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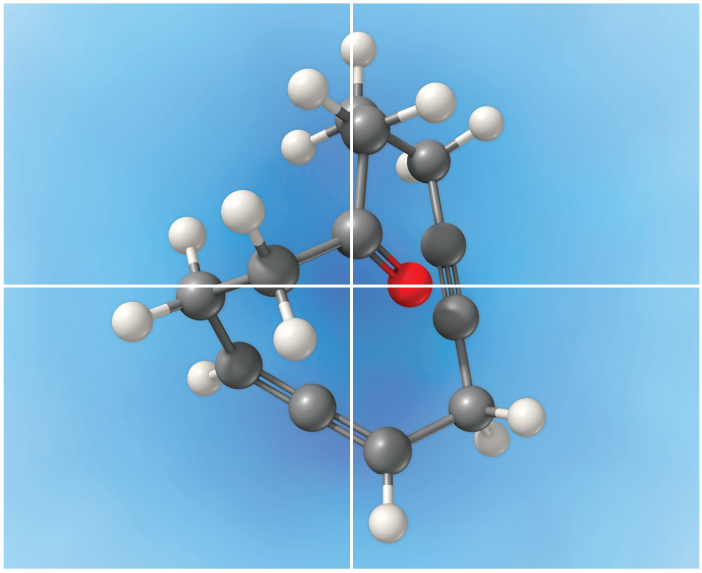

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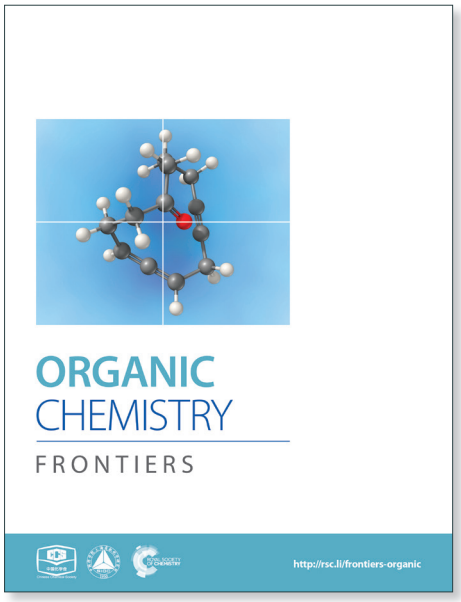


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RESEARCH ARTICLE

# A novel dynamic pseudo[1]rotaxane based on mono-biotin-functionalized pillar[5]arene

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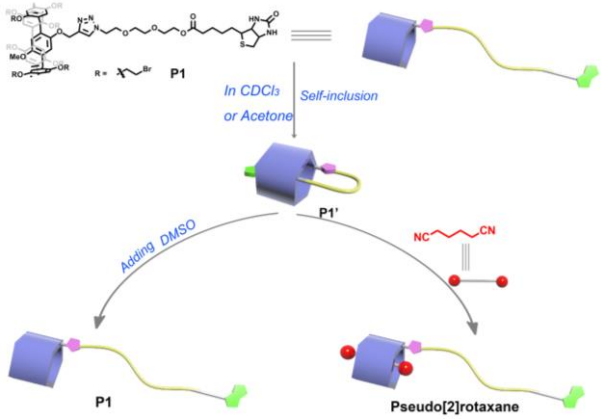
**A mono-biotin-functionalized pillar[5]arene P1 was synthesized by the click reaction, which could form a stable pseudo[1]rotaxane P1' in non-polar or weak-polar solution. Interestingly, the obtained pseudo[1]rotaxane P1' exhibited a dynamic slow disassembly process within NMR timescale upon adding strong-polar solvent or competitive guest. Moreover, this dynamic behavior also had potential application in aqueous solution, which might be used as a switch to turn on or off the bioactivity of biotin moiety.**

Mechanically interlocked molecules (MIMs), for their characteristic topological structures and potential application in molecular machines, have attracted tremendous attention over the past decades.[1-5] Among various types of MIMs, pseudo[1]rotaxane, as the simplest interlocked structure, where the wheel is threaded by its own axle, exhibits fast or slow exchange process between self-included and free structure to external stimuli due to its reversible conversion behavior. So far, many researches have been conducted on this special structure for its potential applications in constructing molecular muscles,[6] molecular switches,[7-9] insulated molecular wires,[10, 11] and fluorescence sensors.[12, 13]

