A pH-Sensitive Peptide-Containing Lasso Molecular Switch

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Abstract: The synthesis of a peptide-containing lasso molecular switch by a self-entanglement strategy is described. The interlocked [1] rotaxane molecular machine consists of a benzometaphenylene[25]crown-8 (BMP25C8) macrocycle surrounding a molecular axle. This molecular axle contains a tripeptidic sequence and two molecular stations: a N-benzyltriazolium and a pH-sensitive anilinium station. The tripeptide is located 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. At acidic pH, the macrocycle resides around the anilinium moiety, whereas it shuttles around the triazolium station after deprotonation. This molecular machinery thus forces the lasso to adopt a tightened or a loosened conformation.

Keywords: [1]rotaxane; lasso; molecular switch; self-entanglement; peptide

1. Introduction

As part of our research concerning the synthesis of interlocked molecular machines, we were interested in the synthesis of lasso compounds. In Nature, lasso molecules, more precisely lasso peptides, can be secreted by bacteria. These compounds contain 16 to 21 amino acid residues which define a stable lasso molecular shape (i.e., a peptide macrocycle covalently linked to a peptide axle which threads the macrocycle) with a very constrained conformation, in comparison with their unthreaded analogues. The macrocycle is threaded by the peptide tail, whose bulky side-chains act as
molecular barriers to trap the macrocycle around the peptide axle in the lasso conformation. The encircled peptide axle then adopts a looped conformation, conferring to the lasso a very compact tridimensional structure, which is responsible for its biological activity. Beyond their remarkable stability against proteolytic degradation, chemical and thermal denaturation [1–3], some lasso peptides proved to inhibit HIV replication [4] or the Gram-negative RNA polymerase [5]. As a particular example of lasso peptide, the ribosomally synthesized [6,7] antimicrobial peptide microcin J25 (MccJ25), which was isolated from *E. coli* in 1992, [8] has been extensively studied during the past years [9–12]. The amino acid sequence was first proposed by Blond et al. in 1999, who proposed a 21-residue, head-to-tail cyclic structure [13]. Four years later, three teams (Montelione and Ebright et al., Craik et al., and Darst et al.) demonstrated concomitantly that the initial proposed covalent and three-dimensional structure was incorrect, and that the peptide was in fact a lasso compound [14–16].

In view of their structural-dependent properties, lasso peptides could be considered as potential scaffold for therapeutic peptides [17]. With this aim, several biosyntheses and structure-activity analysis of a wide range of lasso peptides were realized [18–20]. Recently, Marahiel et al. have highlighted the interest of incorporating a RGD peptide sequence in the turn of the lasso MccJ25 [21]. They substituted the native Gly^{12}Ile^{13}Gly^{14} by the ArgGlyAsp sequence in the loop of the lasso, using site-directed mutagenesis of the precursor protein McjA. Interestingly, they found that the use of a lasso scaffold triggers a specific conformation of the incorporated bioactive peptide sequence, which is responsible for specific and distinct physical, biological and chemical properties, with respect to their linear shape. To date, no lasso peptide has been synthesized using chemical protocols, probably because of the highly difficult synthetic challenge, especially due to the folding of peptides which disturbs the necessary preorganization between the components to be assembled into an interlocked lasso. However, other more or less related works concerning peptides and interlocked molecular architectures such as peptido[2]rotaxanes have already been reported. Leigh et al. have been the first to surround the dipeptide unit GlyGly with a tetramide macrocycle, using hydrogen bond interactions between the peptide amide carbonyl groups of the thread and the NH of the macrocycle [22]. They next extended their efficient five-components hydrogen bond directed clipping strategy to the synthesis and the study of a wide range of dipeptides [23–26]. They also prepared many [2]rotaxane molecular shuttles in which the encircled thread contains different peptide moieties as molecular stations [27–35]. Inversely, some [2]rotaxanes, in which the macrocycles consist of cyclic peptides were reported too [36]. More recently, Moretto et al. employed the Leigh’s strategy to yield an oligopeptide [2]rotaxane shuttle, in which the longest part of the axle is a rigid helical peptide [37]. It is also noteworthy that some biological applications of the peptide rotaxanes emerged. Indeed, Leigh et al. prepared a pentapeptide [2]rotaxane derived from the Met-enkephalin in which the peptide core is protected against peptidase-catalyzed hydrolysis, until it is released by the action of a galactosidase [38,39]. Anderson et al. reported an enzymatic synthesis, using α-chymotrypsin, of a peptide rotaxane based on a cyclodextrin and a peptide thread containing a diazo moiety. They also described the resistance of the obtained peptide rotaxane against enzyme-catalyzed hydrolysis depending on the photoisomerization of the diazo moiety [40]. Meanwhile, Smithrud et al. designed rotaxanes as peptide carriers, using a grafted crown ether and a calix[4]arene, cyclophanne, or cleft as a blocking group [41]. It remains from all these studies that protection of peptides against enzymatic degradation, on one hand, and the
tailoring of the shape of peptides, on another hand, are two main problems to tackle for the conception of new efficient peptide drugs.

Since the properties of a molecule highly rely on its topology, we were interested in combining a lasso molecular architecture that contains a peptide sequence with the molecular machinery. Therefore, we report, in this paper, a first synthetic approach of a lasso molecule containing, in its turn, the simplest GlyGlyGly peptide as a model sequence (Figure 1).

**Figure 1.** Cartoon representation of the molecular lasso targets and of the synthetic strategy used to prepare pH-sensitive peptide-containing lasso molecular switch.

The lasso has also been designed so that it could be possible to act as a pH-sensitive molecular shuttle. Thus, the shuttling of the macrocycle along the threaded axle could trigger a variation in the conformation of the lasso (more or less tightened), hence causing a more or less bent conformation of the peptide. This concept should then be attractive for switching the properties of a peptide part of a lasso after applying an external stimulus.

The molecular machinery described in this paper is based on interactions between a crown ether macrocycle and our already reported system of molecular stations [42]. Indeed, the lasso compound is composed of a benzometaphenylene[25]crown-8 (BMP25C8) [43–46] macrocycle which surrounds a molecular axle containing two molecular stations for the BMP25C8. The anilinium moiety [47–49] is the best molecular station and is used as the molecular template for the rotaxane formation. The N-benzyltriazolium station is of poorer affinity, as long as the anilinium remains protonated. At the protonated state, the lasso lies in a loosened conformation without many constraints for the conformation of the peptide. However, after deprotonation, the BMP25C8 shuttles around the triazolium station, causing a tightening of the lasso and forcing the peptide to adopt a more bent conformation.
2. Results and Discussion

2.1. Synthetic Strategy to Synthesize the Lasso Compounds

The necessary driving force to yield our interlocked lasso molecules lies on the interactions between a crown ether and an ammonium moiety [50,51]. Even though the binding affinities of the molecular stations are lower for the larger macrocycle BMP25C8 than for the smaller DB24C8, the formation of the [1]rotaxane was still possible. In the present paper, the strategy used to interlock the molecular structure is based on a self-entanglement [52] of a “hermaphrodite” molecule (i.e., a molecule containing both a macrocycle as host and an axle holding the anilinium template as guest) (Figure 1). The size of the cavity of the macrocycle is crucial for the successful self-entanglement strategy, especially because the end of the molecular axle is already capped with a bulky stopper, which stops the axle from threading through the macrocycle by this extremity. However, the BMP25C8 macrocycle appears large enough to allow for the rotation of the meta-substituted aromatic ring. This internal rotational movement in the BMP25C8 leads to the threading of the covalently linked anilinium-containing molecular axle, until the macrocycle surrounds the anilinium molecular template. At this stage, the lasso compound remains in equilibrium with the initial un-interlocked hermaphrodite molecule. We can then take advantage of the presence of the triazole moiety, which is located between the macrocycle and the anilinium station, in order to create the second molecular station (i.e., triazolium) on one hand, and especially on the other hand, to incorporate a bulky side-chain that acts as a kinetic molecular barrier [53] and traps the interlocked architecture.

