Rotaxanes

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A Rigid Helical Peptide Axle for a [2]Rotaxane Molecular Machine**

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Rotaxanes are mechanically interlocked molecular architectures in which a central linear molecule (axle) passes through the cavity of a macrocycle (wheel).[1] The axle is held in place by the presence of sterically bulky stoppers at both ends. Oligomeric systems, which typically consist of repeating oxyethylene or methylene units, are often exploited as axles in the construction of rotaxanes.

Peptido[2]rotaxanes, based on various -Gly-Xxx- dipeptide stations in the axle, were first reported by Leigh and co-workers[2] and subsequently by Onagi and Rebek.[3] More recently, the Leigh research group described a rotaxane in which the wheel is able to protect a bioactive pentapeptide axle from peptidase-catalyzed hydrolysis.[4] We are currently investigating the synthesis and properties of a new set of symmetrical and nonsymmetrical peptido[2]rotaxanes with amino acid repeating units (oligopeptide systems) in their axles. Herein we describe our results on a [2]rotaxane shuttle in which the longest part of the axle is a rigid helical peptide, which was planned to act as a track for the reversible motion of a tetramide macrocyclic wheel.

As a first step in this study, we chose two symmetrical axles, each characterized by either two α-aminoisobutyric acid (Aib) residues or by two -$(Aib)_4$- homopeptide ester sequences; the latter sequence is known to generate incipient 3_{10}-helices,[5a,b] that is, secondary structures stabilized by two intramolecular, consecutive $i \leftarrow i+3$ C=O···H–N hydrogen bonds.[5c–e] A fumardiamide-derived central station,[2] two ethoxy linkers, and two 9-fluorenylmethoxycarbonyl (Fmoc) N^{α}-protecting group[6] stoppers completed the chemical structures of the [Fmoc-$(Aib)_n$-O-$(CH_2)_2$-NH]$_2$-FUM (n = 1,4; FUM = fumaric unit) axles. Each step of the synthesis of the axle was performed by using solution methods. The tetramide macrocycle[2,7] developed by Leigh and co-workers was used as the wheel. The fumardiamide unit has multiple hydrogen-bonding sites. In solvents of low polarity an axle containing such a motif is expected to template the formation of the tetramide macrocycle to afford a rotaxane through a five-component clipping reaction. Following the protocol developed by Leigh and co-workers,[2,7] we synthesized a macrocycle (from xylylenediamine and isophthaloyl dichloride) on the fumardiamide station in yields of 78 and 72% for $n = 1$ and $n = 4$, respectively.

The X-ray diffraction structures of the two resulting highly crystalline, peptido[2]rotaxanes (Figure 1 and see the Supporting Information) show that the fumardiamide-diethoxy moiety is an excellent station for the chair conformation of the aromatic tetramide ring,[7] despite the proximity of the bulky Aib residues or -$(Aib)_4$- homopeptide helices.[8] The ring is held in place by two sets of hydrogen bonds from the two NH groups of each of its two isophthaldiamide moieties to each of the two double-acceptor fumardiamide carbonyl groups. The overall architecture is further stabilized by two fluorenyl···isophthaloyl (stopper···macrocycle) face-to-edge interactions and a xylylene···olefin···xylylene (macrocycle···axle···macrocycle) π-stacking interaction. The inner cavity of the macrocycle is roughy rectangular, with van der Waals dimensions of 5.4 × 4.0 Å and 7.4 × 3.4 Å in the mono- and tetrapeptide-based peptido[2]rotaxanes, respectively. The lumen of the wheel is, therefore, significantly smaller than the outer diameter of the -$(Aib)_4$- 3_{10}-helix (about 10 Å).