However, most of these reported pseudo[1]rotaxanes were usually based on traditional macrocycles, such as cyclodextrins and crown ethers. Pillararenes as a new generation of macrocyclic molecules,[14] have received considerable attention and development due to their unique structures, easy modification,[15, 16] and excellent properties in host-guest chemistry.[17-21] Up to now, various pillararene-based interlocked structures have been reported, such as rotaxanes[22-27] and catenanes.[28-30] Whereas, there are only a few reports on the formation of pseudo[1]rotaxanes based on this novel host molecule.[31-34] Therefore, the construction of pillararene-based pseudo[1]rotaxanes through host-guest interactions, especially with stimuli-responsive dynamic behaviors, is of great interest and importance in the application of constructing controllable molecular machines. Recently, a stable pseudo[1]rotaxane based on ester group-containing pillar[5]arene was successfully constructed by Cao, which showed responsiveness to dihalogen alkanes.[31] Moreover, Hou also reported a stable pseudo[1]rotaxane by introducing an amino group to the side chain of pillar[5]arene, which was stabilized by the intramolecular hydrogen bond.[32] On the basis of our previous work on the construction of dynamic pseudo[1]rotaxane based on a urea-modified pillar[5]arene[33] and another mono-functionalized pillar[5]arene bearing Boc end group,[34] we could concluded that the terminal group played a vital role in the dynamic behavior of the formed pseudo[1]rotaxanes. Therefore, we envision that we can design a novel dynamic pillarene-based pseudo[1]rotaxane which could be synthesized by introducing a bioactive biotin moiety to a side chain of pillar[5]arene *via* a flexible ethylene glycol chain to achieve its controllable dynamic self-inclusion behavior. Furthermore, in aqueous solution, we speculate that this dynamic behavior might be used as a switch to turn on or off the bioactivity of biotin moiety.

Herein, a mono-biotin-functionalized pillar[5]arene **P1** was successfully prepared by the efficient click reaction, which could form a stable pseudo[1]rotaxane **P1'** in non-polar or weak-polar solution such as $CHCl_3$ or acetone. Moreover, the obtained pseudo[1]rotaxane **P1'** showed a dynamic slow disassembly process upon the addition of a competitive guest or increasing the polarity of




**Scheme 1.** Schematic illustration of the dynamic behaviors of **P1**.

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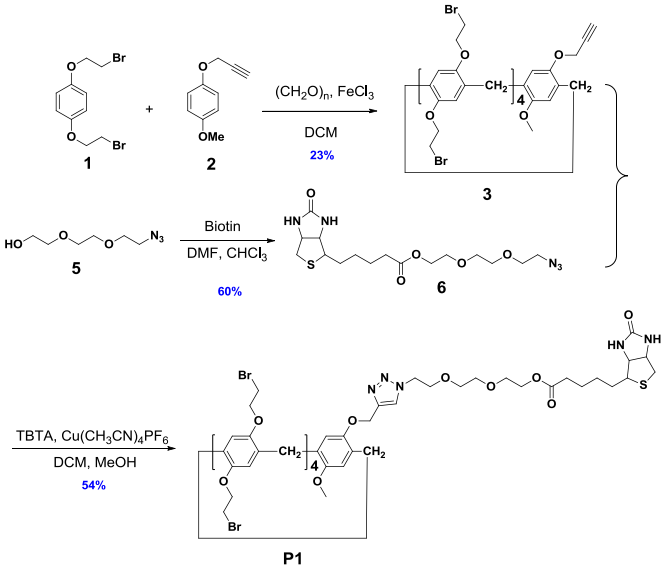
the solution (Scheme 1). More importantly, this dynamic pseudo[1]rotaxane could be applied as a biotin-based switch in aqueous solution, which might have potential applications in drug delivery system.

The target molecule **P1** was synthesized by means of copper(I) catalyzed azide-alkyne cycloaddition (CuAAC).[35, 36] The alkynyl-functionalized pillar[5]arene **3** was prepared according to the typical [4+1]cyclization of monomers **1** and **2**. Meanwhile, the azide-modified biotin derivative **6** was obtained by using commercially available triethylene glycol as the starting material. Firstly, through two steps of typical reaction, a mono-azide-modified glycol **5** was obtained, and then a condensation reaction between compound **5** and biotin was carried out to give the biotin derivative **6**. Finally, the CuAAC reaction between **3** and **6** generated the target molecule **P1** in a moderate yield (Scheme 2).