2.2. Preliminary Results Obtained on a Non-Peptidic Lasso Molecular Switch

We have recently reported the feasibility of the self-entanglement strategy on a non-peptidic molecule [54] (Scheme 1, left side). The hermaphrodite unthreaded molecule 4u, consisting of the BMP25C8 macrocycle linked to the anilinium molecular axle, was prepared in a two-step sequence from the already synthesized alkyne 1 [55]. This alkyne compound possesses a carbamoylated di-tert-butylnilamine moiety, so that no template effect can occur at this time.

The first step was a copper(I)-catalyzed Huisgen [56–59] 1,3-dipolar cycloaddition, also called “CuAAC click chemistry [60,61], between the alkyne 1 and the azido BMP25C8 2 in dichloromethane in the presence of 2,6-lutidine and Cu(MeCN)4PF6. This step led very efficiently to the triazole compound 3, which was then submitted to a decarbamoylation that revealed the anilinium template moiety. A slow equilibrium, on the NMR time scale, between the interlocked lasso 4 and the uncomplexed molecule 4u could then be observed. Since the di-tert-butylnilinium end of the molecular axle is too bulky to thread into the macrocycle, the only way to assemble the lasso architecture is through self-entanglement. It was demonstrated that the best conditions to obtain the lasso 4 was the use of dichloromethane, as a good hydrogen bond promoting solvent, and at high dilution. In these optimal conditions, a maximum ratio 4/4u of 45/55 was measured. Subsequent trapping of the lasso structure was realized by taking advantage of the localization of the triazole moiety between the macrocycle and the anilinium molecular station. The benzylaion of the triazole afforded the lasso 5 in a 27% yield. Here, no more equilibrium between interlocked compound 5 and uncomplexed compound 5u was possible, due to the incorporation of the benzyl moiety on the triazole
which acts as a steric molecular barrier for the BMP25C8. After deprotonation of the lasso 5, we found that the BMP25C8 shuttles toward the triazolium molecular station, thus tightening the lasso structure. This process could be reversed in acidic medium.

**Scheme 1.** Synthesis of the molecular lassos and molecular machinery.
2.3. Extension to the Preparation of a pH-Sensitive Tripeptide-Containing Lasso Molecular Switch

2.3.1. Synthetic Part

We then aimed to incorporate a peptide sequence in the turn of the lasso molecular architecture. The simplest GlyGlyGly tripeptidic sequence was chosen in order to fit with the structural prerequisites for the self-entanglement synthetic strategy. Indeed, no such self-entanglement should be authorized with bulky amino acid side-chains. With the aim to be able to tailor the conformation of the peptide depending on the molecular machinery applied to the lasso, the peptide sequence has been localized the closest to the macrocycle BMP25C8, that is to say in the loop of the lasso. Thus, the azido-containing macrocycle 10 was first synthesized according to a three-step sequence from the commercially available BocGlyGlyGlyOH. Activation of the C-terminal side of the tripeptide using Castro’s reagent BOP [62] in the presence of triethylamine and 2-azidoethylamine [63] afforded the azido tripeptide 8 in quantitative yield. The further decarbamoylation using trifluoroacetic acid provided the ammonium intermediate 9, which was then submitted to a coupling reaction with the carboxylic acid substituted BMP25C8 to yield the azido macrocycle 10. Due to the very poor solubility of compound 10 in dichloromethane and in acetonitrile at room temperature, the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition was accomplished with alkyne 1 in acetonitrile and at a temperature of 70 °C. In these experimental conditions, 90% of the unthreaded compound 11 was isolated. Decarbamoylation of 11 unmasked the anilinium template and allowed for the self-entanglement of the hermaphrodite molecule 12u. The equilibrium between the lasso 12 and its unthreaded analogue 12u was studied in details (see Section 2.3.2.). The subsequent benzylation of the lasso 12 allowed for the trapping of the lasso molecular architecture: it was carried out in dichloromethane at high dilution with respect to 12 and using a very large excess of benzyl bromide. Although the conversion rate for the benzylation of the non-peptidic lasso 4 was quasi quantitative (only 5% of the starting triazole compounds 4/4u were recovered after chromatographic columns), the formation of the benzyl triazolium appears much more tricky in the case of the peptide-containing lasso. Indeed, after 7 days, 33% of the triazole compounds 12/12u were still detected by NMR spectroscopy. Moreover, several chromatographic columns on both silica gel and sephadex yielded to a mixture composed of 17% of the unthreaded benzyltriazolium 13u and 17% of the desired triazolium lasso 13. The separation between these compounds was found to be very tough and only 4% of 13 could be isolated. We were curious about the possibility to realize the benzylation at a higher temperature in order to accelerate the rate of the reaction. Unfortunately, VT 1H-NMR experiments in C2D2Cl4 on 12/12u showed that increasing the temperature results in the dramatic decrease of the proportion of the lasso compound 12 (Table 1, entries 3–7). Despite the poorly efficient reaction of benzylation, the deprotonation of the isolated loosened lasso 13 was carried out efficiently by adding the Hünig’s [64] base DIEA. Deprotonation of the anilinium triggered the shuttling of the BMP25C8 towards the triazolium moiety, hence causing the tightening of the lasso and inducing the more constraint conformation of the tripeptide (see Section 2.3.3.).
2.3.2. Studies of the Equilibrium between the Unthreaded Compound $12u$ and the Lasso $12$

The equilibrium between the unthreaded compound $12u$ and the lasso $12$ highly depends on the polarity of the solvent (Figure 2), on the concentration (Figure 3), and on the temperature.

Figure 2. $^1$H-NMR spectra (600 MHz, 298 K) at $5 \times 10^{-4}$ M of a mixture of compounds $12/12u$ in: (a) CD$_3$OD. (b) CD$_3$CN. (c) CD$_2$Cl$_2$. The lettering and numbering correspond to the proton assignments indicated in scheme 1. The orange color corresponds to the unthreaded molecule $12u$, whereas the green color corresponds to lasso compound $12$. The black assignments correspond to both compounds.