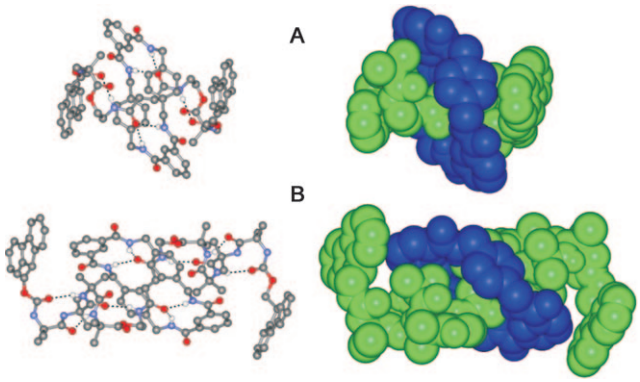

Figure 1. Two representations each of the two X-ray diffraction structures of the symmetrical [Fmoc-$(Aib)_n$-O-$(CH_2)_2$-NH]$_2$-FUM (A: n = 1; B: n = 4) [2]rotaxanes. Left: the carbon, oxygen, and nitrogen atoms are depicted in gray, red, and blue, respectively. The intramolecular C=O···H–N hydrogen bonds are shown as black dotted lines. Right: space-filling representations (macrocycle in blue and axle in green).

Whereas the NH group of each of the two amidoethoxy linkers forms an intramolecular hydrogen bond with the urethane Fmoc-Aib carbonyl group (C_{10} pseudocyclic struc-

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ture) in the Aib rotaxane, this same NH group in the -$(Aib)_4$- rotaxane is hydrogen bonded to the Aib(2) carbonyl group to afford a C_{13} pseudocyclic structure. Moreover, in the -$(Aib)_4$- rotaxane the regularity of the two incipient 3_{10}-helices (one right handed, the other left handed), including the presence of two intramolecular $i \leftarrow i+3$ C=O···H–N hydrogen bonds,[5] is not disturbed by the formation of the macrocycle. Also, in these structures the two helices are almost antiparallel and orthogonal to the fumardiamide-diethoxy station, thereby affording an overall S-shaped conformation of the axle. The breaking points of the axle linearity are given by the *gauche* disposition of two consecutive torsion angles of the -O-CH_2-CH_2-NH- linker moiety.

We then moved to the second step of our study, namely to attempt to create a peptido[2]rotaxane molecular machine. To this end, we envisaged the nonsymmetrical (*E*)-FUM configured axle shown in Figure 2 A. The N terminus is based on a 9-mer peptide with the sequence -D-Leu-$(Gly)_2$-$(Aib)_6$-, followed by an ethoxyamide-FUM unit and a L-Leu residue. The two stations of opposite chirality (-D-Leu-Gly-Gly- and -(*E*)-FUM-L-Leu-) were expected to offer the possibility to also check the system by using CD spectroscopy. The -$(Aib)_6$- sequence is known to fold in a robust 3_{10}-helix.[5,9] We selected the diphenylacetyl and diphenylmethyl amino moieties (at the N and C terminus, respectively) and the aromatic tetramide ring developed by Leigh and co-workers for the stoppers and macrocycle.[2,7]

The results of the TOCSY and ROESY NMR analyses on the (*E*)-FUM axle in CD_3CN solution confirmed the predicted -$(Aib)_6$- helical secondary structure (see Figure S7 and Table S1 in the Supporting Information). By using the protocol developed by Leigh and co-workers,[2,7] the macrocycle self-assembled on the fumardiamide station of the (*E*)-FUM axle (**I**) in 95% yield, thus producing the (*E*)-FUM peptido[2]rotaxane (**III**) (Figure 2). Evidence for the regioselective positioning of the macrocycle is provided by the differences in the CH olefin proton resonances between **I** and **III** (Figure 3 and Figure S9 in the Supporting Information). The axle contains as many as 12 amide (or peptide) bonds which could act as templates for the assembly of the macrocycle. However, in solvents of low polarity such as $CHCl_3$ (the solvent used in the synthesis), most of the carbonyl groups of Aib-rich peptides are so strongly intramolecularly hydrogen bonded that they are not available for templation of the macrocycle. A comparison of the FTIR absorption spectra of **I** and **III** corroborates the structural

