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[3]Foldarotaxane-mediated synthesis of an improbable [2]rotaxane[†]

Victor Koehler,^{‡^a} Maxime Gauthier,^{‡^b} Chenhao Yao,^a Karine Fournel-Marotte,^b Philip Waelès,^b Brice Kauffmann,¹^{b^c} Ivan Huc,^{b^d} Frédéric Coutrot¹^{b*b} and Yann Ferrand^{*^a}

The wrapping of an aromatic oligoamide helix around an active ester-containing [2]rotaxane enforced the sliding and the sequestration of the surrounding macrocycle around a part of the axle for which it has no formal affinity. The foldamer-mediated compartmentalization of the [2]rotaxane shuttle was subsequently used to prepare an improbable rotaxane.

Mechanically interlocked molecules such as rotaxanes are appealing compounds for potential applications in fields as varied as materials science¹ and biology.² A [2]rotaxane consists of a molecular axle that threads through a macrocycle. Bulky stoppering units at each end of the axle sterically ensure the mechanical bond³ by preventing the macrocycle from dethreading. The non-covalent assembly allows for co-conformational degrees of freedom of the interlocked elements with respect to one another. Taking advantage of this, numerous well-thought [2]rotaxanes have been reported in the literature with the aim of operating as molecular shuttles,⁴ through the controlled translational motion of the surrounding macrocycle between sites of interactions (called molecular stations) on the encircled axle.⁵ Most of these co-conformational changes may be triggered by the alteration of the interactions between the macrocycle and its axle thanks to an external stimulus such as a chemical reaction,⁶ photo-irradiation,⁷ variation in solvent polarity⁸ or temperature,9 or recognition of ions.10 Much scarcer are the



examples relative to the control of the macrocycle localization

due to an intermolecular recognition of a neutral compound.¹¹

Berna has reported a rotaxane with two diacylaminopyridinestations, which upon binding of barbital to these stations leads

to a change in the co-conformational behavior of the macro-

cycle. Recently, we reported the formation of a foldarotaxane architecture consisting in a [2]rotaxane, the axle of which is also wrapped by a foldamer helix.¹² We showed that the winding of

the helix around the encircled axle can be altered by the

macrocycle localisation. Here, we investigate the reciprocal

effect. We propose the utilization of aromatic oligoamide helix

1 as a supramolecular auxiliary aimed at compartmentalizing

[2]rotaxane 3-Boc through efficient intermolecular binding

^a Institut de Chimie et Biologie des Membranes et Nano-objets CBMN (UMR5248), Université de Bordeaux, CNRS, IPB, 2 rue Robert Escarpit, 33600, Pessac, France. E-mail: y.ferrand@iecb.u-bordeaux.fr

^b Supramolecular Machines and Archtectures Team, IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France. E-mail: frederic.coutrot@umontpellier.fr;

Web: https://www.glycorotaxane.fr

^c Université de Bordeaux, CNRS, INSERM, UMS3033, IECB, 2 rue Robert Escarpit, 33600, Pessac, France

^d Department of Pharmacy, Ludwig-Maximilians-Universität, Butenandtstr. 5–13, 81377, München, Germany

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[‡] These authors contributed equally to this work.



Fig. 1 (a) Schematic representation of the successive molecular machineries, first between the [2] rotaxanes **3-HPF**₆ and **3-Boc** through deprotonationthen-carbamoylation of the ammonium, then by the displacement and sequestration of the DB24C8 after the entwining of the helix to produce a foldarotaxane. The helix binding induces the displacement of the macrocycle that becomes sequestrated on a part of the axle for which it has no affinity. It follows that the cleavage of the axle can yield a [2] rotaxane. (b) Sequence of the dissymmetrical helix **1** and (c) formulae of rotaxanes **3-HPF**₆, **3-Boc** and **6** and non-interlocked molecular axles and reactants **2**, **4**, **5u-Boc** and **6u**.

a carbamate cleft and an amide cleft (Fig. 1b), capable of binding to carbamate and amide moieties spaced by a seven methylene α,ω -alkane chain with high affinity in chloroform $(K_a > 10^5 \text{ M}^{-1})$.¹⁶ A cleavable NHS ester link was placed between two sections of the encircled axle with the aim of obtaining the shorter [2]rotaxane 6 that is devoid of any formal mutual site of recognition between the DB24C8 and the axle and could not be obtained efficiently by a template-mediated threading-and-capping strategy. In 3-HPF₆, the DB24C8 was mainly localized around the best ammonium station. Deprotonation-carbamoylation of the ammonium caused the gliding of the DB24C8 towards the amide secondary station (see the ESI⁺ for the ¹H NMR evidences related to the localization of the DB24C8 along the encircled axle in 3-HPF₆ and 3-Boc). In 3-HPF₆ and 3-Boc, the DB24C8 was thus localized on the "left" side of the axle with respect to the NHS ester. The NHS moiety not being bulky enough to preserve the mechanical bond, cleavage of the rotaxane axle through aminolysis of the active ester would result in the irremediable loss of the interlocked architecture, therefore providing the free macrocycle along with axle fragments. Before envisioning the cleavage of the NHS ester link, we thus considered the possibility to wrap the foldamer helix 1 around the encircled axle of 3-Boc in order to displace the surrounding DB24C8 around the sole vacant unhindered "right" part of the axle with respect to the NHS.

