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Letter

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Preorganized Aryltriazole Foldamers as Effective Transmembrane Transporters for Chloride Anion

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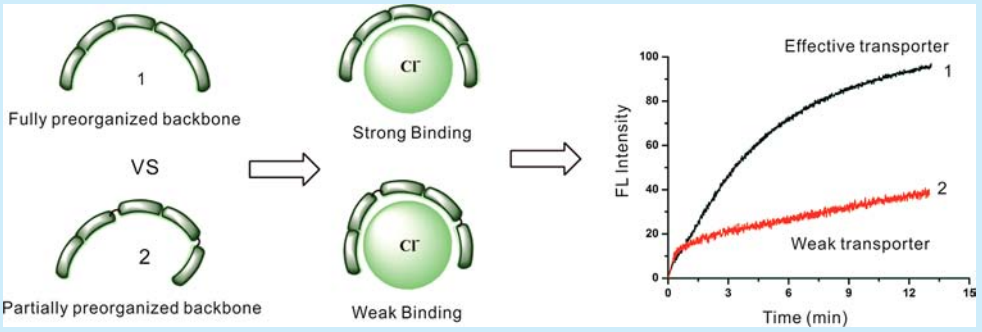
Supporting Information



ABSTRACT: Preorganized aryltriazole foldamers **1** and **2** were designed and synthesized. NMR studies and X-ray analysis demonstrate that **1** adopts a crescent conformation driven by a series of continuous hydrogen bonds at the periphery of the foldamer, whereas **2** displays a coil conformation. NMR titrations reveal that the affinities of fully preorganized foldamer **1** for halogen anions are much stronger that those of partially preorganized foldamer **2**. Furthermore, it is found that such full preorganization makes **1** an effective transmembrane transporter for the chloride anion across a lipid bilayer.

Developing synthetic receptors for selectively binding anions has attracted increasing attention in the past few decades because of their biological importance.[1] Foldamers,[2] artificial analogues of biopolymers having strong a tendency to adopt well-defined conformation, hold great promise as hosts for biologically interesting guests because of their dynamic internal cavities or channels. Prominent examples for foldamers serving as hosts include *m*-phenylene ethynylene oligomers,[3] oligo(*m*-ethynylpyridine)s,[4] hydrogen-bonded oligohydrazide foldamers,[5] capsule-like oligoamide foldamers,[6] oligocholates and metallohelicenes,[7,8] oligoindole-based foldamers, and aryltriazole foldamers.[9−11] However, it is true to say that the systems of foldamer-based anion recognitions are not fully developed yet, despite the fact that a number of synthetic anion receptors[1] and foldamers adopting well-defined helical conformations[2] have been reported so far. The challenge in the field of anion-mediated foldamers is how to efficiently synthesize elegant foldamers with suitable anion-binding sites in the internal cavity.

We have long-term interest in foldamer-based molecular recognitions,[6] particularly in anion recognitions.[11] This is because anions play vital roles in biological processes.[1] It is believed that the metabolic dysregulation of anions is implicated to a number of genetic diseases including most notably cystic fibrosis and the renal disease Bartter's syndrome.[12] Therefore, a number of transmembrane anion transporters have been developed so as to shed light into the mechanisms of these genetic diseases and also to provide potential therapies for treating them.[13] However, examples of foldamer-based anion transporters are extremely rare.[14] Previously, we and others reported a series of chloride mediated aryltriazole foldamers.[10,11] Later, we successfully replaced some triazole moieties with more acidic amides so that the affinities of aryltriazole foldamers for halogen anions were significantly enhanced.[11c] On the other hand, Jeong[9] and Flood[10] utilized an alternative approach to achieve the same purpose by partially rigidifying backbones of foldamers. Encouraged by these results, herein, we report our design and syntheses of the preorganized aryltriazole foldamers (Figure 1) and their functions as transmembrane transporters for chloride. To our delight, we found that the full preorganization makes aryltriazole foldamer **1** a potent anion chelator and an effective transmembrane transporter for chloride anions across a lipid bilayer.