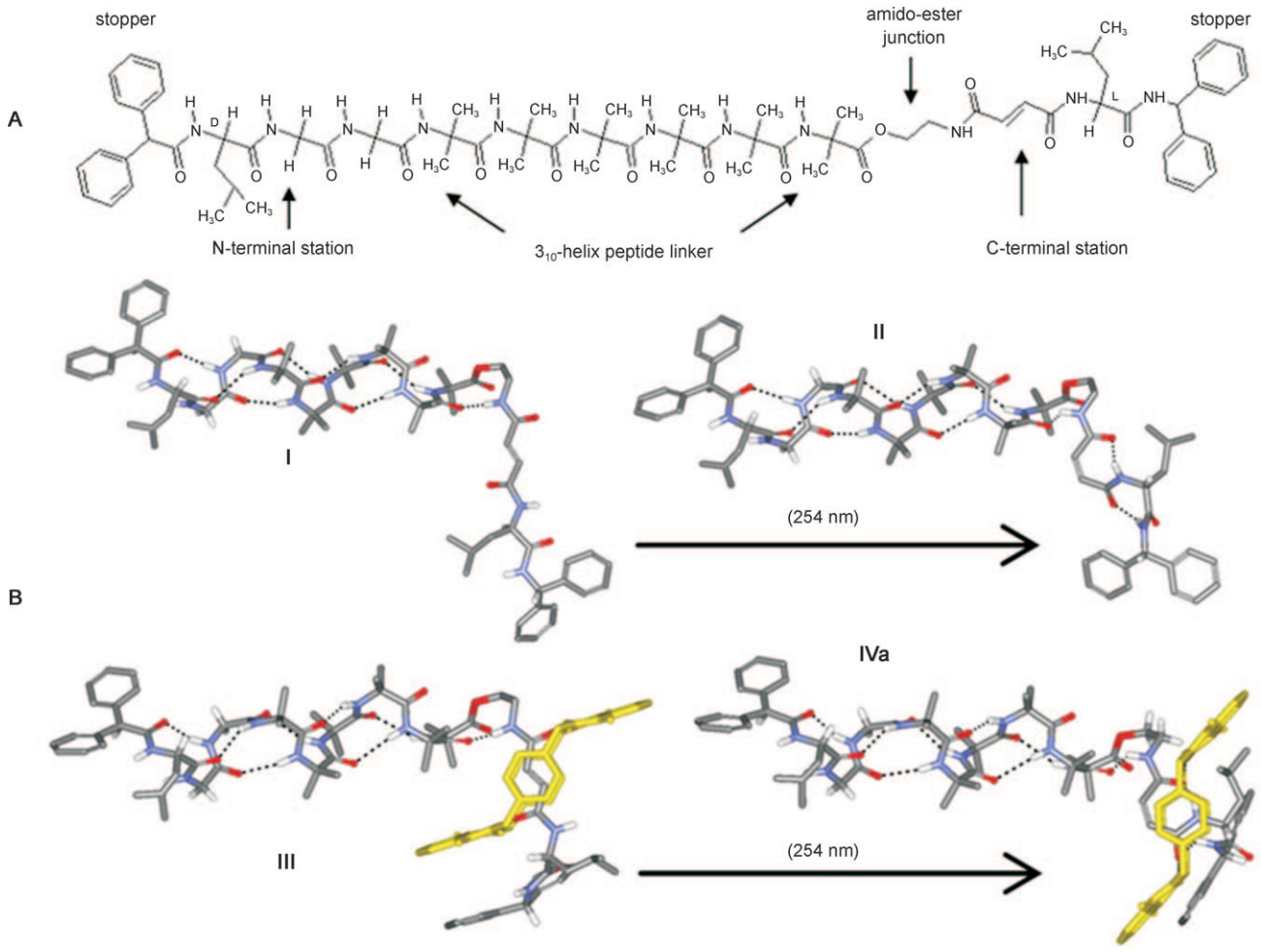

Figure 2. A) Chemical structure of the nonsymmetrical (*E*)-FUM axle **I**. B) Computer generated structures of **I**, the (*Z*)-MAL axle **II**, the (*E*)-FUM peptido[2]rotaxane **III**, and the (*Z*)-MAL peptido[2]rotaxane **IVa**. The wheel is highlighted in yellow.

