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Synthetic chloride transporters with the binding mode observed in a ClC chloride channel†

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A series of synthetic molecules bearing the same hydrogen bonding mode observed in StClC were prepared and their transport ability of chloride ion across a lipid membrane was systematically optimized.

The transmembrane transport of anions across biological membranes is a crucial process to regulate ionic concentrations, maintain cellular volume and pH, transmit electrical signals, *etc*.[1] Dysfunction of anion transport, *e.g*. chloride ion, is therefore associated with serious diseases, such as cystic fibrosis and Bartter's syndrome.[2] There are two distinct pathways, transmembrane channels and mobile carriers, for anions to pass through the lipid membrane. Much effort has been devoted to the synthesis and characterization of small synthetic molecules capable of forming anion channels or functioning as anion carriers,[1,3] based on a variety of molecular scaffolds, such as oligoamides,[4] electron-deficient aryls,[5] steroids,[6] macrocycles,[7] acyclic and cyclic pyrroles,[8] and others.[9] The development of synthetic anion transporters may provide us with an opportunity help to gain insight into the underlying principles of anion transport in biological systems and to find suitable compounds for biomedical applications.

In 2002, MacKinnon and coworkers reported the crystal structure of a ClC chloride channel from *S. typhimurium* (StClC) wherein chloride ion was held by four hydrogen bonds; two with main-chain amide NHs (Ile 356 and Phe 357) and two with side-chain OHs (Ser 107 and Tyr 445) (Fig. 1).[10] Stabilized further by helix dipole interactions, the chloride ion was surrounded by hydrophobic side chains of amino acids. By mimicking the hydrogen-binding mode observed here, we have prepared a series of anion receptors **4a–4h** with different side chains, each of which possesses a well-defined cavity with convergent urea and hydroxyl groups. The receptors strongly bind chloride ion by four hydrogen bonds like in StClC, as unambiguously determined by the crystal structure of a complex between **4f** and tetrabutylammonium chloride (TBACl). In particular, transport experiments reveal that **4h** greatly facilitates the transport of chloride ion across a lipid (POPC) bilayer membrane.

In the design of synthetic molecules capable of transporting anions across a lipid membrane should be considered several factors, including the binding affinity with a target anion, lipophilicity and hydrophilicity for effective partitioning in the lipid-water interphase, and mobility inside the lipid bilayer. Despite much progress for the past several years, the rational design of synthetic anion transporter still remains highly challenging. Our approach therefore relies on the systematic modification of the side chains (R^1, R^2 and R^3) of an initially designed molecule **4a**. Syntheses are outlined in Scheme 1. Ureas **3a–3h** were synthesized by iodination of appropriate anilines,[11] followed by the treatment with triphosgene.[12] Then, Sonogashira reaction[13] of **3a–3h** with the corresponding alcohols gave compounds **4a–4h**.

The binding mode of these receptors with chloride ion was first confirmed by the X-ray analysis. Single crystals of complex **4f**·TBACl were obtained by slow diffusion of heptane into a toluene/CH_2Cl_2 solution containing **4f** and excess TBACl. As anticipated, the chloride ion is held by four hydrogen bonds, two with urea NHs and two with terminal OHs (Fig. 2). Two phenyl rings in the complex are significantly tilted, and a dihedral angle is approximately 66° between two phenyl planes. As the result, two hydroxyls are located back and forth to create a three-dimensional cavity wherein the chloride ion is completely entrapped with hydrogen bond

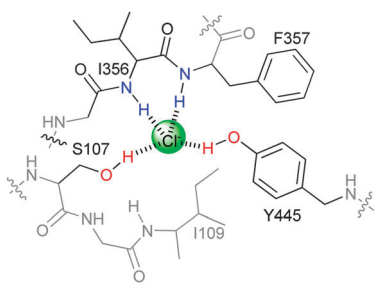

Fig. 1 Schematic representation of the hydrogen-bonding mode observed in the X-ray structure of StClC chloride channel.[10]