Table 1 summarizes the $12/12u$ ratios measured in the different solvents from the best hydrogen bond promoting solvents dichloromethane and tetrachloroethane to the more polar solvent methanol and upon variation of the concentration and temperature. As the equilibrium between $12$ and $12u$ proved to be slow on the NMR time scale, two sets of $^1$H-NMR signals were observed for each unthreaded and lasso compounds. The ratio values reported in Table 1 were obtained by $^1$H- NMR spectroscopy at 600 MHz by integrating the signal of hydrogens H$_{15}$ or H$_{12}$.
Figure 3. \(^1\)H-NMR spectra (600 MHz, 298 K) of a mixture of compound 12/12u in CD\(_2\)Cl\(_2\) at: (a) 5.10\(^{-2}\) M. (b) 5.10\(^{-3}\) M. (c) 5.10\(^{-4}\) M. The lettering and numbering correspond to the proton assignments indicated in scheme 1. The orange color corresponds to the unthreaded molecule 12u, whereas the green color corresponds to lasso compound 12.

Table 1. Ratio between lasso compound 12 and unthreaded molecule 12u depending on concentration, solvent and temperature.

<table>
<thead>
<tr>
<th>Entries</th>
<th>C ((12/12u)) [M]</th>
<th>T (K)</th>
<th>CD(_3)OD</th>
<th>CD(_3)CN</th>
<th>CD(_2)Cl(_2)</th>
<th>C(_2)D(_2)Cl(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.10(^{-2})</td>
<td>298</td>
<td>-</td>
<td>-</td>
<td>35/65</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>5.10(^{-3})</td>
<td>298</td>
<td>-</td>
<td>-</td>
<td>44/56</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>5.10(^{-4})</td>
<td>298</td>
<td>0/100</td>
<td>25/75</td>
<td>47/53</td>
<td>55/45</td>
</tr>
<tr>
<td>4</td>
<td>5.10(^{-4})</td>
<td>318</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38/61</td>
</tr>
<tr>
<td>5</td>
<td>5.10(^{-4})</td>
<td>328</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>29/71</td>
</tr>
<tr>
<td>6</td>
<td>5.10(^{-4})</td>
<td>338</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14/86</td>
</tr>
<tr>
<td>7</td>
<td>5.10(^{-4})</td>
<td>348</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8/92</td>
</tr>
</tbody>
</table>

Obviously, it can be seen from the data reported in Table 1 that the nature of the solvent has a big influence on the 12/12u ratio. At 298 K, the best self-entanglement was noticed in the less polar dichloromethane and tetrachloroethane and at the highest dilution (Table 1, entry 3). At a similar concentration, the proportion of the lasso 12 dramatically declines in the more polar acetonitrile and no presence of the lasso was detected in methanol (Figure 2 and Table 1, entry 3). Eventually, increasing the concentration of the sample in dichloromethane tends to be unfavorable to the interlocked lasso architecture (Figure 3 and Table 1, entries 1–3).
Besides, the equilibrium between 12u and 12 could be highlighted using drift tube ion mobility mass spectrometry (DT IM-MS). By this analytical method, it was possible to separate compounds 12 and 12u, because they have different velocities which are due to their distinct sizes related to their unlike conformational structures. A similar analytical study was utilized by Leigh et al. to distinguish a trefoil knot from its unknotted-macrocycle analogue [65]. Figure 4 shows the DT IM-MS results of various samples of a mixture of compounds 12/12u at the same concentration (5 × 10⁻⁴ M) upon variation of the ratio of a solvent mixture of dichloromethane/acetonitrile. Interestingly, and whatever the polarity of the solvent mixture used for the injection, the lasso compound 12, whose conformation is smaller and more compact, have a lower arrival time distribution (63 scans). On the contrary, the unthreaded molecule 12u consists of a larger ion with less conformational restrictions. As a consequence, it takes more time for this ion to travel through the drift cell (arrival time distribution of 67 scans). Moreover, the same trend in the 12/12u ratio could be detected upon variation of the solvent polarity by this method as that observed by NMR. Indeed, a 12/12u ratio of 18/82 in favor of 12u was measured in the more polar acetonitrile, whereas the ratio was inverted in pure dichloromethane. The consistency of this trend observed from the dichloromethane to the acetonitrile, with respect to the NMR study, suggests that the equilibrium between 12 and 12u is slow on the ion mobility mass spectrometry time scale. The width at half-height of each pick was also measured in pure acetonitrile and dichloromethane: the peak related to the lasso 12 has a slightly narrower distribution, which reveals a more persistent size and shape, in accordance with the more restraint lasso structure. On the contrary, the slightly broadest distribution observed for the peak of the unthreaded molecule 12u exhibits its larger degree of flexibility.

2.3.3. ¹H-NMR Investigation of the Molecular Machinery between Lasso Compounds 13 and 14

The comparison between the ¹H-NMR spectra of the protonated and deprotonated unthreaded molecules 13u, 14u with their respective protonated and deprotonated molecular lassos 13 and 14 allowed to demonstrate the lasso interlocked architecture and the localization of the BMP25C8 around the molecular axle (Figure 5).

In the ¹H-NMR spectrum of the protonated molecular lasso 13, signals for the hydrogen atoms H₁₋₅ of the BMP25C8 appear split because they are facing the two non-symmetrical ends of the molecular axle of the interlocked lasso architecture. Moreover, the localization of the macrocycle around the anilinium station in 13 can be deduced from the direct comparison between the ¹H-NMR spectra of 13 and 13u [Figure 5(a) and (b)]. Indeed, in the lasso 13, the signals for the hydrogen atoms H₂₁ appear at very high chemical shift (δ = 8.60 ppm). Likewise, hydrogens H₂₀ belonging to the anilinium station are shifted downfield with respect to the protonated unthreaded molecule 13u (Δδ = 0.15 ppm), because they interact by hydrogen bonds with the oxygen atoms of the BMP25C8. Concerning the hydrogen H₁ of the BMP25C8, it is dramatically shifted upfield in 13 (Δδ = −0.67 ppm) because it experiences the shielding effect of the anilinium aromatic ring. The same trend is observed for hydrogen atoms H₁₋₅ of the BMP25C8. Eventually, the hydrogen atoms H₁₁ and H₁₅₋₁₉ are all more or less shielded (respectively Δδ = −0.17, −0.15, −0.40, −0.50, −0.63 and −0.30 ppm) because they all undergo the shielding effect of the aromatic rings of the BMP25C8. Among them, it is noteworthy that hydrogen atoms H₁₆₋₁₇₋₁₈ are the more concerned by this effect.
Figure 4. DT IM-MS of the mixture 12/12u in: (a) CH$_3$CN. (b) CH$_3$CN/CH$_2$Cl$_2$ 30/70. (c) CH$_3$CN/CH$_2$Cl$_2$ 20/80. (d) CH$_3$CN/CH$_2$Cl$_2$ 10/90. (e) CH$_2$Cl$_2$. Data shows: the average time distribution (in scan units) at a drift voltage of 40V and the peak area (in %) for the detected molecular ion [M–PF$_6$]$^+$. 
**Figure 5.** $^1$H-NMR spectra (600 MHz, CD$_2$Cl$_2$, 298 K) of: (a) the protonated unthreaded compound 13u. (b) the protonated lasso-based compound 13. (c) the deprotonated lasso-based compound 14. (d) the deprotonated unthreaded compound 14u. The coloring, lettering and numbering correspond to the proton assignments indicated in scheme 1.

