Supramolecular Machines and ARchitectures Team D15

pH-Responsive Lasso-Based Rotaxane Molecular Switches

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■ Introduction.

Stimuli-responsive multi-components molecular machines have been the subject of many efforts during the past decades, especially in designing novel interlocked molecular architectures, new sites of interactions and in improving the efficiency of template-synthesis. Pseudo[1]rotaxanes, which consist of a macrocycle covalently linked to a molecular axle that can be threaded or not in a reversible manner, have been rather intensively studied until now. On the contrary, the synthesis of [1]rotaxanes, where no equilibrium takes place with their non-interlocked analogues, have been the subject of only a few reports. To the best of our knowledge, to date, the only lasso molecular machines reported that exhibit a large amplitude movement (four in total), are all based on the system of molecular machinery reported by our team.[1] These lasso molecular architectures of changeable conformations triggered by external *stimuli* appear to be appealing targets, especially because the properties of molecules are well known to be tremendously relied on their topology. Bioactive molecules found in nature, like lasso-peptides, demonstrate the utility of such interlocked lasso architectures. Indeed, the natural lasso peptides, which consist of 16-21 amino acid residues, share a lasso structure which is responsible of a biological activity as receptor antagonist or enzymatic inhibitor. However, no lasso peptides has been synthesized using chemical tools until now and no lasso molecular machinery has been envisaged so far in order to modify the conformation of a peptide in a lasso structure. Here, we present the synthesis of a new lasso-based molecular switch using a self-entanglement strategy of a "hermaphrodite" molecule [3] (Figure 1), and the extension to the pH-responsive peptide-containing lasso series [4] (Figure 3).

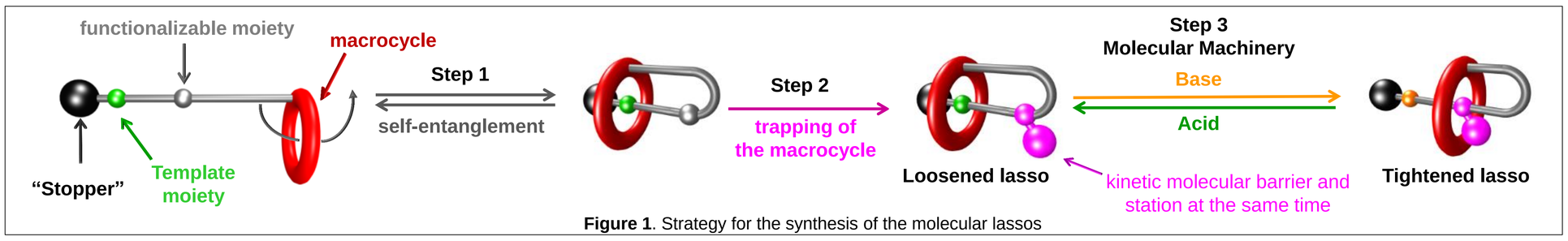


Figure 1. Strategy for the synthesis of the molecular lassos

The lasso molecular structure **4** is composed of a benzometaphenylene-25-crown-8 (BMP25C8) macrocyclic head and a molecular tail containing an anilinium template moiety for the BMP25C8 and terminated by a bulky di-*tert*-butylphenyle extremity (Scheme 1). The choice of the macrocycle is crucial for the efficiency of the [1]rotaxane formation: indeed, it must interact with the molecular anilinium template, on one hand, and must have a size of cavity that allows the self-entanglement strategy. This is the case of the BMP25C8 which holds the tail and the glycol substituents of one aromatic ring in *meta* position to each other. Even though the affinity of the anilinium template appeared to be less good for the BMP25C8 than for the DB24C8, the interlocking is possible, in some specific conditions, by self-entanglement of the non-interlocked hermaphrodite molecule **4u** including the anilinium template and a triazole moiety. The subsequent benzylation of the triazole [2] allowed, at the same time, for the creation of a second site of interactions for the BMP25C8 and for the trapping of the lasso structure by hindering the axle between the anilinium template and the BMP25C8. Variations in pH triggers the tightening or the loosening of the lasso.