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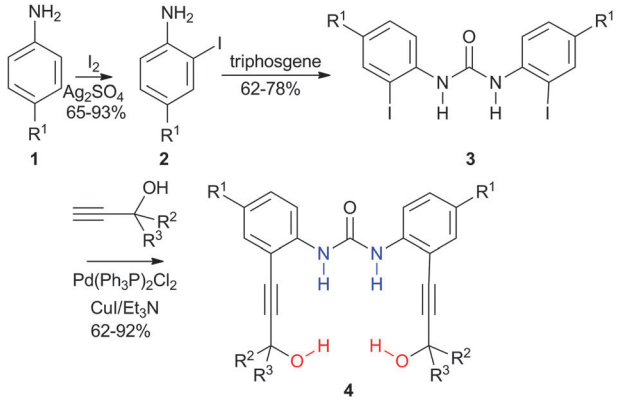

Scheme 1 Syntheses of receptors **4a–4h**.

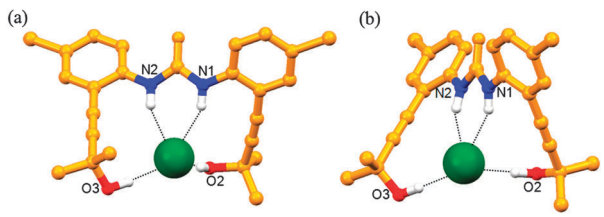

Fig. 2 (a) Front view and (b) side view of the crystal structure of **4f** with tetrabutylammonium chloride. The CH hydrogen atoms and tetrabutylammonium are omitted for clarity, and hydrogen bonds are shown as dashed lines.

distances of 3.32–3.36 Å for N···Cl and 3.12–3.22 Å for O···Cl (see ESI†, Table S2).

More quantitative information on binding between anion receptors **4a–4h** and chloride ion were found in ^{1}H NMR spectroscopy in 1% (v/v) H_2O/CD_3CN. Upon addition of TBACl, the NH and OH signals of **4a–4h** were largely shifted downfield by $\Delta\delta$ = 0.83–0.95 and 0.92–1.06 ppm, respectively, indicative of the formation of strong hydrogen bonds. Based on changes in the chemical shifts of these protons, association constants were determined by nonlinear squares fitting analysis,[14] and the results are summarized in Table 1. Two trends are apparent. First, the magnitudes of the association constants depend on the nature of substituents (R^1) on the phenyl ring. As expected, electron-withdrawing substituents such as ester and chloro groups enhance the association constant because of the increased hydrogen-bonding ability of urea NHs by direct resonance. Second, the binding affinities are essentially identical, regardless of the chain length of esters

Table 1 Association constants (K_a ± 12%, M^{-1}) and Gibbs energy (kcal mol^{-1}) of complex for **4a–4h** with tetrabutylammonium chloride in 1% (v/v) H_2O/CD_3CN at 24 ± 1 °C

Entry	Receptor	K_a (M^{-1})	ΔG (kcal mol^{-1})
1	**4a**	5100	−5.05
2	**4b**	8500	−5.35
3	**4c**	8000	−5.32
4	**4d**	9200	−5.40
5	**4e**	8700	−5.37
6	**4f**	17 000	−5.76
7	**4g**	13 000	−5.60
8	**4h**	17 000	−5.76

(entry 2–5) and the steric bulkiness of substitents (R^2 and R^3) at the propargylic positions (entry 6–8).

