting products were analyzed. Electrospray (ES) mass spectrometry confirmed the existence of the 1:1 complex. A doubly charged molecular ion, corresponding to **[A·1]** lacking PF_6^-, is observed in the spectra (Figure 1). However, no trace of either a 2:1 complex or a singly charged 1:1 complex were evident. These observations suggest strongly that the supramolecular species is indeed a pseudorotaxane involving the cooperative action of the two crown moieties.

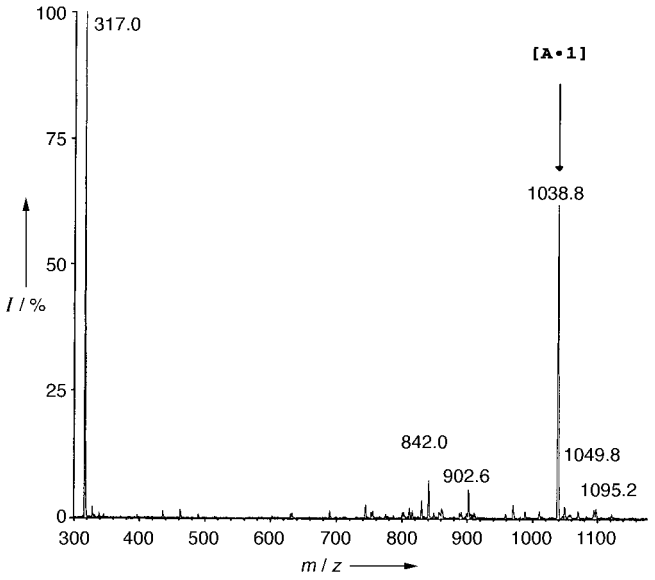


Figure 1. ES mass spectrum of complex **[A·1]**. The peak indicated by the arrow is doubly charged, and corresponds to the mass of the complex lacking two PF_6^- counterions (2078).

NMR spectroscopy further corroborates this conclusion. Differences between the spectra of free and **1**-complexed peptide **A** (Figure 2) support the proposal of increased order within the peptide backbone upon the addition of **1**. Moreover, one can observe characteristic peaks for the benzylic methylene protons of complexed polyammoniums ions around $\delta = 4.7$,[7] along with signals for deshielded α-carbon

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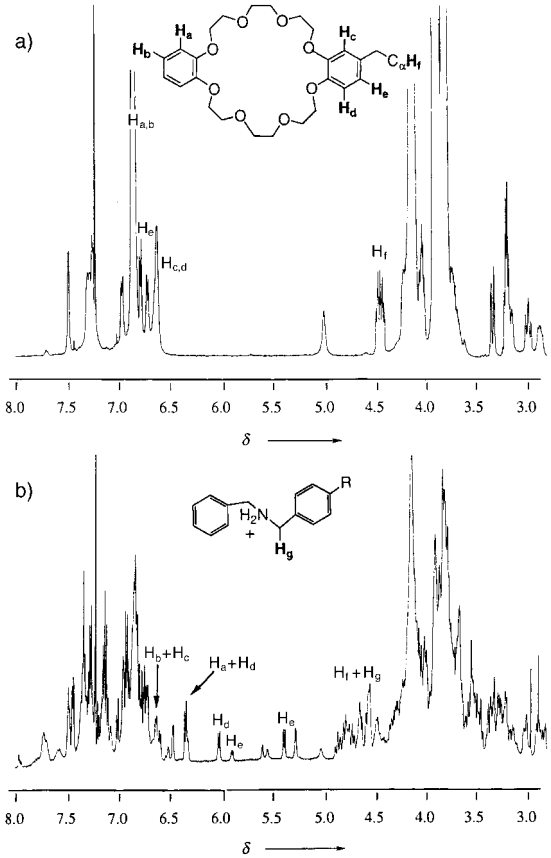