Deprotonation of the loosened lasso 13 led to the tightened lasso 14. The direct comparison of the $^1$H-NMR spectra between the two lasso [1]rotaxanes 13 and 14 reveals the shuttling of the BMP25C8 [Figure 5(b) and (c)]. Unsurprisingly, $^1$H-NMR chemical shifts of hydrogens H$_{20}$, H$_{23}$ and H$_{25}$, which belong to the anilinium moiety, are shifted upfield in the deprotonated lasso 14, due to the deprotonation of the anilinium unit. At the same time, in 14, H$_1$ is shifted downfield ($\Delta \delta = 0.31$ ppm) because it does not experience anymore the strong shielding effect of the aniline extremity. However, this signal is still at a lower chemical shift than those observed in the unthreaded protonated and deprotonated molecules 13u and 14u (respectively $\Delta \delta = -0.36$ and $-0.34$ ppm) [Figure 5(c) and (d)]. This result can be explained by the new localization of H$_1$ which now experiences the shielding effect of the benzyl triazolium moiety, this latter effect being weaker than those of the anilinium aromatic ring. This observation is corroborated by the upfield shift underwent by the other hydrogen atoms of the aromatic rings of the crown ether H$_J$, H$_A$ and H$_B$ (respectively $\Delta \delta = -0.18$, $-0.04$ and $-0.16$ ppm), which experience the same shielding effect from the triazolium ring. Moreover, the displacement of
the BMP25C8 can account for the upfield shift observed in 14 for the hydrogen atoms of the benzyl molecular barrier H$_{28}$, H$_{30}$ (respectively $\Delta\delta = -0.23$, $-0.24$ ppm) and in a lesser extent H$_{32}$ (with respect to 13), due to their localization in the shielding cavity of the aromatic rings of the BMP25C8. Similarly, the hydrogen atoms of the molecular axle located on the other side of the molecular barrier H$_{11}$ and H$_{12}$ are both shielded by the BMP25C8: actually, this latter lies on the triazolium moiety and its aromatic rings can reach the other side of the molecular barrier (respectively $\Delta\delta = -0.23$, $-0.41$ ppm). On the contrary, the hydrogen atoms located between the aniline and the triazolium unit H$_{16}$, H$_{17}$, H$_{18}$ and H$_{19}$ and in a much lesser extent H$_{13}$, are shifted downfield in 14 (respectively $\Delta\delta = 0.62$, $0.73$, $0.82$, $0.22$ and $0.13$ ppm) because they do not experience anymore the shielding effect of the BMP25C8. The direct comparison between the $^1$H-NMR spectra of the tightened deprotonated lasso 14 and the deprotonated unthreaded analogue 14u corroborates the localization of the BMP25C8 [Figure 5(c) and (d)].

**Figure 6.** $^1$H-NMR ROESY experiment (600 MHz, CD$_2$Cl$_2$, 298 K) of the protonated loosened molecular lasso 13. The coloring, lettering and numbering correspond to the proton assignments indicated in Scheme 1.
The ROESY $^1$H-NMR experiments provided complementary and consistent insights into the structure of the lasso molecular switch 13/14 (Figures 6 and 7). The ROESY $^1$H-NMR experiment carried out on the protonated lasso (Figure 6) provided the evidences of the lasso architecture with a localization of the BMP25C8 around the anilinium station (relevant correlation peaks highlighted in cyan). It is particularly true for the correlation peaks between respectively H$_{23}$ and H$_{27}$ of the anilinium site with H$_{C-H}$ of the BMP25C8, or between H$_{I}$ of the BMP25C8 with H$_{20}$, H$_{23}$ and H$_{27}$. Interestingly, no correlation peak between the BMP25C8 and the triazolium site was observed, which is consistent with the localization of the macrocycle around the anilinium moiety.

**Figure 7.** $^1$H-NMR ROESY experiment (600 MHz, CD$_2$Cl$_2$, 298 K) of the deprotonated tightened molecular lasso 14. The coloring, lettering and numbering correspond to the proton assignments indicated in Scheme 1.
After deprotonation (Figure 7), the relevant highlighted cross peaks relative to the loosened lasso disappear, whereas new correlation peaks demonstrate the new main localization of the macrocycle around the triazolium. In particular, new correlation peaks are observed for the hydrogens $H_{C-H}$ belonging to the BMP25C8 with respectively the triazolium hydrogen $H_{13}$, the benzyl barrier $H_{28}$ and $H_{30}$, and the next hydrogen $H_{15}$. At the same time, hydrogen atoms $H_{E-F}$ correlate with $H_{31-32}$ of the aromatic benzyl molecular barrier. The aromatic hydrogen $H_i$ of the BMP25C8 is now correlated with the hydrogen atoms $H_{15}$, $H_{16}$ and $H_{17}$, whereas the other aromatic hydrogens $H_A$ and $H_B$ of the BMP25C8 are correlated with the benzyl barrier $H_{28}$. In a consistent way, the aromatic hydrogen $H_j$ of the BMP25C8 is correlated with $H_{12}$, whereas the last aromatic hydrogen of the BMP25C8 $H_A$ is now correlated with $H_{11}$. The whole NMR studies are consistent with the two main localizations of the BMP25C8 around either the anilinium or the triazolium station upon variation in pH.

Concerning the peptide part GlyGlyGly of the lasso molecule, the overlapping in ROESY spectra between methylene hydrogens $H_2$, $H_5$, $H_8$ and the very split methylene hydrogens $H_{C-H}$ of the BMP25C8 unfortunately prevents us from giving dependable assumptions concerning an eventual conformational change of the tripeptide sequence. However, slight changes in chemical shifts could be observed when comparing the 1D $^1$H-NMR spectra of the protonated loosened lasso 13 with the deprotonated tightened lasso 14. The $^1$H-NMR chemical shift of methylene hydrogens $H_2$ remained almost unchanged in 14 ($\Delta \delta = +0.02$ ppm), whereas $H_5$ and $H_8$ are more or less shielded in 14 ($\Delta \delta = -0.05$ and $-0.18$ ppm). This latter observation can be assigned to the shielding effect of the aromatic of the BMP25C8, corroborating the new localization of the BMP25C8.

