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Conformational Control of Transmembrane Cl^- Transport

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Due to their biological importance, the complexation, sensing, and transport of anions are attracting increasing attention.[1,2] A variety of receptors based on the isophthalamide skeleton (e.g., **1** in Figure 1) have been studied, as these systems are easy to make and are effective anion receptors in organic solution.[3,4] Two strategies to improve the affinity and selectivity of anion receptors are to increase the acidity of hydrogen bond donors and/or to build more rigid scaffolds. The first strategy may, in some cases, result in deprotonation of the receptor by basic anions,[5] whereas the second strategy may require involved syntheses. We report here a host designed to have both enhanced hydrogen bond donor strength and conformational preorganization. Intramolecular hydrogen bonds make isophthalamide **2** a potent anion binder and an effective transmembrane transporter of Cl^-.

Experiment and calculations show that isophthalamides such as **1** prefer a *syn*−*anti* conformation about the 1,3-diamide unit.[6] As this conformation lacks convergent hydrogen bond donors, it is not optimal for anion binding. We reasoned that the −OH groups in **2** should form intramolecular hydrogen bonds with the amide carbonyls to stabilize the *syn*−*syn* conformation and favor the cleft needed for anion binding.[3] Compound **3** is a negative control, as its C4, C6-OMe groups should hydrogen bond with the N−H protons to stabilize the *anti*−*anti* conformation and preclude anion binding.

Figure 2 shows solid-state structures for **2** and **3**.[7] The majority of the molecules in the unit cell of **2** adopt a *syn*−*syn* conformation with intramolecular hydrogen bonds between the hydroxy and carbonyl groups (O···O 2.55−2.57 Å). In this structure, two molecules of **2** in the *syn*−*syn* conformation bind the carbonyl oxygens of a third molecule of the receptor (Supporting Information). Compound **3** exists only in the *anti*−*anti* conformation. The amide and methoxy substituents are coplanar and involved in intramolecular hydrogen bonds (N···O 2.67−2.68 Å). NMR data confirmed that these conformations for **2** and **3** also predominate in solution. In CD_3CN, the ^1H NMR resonance for the OH protons in **2** was far downfield (δ 13.50), consistent with involvement in hydrogen bonding. Moreover, the chemical shift for the aromatic hydrogen between the amide side chains moved progressively downfield (δ 7.80, 8.18, and 8.70) in going from **2** to **1** to **3**, reflecting an increasing preference for conformations wherein the hydrogen is *syn* to the deshielding carbonyls. The NMR signal for the N−H proton in dimethoxy **3** (δ 7.71) was shifted downfield relative to the N−H protons for **1** and **2** (δ 7.10), presumably due to involvement in intramolecular hydrogen bonds. This structural data indicated that **2** is predisposed toward a *syn*−*syn* orientation, whereas **3** favors an *anti*−*anti* orientation.

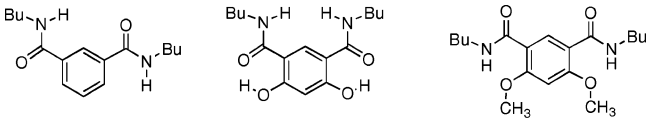

Figure 1. Isophthalamides **1**−**3** and their predominant conformations.

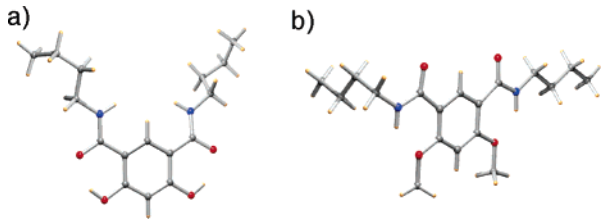

Figure 2. X-ray crystal structures of molecules present in the solid-state structures of (a) **2** and (b) **3**, showing the *syn*−*syn* and *anti*−*anti* conformation for the 1,3-diamide units.

Table 1. Association Constants K_a (M^{-1}) for **1** and **2** Binding Cl^-, Br^-. and I^- (n-Bu_4N^+ Salts) Measured at 298 K in CD_3CN (Errors <10%)

compound	Cl^-	Br^-	I^-
1	195	60	15
2	5230	716	152

Addition of Cl^-, Br^-, and I^- anions to **1** and **2** in CD_3CN resulted in downfield shifts of the N−H and H2 signals, whereas little change was observed for the O−H signal in **2**. These data suggest that **1** and **2** bind anions within the cleft defined by the 1,3-diamide and that the O−H groups in **2** do not interact directly with anions. Addition of Cl^-, Br^-, and I^- to **3** caused no changes in the ^1H NMR, indicating that **3** does not bind anions, presumably because its N−H protons are tied up in intramolecular hydrogen bonds.