Figure 2. 500 MHz NMR spectra ($CDCl_3$) of a) free peptide **A** with labeled signals of the crown ether and b) complex **[A·1]** with labeled signals of the polyammonium guest. This partial assignment is based upon TOCSY experiments.

protons of the crown ether subsituted amino acids. In addition, some small peaks between $\delta = 5.4$ and 6.7 can be assigned to signals of the aromatic protons of the **1**-complexed crown ether that are significantly shifted upfield. Similar observations have already been reported for related systems.[8]

Additional evidence for the formation of a 1:1 pseudorotaxane is provided by circular dichroism (CD) studies. Free peptide **A** in MeCN is characterized by a CD curve (Figure 3a) with a maximum at 190 nm and a minimum at around 206–207 nm, indicative of partly formed helical conformations.[9] Addition of 1 equivalent of **1** results in **[A·1]** complexation, inducing a noteworthy conformational constraint on the peptidic backbone. This is displayed by a dramatic change in the CD curve (Figure 3a) with a maximum at 203 nm and two minima at 194 nm and 233 nm, characteristic of β-turn structures.[10] Upon heating the MeCN solution slow decomplexation occurs (Figure 3b). At 75 °C, peptide **A** appears mainly uncomplexed and displays a CD curve similar to that of the free peptide. Once the solution is cooled to 25 °C, the complex reforms as shown by the CD curve, to recover its original geometry in the process. This result indicates that the formation of the pseudorotaxane is thermodynamically favored. Moreover, addition of two equivalents of dibenzylammonium **2** to peptide **A** had no effect on its conformation, further supporting that conformational changes observed with **1** result from the formation of a pseudorotaxane involving the two crown side chains.

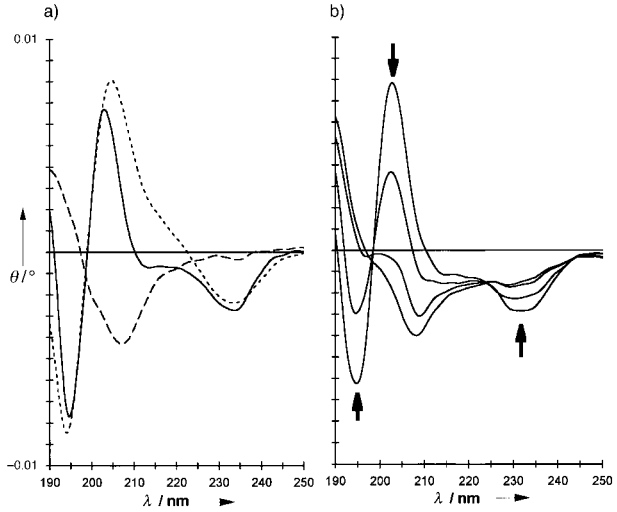

Figure 3. CD studies in MeCN for a) peptide **A** (– – –), complex **[A·1]** (——), and complex **[B·1]** (- - - -) at 25 °C; b) complex **[A·1]** at 25, 45, 65, and 75 °C. The arrows indicate the modifications occuring in the curves upon increasing temperature.

Analysis of the CD data yielded a binding constant (K_a) of 2640 M^{-1} in MeCN for the complexation process.[11] This value is much larger than 420 M^{-1} obtained with dibenzo-24-crown-8 and dibenzylammonium,[7] suggesting a strong cooperative action of the two crown side chains of **A** in the binding phenomenon.

To support the β-turn conformation of the peptidic chain in **[A·1]**, we have designed the analogous heptapeptide **B**, incorporating a proline residue to impose a conformational constraint to the peptidic backbone. Upon addition of **1** to a solution of **B** in MeCN, the CD curve for **[B·1]** superimposes on the curve for **[A·1]** (Figure 3a). The observation of a maximum at 205 nm, and two minima at 194 nm and 233 nm corroborates the proposed turn structure adopted by the peptides once complexed. Modeling studies substantiate the rotaxane-induced conformational change.[12] As seen in Figure 4, when the backbone of **A** is under a type II β-turn