3. Experimental

3.1. General

All reactions were achieved under an atmosphere of argon unless otherwise indicated. Dichloromethane was distilled over P$_2$O$_5$ and was degassed by bubbling Ar for 20 min. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates. Compounds were visualized by dipping the plates in an ethanolic solution of ninhydrine, followed by heating. $^1$H-NMR and $^{13}$C-NMR spectra were obtained from a Bruker Avance III spectrometers (respectively at 400.13 MHz or 600.13 MHz and 100.62 MHz or 150.95 MHz). Chemical shifts of $^1$H-NMR and $^{13}$C-NMR are given in ppm by using CHD$_2$OH or CHDCl$_2$ as references (3.31 ppm and 5.32 ppm respectively for $^1$H spectrum and 49 ppm and 54 ppm respectively for $^{13}$C spectrum). Coupling constants ($J$) are reported in hertz (Hz). Standard abbreviations indicating multiplicity were used as follows: s (singlet), br (broad), d (doublet), t (triplet), q (quartet), m (multiplet). High-resolution mass spectra (HRMS) and mass spectra were recorded on a Q-TOF Micro (water) apparatus.


$\text{Boc-GlyGlyGly-NH-CH}_2\text{CH}_2\text{-N}_3$ (8). 2-Azidoethylamine was first prepared by adding sodium azide (800 mg, 12.2 mmol, 2.3 equiv) to a solution of 2-bromoethylamine hydrobromide (1.08 g, 5.3 mmol, 1 equiv) in H$_2$O (20 mL). The resulting solution was then stirred overnight at 65 °C. After cooling down to RT, NaOH (232 mg, 5.8 mmol, 1.1 equiv) was added to the reaction mixture. The resulting aqueous
solution was saturated with NaCl and extracted with CHCl₃ (3 × 20 mL). The organic layer was dried over anhydrous MgSO₄ and used directly for the next step without being concentrated. The solution containing the 2-azidoethylamine (5.3 mmol, 1.5 equiv) was diluted with CHCl₃ (total volume: 150 mL) before adding successively Boc-glycylglycylglycine (1 g, 3.5 mmol, 1 equiv) and triethylamine (1.43 mL, 10.6 mmol, 3 equiv). The mixture was then stirred until complete dissolution of the tripeptide (15 min). BOP (1.88 g, 4.6 mmol, 1.3 equiv) was added and the mixture was stirred at RT for 18 h. The resulting white precipitate was filtered, washed with CH₂Cl₂ and dried under vacuum. It was used in the next step without further purification (1.25 g, quantitative). Rf: 0.40 (CH₂Cl₂/MeOH 9/1). ¹H-NMR (600 MHz, CD₃OD, 298K): δ ppm = 3.90 & 3.86 & 3.75 (3s, 3×2H, H₂ H₅ H₈), 3.45–3.34 (m, 4H, H₁₁ H₁₂), 1.45 (s, 9H, HCH₃-Boc). ¹³C-NMR (150 MHz, CD₃OD, 298K): δ ppm = 173.6 & 172.2 & 171.9 (C₃ C₆ C₉), 158.8 (C O-Boc), 81.0 (C(CH₃)₃-Boc), 51.3 (C₁₂), 44.9 & 43.8 & 43.4 (C₂ C₅ C₈), 39.9 (C₁₁), 28.7 (CH₃-Boc). MS (ESI): [M + H]+ calcd for C₁₃H₂₄N₇O₅: 358.18, found: 358.19.

TFA, H-GlyGlyGly-NH-CH₂CH₂-N₃ (9). TFA (5 mL) was added to a suspension of the Boc-GlyGlyGly-NHCH₂CH₂N₃ (1.25 g) in CH₂Cl₂ (20 mL), leading to complete dissolution of this compound. After stirring for 15 min at RT, the reaction mixture was concentrated and co-evaporated 5 times with CH₂Cl₂ (20 mL) in order to remove the excess of TFA. The resulting white solid (1.15 g, quantitative) could be used without further purification. Rf: 0.09 (CH₂Cl₂/MeOH 9/1). ¹H-NMR (600 MHz, CD₃OD, 298K): δ ppm = 3.98 & 3.88 & 3.78 (3s, 3×2H, H₂ H₅ H₈), 3.39 (s, 4H, H₁₁ H₁₂). ¹³C-NMR (150 MHz, CD₃OD, 298K): δ ppm = 171.9 & 171.8 & 168.4 (C₃ C₆ C₉), 161.0 (q, J²C-F = 38.3 Hz, CO₂-TFA), 117.1 (q, ²J²C-F = 287.6 Hz, CF₃-TFA), 51.4 (C₁₂), 43.6 & 43.4 & 41.6 (C₂ C₅ C₈), 39.9 (C₁₁). MS (ESI): [M−TFA]+ calcd for C₉H₁₆N₇O₅: 258.13, found: 258.13.

Azido macrocycle (10). The preliminary synthesized carboxylic acid macrocycle [8] (894 mg, 1.82 mmol, 1 equiv) and compound 9 (707 mg, 2 mmol, 1.1 equiv) were dissolved in DMF (20 mL). Then, triethylamine (755 µL, 5.45 mmol, 3 equiv) and BOP (973 mg, 2.36 mmol, 1.3 equiv) were added and the mixture was stirred at RT for 17 h. DMF was evaporated under vacuum and the resulting yellow oil was diluted with CH₂Cl₂ (100 mL), before being successively washed with an aqueous solution of HCl 1M (100 mL), a saturated aqueous solution of NaHCO₃ (100 mL) and brine (100 mL). The organic layer was then dried over MgSO₄ and concentrated to yield a beige precipitate, which was then triturated, washed with Et₂O (200 mL) and dried under vacuum. The compound was pure enough to be used without further purification (1.33 g, quantitative). Rf: 0.63(CH₂Cl₂/MeOH 9/1). ¹H-NMR (600 MHz, CD₃OD, 298K): δ ppm = 7.04 (d, 2H, ²J₄H₄H₅ = 2.1 Hz, H₄), 6.98–6.92 (m, 2H, H₁ H₂), 6.91–6.85 (m, 3H, H₁ H₇ H₈), 4.22–4.16 (m, 4H, H₄H₅), 4.14–4.08 (m, 4H, H₄C), 4.04 (s, 2H, H₂), 3.91 & 3.89 (2s, 2×2H, H₅ H₈), 3.84–3.77 (m, 8H, H₉ H₁₀H₁₁), 3.69 (s, 8H, H₈ H₉ H₁₀), 3.35–3.32 (2m, 2×2H, H₁₁H₁₂). ¹³C-NMR (150 MHz, CD₃OD, 298K): δ ppm = 172.9 & 172.2 & 171.9 & 170.4 (C₄ C₅ C₆ C₇), 161.6 & 150.5 & 136.6 (C₁IV arom BMP25C₈), 123.0 (C₉), 116.8 (C₈A), 108.0 (C₁), 107.5 (C₁), 72.0 & 71.9 (C₁C₂), 71.0 (2s, C₁₀ C₁₁), 70.3 (C₁₂), 69.4 (C₁₃), 51.3 (C₁₁), 44.5 (C₂), 44.0 & 43.4 (C₅ C₈), 39.9 (C₁₁). MS (ESI): [M + H]+ calcd for C₃₃H₄₆N₇O₁₂: 732.32, found: 732.32.