A preliminary experiment was performed to evaluate the ability of **1** to form a foldaxane¹⁷ by wrapping around the carbamate–amide binding site of model axle **2** despite the close proximity of the *O*-benzyl NHS moiety (Fig. 2). Titration of single helix **1** by **2** was carried out in CDCl₃. ¹H and ¹⁹F NMR monitoring revealed changes consistent with the formation $1 \supset 2$ in slow exchange with the free helix on the NMR time scale (Fig. 2a–d and Fig. S4, S5, ESI†). As expected, mixing the racemic helix **1**, that is, the mixture of *P* and *M* enantiomeric conformers, with racemic axle (R/S)-2 led to the emergence of two sets of new resonances corresponding to two pairs



Fig. 2 Excerpts of the ¹H NMR spectra of **1** in CDCl₃ (300 MHz, 298 K, 1 mM) showing the amide resonances of: (a) **1** as a single helix and after the addition of (b) 1 equiv. and (c) 2 equiv. of axle **2**. Amide signals of single helix **1** and the foldaxane $1 \supset 2$ are marked with diamonds and blue circles, respectively. (d) X-Ray structure of $1 \supset 2$. The axle is shown in CPK representation whereas the helix is shown either in tube. (e) Detailed views showing the binding mode in vicinity of the NHS group.

of diastereomeric complexes $[P\cdot\mathbf{1}\supset(R)\cdot\mathbf{2}/M\cdot\mathbf{1}\supset(S)\cdot\mathbf{2}]$ and $[P\cdot\mathbf{1}\supset(S)\cdot\mathbf{2}/M\cdot\mathbf{1}\supset(R)\cdot\mathbf{2}]$ in the same proportion (Fig. S6, ESI†). The absence of diastereoselectivity can be explained by the lack of interaction between helix **1** and the stereogenic centre of the NHS moiety. An apparent association constant $K_a = 8500 \text{ M}^{-1}$ was calculated by direct integration of the NMR resonances of **1** and $\mathbf{1}\supset\mathbf{2}$. Single crystals were obtained by slow diffusion of hexane in a chlorobenzene solution of $\mathbf{1}\supset\mathbf{2}$ and the structure was refined in the orthorhombic *Pbca* space group (Fig. 2d–e). The disorder of the R and s axles in the crystalline structure could be modelled and confirmed the absence of diastereoselectivity as both can be indifferently found in either the *P* or *M* helix. The solid state structure also revealed that the space between the NHS moiety and **1** is kept to the minimum. Therefore, the DB24C8 is not given

the opportunity to reside between the NHS and the helix in the targeted foldarotaxane $1 \supset 3$ -Boc.

We then attempted to predict the outcome of the wrapping of 1 around the fully functional [2]rotaxane 3-Boc using molecular mechanics. For this purpose, the model of $1 \supset 3$ -Boc was built using the Merck Molecular Force Field static (MMFFs) (Fig. 3f). Upon wrapping around the carbamate-amide station of 3-Boc, the bulky helix 1 can displace the DB24C8 away from the amide station and compartmentalize the [2]rotaxane. The resulting foldarotaxane architecture combined both a rotaxane and a foldaxane arrangements. Because the "left" part of the axle is hindered by both the Boc group and 1, the DB24C8 has sterically no other choice than shuttling across the NHS group and being compartmentalized on the "right" part of the axle despite the lack of any formal site of interactions for it. The supramolecular assembly of foldarotaxane $1 \supset 3$ -Boc was then monitored by ¹H NMR (Fig. 3b–e). The formation of $1 \supset 3$ -Boc indicates that the affinity between 1 and 3-Boc is sufficiently large to overcome the interaction between the DB24C8 and the amide.¶ As for $1 \supset 2$, gradual addition of 3-Boc on 1 in CDCl₃ led to the emergence of a new set of resonances in slow exchange on the NMR time scale. Saturation of helix 1 was reached after 144 hours using 2.5 equiv. of 3-Boc. The slowness to reach the thermodynamic equilibrium is attributed to the presence of the bulky ends of the encircled axle, which prevent the helix from freely gliding over them. Additionally, over time, single helix 1 can dimerise back into parallel and anti-parallel double helix $(1)_2$ (Fig. 3a, left). To confirm the efficient foldamer-driven compartmentalization of $1 \supset 3$ -Boc, cleavage of the foldarotaxane axle at its NHS ester reactive site was performed by adding 2.75 equiv. of the bulky 4, 4-diphenylbutylamine. The addition then elimination mechanism allowed for the preservation of the interlocked [2]rotaxane