The N^2/N^3 atoms in triazole provide ideal hydrogen bond acceptors. The formation of hydrogen bonds with the triazole's N^3 atom were recently demonstrated by Flood.[10] On the other hand, the NH in amide is widely used as a hydrogen bond donor in the construction of foldamers. Accordingly, we

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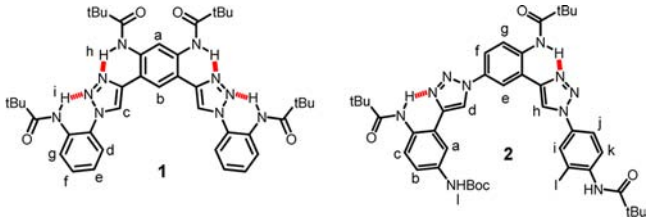

Figure 1. Structures of preorganized foldamers **1** and **2**.

designed pentad **1**, in which the amide moieties were introduced into aromatic rings adjacent to triazoles so as to form four intramolecular hydrogen bonds with the triazoles' N^2/N^3 atoms, respectively. We expected that these intramolecular hydrogen bonds would eventually result in a full backbone-rigidified foldamer with enhanced affinity for halogen anion. In addition, a partial preorganized foldamer **2** has also been designed for comparison. The synthetic routes and full characterization data for **1** and **2** are provided in the Supporting Information (SI).

The preorganization in the aryltriazole foldamer **1** was first confirmed by the X-ray analysis. Single crystals of **1** were obtained by evaporating its solution in dichloromethane/ethyl acetate. As expected, each triazole is held by two intramolecular hydrogen bonds between the amides NH and the triazole's N^2/N^3 atoms, respectively, as shown in Figure 2. However, the

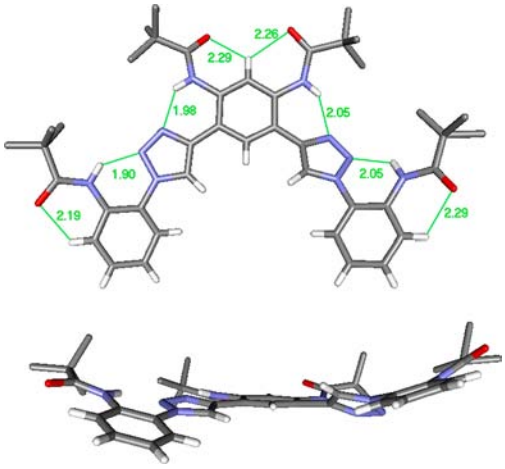

Figure 2. Top and side views of the crystal structure of **1**. The distances of hydrogen bonds were labeled and highlighted in green. The protons in *tert*-butyl groups were deleted for clarity.

distances of hydrogen bonds involved in one triazole (about 2.05 Å) are a little longer than those in the other, leading to the tilt-up of one triazole. In addition, four weak hydrogen bonds between the amide's carbonyl groups and adjacent CHs in the aromatic rings are also observed. Taken together, the backbone of aryltriazole foldamer **1** is greatly rigidified by these hydrogen bonds, which consequently results in the terminal aromatic rings tilting up and down according to the central aromatic ring (side view in Figure 2). Efforts on obtaining crystals of **2** and complexes **1**TBACl and **2**TBACl failed.