Compound (11). To a solution of the azido macrocycle 10 (165 mg, 0.225 mmol, 1 equiv) and the preliminary synthesized alkyne 1 [9] (103 mg, 0.25 mmol, 1.1 equiv) in dry CH₃CN (8 mL) at 70 °C,
were added successively Cu(CH₃CN)₄PF₆ (84 mg, 0.225 mmol, 1 equiv) and 2,6-lutidine (3 μL, 0.03 mmol, 0.1 equiv). The mixture was stirred for 24 h at 70 °C. After filtration, the solvent was evaporated under vacuum. The crude was then purified by chromatography on a silicagel column (solvent gradient elution CH₂Cl₂ to CH₂Cl₂/MeOH 80/20) to give compound 11 (234 mg, 90%) as a beige powder. R_f 0.65 (CH₂Cl₂/MeOH 9/1). 1H-NMR (600 MHz, CD₂Cl₂, 298K): δ (ppm) = 7.99 (br t, 1H, H1), 7.70 & 7.46 (2 br t, 2 × 1H, H 4 H7), 7.36 (s, 1H, H13), 7.26 (s, 1H, H25), 7.11 (br t, 1H, H10), 7.01–6.95 (m, 4H, H23 HJ), 6.92–6.86 (m, 4H, H4 H8), 6.83 (s, 1H, H1), 4.32 (t, 2H, 3JH12-H11 = 5.3 Hz, H12), 4.18–4.13 (m, 4H, HH), 4.12–4.07 (m, 4H, HC), 4.00 (d, 2H, 3JH2-H1 = 5.1 Hz, H2), 3.86–3.74 (m, 12H, H5 H8 HD HG), 3.67 (s, 8H, HE HF), 3.62–3.56 (m, 2H, H 11), 3.36 (t, 2H, 3JH20-H19 = 7.3 Hz, H20), 3.20 (2s, CE CF), 3.14 (m, 4H, H16 H17 H18), 1.76–1.69 (m, 2H, 3JH20-H19 = 5.8 Hz, H20), 1.63–1.54 (m, 2H, H16), 1.41–1.32 (m, 2H, H18), 1.35–1.22 (m, 2H, H17), 1.29 (s, 8H, HE HF), 0.81 (br s, 2H, H21), 8.19 (br t, 1H, H1), 7.69–7.62 (m, 1H, H4 or H7), 7.56 (s, 1H, H13), 7.52 (br t, 1H, H25), 7.39 (d, 2H, 4JH23-H25 = 1.2 Hz, H23), 7.19 (t, 1H, 3JH10-H11 = 5.8 Hz, H10), 7.03 (d, 2H, 4JH1-HH = 1.7 Hz, H1), 6.96–6.92

Compounds (12/12u). TFA (2.5 mL) was added to a solution of compound 11 (156 mg, 0.14 mmol, 1 equiv.) in CH₂Cl₂ (10 mL). After stirring for 30 min at RT, the reaction mixture was concentrated and co-evaporated 5 times with CH₂Cl₂ (20 mL) in order to remove the excess of TFA. The residue was then diluted in CH₂Cl₂ (10 mL). To this solution was added NH₄PF₆ (114 mg, 0.7 mmol, 5 equiv) and H₂O milliQ (5 mL): the biphasic solution was stirred vigorously for 30 minutes. The aqueous layer was then extracted with CH₂Cl₂ (3 × 5 mL) and the combined organic layers were dried over MgSO₄ and concentrated to give compounds 12/12u (162 mg, quantitative) as a beige powder. R_f 0.52 (CH₂Cl₂/MeOH 9/1). HRMS (ESI): [M+H−PF₆]²⁺ calcd for C₅₅H₈₂N₈O₁₂: 523.3026, found: 523.3007.

Unthreaded Compound (12u). 1H-NMR (600 MHz, 5.10−2 M in CD₂Cl₂, 298K): δ (ppm) = 8.63 (br s, 2H, H21), 8.19 (br t, 1H, H1), 7.69–7.62 (m, 2H, H4 H7), 7.56 (s, 1H, H13), 7.52 (br t, 1H, H25), 7.39 (d, 2H, 4JH23-H25 = 1.2 Hz, H23), 7.19 (t, 1H, 3JH10-H11 = 5.8 Hz, H10), 7.03 (d, 2H, 4JH1-HH = 1.7 Hz, H1), 6.96–6.92
(m, 2H, H_A), 6.90–6.86 (m, 2H, H_B), 6.20 (br t, 1H, H_1), 4.43 (br t, 2H, H_12), 4.24–3.99 (m, 8H, H_C), 4.24–3.99 (m, 8H, H_H), 3.94–3.67 (m, 12H, H_5 H_8 H_12), 3.73–3.46 (m, 8H, H_E), 3.67–3.56 (m, 2H, H_11), 3.56–3.48 (m, 2H, H_20), 2.41 (t, 2H, J_H15-H16 = 8 Hz, H_15), 1.62–1.52 (m, 2H, H_19), 1.38–1.29 (m, 2H, H_16), 1.27 (s, 18H, H_27), 1.01–0.93 (m, 2H, H_17), 0.87–0.80 (m, 2H, H_18). 13C-NMR (150 MHz, 5.10−2 M in CD2Cl2, 298K): δ (ppm) = 172.4 & 171.2 & 169.1 (4s, C K C3 C6 C9), 160.1 & 149.1 & 135.7 (C IV arom BMP25C8), 154.2 (C 24), 147.8 (C 22), 124.9 (C25), 123.9 (C_A), 117.3 (C23), 113.2 (C_B), 108.2 (C_I), 106.8 (C_J), 71.9 & 71.3 (C_E CF), 70.7 & 70.6 (C_D CG), 69.1 & 68.6 (C C CH), 52.3 (C_B), 50.2 (C12), 45.3 (C2), 44.2 & 44.0 (C5 C8), 39.9 (C11), 35.7 (C26), 31.6 (C27), 30.2 (C16), 29.4 (C17), 29.3 (C19), 26.2 (C18), 25.4 (C13).

