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Synthesis of a water-soluble pillar[6]arene dodecaamine and its selective binding of acidic amino acids in water

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ABSTRACT

A water-soluble pillar[6]arene dodecaamine has been synthesized. ^{1}H NMR and fluorescence studies indicate that pillar[6]arene dodecaamine could selectively and strongly bind acidic amino acids, *i.e.* glutamic acid and aspartic acid in water. And the complexation behavior of pillar[6]arene dodecaamine towards acidic tripeptide glutathione and short chain length (C_3 to C_8) dicarboxylic acids in water is also investigated.

The recognition of biologically relevant substrates with synthetic receptors is a topic of great interest in supramolecular chemistry.[1] In this respect, numerous efforts have been devoted to the design and synthesis of macrocyclic receptors with specific properties and functions revealing their affinity and selectivity towards biologically relevant molecules.[2] Along with the crown ethers,[3] cyclodextrins,[4] calixarenes[5] and cucurbituril,[6] the pillararenes are one of the most important categories of macrocyclic receptors. Pillararenes,[7] as a new class of synthetic macrocycles, have received much attention owing to its intriguing and versatile host–guest binding properties and many potential applications in chemistry, biology and materials science.[8] Pillararene-based molecular recognition in organic media has been mostly investigated due to the inherent poor solubility of pillararene analogues in aqueous medium. To combat that, anionic,[8c,j,9] cationic[10] and neutral[11] water-soluble pillararenes have been synthesized and shown to act as scaffolds to various cationic, anionic and biologically significant guests.

Amino acids are the basic structural building blocks of proteins and other biomolecules. The acidic amino acids, glutamic acid and aspartic acid, play critical roles in the central nervous system as excitatory neurotransmitters. Concentration changes of glutamic acid and aspartic acid in specific regions of the brain are closely related to Parkinson's disease.[12] Therefore, the selective recognition of acidic amino acids has a wide range of potential applications in the field of biomedicine. At present there were many reports on selective recognition of basic amino acids by anionic macrocycles.[8a,13] However, to the best of our knowledge, the pillararene-based selective acidic amino acids recognition in water has not been reported yet. Herein, we report the facile synthesis of a new water-soluble pillar[6]arene dodecaamine **1** and its selective binding of acidic amino acids, *i.e.*, L-glutamic acid (Glu) and L-aspartic acid (Asp) in water. Furthermore, its molecular recognition with acidic tripeptide glutathione (GSH) and short chain length (C_3 to C_8) dicarboxylic acids were also investigated.

The synthetic approach depicted in Scheme 1 outlines the preparation of pillar[6]arene dodecaamine. Compound **4** was first prepared from commercially available 1,4-bis(2-hydroxyethoxy) benzene according to previously reported procedure.[10a] Treatment of **4** and metaformaldehyde with boron trifluoride diethyl etherate in chloroform at room temperature gave **3** in 25% yield. Pillar[6]arene derivative **3** was then reacted with sodium azide in *N*,*N*-dimethylformamide (DMF) at 100 °C to produce **2** in 89% yield. Palladium-catalyzed hydrogenation of **2** in methanol at 50 °C afforded **1** as a colourless solid in 99% yield. The structure of pillar[6]arene dodecaamine **1** was confirmed[14] by ^{1}H NMR, ^{13}C NMR and high-resolution mass spectrometry (HRMS).

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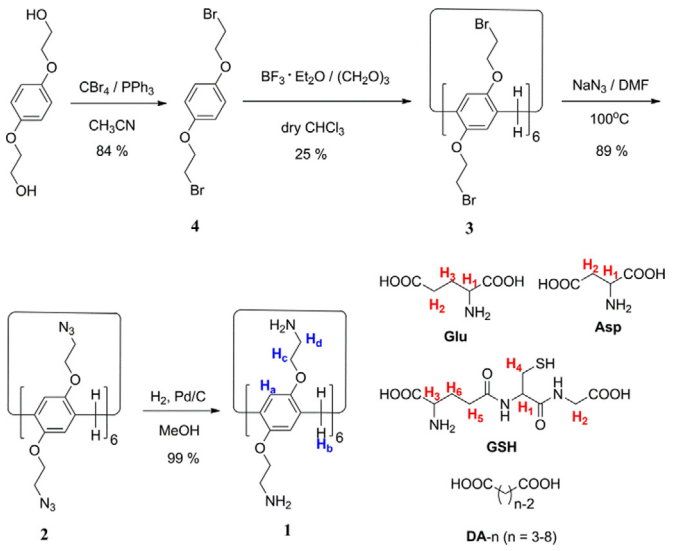

Scheme 1. Synthesis of water-soluble neutral pillar[6]arene dodecaamine **1** and structural illustration of Glu, Asp, GSH and short chain length (C_3 to C_8) dicarboxylic acids.