The labeled protons and solution structures of **1** and **2** were assigned and characterized by HSQC, HMBC and 2D NOESY experiments in CD_2Cl_2 and $CDCl_3$, respectively (see the SI). In the case of **1**, as shown in Figure 3A, the amide protons are deshielded at 10−11.5 ppm, as is expected for a hydrogen-bonded structure. Such a series of continuous hydrogen bonds at the periphery of foldamer block free rotation of triazoles and aromatic rings. In such a situation, only NOE correlations between triazole proton Hc and adjacent protons Hb and Hd are observable (SI). These NMR data further confirm that **1** adopts a crescent conformation as observed in the solid phase. However, in the case of **2**, although similar hydrogen bonds between amides and triazoles are observed (Figure 3B), such hydrogen bonds are unable to fully rigidify the backbone of **2**. The free rotations between the triazole moieties and adjacent aromatic rings were confirmed by 2D NOESY experiment (SI), implying an unfolded conformation for **2**.

The NMR titrations of **1** with the chloride anion as shown in Figure 3A are representative of the features observed for Cl^-, Br^- and I^-. The titrations of chloride anions cause all of the inner protons expected to bind chloride to significantly shift downfield by great amounts (SI). For example, the proton Hc shifts downfield by the largest amount up to 2.21 ppm (Figure 3C and Table S1, SI). In addition, the chemical shifts of the protons of amides display slight variations upon the titrations of halide anions, indicating that the protons in the cavity primarily serve in binding the halide anions. In the case of bromide and iodide anion titrations, similar but weaker downfield shifts than those in the case of chloride were observed for all of the inner protons. The largest downfield shifts of the proton Hc are 2.06 and 1.70 ppm for Br^- and I^-, respectively (Figure 3C and SI). The binding stoichiometries for all anions were determined to be 1:1 by Job's plot (SI). The binding constants of **1** for Cl^-, Br^- and I^- anions were calculated on the basis of the NMR titrations by the WinEQNMR program[15] to be 757, 367, and 134 M^{-1}, respectively (Table S1, SI). The binding affinities (K) and largest changes in chemical shifts ($\Delta\lambda$) of Hc appear in the order of $Cl^- > Br^- > I^-$ as a result of the electrostatic nature of hydrogen-bonding interaction.

In the case of **2**, the representative NMR titration spectra are shown in Figure 3B. The tendencies in the variations of the chemical shifts of **2** are similar to those of **1** upon the addition of halide anions. The protons of triazoles significantly move downfield. For example, Hh shifts downfield by the amount of 1.80, 1.69, and 1.35 ppm for Cl^-, Br^-, and I^-, respectively (SI). The affinities of **2** for Cl^-, Br^- and I^- were extracted from NMR data to be 91, 109, and 86 M^{-1}, respectively (Table S1, SI). These values are much smaller than those in the case of **1** and appear in the order of $Br^- > Cl^- > I^-$ in the contrary to the foldamer **1**. The differences in the order presumably are caused by the flexible backbone of **2**, which is just right for Br^-. The largest difference in the affinity is found for the chloride anion up to about 8 times. Such differences in the affinities presumably originate from the extent of preorganization in the backbones of ary-triazole foldamers.

The halogen anion-binding properties of **1** and **2** prompted us to investigate the capacity of these molecules of transporting halogen anions across lipid bilayer. Thus, the Cl^- transport behaviors of the new aryltriazole foldamers **1** and **2** were first studied by imposing a Cl^- gradient across the lipid membrane of the large unilamellar vesicles (LUVs) made from the egg yolk L-α-phosphatidylcholine (EYPC). A suspension of vesicles entrapping the Cl^--sensitive dye lucigenin was added to a buffer containing KCl to produce a KCl gradient.[16] The transport of Cl^- into the vesicles was assessed by monitoring the fluorescence intensity of lucigenin. Adding **1** and **2** [molar ratio relative to lipid (x) = 0.75%] to the above vesicle suspension caused a small change (25%) in fluorescence