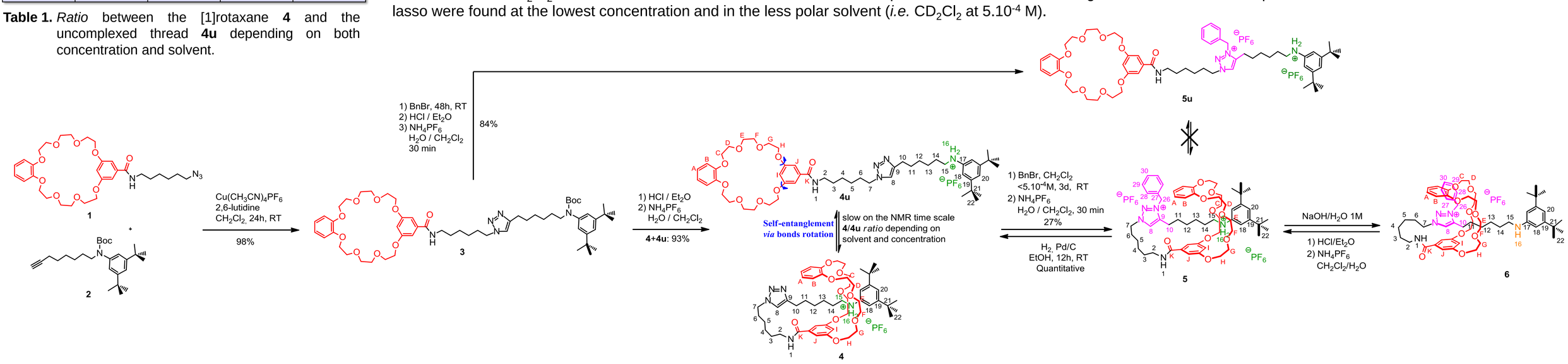
■ Synthesis.

The uncomplexed thread **3** was almost quantitatively prepared from the previously synthesized macrocycle azide **1** and the *N*-Boc protected aniline alkyne **2** using the Copper(I)-catalyzed Huisgen alkyne-azide 1,3-dipolar cycloaddition in the presence of $Cu(MeCN)_4PF_6$ (1 equiv) and 2,6-lutidine (0.1 equiv) (Scheme 1). The subsequent removal of the N-Boc protection in acidic medium revealed the anilinium moiety, which could then freely interact with the BMP25C8 macrocycle. This afforded a mixture of uncomplexed thread **4u** and pseudo[1]rotaxane **4** in a 93% yield. At this stage, the presence of the bulky di-*tert*-butyl anilinium extremity prevented the tail of the molecule from threading the macrocycle. However, the *meta* substitution of the aromatic ring of the macrocycle allowed for its rotation around the two carbon-oxygen σ bonds (blue arrows in Scheme 1), causing the self-entanglement of **4u**. Interestingly, this self-entanglement equilibrium between **4** and **4u**, could be displaced either upon variation in solvent polarity or concentration (Table 1).

4/4u	DMSO-*d*6	CD_3OD	CD_3CN	CD_2Cl_2
5.10^{-2}M	-	-	-	23/77
5.10^{-3}M	0/100	4/96	14/86	36/64
5.10^{-4}M	-	-	-	45/55

Table 1. *Ratio* between the [1]rotaxane **4** and the uncomplexed thread **4u** depending on both concentration and solvent.

The *ratio* **4**/**4u** was easily determined by ^{1}H NMR since the equilibrium between pseudo[1]rotaxane **4** and uncomplexed thread **4u** appeared to be slow on the NMR time-scale, therefore exhibiting one family of ^{1}H NMR signals for each compound. Unsurprisingly, in the more polar DMSO-*d*6 solvent, no self-entanglement was observed, due to the high solvation of the anilinium template by DMSO, preventing from any kind of interactions between the BMP25C8 and the anilinium template. Traces of pseudo[1]rotaxane **4** were detected in CD_3OD, whereas the amount of interlocked compound **4** becomes not negligible in CD_3CN and significant in the less polar solvent CD_2Cl_2. Variation of the concentration in CD_2Cl_2 was then realized in order to determine the best parameters for the self-entanglement of **4**. The best experimental conditions for the formation of the lasso were found at the lowest concentration and in the less polar solvent (*i.e.* CD_2Cl_2 at 5.10^{-4} M).



Scheme 1. Synthesis of the molecular lassos and molecular machinery

The benzylation of the triazole moiety of **4** was then achieved, with the aim to incorporate a bulky molecular barrier that traps the macrocycle around the thread, thus preventing the structure from any self-disentanglement, and to create a second molecular station for the BMP25C8. To favour the formation of the lasso structure, the triazole moiety was benzylated at a very low concentration (<5.10^{-4}M) by slowly adding over a period of 24h a solution of **4**/**4u** in CH_2Cl_2 to a very large excess of benzyl bromide dissolved in dichloromethane. After stirring for further 48h, benzyl triazoliums **5** and **5u** were recovered separately after chromatographic columns respectively in 27% and 38% yield.