The host–guest complexation between the host **1** and native L-α-amino acids was then investigated by ^{1}H NMR spectroscopy. As shown in Fig. 1b, the 1:1 mixture of **1** and Glu in D_2O (pD = 6.0) had substantial upfield shifts and broadening effects for the Glu protons (H_{1-3}) compared to free Glu ($\Delta\delta$ = −0.14 to −0.28 ppm) (Fig. 1c), indicating a strong threaded host–guest complex formation. And the presence of only one set of peaks for the solution of **1** and Glu (Fig. 1b) suggests the host–guest complex formation is a fast exchange process on the NMR time scale. The downfield shift of aromatic proton H_a of **1** (Fig. 1b) compared to free **1** ($\Delta\delta$ = +0.10 ppm) (Fig. 1a), caused by deshielding gives an additional support for interpenetrated complex formation. The host–guest interaction in water can be further confirmed by 2D NOESY, which has a maximal observation limit at a spatial proximity of 5 Å.[15] From the 2D NOESY spectrum of a solution of **1** and Glu (Figs. 2 and S11), intermolecular correlations were observed between aromatic proton H_a of **1** and methylene protons H_{1-3} of Glu, which also confirmed the interpenetrated geometry.

Subsequently another acidic amino acid Asp was studied for host–guest complex formation with **1**. A 1:1 mixture of **1** and Asp in D_2O (pD = 6.0) was examined by ^{1}H NMR spectroscopy

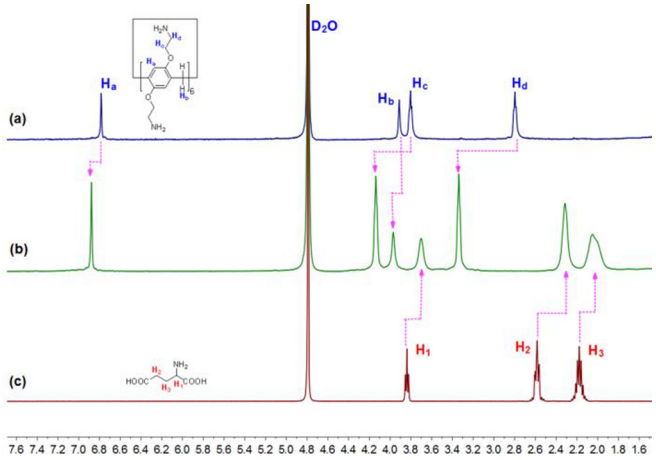

Fig. 1. ^{1}H NMR spectra (400 MHz, D_2O, 293 K) of (a) 1.00 mM **1**, (b) 1.00 mM **1** + 1.00 mM Glu, and (c) 1.00 mM Glu at pD 6.0.

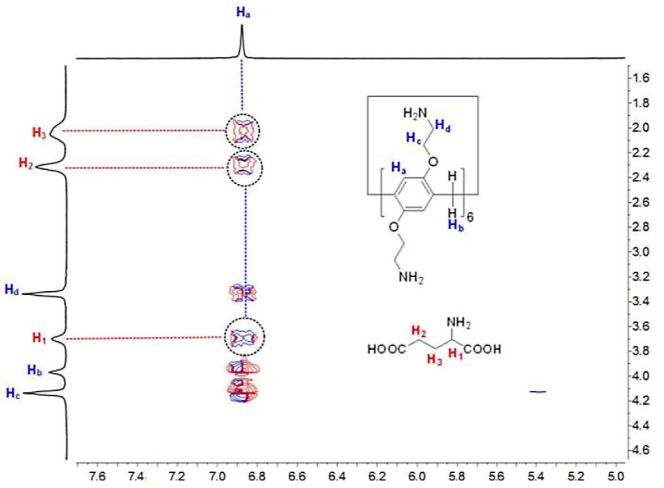

Fig. 2. Partial 2D NOESY NMR spectrum of Glu⊂**1** (400 MHz, D_2O, 298 K, mixing time = 300 ms), [**1**] = 1.00 mM. [Glu] = 1.00 mM.