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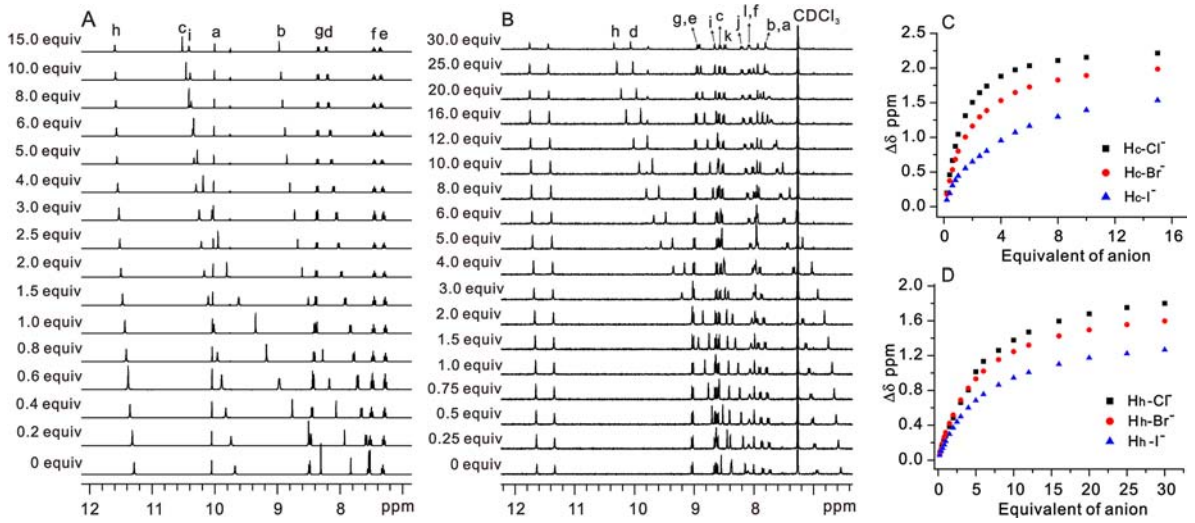

Figure 3. Changes in the ^{1}H chemical shift of **1** and **2**. (A) Partial ^{1}H NMR (298 K, 400 MHz) spectral changes of **1** in CD_2Cl_2 upon addition of TBACl. (B) Partial ^{1}H NMR spectral changes of **2** in $CDCl_3$ upon addition of TBACl. (C) Changes in ^{1}H chemical shift for the triazole (Hc) of **1** in CD_2Cl_2 with increasing concentrations of different anions. (D) Changes in ^{1}H chemical shift for the triazole (Hh) of **2** in $CDCl_3$ with increasing the various concentrations of different anions; [**1**] = [**2**] = 2 mM.

intensity (Figure 4a). However, adding their mixtures with valinomycin (0.002 mol % relative to lipid), an efficient K^+

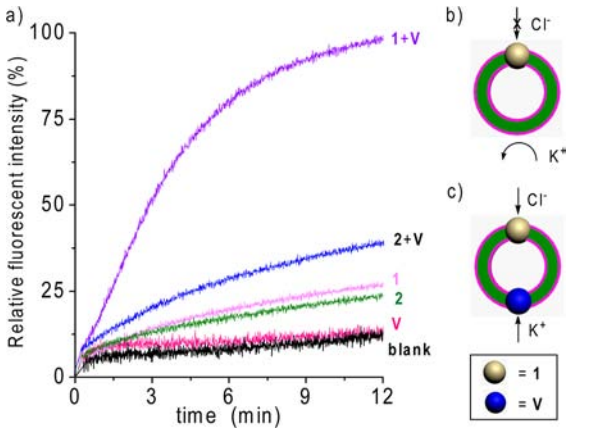

Figure 4. (a) Changes in fluorescence intensity (λ_{ex} = 372 nm, λ_{em} = 503 nm) of vesicles with time after addition of **1** or **2** (x = 0.75%) and/or valinomycin (x = 0.002%). The vesicles loaded with lucigenin (2.0 mM) buffered at pH 7.0 with HEPES (10 mM) and suspended in KCl (100 mM) solution (buffered to pH 7.0). (b, c) Schematic representation of blockage and permit transport of Cl^- in the absence and presence of valinomycin (V).