[1]rotaxane (13) A solution of compound 12 (162 mg, 0.14 mmol, 1 equiv) dissolved in CH2Cl2 (10 mL) was added dropwise over 24 h to a solution of benzyl bromide (55 mL) and CH2Cl2 (218 mL). The mixture was stirred for further 6d at RT. CH2Cl2 was then evaporated and the excess of benzyl bromide was removed by filtration on a silica gel column (solvent gradient elution CH2Cl2 to CH2Cl2/MeOH 80/20). The remaining solid (224 mg) was then diluted in CH2Cl2 (16 mL). To this solution was added NH4PF6 (133 mg, 0.82 mmol, 5 equiv) and H2O milliQ (8 mL): the biphasic solution was stirred vigorously for 30 minutes. The aqueous layer was extracted with CH2Cl2 (3 × 20 mL) and the combined organic layers were dried over MgSO4 and concentrated under vacuum. The crude was purified twice by chromatography on a silica gel column (CH2Cl2 to CH2Cl2/MeOH 80/20) to give several mixed fractions and the isolated [1]rotaxane 13 (7 mg, 4%) as a colourless oil. Rf 0.86 (CH2Cl2/MeOH 9/1). 1H-NMR (600 MHz, CD2Cl2, 298K): δ (ppm) = 8.60 (br s, 2H, H21), 8.16 (s, 1H, H13), 7.56 (br t, 1H, H_1), 7.52 (s, 1H, H23), 7.41 (s, 2H, H2), 6.94–6.88 (m, 2H, H_A), 6.88–6.83 (m, 2H, H_B), 6.20 (s, 1H, H_I), 5.64 (s, 2H, H28), 4.70–4.63 (m, 2H, H12), 4.24–4.18 & 4.18–4.12 & 4.10–4.04 (m, 8H, H_H), 4.01–3.95 (m, 2H, H_2), 3.92–3.73 (m, 12H, H5 H_8 H_D H_G), 3.78–3.73 (m, 2H, H_11), 3.73–3.41 (m, 8H, H_E H_F), 3.51–3.41 (m, 2H, H_20), 2.51 (t, 2H, J_H15-H16 = 7 Hz, H15), 1.48–1.41 (m, 2H, H_19), 1.31–1.21 (m, 2H, H_16), 1.29 (s, 18H, H_27), 0.88–0.80 (m, 2H, H_17), 0.75–0.67 (m, 2H, H_18). HSQC 13C-NMR (150 MHz, CD2Cl2, 298K): δ (ppm) = 129.9 (C31 C32), 129.6 (C13), 128.5 (C30), 124.7 (C25), 122.5 (C_A), 117.3 (C23), 113.1 (C_B), 108.8 (C_I), 106.4 (C_J), 70.6 & 69.7 (C_D CG), 68.8 & 68.5 (CH C_C), 55.2 (C28), 53.8 (C12), 52.3 (C20), 44.9 (C2), 43.5 (C5 C8), 39.3 (C11), 31.8 (C27), 28.8 (C17), 27.6 (C19), 27.1 (C16), 25.8 (C18), 23.8 (C15). HRMS (ESI): [M-PF6]− calcd for C62H88F6N8O12P: 1281.6164, found: 1281.6177.

[1]Rotaxane (14). To a solution of the [1]rotaxane 13 (3 mg, 2.1 µmol, 1 equiv) in CD2Cl2 (0.6 mL) was added 74 µL of a 1% solution of DIEA in CD2Cl2 (4.2 µmol, 2 equiv.). Rf 0.73(CH2Cl2/MeOH 9/1). 1H-NMR (600 MHz, CD2Cl2, 298K): δ (ppm) = 8.29 (s, 1H, H13), 7.33–7.28 (m, 1H, H32), 7.28–7.24 (m, 2H, H_1), 7.20 (br t, 1H, H_I), 7.12 & 7.08 (2 br t, 2H, H_H), 7.03 (d, 2H, J_H90-H91 = 7.4 Hz, H90), 6.93 (br t, 1H, H10), 6.88–6.84 (m, 2H, H_A), 6.83 (s, 2H, H_2), 6.76 (s, 1H, H23), 6.73–6.68 (m, 2H, H_B), 6.51 (s, 1H, H_I), 6.48 (m, 2H, H23), 5.41 (s, 2H, H28), 4.26 (t, 2H, J_H12-H11 = 6.3 Hz, H12), 4.19–4.14 & 4.10–4.04 (m, 4H, H_H), 4.03–3.97 & 3.94–3.89 (m, 4H, H_C), 4.00 (d, 2H, J_H22-H21 = 5.3 Hz, H2), 3.86–3.66 (m, 12H, H5 H_8 H_D H_G), 3.71–3.55 (m, 8H, H_E H_F), 3.55–3.50 (m, 2H, H_17), 3.15 (t, 2H, J_H20-H19 = 6.5 Hz, H20), 3.07–3.01 (m, 2H, H_15), 1.95–1.82 (m, 2H, H16), 1.69–1.62 (m, 2H, H19),
1.60–1.54 (m, 2H, H17), 1.56–1.49 (m, 2H, H18), 1.28 (s, 18H, H27). HSQC 13C-NMR (150 MHz, CD2Cl2, 298K): δ (ppm) = 129.4 (C31 C32), 129.3 (C13), 129.1 (C30), 121.9 (C4), 113.8 (C8), 112.1 (C25), 108.3 (C1), 107.8 (C2), 105.1 (C1), 71.8 & 71.5 & 69.9 (C6 C7), 70.9 & 70.8 & 68.4 (CD CE), 69.8 & 69.7(C1C), 68.6 & 68.5 (C1H), 54.3 (C28), 52.3 (C12), 44.7 (C20), 44.4 (C2), 44.6 & 43.4 (C5 C8), 38.5 (C11), 31.1 (C27), 30.5 (C19), 29.9 (C17), 27.8 (C18), 26.6 (C16), 23.3 (C15). HRMS (ESI): [M+H]+ calecd for C62H88F6N8O12P: 1281.6164, found: 1281.6144.