(Fig. S8) and similar upfield shifts along with peak broadening were observed for the Asp protons (H_{1-2}) compared to free Asp, indicating a strong threaded host–guest complex formation. Furthermore, NOE correlations were observed between aromatic proton H_a of **1** and methylene protons H_{1-2} of Asp, confirming that these protons were located in the cavity of **1** to form an interpenetrated geometry (Fig. S12). Next, the host–guest complexation of **1** with other 17 naturally occurring amino acids was also performed in D_2O (pD 6.0) by ^{1}H NMR spectroscopy (Figs. S9 and S10). The corresponding chemical shift changes ($\Delta\delta$) of host **1** protons in the presence of amino acids guests were listed in Table S1. As shown in Table S1, there were observed no noticeable chemical shift changes in their ^{1}H NMR spectra when 1.0 equiv. of other 17 naturally occurring amino acids were added (Table S1), respectively, which indicated that among 20 native L-α-amino acids, only acidic amino acids, Glu and Asp, could effectively bind to the pillar[6]arene dodecaamine **1** to form deep inclusion complexes. According to the pK_a values of the branched carboxyls of the two acidic amino acids (Glu: 4.25; Asp: 3.65) and the pI (isoelectric point) values of the two acidic amino acids (Glu: 3.22; Asp: 2.77), it can be concluded that both the branched carboxyls and the α-carboxyls of the two acidic amino acids should be in the deprotonated form at pH 6.0. Conversely, the α-amino groups of the two acidic amino acids should be in the protonated form (NH_3^+) at pH 6.0. Therefore, we deduce that the interaction mechanism of **1** with the two acidic amino acids is that the acidic amino acids with two carboxylate anions could bind positively charged **1** bearing ammonium groups in aqueous solutions at pH 6.0, where the cooperative electrostatic attraction forces between two carboxylate anions of the two acidic amino acids and two cationic portals of the host **1** play a dominant role in the present host–guest complexation. In addition to electrostatic interactions, it seems also reasonable to assume that other noncovalent interactions, such as cation–π[16] interactions between the cavity-included NH_3^+ groups of the two acidic amino acids and aromatic rings of **1**, as well as N–H···π[17] interactions between ammonium hydrogen atoms of the two acidic amino acids and π-plane of **1**, may contribute to the stabilization of these inclusion complexes. We also recorded the ^{1}H NMR spectroscopy of Glu in the absence and in the presence of **1** in water at pH 4.0 (Fig. S14). Differing from the case at pH 6.0, no obvious chemical shift changes of Glu protons were found except a slightly downfield shift of H_1 (Fig. S14b) compared to free Glu at pH 4.0, just caused by electrostatic attraction force between negatively charged α-carboxylate anion and

positively charged **1**. The difference can be explained by the fact that the branched carboxylic group of Glu is in the protonated form at pH 4.0, which demonstrated that the above-mentioned cooperative electrostatic attraction forces are the decisive driving forces in these inclusion complexes.

Investigations of the host–guest complexation between **1** and the two acidic amino acids employing Job plots[18] (Figs. S15 and S17) using UV–vis absorption data indicated the formation of 1:1 complexes. The quenching of fluorescence intensity (Figs. S16 and S18) was found to be significant enough that we could quantitatively measure (Table 1) the binding behaviors of Glu and Asp with host **1**. By using the Benesi-Hildebrand equation,[19] the association constants (K_a) were calculated to be $(1.00 \pm 0.01) \times 10^6\ M^{-1}$ and $(0.98 \pm 0.03) \times 10^6\ M^{-1}$ for Glu and Asp, respectively (Figs. S16 and S18).

To extend the scope of the host properties and to elucidate the affinity and selectivity of **1**, the binding of **1** to an acidic tripeptide containing the Glu residue (GSH) and aliphatic dicarboxylic acids of different lengths (DA-*n*, *n*: carbon number, *n* = 3–8) was further examined. Fig. 3 shows the ^{1}H NMR spectra of the 1:1 mixture of **1** and GSH in D_2O (pD = 6.0), from which the noticeable upfield chemical shifts of GSH proton resonances H_{1-6} were observed upon addition of **1** due to the shielding effect of the electron-rich cavities of **1** for GSH, indicating the inclusion of GSH into **1** cavity. Besides, NOE correlations were observed between aromatic proton H_a of **1** and methylene protons H_{1-6} of GSH, confirming that GSH threaded through the cavity of **1** (Fig. S13). The stoichiometry for the complex of **1** with GSH was confirmed to be 1:1 in solution by Job plots (Fig. S19). Additionally, The K_a value of GSH with **1** was calculated to be $(1.20 \pm 0.50) \times 10^6\ M^{-1}$ by using curve-fitting analysis (Fig. S21). Similar to acidic amino acids, DA-*n* (*n* = 3–8) also form deep inclusion complexes with **1**, which can be confirmed by ^{1}H NMR spectra of DA-*n* (*n* = 3–8) in D_2O (pD = 6.0) recorded in the absence and in the presence of 1.0 equiv. of the host (Figs. S24–S29). But their binding abilities are considerably different. Compared with Asp and Glu, DA-4 and DA-5 (both without the ammonium moiety) have a dramatically lower affinity with the host **1**, respectively (Table 1). This may be attributed to the additional cation–π and N–H···π interactions between the two acidic amino acids and **1**. Additionally, the K_a values of DA-*n* (*n* = 3–8) with **1** which were calculated by using curve-fitting analysis (Fig. S23) increase with increasing chain length of the DA-*n* (Table 1). This hike in K_a values might be explained by the increase in the number of methylene groups resulting in greater hydrophobic effect.