**Scheme 2.** The synthesis route of **P1**.

The inclusion behavior of **P1** was firstly studied by $^1$H NMR spectroscopy in different solvents (Fig. 1). From the $^1$H NMR spectra, the obviously upfield chemical shifts of the protons ($H_{a–e}$) on the biotin group could be observed in $CDCl_3$ compared with that in DMSO-$d_6$, indicating the inclusion of the biotin group into the cavity of the pillar[5]arene, which resulted in the formation of the inclusion structure **P1'**. Similar upfield shift phenomena was also observed from the spectrum using acetone-$d_6$ as the solvent, indicating that the polarity of acetone could not destroy the inclusion

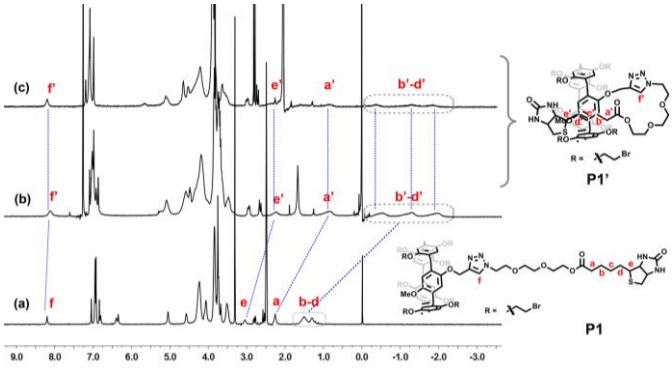



**Fig. 1** $^1$H NMR spectra (300 MHz, 298 K) of **P1** (15 mM) in different solvents: (a) in DMSO-$d_6$, (b) in $CDCl_3$, and (c) in acetone-$d_6$.

structure of **P1'**. However, the signals of the protons of **P1'** in $^1$H NMR spectra tend to be normal in strong polar solvents, such as DMSO-$d_6$, which meant that the inclusion behavior of **P1** was completely destroyed in DMSO-$d_6$. The above results showed that this inclusion structure formed by **P1** was stable in non-polar and weak-polar solvents, but it was instable in strong polar solvents. This was consistent with the results in previous studies that strong-polar solvent would destroy the driving force of the formed inclusion structures.[31, 33]

To investigate whether the inclusion behavior was intramolecular or intermolecular, the host-guest interaction between **3** and **6** was firstly studied. As shown in Fig. S12 (ESI†), the $^1$H NMR spectra of **6** with the addition of different equivalents of **3** showed that the chemical shifts of $H_{a–e}$ on **6** gradually shifted upfield, exhibiting a fast exchange process within NMR timescale. However, only a slightly upfield shift (0.15 ppm) of protons $H_{a–e}$ on **6** could be observed when 1.0 equiv. of **3** was added, which indicated that the interaction between **3** and **6** was very weak. Subsequently, variable concentration $^1$H NMR spectroscopy of **P1** in $CDCl_3$ was carried out (Fig. 2). It was found that the aggregates formed by **P1** were very stable even in very dilute $CDCl_3$ solution (5 mM). As the concentration increased, the proton resonances did not exhibit obvious changes even at a high concentration of 80 mM, suggesting that **P1** did not form intermolecular complexes in $CDCl_3$.[33] Therefore, it could be concluded that a stable pseudo[1]rotaxane **P1'** was formed in chloroform.

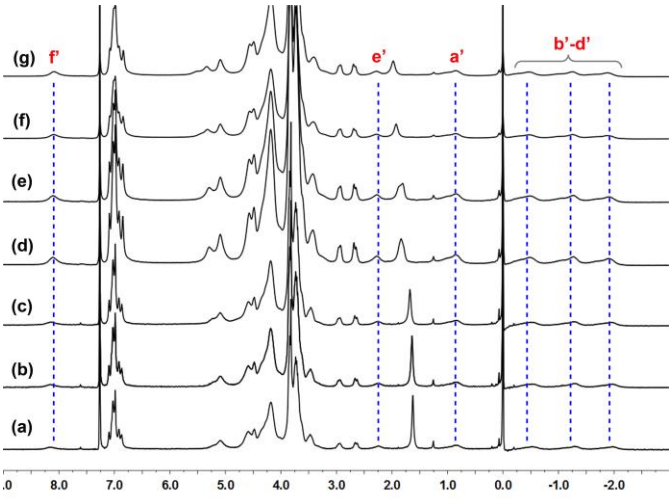



**Fig. 2** $^1$H NMR spectra (300 MHz, 298 K) of **P1** at variant concentrations in $CDCl_3$: (a) 5 mM, (b) 10 mM, (c) 15 mM, (d) 20 mM, (e) 40 mM, (f) 60 mM, and (g) 80 mM.