Next, the chloride transport abilities of **4a–4h** across a lipid bilayer membrane were compared by a fluorescence assay in large unilamellar vesicles (LUVs) comprising 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC).[15] The LUVs were loaded with $NaNO_3$ (200 mM) and a chloride-sensitive dye lucigenin (1 mM), and suspended in a phosphate buffer solution (pH = 7.2) containing $NaNO_3$ (200 mM) and NaCl (30 mM). The flow of chloride ion into the vesicle was monitored by addition of a DMSO solution of **4a–4h** (1 mol% relative to POPC), based on the gradual decrease of fluorescence intensity of the internal lucigenin. After 400 s, detergent was added to lyse the vesicles and final fluorescence intensity was set as I_f. The results were shown in Fig. 3.

The activity of the chloride transport is negligible without any anion receptor or in the presence of an initially prepared compound **4a** with R^1 = *t*-butyl. We first attempted to optimize the transport activity using a series of the esters **4b–4e** which bear different types of alkyl groups; straight (butyl, octyl), a branched (dimethyloctyl), and a more hydrophilic chain (butoxyethoxyethyl). Although there were small differences in the transport efficiency between the esters, the improvement was not satisfactory at all. Then, the ester functionality was replaced by chloro substituent to give compound **4f** which delightfully showed noticeable improvement of the transport activity. In recent years, Gale *et. al.* demonstrated nicely that the fluorinated substituents (*e.g.* CF_3) greatly increased the transport ability of an anion receptor

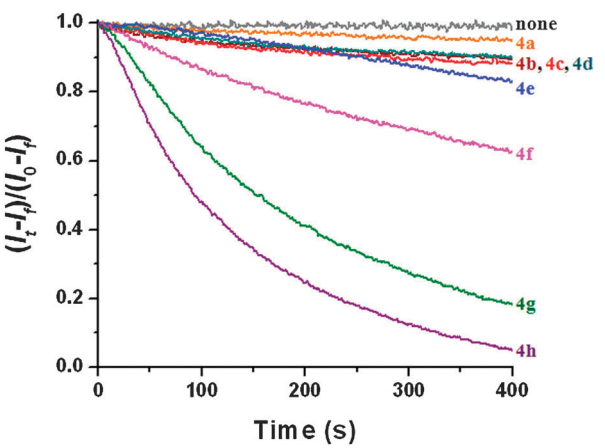

Fig. 3 Chloride influx through vesicles containing 200 mM $NaNO_3$, 1 mM lucigenin and 10 mM phosphate buffer pH 7.2.

across lipid membranes.[16] Further dramatic improvement was observed when dimethyl side chains (R^2 and R^3) were changed to more lipophilic groups, diethyl (**4g**) and methyl isobutyl unit (**4h**). The binding affinities of chloro substituents **4f–4h** are essentially identical, and therefore the large enhancement of the transport activity of **4g** and **4h** is possibly attributed to the increased lipophilicity and mobility; that is, longer alkyl chains such as isobutyl may wrap around chloride ion more completely, thus facilitating the diffusion of the complex in the lipid membrane.[17]

To reveal the transport mechanism, the transport experiment was also carried out under identical conditions except using vesicles loaded with Na_2SO_4, instead of $NaNO_3$. Even compound **4h** showed no activity of transporting chloride ion into the vesicles containing Na_2SO_4 (ESI†, Fig. S10), implying that the transport occurs by Cl^-/NO_3^- exchange mechanism. In addition, no difference in the transport rate was found when potassium chloride was used instead of sodium chloride (see ESI†, Fig. S11), indicative of an antiport mechanism. Finally, the transport rate of chloride ion was found to be linearly proportional to the concentration of **4h** (see ESI†, Fig. S12 and S13), suggesting that **4h** function as a molecular carrier for chloride ion, not forming a channel.

In conclusion, we have demonstrated that the chloride transport across a lipid membrane can be drastically improved by the systematic modification of synthetic receptors with the bonding motif observed in a ClC chloride channel. In particular, the chloro substituent greatly enhances the efficiency of the chloride transport, which can be further improved by the incorporation of longer alkyl chains around the binding site possibly due to the increased lipophilicity and mobility of the chloride complex in the lipid membrane.

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