architecture because the DB24C8 was localized between the diphenyl stopper and the NHS ester. Indeed, at each stage of the mechanism, the ending groups were always large enough to ensure the mechanical bond. The aminolysis was followed by ¹H NMR monitoring of the amide region of $1 \supset 3$ -Boc (Fig. 4b-e). The resonances of the foldarotaxane $1 \supset 3$ -Boc progressively disappeared to be replaced by those of foldaxane $1 \supset 5u$ -Boc. Reaction was complete after 18 h. Recycling GPC was used to remove the high molecular weight foldamercontaining species (e.g. $1 \supset 5u$ -Boc, 1) from the smaller components of the reaction. Further HPLC analysis on reverse phase was carried out and indicated the presence of the expected [2]rotaxane 6. By-products 5u-Boc, 6u and DB24C8 were also detected: they resulted from the cleavage of the excess amount of rotaxane 3-Boc used for the formation of the foldarotaxane $1 \supset 3$ -Boc. Purification on silica gel chromatography allowed for the isolation of the improbable [2]rotaxane 6 in a 87% vield.

In summary, we reported herein the use of a foldamer helix to compartmentalize a [2]rotaxane molecular shuttle. Because of its greater affinity, helical foldamer 1 binds to axle of rotaxane 3-Boc by displacing the macrocycle away from the amide. Models suggest, and covalent trapping experimentally confirms, that the macrocycle can only be located around the diphenylheptanoate part in $1 \supset 3$ -Boc. To the best of our knowledge, this is the first utilisation of a foldamer helix as a supramolecular auxiliary in order to displace and sequestrate the surrounding macrocycle of a [2]rotaxane around a part of the encircled axle for which it has no formal site of interaction. This study opens the way to the design of novel molecular shuttles able to actuate through interdependent motion of foldamer and macrocycles along a same molecular axle. Well-designed foldamer-assisted molecular pump might also be envisaged.



Fig. 3 (a) (*left*) Equilibrium between double helix $(1)_2$ and single helix **1**. Note that, as sequence **1** is dissymmetrical, $(1)_2$ can be either parallel or antiparallel. (*right*) The formation of the foldarotaxane $1 \supset 3$ -Boc requires the unfolding and refolding of the helix around the molecular axle. The binding of **1** forces the translocation of the macrocycle (DB24C8) through the NHS moiety (blue pentagon). (b–e) Kinetic monitoring of the folding of single helix **1** (6 mM) around axle **3-Boc** (15 mM) in CDCl₃ followed by ¹H NMR (700 MHz, 298 K) and showing the amide resonances of the helix; (b) initial single helix **1**; (c) 18 h after the addition of **3-Boc**; then after (d) 72 h and (e) 144 h. For the equilibration procedure see the ESI.† Amide signals of **1**, (**1**)₂ and **1** \supset **3-Boc** are marked with diamonds, triangles and red circles, respectively. (f) Energy-minimized molecular model, built using MMFFs of the foldarotaxane **1** \supset **3-Boc**. The various functional groups are colour coded as in Fig. 1. Side chains and non-polar hydrogen atoms of the helix have been removed for clarity.



Fig. 4 (a) Cartoon illustrating the aminolysis of $1 \supset 3$ -Boc that leads to rotaxane 6. Parts of the ¹H NMR spectra (700 MHz, 298 K) showing the amide resonances of $1 \supset 3$ -Boc (6 mM) in CDCl₃: (b) the initial $1 \supset 3$ -Boc foldarotaxane; and in the presence of the amine 4 (16.5 mM) after: (c) 1 h, (d) 3 h 30 min and (e) 18 h. The resonances of $1 \supset 3$ -Boc and $1 \supset 5$ u-Boc are marked with red and green circles, respectively. HPLC chromatograms (C8 column, H₂O + 0.1% TFA/MeCN + 0.1% TFA -60 : 40 to 0 : 100, 293 K, 254 nm) of: (f) the mixture of the different components obtained after the aminolysis $1 \supset 3$ -Boc at the exclusion of $1 \supset 5$ u-Boc that has been previously removed by preparative GPC. (g) Rotaxane 6 after purification using silica chromatography. (h) Electrospray ionization mass spectrum (ESI-MS) of the rotaxane 6. The star and the cross denoted an artefact of the mass spectrometer and an undetermined *m*/*z* value of [6 + 28], respectively.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

§ Improbable rotaxanes, also termed "impossible" rotaxanes in the literature, cannot be obtained through template-based synthetic methods because of the absence of formal mutual site of interaction. They are instead prepared using a mechanically interlocking auxiliary, or through active template or covalent-bond driven method, see ref. 13. ¶ It was not possible to determine a precise affinity constant for the complex as the NMR was too crowded to allow the correct integration of the different species resonances.

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