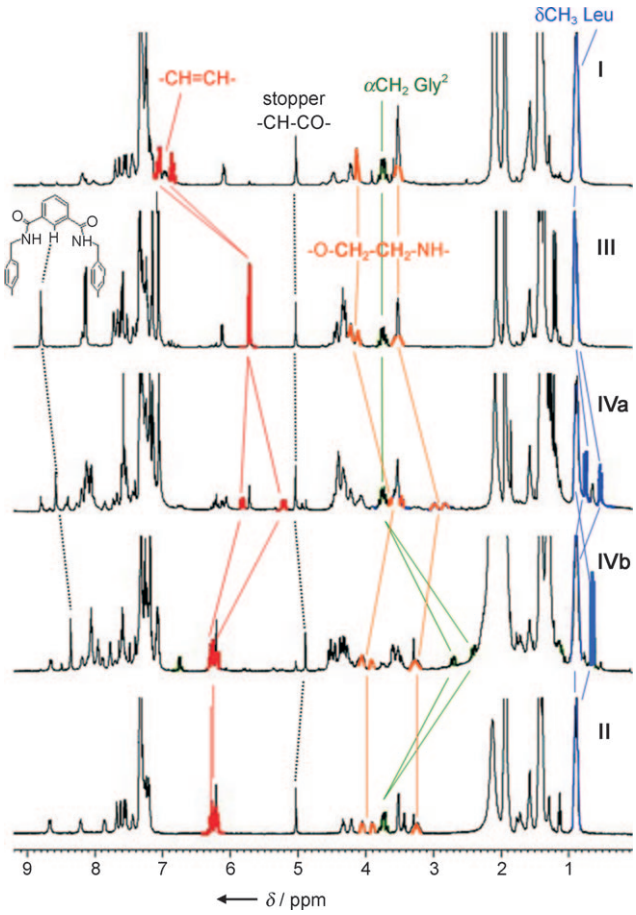

Figure 3. ^{1}H NMR spectra (400 MHz, CD_3CN, 318 K) of the (*E*)-FUM axle **I**, (*E*)-FUM peptido[2]rotaxane **III**, (*Z*)-MAL peptido[2]rotaxane **IVa**, (*Z*)-MAL peptido[2]rotaxane **IVb**, and (*Z*)-MAL axle **II**.

assignment of the latter, as it highlights the contribution of the four macrocyclic hydrogen-bonded NH groups to the absorption band at about 3320 cm^{-1} (see Figure S5 in the Supporting Information). In agreement with all of the NMR and FTIR absorption data collected, we propose that **I** and **III** adopt the 3D structures shown in Figure 2B. Moreover, from our NMR findings it is evident that the macrocycle assembled onto the axle does not affect the helical domain of the axle.

We then applied photon stimuli (254 nm) and switched the fumardiamide unit of **I** and **III** to a maleidiamide. We obtained the (*Z*)-MAL (MAL = maleic) axle (**II**; 100% yield in 15 min) and the (*Z*)-MAL peptido[2]rotaxane (**IVa**; 49% yield in 45 min); in the latter case the wheel is on the maleidiamide station. Comparisons of the ^{1}H NMR spectra of: 1) axles **I** and **II**, and 2) peptido[2]rotaxanes **III** and **IVa** (Figure 3 and Table S1 in the Supporting Information) show clearly the differences between the *E* and *Z* olefin CH proton dyads. Moreover, the resonances corresponding to the protons of the two methylene groups of the amidoethoxy linker are remarkably downfield shifted in the case of the (*Z*)-MAL peptido[2]rotaxane **IVa** compared to its (*E*)-FUM counterpart **III**. Therefore, in the former the macrocycle is located over the *Z* olefin unit through multiple intercomponent hydrogen bonding. Again, the presence of the macrocycle on the axle does not alter the helical stretch of the axle.

To activate the molecular machine we heated **IVa** to 50 °C for 60 minutes in CH_3CN solution. This results in the macrocycle wheel moving to the -D-Leu-Gly-Gly- N-terminal station, thus affording the (*Z*)-MAL peptido[2]rotaxane (**IVb**) (Figure 4) in 90% yield. The ^{1}H NMR data supporting