In addition, diffusion-ordered $^1$H NMR spectroscopy (DOSY) experiments were performed, and the diffusion coefficients of pseudo[1]rotaxane **P1'** in $CDCl_3$ at both low and high concentrations were recorded. From the DOSY spectra (Fig. S13 and Fig. S14, ESI†), only one set of signals could be observed at both low concentration (10 mM) and high concentration (45 mM) of **P1'**, which further indicated that the inclusion behavior of **P1** was not intermolecular. Then, the Stokes-Einstein relation was used to calculate the radius of **P1'** in $CDCl_3$, which was calculated to be 9.98 Å (Table S1, ESI†). Furthermore, theoretical calculation was carried out to optimize the structure of **P1'** by employing the Gaussian 09 program package.[38] The result showed that the calculated diameter

of the pseudo[1]rotaxane **P1'** was nearly 18.12 Å ($r$ = 9.06 Å) (Fig. S15, ESI†), which was in good agreement with the result obtained from DOSY experiment. Therefore, based on the above observations, we could clearly confirm the self-inclusion behavior of **P1** in chloroform, resulting in the formation of pseudo[1]rotaxanes **P1'**.

Subsequently, we attempted to know the dynamic behavior of this pseudo[1]rotaxane **P1'**. Initially, the effect of solvent polarity on this disassembly process was investigated. As shown in Fig. 3, it was found that, with the increase of the solvent polarity by gradually adding DMSO-$d_6$ into the $CDCl_3$ solution of **P1**, the peaks for $H_{a'-e'}$ of the self-inclusion structure **P1'** disappeared gradually, and meanwhile the peaks for $H_{a-e}$ of the un-entangled structure **P1** appeared and strengthened gradually. This observation suggested that the self-inclusion structure was gradually destroyed, presenting a slow exchange process within the NMR timescale. When the ratio of the mixture DMSO-$d_6$/$CDCl_3$ was increased to 1:1 (*v*/*v*), most of the self-inclusion structure **P1'** was transformed to the un-entangled form **P1**.

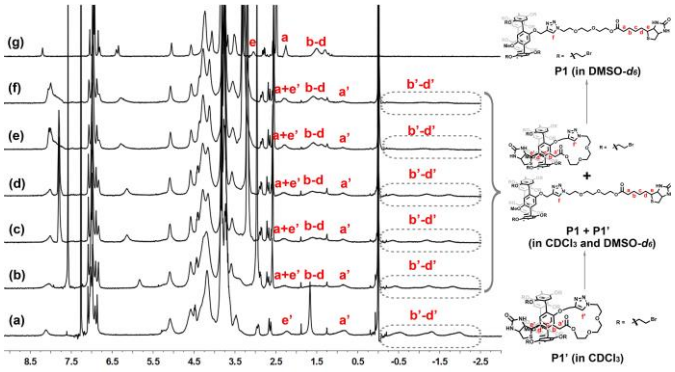



**Fig. 3** $^1$H NMR spectra (300 MHz, 298 K) of **P1** (15 mM) in mixed solvents $CDCl_3$/DMSO-$d_6$ with different ratios (*v*/*v*): (a) 5:0, (b) 5:1, (c) 5:2, (d) 5:3, (e) 5:4, (f) 5:5, and (g) 0:5.

Similar dynamic disassembly process of pseudo[1]rotaxane **P1'** could also be observed if a competitive guest was added to the **P1'** solution. Since hexanedinitrile shows strong host-guest binding affinity with pillar[5]arene,[39] herein, hexanedinitrile was chosen as a typical competitive guest to investigate the dynamic behavior of **P1'** (Fig. 4). It was found that with the addition of hexanedinitrile to the solution of **P1'** in $CDCl_3$, the protons $H_{b'-d'}$ of biotin group attributed to the self-inclusion structure **P1'** disappeared gradually, and a new peak assigned to the **P1**⊃hexanedinitrile complex appeared, which