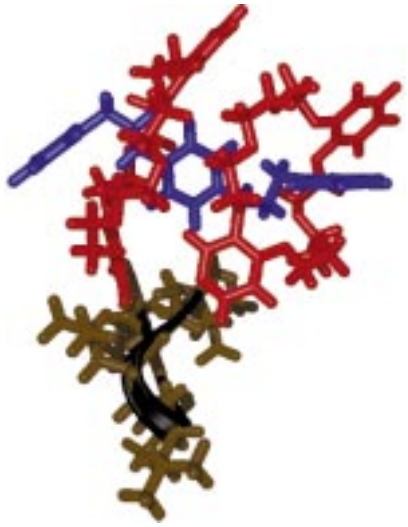

Figure 4. Representation of pseudorotaxane complex **[A·1]** after potential energy minimization.[12] Crown ethers, diammonium **1**, and peptide chain are shown in red, blue, and tan respectively. The black ribbon emphasizes the β-turn structure between ^{4}Leu–^{5}Leu of the central triad.

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structure, the two crown moieties are nicely oriented to form a threaded complex with **1**. On the possible turn structures, the one having ^{4}Leu-^{5}Leu at $i+1$, $i+2$ positions gave the most stable structure for the complex.[13]

In conclusion, we have prepared peptidic pseudorotaxanes **[A · 1]** and **[B · 1]** through self-assembly under thermodynamically controlled conditions. These supramolecular systems display thermoregulated optical properties that could make them useful for the development of optical devices. Future work will focus on the applications of these supramolecular systems, as well as on the preparation of receptors with different amino acids by a combinatorial approach, permitting the molecular engineering of novel materials with unique properties.

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[13] It is likely that the most stable structure of **[A · 1]** shown in Figure 4 is accompanied by one or more other β-turn complexes in solution. This would explain the difficulties in crystallizing **[A · 1]** and discrepancies in the NMR integrations.

Application of Combinatorial Procedures in the Search for Serine-Protease-Like Activity with Focus on the Acyl Transfer Step**

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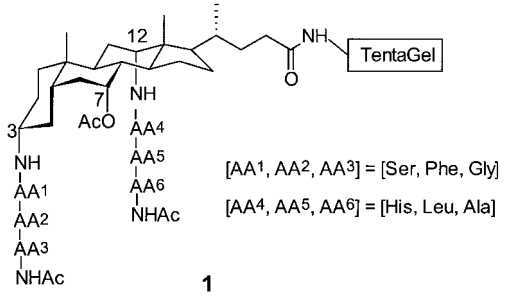
The attainment of the selectivities and efficiencies of enzymes remains a major goal in organic chemistry.[1] The efficiency of the serine protease α-chymotrypsin in cleaving peptide bonds, relative to the uncatalyzed hydrolysis, serves as an illustrative example.[2] Although notable progress has been made in the development of synthetic hydrolases,[3] the real challenge, the cleavage of nonactivated amides, still remains. Several years ago we embarked on a long-term program aimed at the development of synthetic hydrolases with the focus on gaining a better understanding of the enzymatic process. In particular we developed non-peptidic organic molecules possessing an array of functional groups in a suitable geometry for eventual hydrolytic activity.[4] In contrast to that work, in which a carefully designed molecule was targeted, we describe herein the first results of a study in which serine-protease activity is searched by combinatorial techniques.[5]

Our long term goal is to develop a system that is characterized by three independent peptidic chains, each containing one of the three active residues[6] of the classic triad (Ser, His, and Asp), and possessing serine-protease-like activity. As a first step towards this goal we describe here: 1) the mix-split synthesis of the library **1** containing 729 (3^6) members, in which the two most important catalytic residues



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Ser and His are each incorporated into one of the two tripeptidic chains generated on a steroidal scaffold, 2) the screening of the solid-phase-bound library for reaction with the test substrate **2** (NF31) as a model for the first step of the

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