■ Molecular Machinery between Lasso Compounds 5 and 6.

Variation in pH was then envisaged to tighten and loosen the lasso structure. At acidic pH, the BMP25C8 resides around the best anilinium molecular station. This was confirmed by the direct comparison between the ^{1}H NMR spectra of the non-interlocked thread **5u** and the [1]rotaxane **5** (Figure 2, a-b). Upon deprotonation of the [1]rotaxane **5**, the BMP25C8 moves from its initial anilinium localization towards the triazolium station, thus triggering the tightening of the lasso structure. It is noteworthy that absolutely no uncomplexed thread **6u** was observed after deprotonation, which corroborates the already discussed fact that no self-disentanglement can be possible when a benzyle gate is present on the axle. The protonated loosened and the deprotonated tightened lasso structures can be evidenced by the direct comparisons of the ^{1}H NMR spectra between the protonated lasso **5** and its unthreaded analogue **5u** (Figure 2, a-b), between the lassos **5** and **6** (Figure 2, b-c) and between the deprotonated lasso **6** and its unthreaded analogue **6u** (Figure 2, c-d).

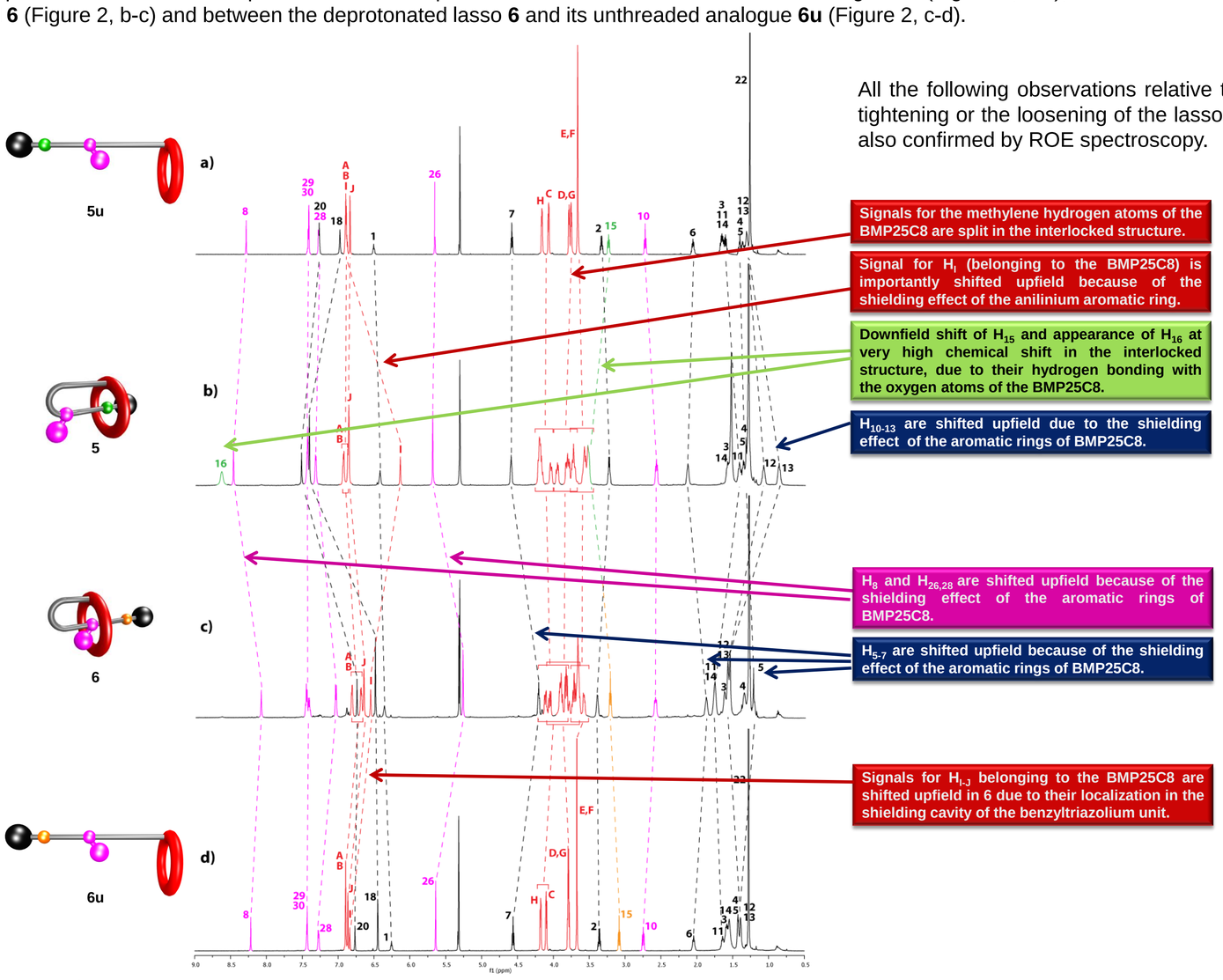


Figure 2. ^{1}H NMR spectra (600 MHz, CD_2Cl_2, 298 K) of: (a) the protonated non-interlocked thread **5u**; (b) the protonated loosened lasso-based compound **5**; (c) the deprotonated tightened lasso-based compound **6**; (d) the deprotonated non-interlocked thread **6u**. The colouring, lettering and numbering correspond to the proton assignments indicated in Scheme 1.