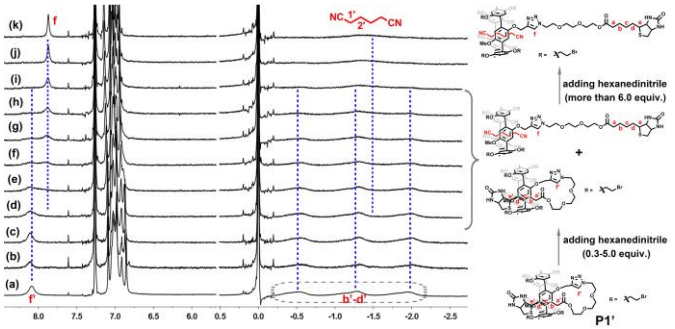



**Fig. 4** Partial $^1$H NMR spectra (300 MHz, 298 K, $CDCl_3$) of **P1'** (10 mM) with different equivalents of hexanedinitrile: (a) 0 equiv., (b) 0.3 equiv., (c) 0.6 equiv., (d) 1.0 equiv., (e) 1.5 equiv., (f) 2.0 equiv., (g) 3.0 equiv., (h) 4.0 equiv., (i) 5.0 equiv., (j) 6.0 equiv., and (k) 7.0 equiv.

clearly revealed that the self-inclusion structure **P1'** was destroyed gradually and a pseudo[2]rotaxane was formed between **P1** and hexanedinitrile. Moreover, upon adding hexanedinitrile, the proton $H_{f'}$ of triazole group split into two different sets of peaks, the one in the original position which assigned to the self-inclusion structure **P1'** disappeared gradually, while the other that derived from the **P1**⊃hexanedinitrile complex shifted upfield and strengthened gradually. According to the previous work,[13, 40] we speculated that hydrogen bonding might be formed between the triazole group and the glycol unit in the pseudo[1]rotaxane **P1'**, which might play an important role in stabilizing the self-inclusion structure.

As we know biotin was a bioactive molecule, which could be used as tumor-targeted molecule for drug delivery.[41, 42] Based on the above results, we noted that the biotin group would slip out from the cavity of pillar[5]arene in **P1'** if a competitive guest was added to the solution. So we wondered if this phenomenon could still be observed in aqueous solution. Once such dynamic self-inclusion and disassembly processes could be realized, the formed pseudo[1]rotaxane would be used as a switch to turn on or off the bioactivity of biotin. Along this line, a water-soluble biotin-functionalized pillar[5]arene **P2** was synthesized through a simple reaction of **P1** with trimethylamine. From the NMR spectra (Fig. 5), it was found that the protons $H_{b'-d'}$ on the biotin group shifted up-field obviously, indicated that the self-inclusion structure **P2'** could also be formed. Then, sodium caprylate (**SC**), the competitive guest was added to the **P2'** solution. To our delight, a similar disassembly phenomenon like **P1'** was also observed, where the biotin group left the cavity of pillar[5]arene in **P2'**, and the protons $H_{1-7}$ on **SC** showed slightly up-field shifts, indicating a host-guest complex between **P2** and **SC** was formed. The above study gave us very useful guideline for our further studies on the design of biotin-based drug delivery system.

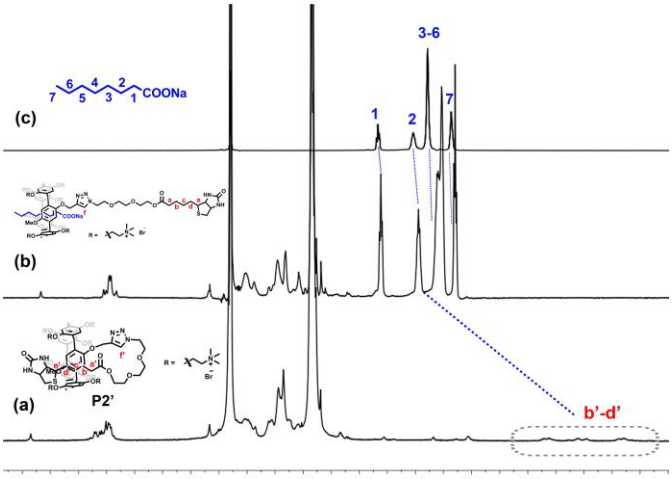



**Fig. 5** $^1$H NMR spectra (300 MHz, 298 K, $D_2O$) of (a) **P2'** (2 mM), (b) **P2'** (2 mM) + **SC** (20 mM), and (c) **SC** (20 mM).

## Conclusions

In summary, a mono-functionalized pillar[5]arene **P1** using biotin as the end group was successfully synthesized through a CuAAC reaction, which could form a stable pseudo[1]rotaxane **P1'** in non-polar and weak-polar solutions. However, this self-

inclusion structure showed a slow dynamic disassembly process upon adding strong-polar solvent or competitive guest, resulting in the formation of un-entangled structures. Moreover, this dynamic self-inclusion behavior could be used as a biotin switch to turn on or off the bioactivity of biotin, which gave us constructive guidelines for the following study on the design of biotin-based drug delivery system.

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