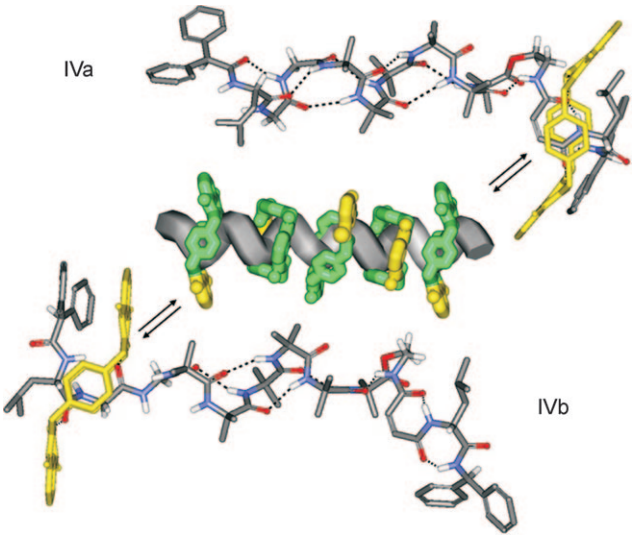

Figure 4. Reversible interconversion of the peptido[2]rotaxane **IVa** (top) and **IVb** (bottom). The wheel is highlighted in yellow. The proposed mechanism for the concomitant rotation and translation of the wheel along the helical axle is depicted schematically in the center.

this conclusion (Figure 3 as well as Table S1 and Figure S10 in the Supporting Information) are as follows: 1) The resonances of the olefin CH protons of **IVb** and those of the protons of the two methylene groups of the amidoethoxy linker move back to the same positions as those of the corresponding axle **II**; 2) the Gly^2 NH and αCH protons, as well as the D-Leu NH proton are downfield shifted with respect to the corresponding protons of **II** and **IVa**. Our interpretation of the NMR results is corroborated by the switchable CD response of the two translational isomers **IVa** and **IVb** of (*Z*)-MAL peptido[2]rotaxane (see Figure S4 in the Supporting Information). In particular, the induced ellipticities at about 230 nm, where the benzamido chromophore of the achiral wheel absorbs strongly,[10] are quite different, being close to zero for **IVb** (with the wheel on the D-Leu-containing station) and very strongly negative for isomer **IVa** (with the wheel on the L-Leu maleidiamide station).[2d] It is noteworthy that this motion of the wheel can be reversed (Figure 4) by heating **IVb** in either $CHCl_3$ or 1,1,2,2-tetrachloroethane solution at 55 °C for three days, which results in a 95% conversion into **IVa**, as demonstrated by the HPLC profiles (see Figure S3 in the Supporting Information). Our long-standing experience on medium-sized 3_{10}-helical peptides based entirely on the strongly helicogenic C^{α}-tetrasubstituted α-amino acid Aib,[5d,e] along with literature data on a variety of peptides from this series from other research groups,[11] make us quite confident that the overwhelmingly preferred conformation (3_{10}-helix) of the -CO-$(Aib)_6$-O- sequence used in this study is extremely resistant to melting, particularly under the rela-

tively mild experimental conditions (heating to 50–55 °C in solvents of low polarity, such as $CHCl_3$ or CH_3CN) required for activating our peptido[2]rotaxane molecular machine. Our results also suggest that changing the solvent from $CHCl_3$ to CH_3CN results in modification of the relative stabilities of the two stations (*Z*)-MAL diamide and -D-Leu-Gly-Gly- in **IVa** and **IVb**, respectively. Acetonitrile, which is known to interact significantly with amide or peptide groups,[12] seems to destabilize the expansion of the -$(Aib)_6$- helical structure to the N-terminal -Gly-Gly- dipeptide unit, which we believe takes place in $CHCl_3$. The more extended, non-intramolecularly hydrogen-bonded, 3D structure of this dipeptide unit that is present in CH_3CN would favor its role as a station for the macrocycle. This interpretation would also explain the observation that under the experimental conditions ($CHCl_3$ solution) we used for assembling the peptido[2]rotaxane the wheel is formed on the olefin station, not on the potentially competitive -D-Leu-Gly-Gly- station.

In summary, we have de novo designed, synthesized, and characterized the 3D structure of the first peptido[2]rotaxanes with a rigid helix as part of their axle. More importantly, we have also constructed a reversible molecular device based on one such rotaxane. In this case: 1) the wheel switches almost quantitatively between two, unambiguously identified, stations on changing the solvent, and 2) by virtue of the size of the inner cavity of the wheel relative to the outer diameter of the helix, a rotation of the wheel might occur concomitantly with its translation along the axle. We are currently using a combination of spectroscopic methods and theoretical calculations to investigate the mechanism of the wrapping motion in this molecular machine in detail.

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