In conclusion, we have successfully synthesized a new water-soluble pillar[6]arene dodecaamine **1**, and investigated the inclusion complexation between **1** and 20 naturally occurring amino acids in water. This water-soluble pillar[6]arene exhibits strong binding affinities towards acidic amino acids, *i.e.*, Glu and Asp (with the K_a values in the order of magnitude of $10^6\ M^{-1}$ in water) against other α-amino acids. The cooperative electrostatic interactions between two carboxylate anions of the two acidic amino acids and two cationic portals of the host **1** are the decisive driving forces in these electrostatic inclusion complexes. Additionally, cation–π and N–H···π interactions between cationic ammonium sites of both the two acidic amino acids and aromatic rings of **1** may also play an important role in the host–guest binding. Further studies of the complexation of **1** with tripeptide GSH and short chain length (C_3 to C_8) dicarboxylic acids show that **1** can also be able to form inclusion complex with both GSH and short chain length (C_3 to C_8) dicarboxylic acids. Compared with Asp and Glu, short chain length (C_3 to C_8) dicarboxylic acids guests have a lower affinity (with the K_a values in the order of magnitude from 10^3 to $10^4\ M^{-1}$) with the **1** host. These selective recognitions of **1** toward acidic amino acids may provide reference in design amino acid molecular machines as well as biological applications.

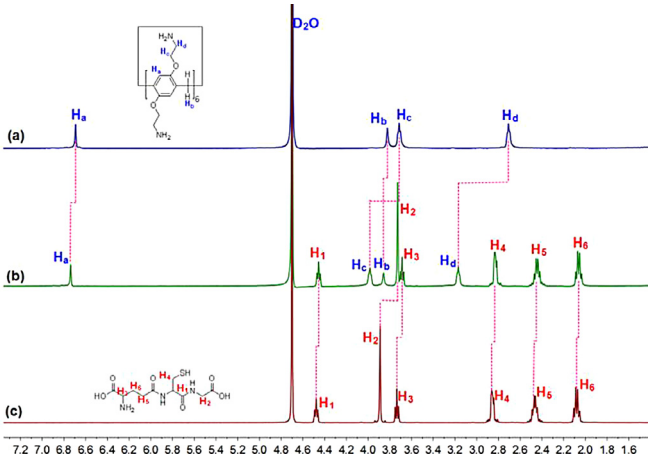

Fig. 3. ^{1}H NMR spectra (400 MHz, D_2O, 293 K) of (a) 1.00 mM **1**, (b) 1.00 mM **1** + 1.00 mM GSH, and (c) 1.00 mM GSH at pD 6.0.

Table 1
Association constants[a] (K_a) for 1:1 inclusion complexation of the guests with host **1** at 298 K.

Guest[b]	K_a (M^{-1})
L-Glu	$(1.00 \pm 0.01) \times 10^6$
L-Asp	$(0.98 \pm 0.03) \times 10^6$
GSH	$(1.20 \pm 0.50) \times 10^6$
DA-3	$(6.08 \pm 0.83) \times 10^3$
DA-4	$(8.61 \pm 0.70) \times 10^3$
DA-5	$(8.88 \pm 0.79) \times 10^3$
DA-6	$(9.69 \pm 0.99) \times 10^3$
DA-7	$(3.69 \pm 0.16) \times 10^4$
DA-8	$(5.97 \pm 0.36) \times 10^4$

[a] The K_a values were determined in H_2O at pH 6.0 by fluorescence titration methods.
[b] For other 17 native amino acids, the K_a values were too small to be calculated.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tetlet.2017.10.025.

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19. Take Glu as an example, fluorimetric titrations of **1** (1.0×10^{-5} M, $\lambda_{excitation}$ = 290 nm) with Glu were carried out in H_2O at pH 6.0 at 25 °C. The data at high $[Glu]_0$ were analyzed using the modified Benesi-Hildebrand equation $I_0/(I - I_0) = \{a/(b - a)\}\{(1/K_a)[Glu]^{-1} + 1\}$,[20] where a and b are constants while I and I_0 are the fluorescent emission intensities at $\lambda_{emission}$ = 326 nm with various initial concentrations of Glu and in its absence, respectively. The association constant K_a was obtained from the ratio of the y-intercept to the slope of the plot and its error was based on the errors of the y-intercept and the slope. More details are provided in the Electronic Supplementary data.
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