3.3. Synthesis and Characterizations of the Uncomplexed Threads 13u and 14u

Protonated Unthreaded Compound (13u): Compound 11 (110 mg, 0.096 mmol, 1 equiv) was dissolved in benzyl bromide (3.4 mL, 300 equiv) and the mixture was stirred for 6d at RT before being directly filtered on a silicagel column to remove the excess of benzyl bromide (solvent gradient elution CH2Cl2 to CH2Cl2/MeOH 85/15). To a solution of the residue in CH2Cl2 (4 mL) was then added TFA (1 mL). The mixture was stirred for 30 minutes before being concentrated and co-evaporated 5 times with 20 mL of CH2Cl2 in order to remove the excess of TFA. The residue was then diluted in CH2Cl2 (20 mL). To this solution was added NH4PF6 (78 mg, 0.48 mmol, 5 equiv) and MilliQ H2O (10 mL): the biphasic solution was stirred vigorously for 30 minutes. The aqueous layer was extracted with CH2Cl2 (3 × 5 mL) and the combined organic layers were dried over MgSO4 and concentrated to give compound 13u (106 mg, 80%) as a white foam. Rf 0.86 (CH2Cl2/MeOH 9/1). 1H-NMR (600 MHz, CD2Cl2, 298K): δ (ppm) = 8.33 (s, 1H, H13), 7.67 (br t, 1H, H1), 7.48 (br t, 1H, H25), 7.43 & 7.28 (2 br t, 2H, H41 H7), 7.42–7.39 (m, 3H, H31 H32), 7.30–7.26 (m, 2H, H30), 7.25 (d, 2H, JHH-HH= 1.4 Hz, H23), 7.04 (br t, 1H, H10), 6.99 (d, 2H, JHH-HH= 2.1 Hz, H2), 6.90–6.86 (m, 5H, H4 H7), 6.75 (d, 2H, JHH-HH= 1 Hz, H23), 6.43 (d, 2H, JHH-HH= 2 Hz, H2), 6.39 (d, 2H, JHH-HH= 2 Hz, H2), 6.35 (d, 2H, JHH-HH= 2 Hz, H2), 6.00 (d, 2H, JHH-HH= 2 Hz, H2), 5.64 (s, 2H, H28), 4.67 (t, 2H, JHH-HH= 5 Hz, H2), 4.19–4.14 (m, 4H, HH), 4.11–4.06 (m, 4H, CC), 4.04 (d, 2H, JHH-HH= 5 Hz, H2), 3.88 (d, 2H, JHH-HH= 5 Hz, H5 or H8), 3.83 (d, 2H, JHH-HH= 5 Hz, H8), 3.81–3.75 (m, 8H, HD HG), 3.76–3.73 (m, 2H, H11), 3.67 (s, 8H, HE HF), 3.31 (t, 2H, JHH-HH= 2 Hz, H2), 2.66 (t, 2H, JHH-HH= 2 Hz, H2), 1.78–1.70 (m, 2H, H19), 1.69–1.62 (m, 2H, H16), 1.38–1.30 (m, 4H, H17 H18), 1.28 (s, 18H, H27). 13C-NMR (150 MHz, CD2Cl2, 298K): δ (ppm) = 171.9 & 171.2 & 171.0 & 169.4 (CK C3 C6 C9), 160.8 & 149.2 & 135.7 & 135.5 (CIV arom BMP25C8), 154.3 (C24), 151.1 (C22), 131.8 (C29), 130.0 (C13 C31 C32), 128.8 (C30), 124.3 (C25), 122.4 (C8), 117.2 (C23), 115.4 (Ca), 107.4 (C4), 107.3 (C1), 71.1 (CE CF), 70.3 & 70.0 (CD CO), 69.2 (C6), 68.9 (C7), 55.4 (C28), 54.0 (C20), 53.9 (C12), 44.5 (C2), 43.8 & 43.4 (C5 C8), 39.4 (C11), 35.6 (C26), 31.4 (C27), 26.3 (C16), 28.1 & 25.9 (C17 C18 C19), 23.6 (C15). HRMS (ESI): [M−PF6]+ calecd for C62H88F6N8O12P: 1281.6164, found: 1281.6173.

Deprotonated Unthreaded Compound (14u): A solution of the thread 13u (48 mg, 0.034 mmol, 1 equiv) in CH2Cl2 (10 mL) was washed with an aqueous solution of 1 M NaOH (5 mL). After separation and further extraction of the remaining aqueous layer with CH2Cl2 (10 mL), the combined organic layer were dried over MgSO4 and then evaporated to obtain the deprotonated unthreaded compound 14u (43 mg, 99%) as a colourless oil. Rf 0.73 (CH2Cl2/MeOH 9/1). 1H-NMR (600 MHz, CD2Cl2, 298K): δ (ppm) = 8.25 (s, 1H, H13), 7.47 (t, 1H, JHH-HH= 6 Hz, H2), 7.44–7.40 (m, 3H, H31 H32), 7.42 (br t, 1H, H41 H7), 7.22 (t, 1H, JHH-HH= 5 Hz, H2), 7.30–7.26 (m, 2H, H30), 7.11 (t, 1H, JHH-HH= 5 Hz, H10), 6.98 (d, 2H, JHH-HH= 2 Hz, H2), 6.91–6.87 (m, 4H, H41 H8), 6.85 (br t, 1H, H41), 6.75 (br t, 1H, H25), 6.43 (d, 2H, JHH-HH= 1 Hz, H23), 5.64 (s, 2H, H28), 4.63 (t, 2H, JHH-HH= 5 Hz,
H_{12}), 4.20–4.16 (m, 4H, H_{10}), 4.11–4.07 (m, 4H, H_{11}), 4.00 (d, 2H, J_{H2-H1} = 5.5 Hz, H_{2}), 3.83–3.74 (m, 12H, H_{5} H_{8} H_{12} H_{9}), 3.71–3.65 (m, 2H, H_{11}), 3.69 (s, 8H, H_{E} H_{F}), 3.06 (t, 2H, J_{H20-H19} = 7 Hz, H_{20}), 2.71 (t, 2H, J_{H15-H16} = 7.8 Hz, H_{15}), 1.64–1.54 (m, 2H, H_{16}), 1.58–1.49 (m, 2H, H_{19}), 1.40–1.28 (m, 4H, H_{17} H_{18}), 1.29 (s, 18H, H_{27}). \textsuperscript{13}C-NMR (150 MHz, CD_{2}Cl_{2}, 298K); \delta (ppm) = 172.1 & 171.0 & 170.8 & 168.9 (CK C_{3} C_{6} C_{9}), 160.8 & 149.4 & 135.7 (CIV arom BMP25C_{8}), 152.2 (C_{24}), 148.8 (C_{14}), 145.2 (C_{22}), 131.8 (C_{29}), 130.2 & 130.1 & 130.0 (C_{13} C_{31} C_{32}), 128.6 (C_{30}), 122.2 (C_{8}), 115.4 (C_{A}), 112.2 (C_{25}), 107.9 (C_{23}), 107.3 (C_{I}), 107.0 (C_{I}), 71.3 (2s, C_{E} C_{F}), 70.3 & 70.2 (C_{D} C_{G}), 69.3 (C_{C}), 68.9 (C_{H}), 55.5 (C_{28}), 54.1 (C_{12}), 44.8 (C_{2}), 44.5 (C_{20}), 44.4 & 43.6 (C_{5} C_{8}), 39.2 (C_{11}), 35.2 (C_{20}), 31.8 (C_{27}), 29.9 (C_{19}), 27.4 (C_{16}), 29.2 & 27.1 (C_{17} C_{18}), 23.9 (C_{15}). HRMS (ESI): [M + H]\textsuperscript{+} calcd for C_{62}H_{88}F_{6}N_{8}O_{12}P: 1281.6164, found: 1281.6168.

4. Conclusions

We have reported the synthesis of a new lasso molecular switch containing a peptide part, using a two-step sequence strategy: (1) the self-entanglement of a hermaphrodite molecule, (2) the trapping of the interlocked pseudo lasso structure with a molecular barrier. 1D and 2D \textsuperscript{1}H-NMR spectroscopies, as well as DT IM-MS studies provided information about the self-interlocking of the molecular lasso and about the pH-dependent molecular machinery. In the protonated state, the lasso remains loosened because the BMP25C_{8} resides around the best anilinium molecular site. However, deprotonation of the anilinium triggers the shuttling of the BMP25C_{8} towards the triazolium station, resulting in a more tightened conformation. It appears consistent to say that the peptide part of the lasso, which is located between the macrocycle and the triazolium unit (that is to say in the loop of the lasso), does not play any role in the molecular machinery: however, it undergoes a conformational change from a more extended and flexible shape at acidic pH to a more constraint bent conformation at basic pH. To the best of our knowledge, this is the first example of a pH-sensitive lasso molecular switch incorporating a peptide backbone. Further investigations towards the structure-activity relationship of such a peptide-containing molecular machine are in progress.

Supplementary Materials


Conflicts of Interest

The authors declare no conflict of interest.

References


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