Association constants for **1** and **2** toward Cl^-, Br^-, and I^- were determined by ^1H NMR titration experiments done in CD_3CN, using the EQNMR software to fit the curves to a 1:1 binding model (Table 1).[8] Preorganization of the amides in **2** with intramolecular OH−O═C H-bonds significantly improved the anion affinity for this receptor (5230 M^{-1} for Cl^-), relative to the unsubstituted isophthalamide **1** (195 M^{-1} for Cl^-). Furthermore, host **2** showed increased selectivity toward binding Cl^- over other halide anions.

We next investigated the transport of Cl^- across bilayer membranes by **1**−**3**, using an assay wherein a Cl^--sensitive dye was encapsulated within phospholipid liposomes. Figure 3 shows data for Cl^- transport across egg-yolk phosphatidylcholine (EYPC) liposomes in the presence of **1**−**3** (2 mol %) at 25 °C. Liposomes (100 nm) containing the fluorescent dye, lucigenin (1 mM),[9] were prepared in a solution of 100 mM $NaNO_3$, 10 mM sodium

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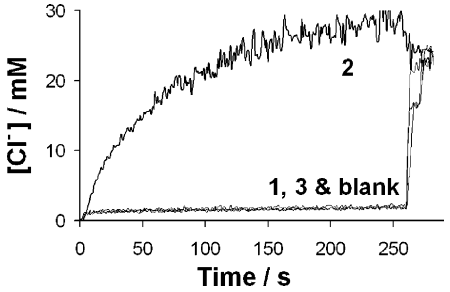

Figure 3. Chloride transport across EYPC liposomes containing lucigenin in a 100 mM $NaNO_3$/10 mM sodium phosphate buffer (pH 6.4). The Cl^- concentration was determined from lucigenin's fluorescence. Compounds **1**−**3** were added to give a 2:100 ligand/lipid ratio. At $t = 0$ s, NaCl was added to give an external Cl^- concentration of 25 mM. Lucigenin fluorescence was converted to Cl^- concentration using the Stern−Volmer constant determined under the assay conditions. The traces shown are the average of three trials.

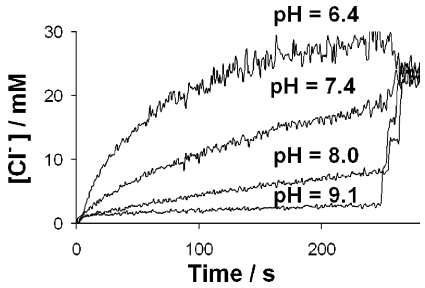

Figure 4. Transmembrane Cl^- transport by **2** as a function of pH. Experiments were done using EYPC liposomes with lucigenin (1 mM) in a 100 mM $NaNO_3$/10 mM sodium phosphate buffer at various pH. Compound **2** was added to give a 2:100 ligand/lipid ratio. At $t = 0$ s, NaCl solution was added to give an external Cl^- concentration of 25 mM. Lucigenin fluorescence was converted to Cl^- concentration using the Stern−Volmer constant determined under the assay conditions. All traces shown are the average of three trials.

phosphate (pH = 6.4). Isophthalamides **1**−**3** in DMSO were added to the liposome solution, followed by addition of NaCl to give an extravesicular [Cl^-] concentration of 25 mM. Movement of Cl^- into the EYPC liposomes quenched the lucigenin fluorescence. We found that **2** showed significant Cl^- transport activity into EYPC liposomes, whereas **1** and **3** were inactive.

It is not yet clear why **2** is such an effective Cl^- transporter, whereas unsubstituted isophthalamide **1** is inactive in transporting anions across phospholipid bilayers. Nonetheless, the putative involvement of the intramolecular OH−O═C hydrogen bond in anion binding and transport by **2** suggested to us that Cl^- carrier activity might be modulated by pH. We reasoned that deprotonation of one of the phenols in **2** should shift the conformational equilibrium about a single amide bond from *syn* to *anti* and thus impact Cl^- binding and transmembrane transport.

Figure 4 shows the efficiency of Cl^- transport as meditated by **2** across EYPC liposomes as a function of extravesicular pH. Chloride transport by **2** decreases with increasing pH, being essentially nil at pH = 9.1. This systematic decrease in transport activity with increasing pH is consistent with deprotonation of a phenol whose pK_a is near 8.[10] Transmembrane transport of Cl^- by **2** can clearly be modulated by controlling external pH.

In conclusion, preorganization of a structurally simple receptor with intramolecular hydrogen bonds enhanced both the affinity and selectivity of anion binding. Remarkably, the 4,6-dihydroxyisophthalamide **2** is also a potent transmembrane Cl^- transporter, whose function can be readily controlled by pH. We are currently trying to delineate the structure−function relationships that make this simple isophthalamide **2** such an effective membrane transport agent for chloride anion.

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Supporting Information Available: Experimental procedures, crystallographic data, and details of anion binding experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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