carrier,[17] caused the intensity to increase by 97% and 39%, respectively, within 12 min. In contrast, the same amount of valinomycin itself resulted in only an 11% increase in fluorescence intensity. These results support the conclusion that **1** and **2** can transport Cl^-. The transport ability can be obviously enhanced by valinomycin. The enhancement of **1** is more remarkable than that of **2**, demonstrating that **1** exhibits higher transport ability. This is not unexpected because **1** possesses higher affinity to Cl^- than **2** does. Considering that **1** and **2** have a similar backbone, it is reasonable to believe that this is a result of their differences in binding affinities to Cl^-, although the better binding does not always lead to effective transport, which is the so-called "Goldilocks principle". Such a phenomenon was observed in the Matile's system[13g] but not in the present case. Our results demonstrate that the affinities of the foldamers are just right for effective transport. The transport experiments of **1** and **2** for Br^- and I^- were also conducted by using the same methods. However, the transport was not measurable, since Br^- and I^- can pass through the lipid bilayer.

It has been established that ion transmembrane transport can be achieved via carrier[13b] or channel mechanism.[18] To further investigate the transport mechanism, Cl^- transport experiments were also carried out by using LUVs composed of EYPC and cholesterol (2:1 in molar ratio). It has been reported that cholesterol decreases the fluidity of a lipid bilayer[19] and that this would have a negative effect on the transport ability of a carrier,[20] which is largely dependent on movement within the bilayer. The Cl-transport activities of **1** and **2** in 2:1 EYPC:cholesterol vesicles show a moderate decrease in comparison with these in EYPC vesicles under the same conditions (see the SI), implying the mobile carrier mechanism. The patch clamp experiments on planar lipid bilayer were also performed. By premixing **1** or **2** with the lipids and painting to form a planar lipid bilayer, no conductance signal was observed, which further confirms the carrier mechanism.

To quantitatively measure the activity of carrier **1**, transport experiments were also carried out under different carrier concentrations. It was found that the transport ability, defined as the relative fluorescence intensity of lucigenin after 12 min of adding **1**, was strongly dependent on x. As x increased from 0 to 0.2%, the intensity increased significantly. Further increasing x caused only a slight increase in intensity (Figure 5). The effective concentration needed for achieving 50% (EC_{50}) transport ability and the Hill coefficient (n) were calculated to be 0.09% and 1.3,[21] respectively. Such a low EC_{50} indicates that the new foldamer is an efficient transporter for Cl^-. The Hill coefficient value closes to a unit, demonstrating that **1** transports Cl^- in a single-molecular-manner.[21]

In summary, the conformations of aryltriazole foldamers can be preorganized by the incorporation of hydrogen bonds at the periphery of foldamers, but the extent of preorganization is greatly dependent on the number of hydrogen bonds, which poses a great effect on the affinity of foldamer for anions. The

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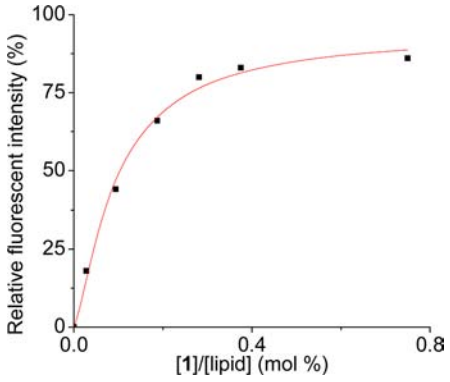

Figure 5. Changes in fluorescent intensity at t = 12 min with the molar ratios of **1** to lipid (x). The solid line represents the plot for fitting the data with the Hill equation.

full preorganization on the backbone of **1** significantly enhances its affinities for halogen anions, making it more effective to transport a chloride anion across a lipid bilayer. Further studies may include use of such a preorganized backbone as a platform for incorporation of a switch to control the capture and release of halogen anions.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra of **1** and **2** and X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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