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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 machines,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 rotaxanes22-27 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]arene33 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 pillararene-based pseudo[1]rotaxane which could be synthesized by introducing a biactive 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 CHCl3 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
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). 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).

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$_6$) with the addition of different equivalents of 3 showed that the chemical shifts of H$_6$ 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$_6$ 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$_3$. Therefore, it could be concluded that a stable pseudo[1]rotaxane P1$'$ was formed in chloroform.

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. The result showed that the calculated diameter of P1$'$ is in good agreement with the experimental value.
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 CDCl₃ solution of P1, the peaks for H_d-e of the self-inclusion structure P1' disappeared gradually, and meanwhile the peaks for H_d-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/CDCl₃ was increased to 1:1 (v/v), most of the self-inclusion structure P1' was transformed to the un-entangled form P1.

Fig. 3 ¹H NMR spectra (300 MHz, 298 K) of P1 (15 mM) in mixed solvents CDCl₃/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, 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 CDCl₃, the protons H_d-e of biotin group attributed to the self-inclusion structure P1' disappeared gradually, and a new peak assigned to the P1'±hexanedinitrile complex appeared, which 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_g 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.31, 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_d-e on the biotin group shifted upfield 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_d-e 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 ¹H NMR spectra (300 MHz, 298 K, D₂O) 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-assembly 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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Notes and references