■ Extension to the synthesis of a peptide-containing lasso molecular switch.

The self-entanglement strategy was then extended to the preparation of a model peptide-containing lasso molecular switch. [4] (Figure 3)

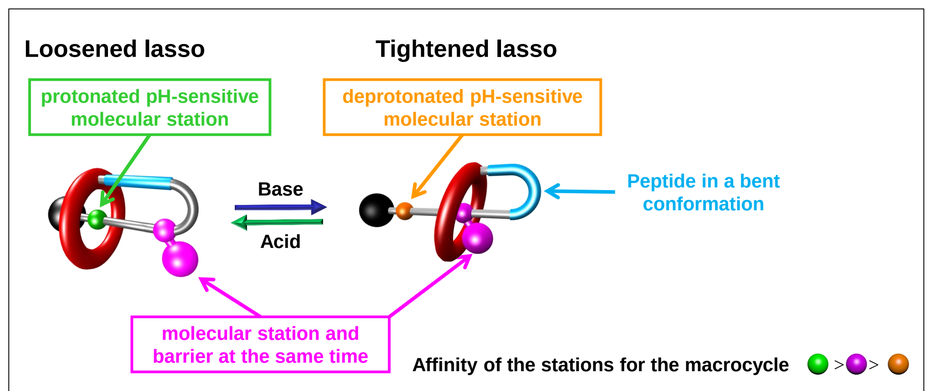
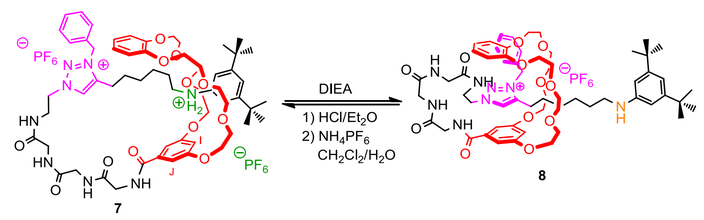


Figure 3. Extension to the synthesis of a peptide-containing lasso molecular switch

In this interlocked [1]rotaxane molecular machine, a model GlyGlyGly tripeptide sequence has been inserted between the macrocycle and the triazolium station, so that its conformation can be tailored depending on the shuttling of the macrocycle from one station to the other. It is noteworthy that the peptide moiety, located in the loop of the lasso, does not play any role in the molecular machinery.



Scheme 2. Molecular machinery on the peptide-containing molecular lassos

To the best of our knowledge, this is the first example of a pH-sensitive lasso molecular switch incorporating a peptide backbone. The extension of this concept to peptide sequences of interest (like RGD for example) is in progress.
Tailoring the conformation of the peptide depending on pH could give rise to the fine tuning of the activity or the affinity of a peptide for its enzyme or receptor.

References

[1] Eric Busseron, Frédéric Coutrot, *Chem. Eur. J.* **2008**, *14*, 4784-4787.
[2] Eric Busseron, Frédéric Coutrot, *J. Org. Chem.* **2013**, *78,* 4099-4106
[3] Caroline Clavel, Camille Romuald, Emile Brabet, Frédéric Coutrot, *Chem. Eur. J.* **2013**, *19,* 2982-2989
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